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VALUE ADDITION OF LEATHERS FROM ETHIOPIAN CATTLE HIDES: STRATEGY FOR QUALITY IMPROVEMENT

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Although the raw cow hide supplies for tanners are not small, producing quality leather is difficult, which can compete in international market. From the total export of finished leather in the year 2011/2012 only 10.7% is the share for finished cow leathers. Main types of problem that alter production of quality cow leather are both anti- mortem defects (scratches, rub mark, or horn rake pox, brand mark, wound etc) and post-mortem defects (fly cuts, fleshing cuts and putrification) that degrade the value. Surface defects mainly scratches and fly cuts largely and wound in certain extent are major drawbacks for not having quality product. To have an insight about Ethiopian cow hide raw material and to standardize a process technology both chemical characterization such as hydroxyproline content, fat content, nitrogen content, moisture content and chromic oxide content and physical characterization such as tensile strength, tear strength, % elongation, grain crackiness and rub fastness were carried out. Also histology of the raw material, Scanning Electron Microscope (SEM) analysis at crust stage, hair pore count using stereo-microscope of the crust and organoleptic properties at crust stage and after finishing were carried out in comparison with Indian cow leather.

A newer approach of drum up-gradation using pigment and protein filler and modern type of finishing i.e. cationic compact finish were used to achieve the overall goal of producing quality leather. The results from the physical tests revels that grain crackiness of the experimental leathers is not affected by the use of pigment and filler in drum. The experimental leather showed better defect coverage and had better organoleptic properties. In the present study, promising results were found that can be used to add value to Ethiopian cow upper leathers to be salable in the world market.

Key Words: Hide, leather, Organoleptic properties, Histology, Scanning Electron Microscope.

1.   INTRODUCTION
Ethiopia is one of the leading countries that have the largest livestock populations in the world providing a strong raw material base for the leather industry. Its livestock population is estimated at 50 million cattle, 25 million sheep and 23 million goats. About 80% of all hides and skins entering the formal market come from rural areas where they are collected by private traders. The remaining 20% are derived from slaughtering facilities found in major town and cities. About 15.5 million pieces of sheep and goat skins and 1.2 million pieces of cattle hides are supplied to the tanneries per annum (FAO, 2011).

Leather sector is among the one that deserves a particular emphasis in Ethiopia and identified as a potentially competitive in the global market. The five year growth and transformation plan (GTP-I)aims at raising the earnings from the sector US$ 76 million end of 2009/10 to US$ 497 million in 2014/15 (Ministry of finance and economic development, Addis Ababa, November 2010.)

In the case of cow hide finished leather many quality issues are bottle necks not to sell in the world market. Due to quality problem (defects, like wound, insect bite, fly cut and processing defect) most tanneries sell for local shoe manufacturing company in contrary with the aim of earning foreign currency from export(Urgessa, 2013).

2.   MATERIALS AND METHODOLOGY

The materials used for this research works are wet-salted cow hides (both Ethiopian and Indian), different leather chemicals from beam-house to crusting(Annex 1 &2) and finishing chemicals ,leather machineries like drum, fleshing machine, samming machine ,shaving machine setting out machine, overhead drier, stacking machine, hand spray-booth and Embossing machine, and different instruments like scanning electron microscope, kjeldhal apparatus, fume hood, soxlet apparatus, uv-vis spectroscopy and different laboratory chemicals like, sulphuric acid, nitric acid, diethyl ether, hydrochloric acid.

The methodologies of the research are summarized as bellow:-

Wet salted raw hide

Soaking

- Histology
- Fat content
- Nitrogen

Limed pelt

- De-liming
- Bating
- Degreasing
- Pickling

Tanning (Wet- blue leather)

- Chrome content

Post-tanning (re-tanning, dyeing, fatliqouring)

- Defect coverage analysis
- Physical testing(strength analysis)
- SEM analysis

Finishing (Finished Leather)

- Defect coverage analysis
- Physical test (rub fastness)
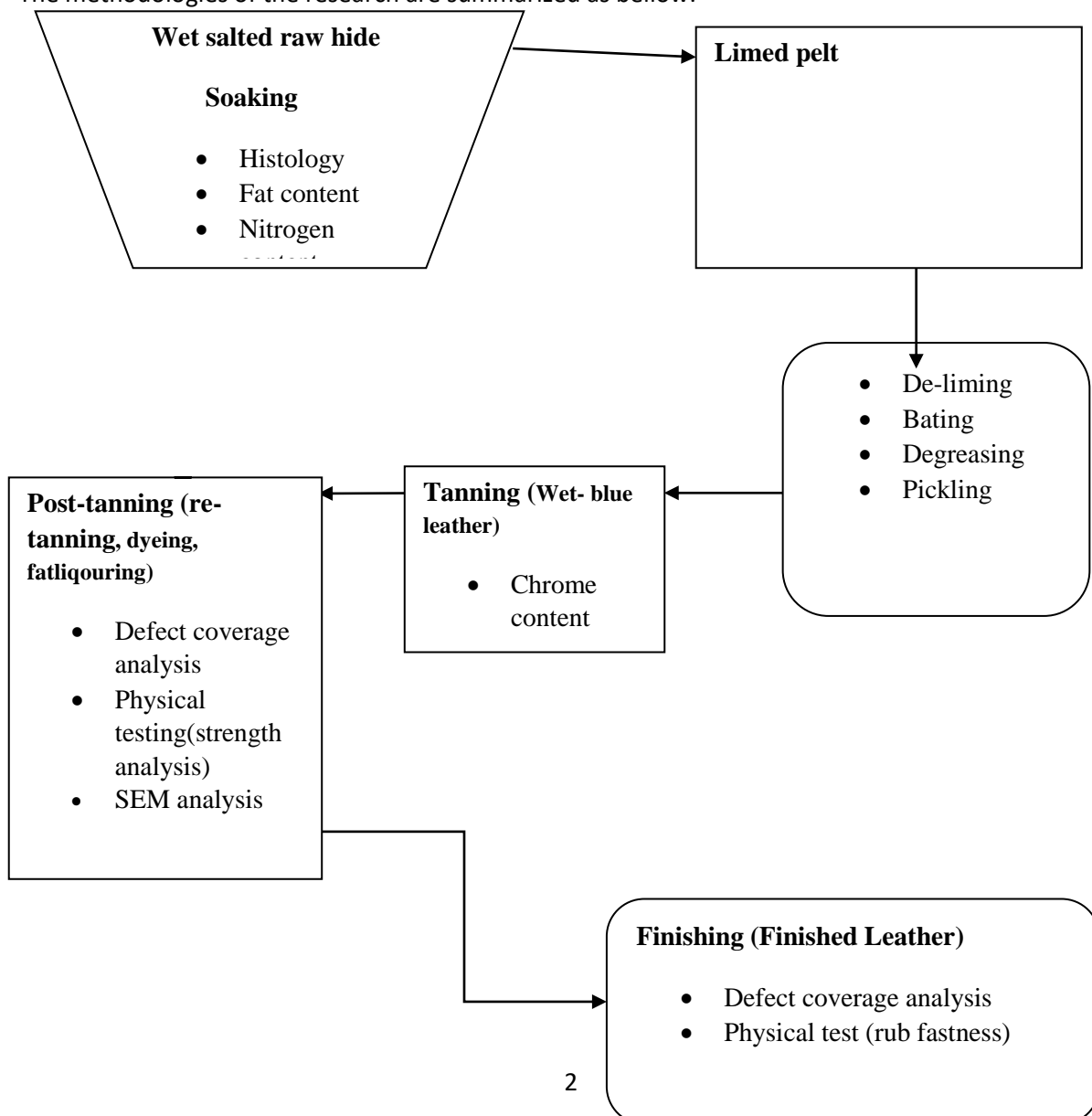
3.   RESULTS AND DISCUSSION

3.1 Determination of Hydroxyproline in raw stage of both Ethiopian and Indian cow hides

20 mg of both Ethiopian and Indian wet salted cow hides were weighed after main soaking from butt, neck and belly area, and the hair was carefully removed by blade before weighing and the experiment was conducted based on the procedure mentioned earlier. The standard graph was prepared using 10mg/100mL stock solution. Different concentrations of hydroxyproline used and the respective OD (optical density or absorbance) values at 557 nm are provided in Table 1.

Table-1 concentration of hydroxyproline in the solution.

µg/1000µl	OD (optical density or Absorbance) Value
2	0.114
4	0.241
6	0.432
8	0.576
10	0.695
12	0.856
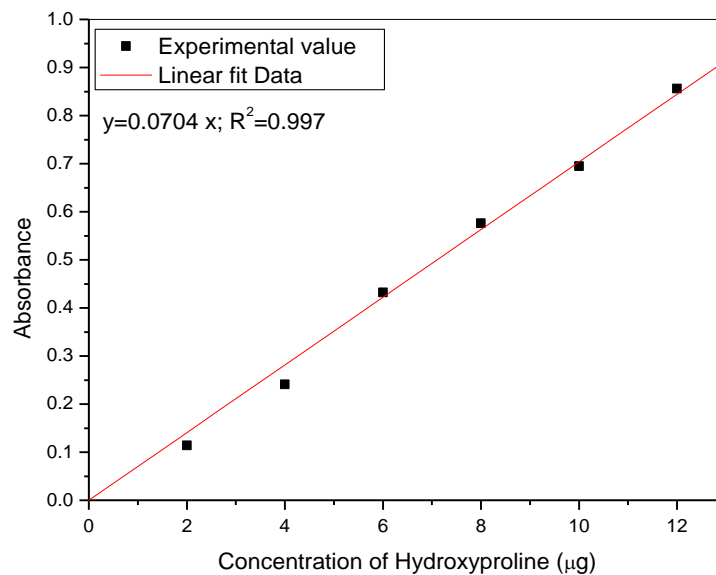
The calibration/standard graph for determining the hydroxyproline content in the hide sample is provided in Fig1.

Fig 1: Calibiration/standard graph, hydroxyproline content determination

Table 2: Concentration of Hydroxyprolin in mg/mg of dry tissue.

Samples	% moisture	Dry weight of the hides (mg)	OD(optical density or Absorbance) at 557 nm	Hydroxyproline concentration (mg/mg of dry hide)	Collagen content (%)
E butt	51.68	9.664	0.177	0.063	47.3
E neck	51.68	9.664	0.153	0.055	41.5
E belly	51.68	9.664	0.132	0.047	35
I butt	52.34	9.532	0.136	0.048	36.4
I neck	52.34	9.532	0.096	0.034	26
I belly	52.34	9.532	0.112	0.04	30.2

Where E-stands for Ethiopia and I-for India

It could be observed from the table that the average hydroxyproline content of the Ethiopian cow hides, taking in to account the values from neck, belly and butt was **0.055mg** of hydroxyproline per mg of hides taken (dry weight). Similarly, the average hydroxyproline content of the Indian cow hides, taking in to account the values from neck, belly and butt was **0.041mg** of hydroxyproline per mg of hides taken (dry weight). Thus, it could be seen that the collagen content (hypo conc. multiply by 7.54) of Ethiopian cow hide (41.5%) is higher than that of the counterpart Indian cow hide (30.7%) and it is possible to conclude that the former has more reactive site than the later one.

3.2 Nitrogen percentage estimation of Ethiopian and Indian raw cow hides

0.5 gm of both Ethiopian and Indian hides was cut for the determination of nitrogen content after main soaking and removing the hair by blade carefully. The nitrogen content of both raw hide samples was determined using the procedure mentioned before and the following average results were found.

$$\text{Nitrogen,} \%\text{Nitrogen} = \frac{14.01 x 0.1 N x (Tv - Bv) x 100}{W X 1000}$$

Where TV- Titrant volume (volume of HCl consumed by the sample solution)
BV-blank volume (volume of HCl consumed by blank solution)
W- Weight of the sample before digestion.

Table 3: volume of HCl used for titration and %N of raw hides

S. No.	Sample	Dry weight of the sample(g)	Titrant volume (TV) in ml	Nitrogen (%)
1	E butt	0.2416	33.1	18.61
2	E belly	0.2416	29	16.24
3	E neck	0.2416	25.6	14.27
4	I butt	0.2383	33	18.81
5	I belly	0.2383	26.5	14.99
6	I neck	0.2383	25.4	14.35

Where-E-stands for Ethiopia and I-stands for India

It could be observed from the table that the average % nitrogen content of the Ethiopian cow hides, taking in to account the values from neck, belly and butt was 16.37% (dry weight). Similarly, the average % nitrogen content of the Indian cow hides, taking in to account the values from neck, belly and butt was 16.05% (dry weight). Thus, it could be seen that the hide substance (%N multiplied by 5.62 as conversion factor) content of Ethiopian and Indian cow hide is 92 and 90.2%, respectively. From the calculations, it could be inferred that increased hide substance value in Ethiopian cow hides reveals that it has more protein fiber content than that of Indian cow hides, which is in concordance with the earlier collagen content results.

3.3 Fat Content Determination of the Raw Material

5g of both Ethiopian and Indian raw hide from butt portion respectively were taken after carefully removing the hair by blade. Using soxhlet apparatus the sample was allowed for the extraction of fats by using petroleum ether as a solvent for 5-hour at 70oC, the experiment was done in triplicate.

The weight of empty round bottom flask was measured and recorded, after extraction and fully evaporation of the solvent the weight was recorded and the following result were found.

$$\% \text{ fat} = \frac{weight\ of\ fat}{dry\ weight\ of\ the\ sample} X100$$

Table 4: fat content of raw hides

Sample name	Dry weight of the samples(g)	% of fats
Ethiopian raw hide	2.416	3.146
Indian raw hide	2.383	2.291

From the result of % fat it is clear that Ethiopian hide has more amount of fat content than the counterpart of Indian cow hides. Hence, it is required to do proper degreasing before tanning. Degreasing becomes a critical operation to have optimum penetration of post tanning chemicals so as to gain good finished product, which can fulfill buyer's requirement.

3.4 Process recipe from raw to dyed crust, Experiment 1

When the hide is processed in to leather, the subsequent removal of non-collagen components may create empty space or voids in the structure between fibrous layers, particularly between the grain and corium. These voids or open spaces may be exacerbated or enlarged by a number of leather processing factors such as bacterial degradation in the raw stack; excessive opening up in the beam-house and mechanical stressing this can produce a very loose structure called **Looseness(Cheng – Kung Liu-2009)**.

To assess whether Ethiopian cow hide has the problem of looseness or not Ethiopian wet salted raw hide and Indian wet salted raw hide were processed as upper crust employing conventional process (**Annex 1**). This trail was performed by skipping degreasing process in-order to see the effect of fat on the quality of wet blue as well it's consequence on post tanning process. An assessment on the wet blue leather shows that pink colors was developed due to reaction between fat and chrome salt. The crust also suffers with improper dye uniformity and less substance due to lack of penetration of

re-tanning material because of the presence of fat. The organoleptic study reveals that no looseness found on the crust from Ethiopian hide.

3.5 Organoleptic properties

The properties of the cow dyed crust leather, such as general appearance, grain tightness, smoothness, fullness, softness, roundness and the uniformity of colors were assessed by experienced experts. The grade of 0-10 was fixed for each properties and the result are provided in Fig 2.

Fig 2   Organoleptic properties of both Indian and Ethiopian dyed crust
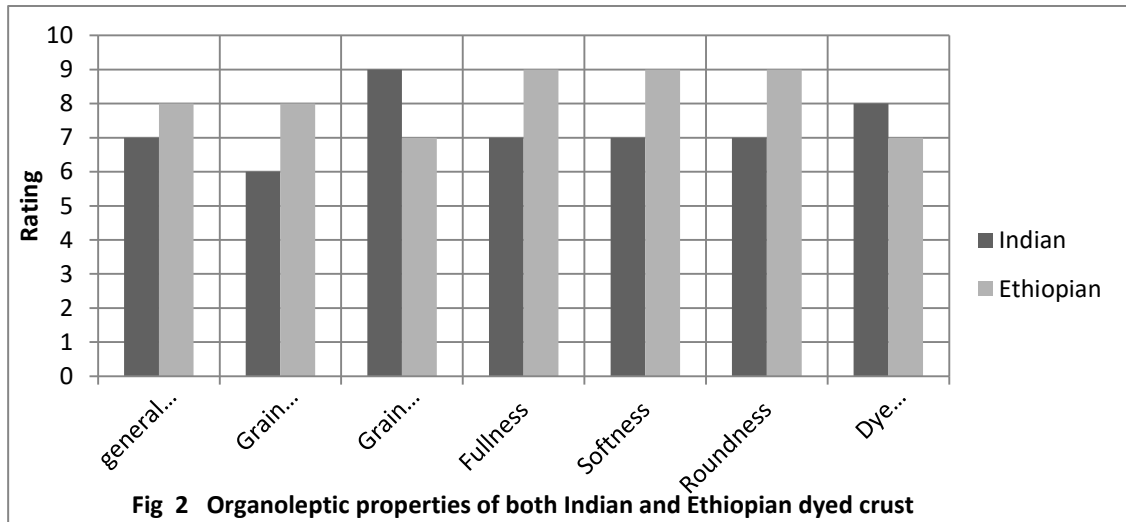
During comparison of the organoleptic properties for both crusts of Indian and Ethiopian cow leathers, dye uniformity is less for Ethiopian crust; this is mainly due to absence of degreasing in this trial. To overcome the problem, an experiment (**Experiment 2**) involving degreasing was suggested (**Annex-2**). In the case of grain smoothness it is better for Indian cow leathers owing to fine and more number of hair pore, which gives smooth grain, however Ethiopian cow has less number of hair pore, which results in less smoother surface when compared to the counterpart. In contrary, other properties for Indian crust are less mainly due to poor substance raw material. For Indian raw hide to be poor in substance different reason may be raised such as breed, climate, age etc.

3.6 Grain surface pattern study

In-order to study the surface finesse or coarseness of the Hide, grain surface pattern was studied using stereo microscope. Samples were cut from butt portion of both Ethiopian and Indian crust leather. The grain surface pattern for the crust from experiment-1 was observed through stereo microscope, 40 X magnifications were used, and the following result was found.
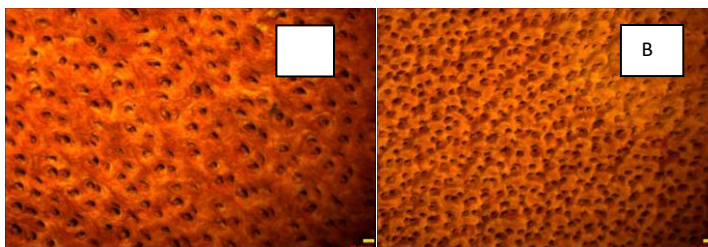

Fig. 3: Microscopic image of crust, Ethiopian (A) and Indian (B)

Dyed crust leathers were observed under the microscope and the number of hair pores was counted at three place and the average results are provided in Table. It could be observed that the Indian cow hides have more hair pores than the Ethiopian cow hides.

Table 5: Hair pore count

Crust leather	Average hair pore per sq.inch
Ethiopian crust	10,533+2
Indian crust	26,784+1

The number of hair pore for Ethiopian crust is lower than that of Indian per sq.inch indicating that it has not so smooth grain compared with Indian. From this result the type of choose-able product that can be made out of our raw material is upper and leather goods.

3.7 Histological Examination of the Hide at Raw stage

Samples for histological analysis were cut from butt portion of both Ethiopian and Indian raw hide after soaking and liming stage before fleshing. Hair is carefully removed by blade from raw sample, Samples before staining were preserved using 10% formalin solution. H and E (hematoxylin and Eosin) staining was done at Indian veterinary college and the following microscopic image was presented.

Ethiopian (C) and Indian (D) **raw hide**, **Entire cross section,** thickness of the slice sample was 25micron and the image was focused with 40x magnification of light microscope.
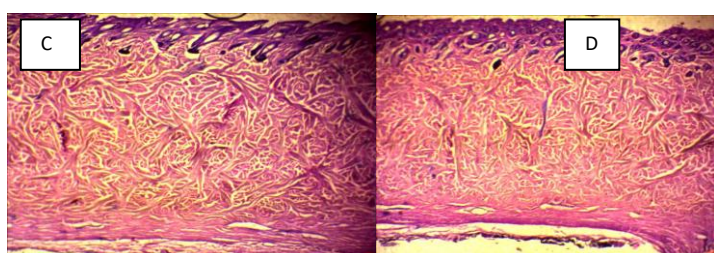
Fig-4: histological image of raw, entire cross section

The thickness for grain, corium and flesh were measured at three different region of the histological image using paint software and average of the three results were taken and converted to mm unit

Table-6-thickness of the grain, corium and flesh as well as grain –corium ratio of both Ethiopian and Indian hide at raw stage

Sample origin	Grain thickness (mm)	Corium thickness (mm)	Flesh thickness (mm)	Total thickness(mm)	Grain to corium ratio
Ethiopian raw hide	0.41	1.87	0.41	2.68	0.22
Indian raw hide	0.42	1.69	0.44	2.54	0.25

3.8 Surface image of the crust using SEM

Surface Image of crust leather at butt portion was observed using scanning Electron microscope using 100 x magnifications, Ethiopian (K) and Indian (S).
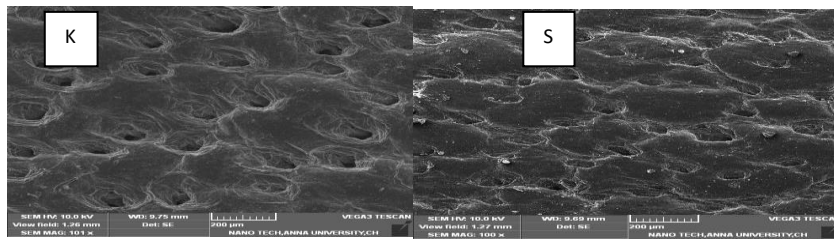
Fig-5: SEM image, crust surface

The surface image of the crust clearly shows that surface of the crust leather for Ethiopian has less number of hair pores and it seems course surface, whereas Indian crust has more number of hair pores and fine surface. The result of SEM complies with the result of grain surface pattern of stereo microscope study.

3.9 Process Recipe from Raw to Crust, Experiment-2

After a clear idea was developed about the nature of Ethiopian cow hide from the previous study in comparison with the counterpart Indian cow hide at raw, lime, wet-blue and crust stage it was arrived at to a conclusion that the main problem, which should be address is avoiding surface blemishes to upgrade the finished leather.

For the idea of upgrading the leather starting from the crusting stage cationic liquid pigment (2k black) and wet end protein filler (sellcomfill) were used to make upper leather ("http://sellamchemicals.in/,").

1% pigment (2k black) + 1% filler(sellcomfill) were used after fixation of all re-tanning chemicals before cationic topping for the experimental leather. To assess the degree of surface defect coverage for the experimental crust leather, samples were cut from butt portion of both experimental and control crust and observed using stereo microscope and the micrograph were presented as follow:-
Microscopic image for the control crust (P) in comparison with the experimental (Q), with 2x10 magnifications.
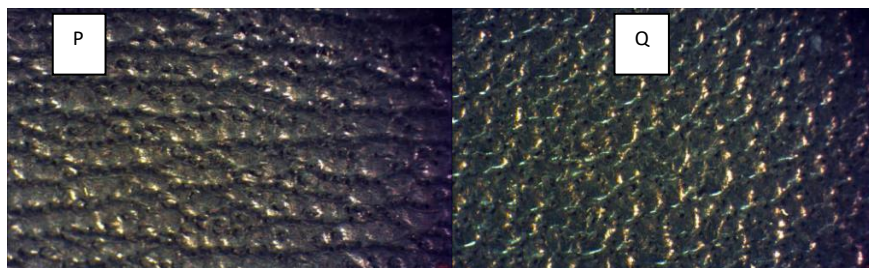

Fig-6: surface image of crust using stereo-microscope, control (P) and experimental (Q)

The micrograph image shows that a better coating of the surface was achieved in the case of experimental crust leather due to filler wax and pigment resulting in better defect coverage.

3.10 Organoleptic study for the crust from experiment-2

8

The following properties are assessed by experienced experts for the dyed crust.

Fig -7 : organoleptic properties for the dyed crust from experiment -2
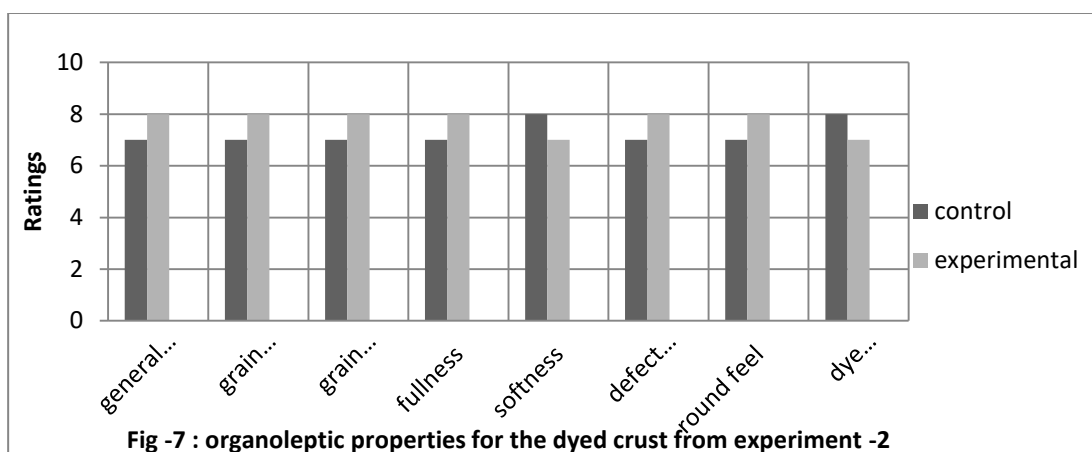
3.11 Grain crackiness test results

Dyed crust from trail-2, both experimental and control as well Indian crust were tested for the grain crackiness and the following results were found.

Table7: physical test result for the crust leather.

Sample ID	Load(kg)	Distance(mm)
Ec1	42	6.08
Ec2	23	5.2
Ec3	30	5.9
means	**31.67**	**5.73**
Eexp1	19	5.44
Eexp2	42	7.34
Eexp3	28	6.10
means	**29.67**	**6.3**
Ic1	22	5.27
Ic2	24	5.53
Ic3	26	5.27
means	**24**	**5.36**

The physical test result of ball burst (grain crackiness) shows that the chemicals used to avoid surface blemishes have not adverse effect on the grain strength of the experimental crust leather.

Where: - Ec- Ethiopian crust control

Eexp-Ethiopian crust experimental

Ic- Indian crust conventional process

3.12 Finishing formulation for the dyed crust, Experiment-2

Both experimental and control crusts are finished using **cationic compact resin based** from **Sellam chemical company ("http://sellamchemicals.in/,")**and the formulations are as follow:-

Table 8: cationic compact resin based finishing formulation

Chemicals	Solutions(in ml)						processes
	1	2	3	4	5	6	
1. Clearing coat							2x spray

Anionic dye solution	50		30			Allow to dry
Water	900	350	540	495	895	
IPA	50	50	30			
2. Cationic base coat						3xspray,Roller haircell@100°c and 120 pressure
SELCOM MK 6757		500				
CATIONIC PIGMENT		100				
3. Anionic season						2xspray, plate Haircell@80°c,150 pressure
SELLCOM CA 1722			300			
ANIONIC PIGMENT			100			
4. Fixing						2xspray,Sandblast plate @80°c,100pressure
MEDIUM SHINE LACQOR				200		
MATT LACQOR				300		
CROSS-LACQOR				5		
5. Top lacquer						1x spray plain plate @80°c,100pressure
SELKHEM KW 0555					100	
SENSOL SW					5	

- SELCOM MK 6757 - cationic compact resin
- SELCOM CA 1722 - anionic compact resin
- SELKHEM KW 0555 -cationic wax
- SENSOL SW- silicon Wax

3.13 Organoleptic properties for the finished leather

Experienced experts were assesed physically the following properties for both control and experimental finished leather. The average results were found and presented as follow:-

Fig-10: Organoleptic properties for finished leather from experiment-2
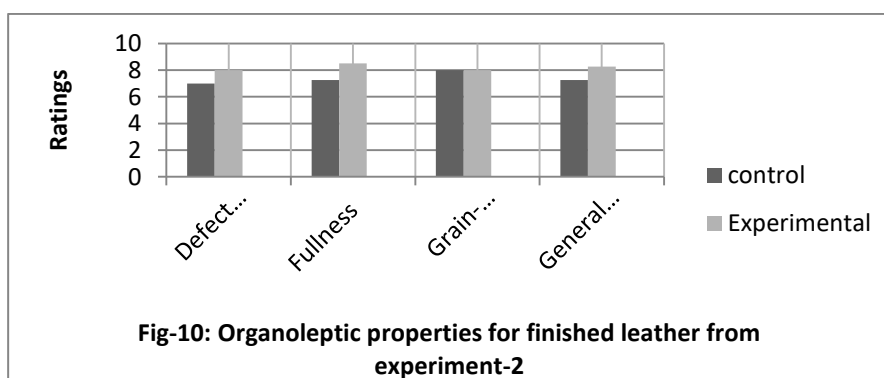
The major target of covering the defect as well as fullness and general appearance of the experimental finished leather was improved well compared to the control one with less number of coatings. Such types of finishes are viable for upgrading lower ends with keeping the natural look of the leather.

4.   Conclusion

Value addition on finished leathers from Ethiopian cow hides in terms of upgrading lower ends is possible. The chemical and physical characterization as well as study of the surface of Ethiopian cow hide leather found that it has coarse surface with less number of hair root that makes the material good for upper leather than clothing leather compared to Indian cow hide.

In the present project, drum based up-gradation of the cow leathers have been established. Drum based pigments and fillers have been used during the post tanning stage and the leathers have been upgraded. Also, cationic compact resin based finishing techniques have been used in order to have upgraded full grain finished leathers. This cationic finishing system provides good selection with least number of coating and easy unit operations for the finishing technician.

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6.   Annexes

6.1 Annex 1 Process recipe from raw to dyed crust, Experiment-1

Two sides, one from Ethiopian (R) and one from Indian(R) wet salted raw cow hide was processed and the recipe was as follow:-

Soaking, liming and re-liming are done using pits.

process	Percentage	Chemicals	duration	parameter
soaking	up- to covering	Water		Hand check for full

	the hide			wetting of the hide in
	0.5	preservative	2-days	the neck and butt
	0.5	Wetting agent		portion.
Drain/ wash				
Liming	200	Water		
	10	Lime	1-day	
	3	Sodium-sulphide		
Un-hairing was done using beam				
Reliming	1	Lime	2-days	Checking the plumpness by hand
Drain/wash				
Fleshing, using fleshing machine and scudding by manually using knife.				
De-liming	100	Water	1.5 hour	Check cross-section for completion of de-liming using phenolphthalein indicator, colorless indicates complete de-liming.
	6	Ammonium chloride		
Drain /wash				
Bating	100	Water		Check completion by thumb test
	1	Alkali bate	1-hour	
Drain/wash				
Pickling	100	Water		
	10	Salt	20-minute	Baume check,6-7
	0.5	Formic acid (on to 5 dilution)	20-minute	
	0.7	Sulphuric- (one to ten dilution)	3x20-minute	PH=2.5-2.8
Leave overnight in the pickle bath, morning run 10-minute ,half drain and tanning started				
Tanning	50	Pickle bath		
	4	Basic chromium sulphate	1-hour	
	0.5	Sodium formate		
	4	Basic chromium sulphate	1-hour	
	0.5	Sodium formate		
Basification	50	Water		
	0.5	Sodium formate	20-minute	
	2	sodium-bicarbonate	3x20-minute	PH=3.8-4.0
Drain/pile for 2-days,sam,split,shave,for post-tanning process, shaved thickness=0.9-1.0mm, for **softy upper**				
Acid wash	200	Water		

	0.5	Acetic acid	20-minute	
	0.5	Wetting agent		
	Drain /wash			
Re-chroming	100	Water		
	3	Chrome syntan		
	5	Basic chromium sulphate	30-minute	
	2	Fish oil		
Basification	1	Sodium formate	20-minute	
	1.5	Sodium-bicarbonate	3x20-minute	PH=4.0-4.2,L/O/N
Morning,Run-20-minute,Drain/wash				
Neutralization	100	Water		
	2	Neutralizing syntan	20-minute	
	1.5	Sodium formate	20-minute	PH=4.8-5.0
	2	Sodium-bicarbonate	3x20'	
Drain/wash				
Re-tanning	100	Water	20-minute	
	3	Acrylic syntan (Relugan RE)		
	6	Mimosa		
	4	Brown dye	1-hour	
	3	Replacementsyntan (retinal FB18)		
	4	Melamine syntan (MR 70)		
Fatliqouring	100	Water	20-minute	
	3	Fish oil		
	4	Sulphited fatliqour (EA1)		
	3	Semi-synthetic (EXP)	40-minute	
Fixing	2	Formic acid	2x20'	
Drain				
Top-dye	100	Water		
	2	Brown dye	20-minute	
Fixing	1	Formic acid	20-minute	

Drain/wash/pile, morning set/over head dryer/stacking.

6.2 Annex-2 Process recipe from raw to dyed crust, Experiment-2, (best recipe)

One side (1L, left-side), from Ethiopian wet salted raw cow hide was processed and the recipe was as follow:-

Weight= 10kg

Soaking, liming and re-liming were done using pit

process	percentage	chemicals	duration	parameter
soaking	up- to covering the hide	Water	2-days	Hand check for full wetting of the hide in the neck and butt portion.
	0.5	preservative		
	0.5	Wetting agent		
Drain/ wash				
Liming	200	Water	1-day	
	10	Lime		
	3	Sodium-sulphide		
Un-hairing using beam				
Re-liming	1	Lime	2-days	Checking the plumpness by hand
Drain/wash				
Fleshing, using fleshing machine and scudding by manually using knife beam. taking fleshed weight, Ethiopian=15kg				
Deliming	100	Water	1.5 hour	Check cross-section for completion of de-liming using phenolphtalin indicator, colorless indicates complete deliming.
	6	Ammonium chloride		
Drain /wash				
Bating	100	Water	1-hour	Check completion by thumb test
	1	Alkali bate		
Drain/wash				
Pickling	100	Water		
	10	Salt	20'	Baume check,6-7
	0.5	Formic acid(ont to 5 dilution)	20'	
	0.7	Sulphuric- (one to ten dilution)	3x20'	PH=2.5-2.8
Depickiling	100	Water		
	1	Sodium bicarbonate	3x20'	PH=4.5-5,
Degreasing	**Dry float**			
	0.5	**Degreasing agent(Tergon ND 01)**	**1-hour**	**drain,2 times wash with salt and drain**
pickling	100	Water		
	10	Salt	20'	PH= 2.5-2.8
	0.5	Formic acid	20'	
	0.7	Sulphuric acid	3x20'	
Leave overnight in the pickle bath, morning run 10-minute ,half drain and tanning started				
Tanning	50	Pickle bath		

	4	Basic chromium sulphate	1-hour	
	0.5	Sodium formate		
	4	Basic chromium sulphate	1-hour	
	0.5	Sodium formate		
Basification	50	Water		
	0.5	Sodium formate	20'	
	2	sodium-bicarbonate	3x20'	PH=3.8-4.0

Drain/pile for 2-days,sam,split,shave,for post-tanning process, shaved thickness=1.2-1.3 mm, and shaved weight 2.7kg for Upper leather

Acid wash	200	Water	20'	
	0.5	Acetic acid		
	0.5	Wetting agent		
	Drain /wash			
Re-chroming	100	Water	30'	
	3	Chrome syntan		
	5	Basic chromium sulphate		
	1	Cationic fatliqour(OK)		
Basification	0.5	Sodium formate	20'	
	1.5	Sodium-bicarbonate	3x20'	PH=3.8-4.0

Drain/wash/pile the leather was summed and cut in to two,$1L_1$ and $1L_2$, and weight was measured,$1L_1$=1.7kg and $1L_2$=1.9kg, $1L_1$ used for the **experimental trial-2** and $1L_2$ used as control.(the post tanning process are done in different drum)

Neutralization	100	Water	20'	PH= 4.8-5.0
	0.5	Sodium formate		Drain/wash
	1.5	Sodium-bicarbonate	3x20'	
Re-tanning	150	Water	20'	
	5	Acrylicsyntan (Relugan RE)		
	4	Black dye	40'	Check-penetration, by cutting the cross-section, it was penetrated well.
	7	Mimosa		
	3	Replacement syntan (FB-18)		
	5	Melamine syntan(MR-70)		
Fat-liquoring	100	Water	20'	
	2	Fish oil(BLSFO)		
	4	Sulphited fat-liqour		

	2	Semi-synthetic fat-liqour	40'	
Fixation	2	Formic- acid	2x20'	Drain/wash
Top-dye	100	Water		
	2	Black-dye	20'	
fixation	1	Formic acid	2x20'	Drain/wash
For the control,1L$_2$,cationic topping				
Cationic topping	100	Water	20'	Drain/wash/pile/set/hook/stack/ trim
	1	Cationic fatliqour		
For the experimental,1L$_1$,after top dyeing and fixation, drain/ wash and follow with next process				
Pigment + filler	**200**	**Water**	**30'**	**Mix the filler with the liquid pigment and add as the drum running.**
	1	**Liquid pigment(2k black)**		
	1	**Sellcomfill 6718**		
fixation	1	Formic acid	20'	drain/wash
Cationic topping	100	Water	20'	
	1	Cationic fatliqour		
Drain/wash/pile/Set/hook/stack/trim				

ISOLATION AND IDENTIFICATION OF HALOTOLERANT ORGANISMS FOR THE PRODUCTION OF PROTEASE ENZYME FOR THE TREATMENT OF HYPER-SALINE SOAK LIQUOR DISCHARGED FROM TANNERIES

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This present study mainly focuses on the enzymatic degradation of soluble bio molecules (proteins) present in soak liquor that contains high salt concentration ranging 4-7% of NaCl that are discharged from tanneries. The halophilic organisms were isolated from different sources acclimatized with soak liquor and screened for their proteolytic activity at saline medium. The hydrolytic protease was produced from two selected halophilic microbes which were isolated from SL acclimatized tannery soil. The biochemical characteristics and 16SrDNA analysis were done for the identification of organisms. The optimization studies on protease enzyme production were done and the optimum time was obtained at 48 h; with corresponding pH, 7.0; Temperature 30°C, substrate concentration 2% and salinity was found to be 4% resulted in the maximum yield of protease production. The produced enzyme solution was purified by dialysis and the specific activity was evaluated. In addition to it, the stability of protease was determined by varying the pH, temperature, TDS and metal ions. The degradation was carried out at different time intervals and complete degradation of protein content present in the soak liquor was obtained at 90min. The degradation efficiency was evaluated by the conversion of proteins into amino acids. The instrumental analysis such as UV-Visible spectroscopy were performed to confirm the enzymatic degradation of proteins into amino acids in hyper saline SL and which may favour the effective treatment of soak liquor by further unit operations.

Key words: Soak liquor, alkaline protease, halophilic microbes, proteins, enzymatic degradation

1. Introduction

The manufacturing of animal products for human consumption (meat and dairy products) or for other human needs (leather), leads inevitably to the production of waste which are released into receiving waters throughout the world.An important environmental impact of the animal processing industry results from the discharge of wastewater because most processes in slaughterhouses, tanneries and dairy plants require the use of water. Discharge of wastewater to surface waters affects the water quality in three ways. Firstly, the discharge of biodegradable organic compounds (BOC's) may cause a strong reduction of the amount of dissolved oxygen, which in turn may lead to reduced levels of activity or even death of aquatic life, followed by the amount of macro-nutrients (Cr, N, P) may cause eutrophication of the receiving water bodies. Excessive algae growth and subsequent dying off and mineralisation of these algae, may lead to the death of aquatic life because

of oxygen depletion. Agro-industrial effluents may contain compounds that are directly toxic to aquatic life **(Pamela, 2009).**

The meat processing industry is large, common to many countries and generates large volumes of wastewater, which requires considerable treatment if its release to the environment is to be sustainable **(Johns, 1995)**. For economic and environmental reasons, it is therefore necessary to find new ways to obtain increased value of slaughterhouse by-products. For example, these by-products exhibit a high protein content, between 15 and 20% (w/w), with many essential nutrients such as amino acids, minerals, vitamins and fatty acids, **(Liu, 2002).** Dairy wastewater is distinguished by the high BOD and COD contents, high levels of dissolved or suspended solids including fats, oils and grease, nutrients such as ammonia or minerals and phosphates and therefore require proper attention before disposal **(Sarkar et al., 2006)**. Dairy wastewater generally does not contain conventional toxic chemicals like those listed under EPA's Toxic Release Inventory. However, it has high concentration of dissolved organic components like whey proteins, lactose, fat and minerals **(Mukhopadhyay et al., 2003).** These protieneous materials of the dairy wastewater were found to be severe foulant for the existing membrane materials **(Madaeni S & and Mansourpanah, 2004)**, with the use of the newer membrane materials which are less prone to fouling were used. To control the fouling and to improve the productivity and life of membranes, use of coagulant and adsorbent before membrane application were done in primary and secondary effluent treatment and in sewage effluent treatment **(Abdessemed, 2002)& (Kim et al., 2002)**

Tanneries are wide common industry all over the world. They contribute to one of the major industrial sectors of India **(Senthilkumar & Balaji, 2011). .** The tanning process aims to transform skins in stable and imputrescible (not capable of putrefaction) products namely leather, these processing of the skin releases pollutants that substantially contributes to the pollution of the environment.Making of leather involves many wet processing stages, which may be broadly categorized in to pre-tanning and post tanning stages, there are four main groups of sub-processes required to make finished leather: beam house operation, tan yard operation, retanning and finishing**(Sundar, V. et al., 2001).** During the tanning process at least about 300 Kg chemicals (lime salt etc) are added per ton of hides. The preparatory stages are when the hide or skin is prepared for tanning. Many steps are involved in pre-tanning operations**(Thanikaivelan et al., 2005)** that includes preservation, soaking, liming, dehairing, fleaing, deliming, bating, degreasing **(Kamini et al., 1999)**, pickling, depickling. Proteases have evolved multiple times, and different classes of protease can have performed. Particularly, extracellular bacterial proteases are important for the hydrolysis of waste proteins and enable the bacteria to absorb and utilize hydrolytic products **(Srinivasan *et al.*, 2009)** by growing easily under extreme pH and temperature conditions. Recently, bacterial alkaline proteases have received attention as a viable alternative for bioremediation of protein rich tannery waste and their use in treatment of raw hide by replacing the hazardous chemicals especially involved in soaking, dehairing and bating of hides prior to tanning to produce quality leather without causing environmental pollution **((Sivakumar et al., 2005) Dodia, M. et al.,** have reported bacterial alkaline protease as an important proteases used in leather processing. He also reported the production of protease and its application as a depilating agent, the production of a salt tolerant protease and its application in tannery saline wastewater treatment. Several bacterial species, belonging to a variety of genera such as ***Bacillus, Pseudomonas, Aeromonas, staphylococcus***, etc. Are reported to produce alkaline protease having diverse industrial applications **(Dodia, M. et al., 2008).** Proteases are generally active at neutral and alkaline pH, with an optimum between pH7 and

11. Alkaline proteases secreted by both neutrophilic and alkalophilic *Bacilli* are of interest because they represent the major source of commercially produced pro-teo-lytic enzymes. The main industrial application for alkalophilic proteases are in the leather tanning process. Most of the alkaline protease producers are *Bacillus* sp.

The present study focuses mainly on the isolation & purification of protease producing microorganism for the degradation of the protein content present in the soak liquor using the protease enzyme produced from the bacterial strains.

2. Materials and methods

2.1. Materials

All the chemicals used in this study such as Sodium carbonate, sodium bicarbonate, casein, trichloro acetic acid, NaOH, Ninhydrin bought from Hi-media. Protease enzyme used in this study was produced from *Bacillus cereus.*

2.2. Isolation and identification of protease producers at saline medium

The microorganism for the present investigation was isolated from different tannery samples. Among the many strains which were streaked onto the skim milk agar plates containing 5% (w/v) skim milk,2% agar and 10%NaCl, the best producer with a good halo around the colony was selected. The clear zones around the colonies indicated the hydrolysis of casein by extracellular protease. Individual bacterial colonies were screened for protease production on skim milk agar medium. Nine cultures were streaked on the skim milk agar plates and incubated at 37^0 C for 24-48hrs. The isolate having maximum clearance zone were selected for studies. Nine bacterial colonies showing maximum clearance were treated with skim milk agar containing 1% 2%3% 4% of NACL and incubated at 37^0C for 24-48 hrs. Seven bacterial cultures shows zone of hydrolysis up to 4% NaCl. Best two bacterial strains having maximum zone of clearance in 1% 2% 3% 4% NaCl were selected for production. For molecular identification Genomic DNA was extracted by CTAB protocol, for all the strains, 16S rRNA gene was amplified by universal primers (27F, 1492R). Purified samples were sequenced in ABI 3130 Genetic Analyser. Sequences were then submitted to Gen bank and accession number was assigned for each strain.

2.4. Optimization studies

Optimization of time were done for the selected bacterial strains at different time intervals 12h, 24h, 36h, 48h, 60h for the estimation of protein, protease, amino acid .The effect of PH on the rate of protease catalysed reaction was determined using different pH values ranging from 3.0 to 10.0 and the protein, protease activity and amino acid were measured under standard assay conditions. The effect of temperature on protease activity was studied by incubating the reaction mixture at different temperature ranging from 20-60^{0}C using BSA as substrate. Then the standard assays are measured for further studies.

2.5. Production extraction and purification of bacterial culture

The production media containing 500ml of nutrient broth, 1.5% of NaCl and it is autoclaved then 0.5% of BSA is to the sterile media and 50ml of inoculums is added and incubated at above optimised conditions. The production media after 48hrs is centrifuged at 5000rpm for 15 min. Supernatant is collected and 2 volumes of cold acetone is added to the supernatant and it is incubated at 20°C for

24hrs.After the overnight incubation the precipitated cultures are centrifuged at 5000rpm for 15 min pellets are collected and it is mixed in 0.2M Phosphate buffer (pH 7) and stored at 4^oc.

2.7. Analytical Methods

Proteins present in the soak liquor was determined using Lowry's method **(Lowry et al., 1951)** and the amino acids present in the sample was estimated using Ninhydrin reagent .Protease activity was determined using 1.9 ml of Casein solution (1%) was added to all test tubes marked as control and Test samples. 3 ml of TCA solution (5%) was added to the to the tubes marked as control. All the test tubes were pre-incubated for 10min at 40º C .0.1ml of suitably diluted enzyme was added to all the test tubes and then incubated for 10min at 40º C for the enzyme react with the substrate. After incubation TCA was added to all the test tubes to stop the reaction. The precipitate was filtered through Whattman filter paper and the absorbance was taken for the filtrate at 280 nm. This was done with reference to Anson method **(Anson, 1939)**with minimal modification.. SDS-PAGE for the identification of molecular weight of the protein was done followed by instrumentation analysis by FT-IR, TGA&DSCA were done for the characterization of the purified enzyme.

2.8. Degradation Studies

2.8.1. Preparation and degradation Studies for protein content of soak liquor

1 kg of animal hide soaked in 3 L of water for overnight and left it for settling for about 2 h, and then it was taken after the primary clarification using AlCl3.Degradation of protein present in the soak liquor by *Bacillus cereus* was optimized with respect to time, pH and temperature.

2.9. Instrumentation techniques

Thermo Gravimetric Analysis (TGA) and Differential Scanning Calorimetric analysis (DSC) were carried out under a nitrogen atmosphere from 30 to 800°C with a temperature gradient of 10 °C min^{-1} and scans were recorded using a TGA Q50 (V20.6 Build 31).The protease enzyme sample was mixed with KBr of spectroscopic grade and made in the form of pellets at a pressure of about 1 MPa , The samples were scanned in the spectral range of 4000 to 400 cm^{-1} using FT-IR .The degradation of soak liquor was studied by UV visible absorption spectra using CARY 5E UV–VIS-NIR Spectrophotometer, USA. The fluorescence spectrophotometer study was carried out to determine the excitation and emission characteristics of both soak liquor and the degraded products, in range of λ 200-800nm (Cary Eclipse, USA).

3. Results and Discussion

3.1. Bacterial isolation and identification for Protease activity at saline medium

The serial dilution method was used to isolate microorganisms from the tannery soil, tannery sludge, sewage sludge, aeration tank, acclimatized soak liquor sample. The above samples are plated on skim milk agar plates by pour plate method.15 organisms which produce protease enzyme shows zone of hydrolysis on skim milk agar plates and those organisms are screened for protease activity **Fig.1(a&b).**
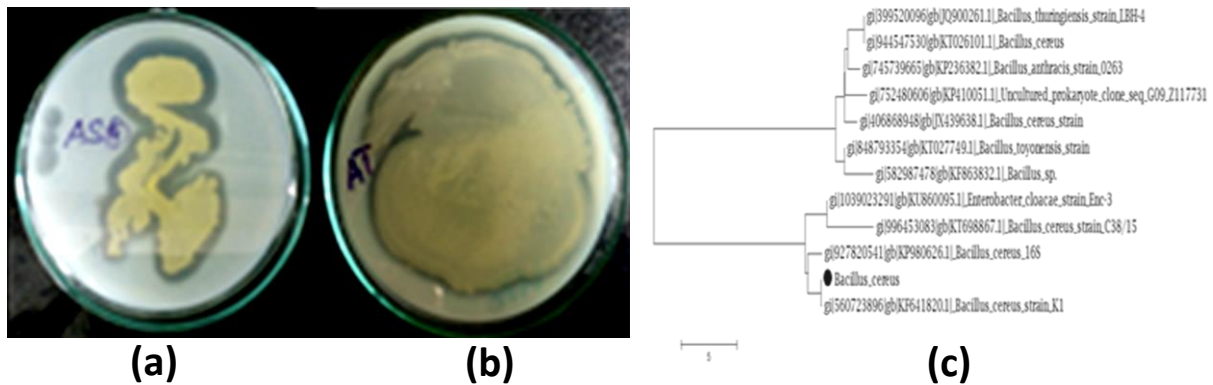
Fig 1(a) Isolation of organisms from (a) Acclimatized soak liquor sample (b) Aeration tank (c) Phylogenetic tree analysis of *Bacillus cereus*

3.2. Screening of microorganisms in Skim Milk agar at saline medium.

Skim milk agar containing 1% 2% 3% 4% of NaCl were sterilized and the isolates were streaked on the sterile agar.7 isolated organisms showing maximum zone of clearance were screened at different NaCl concentrations. The protease producers were confirmed by observation of zone of hydrolysis around the colonies. Best two bacterial strains having maximum zone of clearance in 1% 2% 3% 4% NaCl were selected for production **Fig.2(a-h)**.
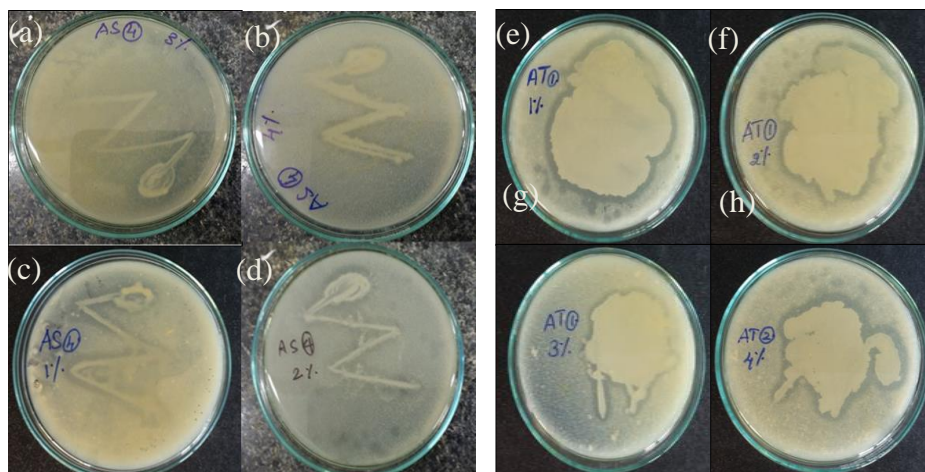

Fig 2 (a), (e)1% NaCl; (b), (f)2%Nacl (c), (g)3%Nacl ;(d), (f)4%Nacl for the AS4 and AT1 samples

3.3 Optimization studies

The characteristic culture growth conditions such as time, pH, temperature, were optimized for the production of protease from AT1 and AS4 bacterial cultures. The optimum time for the bacterial cultures AT1and AS4 was found to be at 48h **Fig 3(a)**. The protease activity is maximum at 48h in reference to the formation of amino acids from **Fig 3(b)**, from **Fig 3(c)** maximum protease activity was found at 48hr (6.5 mg/ml) at a temperature of 30℃ for AS4 bacterial culture. After 48 hr the activity is decreased little for both the bacterial isolates. **Fig 4(a)** The effect of pH (3-10) on the degradation of protein using the isolated bacterial strains AS4 and AT1 was studied. **Fig 4(b).** temperature, 30℃Cand at pH 7 .**Fig 4(c)**shows degradation of protein with maximum protease activity in reference to amino acid formationat pH7. The degradation of protein using bacterial strains was

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active at alkaline pH of 7 with the amino acid formation of about 117µg/ml and 125 µg/ml. **Fig 5(a)** shows the optimum temperature for protease activity was 20 and 30˚C AT1 and AS4. The enzyme activity increased with increasing temperature and peaked at 30°C was shown in **Fig 5(b)** at optimum temperature there is maximum formation of amino acids with reference to increased protease activity,after which it dropped down at 50˚C was shown in **Fig5 (c)** Exposure to 50°C or higher, caused thermal denaturation of the protease. These results reveal that the enzyme is moderately thermophilic in nature.
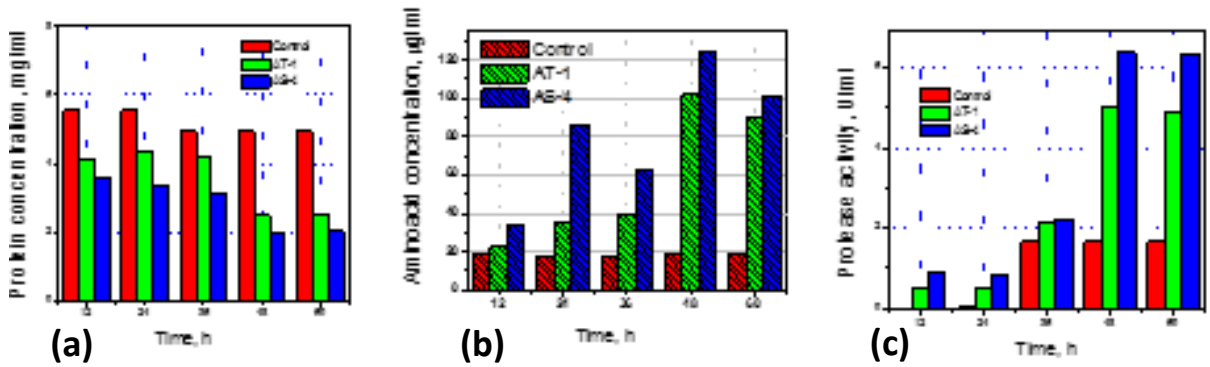

(a)   (b)   (c)

Fig 3 Optimization of time for (a) the degradation of protein (b) formation of amino acids and (c) the protease activity of the organisms AT-1 and AS-4

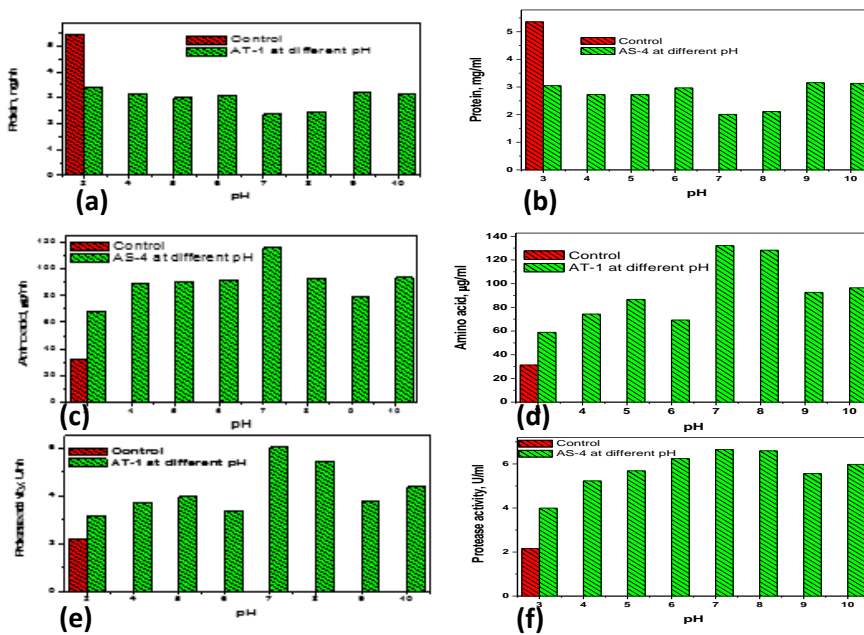(a)   (b)   (c)   (d)   (e)   (f)

Fig 4 Optimization of pH(a) and (b) for the degradation of protein (c) and (d) for the formation of amino acids , (e) and (f) for protease activity by the organisms AT-1 and AS-4
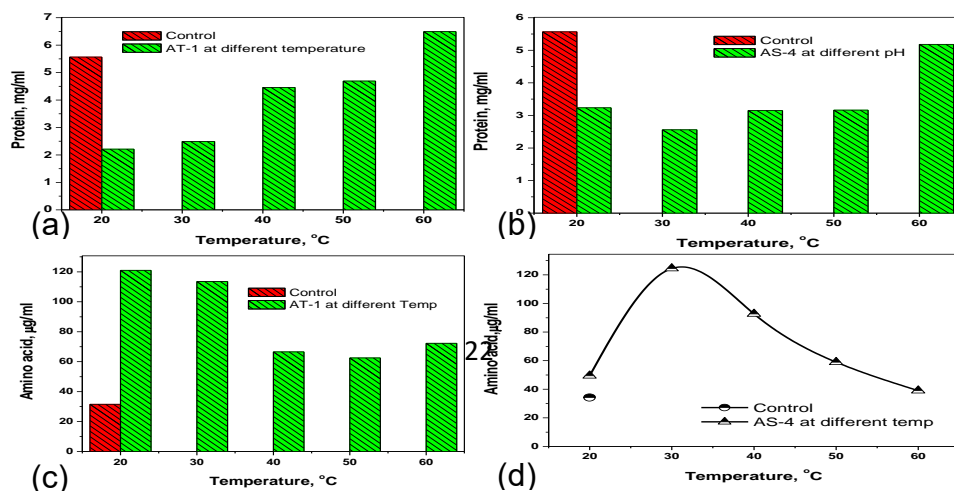

(a)   (b)   (c)   (d)

Fig 5 Optimization of temperature (a) and (b) for the degradation of protein (c) and (d) for the formation of amino acids, (e) and (f) for protease activity by the organisms AT-1 and AS-4

3.4. Characterization of enzyme

Molecular weight of the extracted enzyme was found to be 68 KDa for *Bacillus cereus* which was determined using SDS-PAGE**Fig.6(a)**. The FTIR spectra of protease enzyme was shown in Fig. 2 It show a broad envelope ranging from 3600 cm−1 to 3100 cm−1 which corresponds to the N–H stretching vibrations of peptide whereas N-H bending was around 1640 cm^{-1} for primary amine **(Saranya & Sekaran, 2015)**. The peak at 1402 and 992 cm−1 corresponds to O-H bend in carboxylic acid and its C-N stretching around 1112 cm-^{1} from **Fig.6(b)**
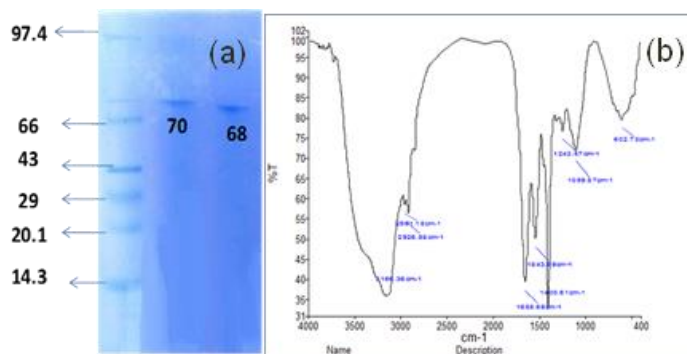

Fig 6(a) Identification of molecular weight of protease using SDS-PAGE (b) FT-IR spectrum of protease isolated from the Organism AT-1

The presence of peptide bond in protease enzyme was confirmed through the amide stretching at 1640 cm-^{1}TGA thermo gram of extracellular protease enzyme showed 1.03% weight loss at the temperature 222.9°C due to the removal of moisture **Fig 7(a)**. Thereafter, 6.37% of weight loss occurred at 305.83°C, where the actual stable compounds are degraded. After this decomposition, there was a decrease in weight loss of 18.39 % observed at 475.26°C 17.31% o and the final residue of 77.02 % reveals the thermal stability of the enzyme at the end of the scan (800°C) from **Fig 7(b)** **(Fan C, Zhang Y, Tang Z, & Wang J, 2015)**
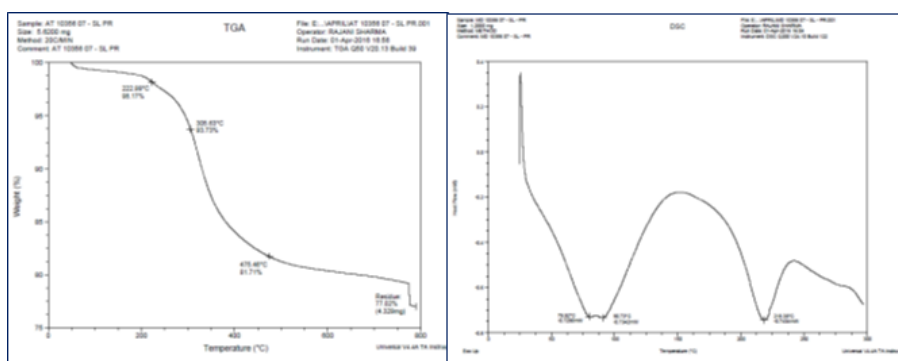
(a)   (b)

Fig 7(a) TGA and (b) DSC thermo gram of protease isolated from the Organism AT-1

The TGA of Protease enzyme from *AT-1 and AS-4 species* showed better stability behaviour. The DSC spectrum of protease enzyme showed three thermal transitions by showing the endothermic peaks appear at 79.92, 90.73°C and 218.38°C **(Ó'Fágáin, 2003).**

3.5. DEGRADATION STUDIES

The optimum time for the degradation of protein was observed to be 60mins, this supports the hydrolysis of proteins which was (200mg/L) initially present was broken down into these constituent amino acid molecules which was found to be (78mg/L) **Fig.8(a)**. The effect of pH (3-10) on the degradation of protein using the isolated bacterial strains AS4 and AT1 was studied **Fig.8(b)**. The activity of the protease enzyme increased with increase in pH and it was observed that maximum amount of amino acid formed was 117µg/ml for AS4 and 125µg/ml for AT1 at time, 48h, temperature, 30°Cand at pH 7 . shows degradation of protein with maximum protease activity in reference to amino acid formationat pH7. The degradation of protein using bacterial strains was active at alkaline pH of 7 with the amino acid formation of about 117µg/ml and 125 µg/ml **Fig.8(c)**. The ideal temperature for the degradation of the of the proteins was observed to be 30°C. The initial amount of protein content was found to be 150(mg/L) which hydrolysed effectively into 40(mg/L) of amino acids.
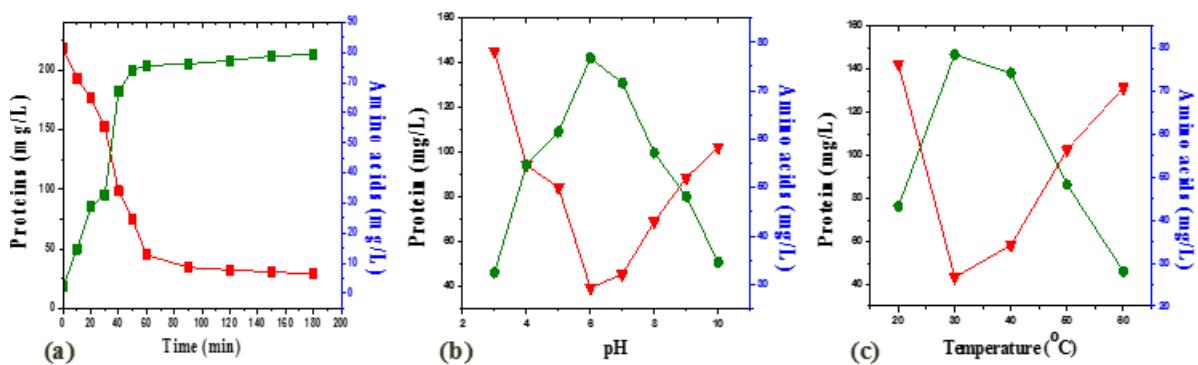

Fig. 8 Optimization of (a) time (b) pH (c) Temperature for the degradation of protein content of soak liquor

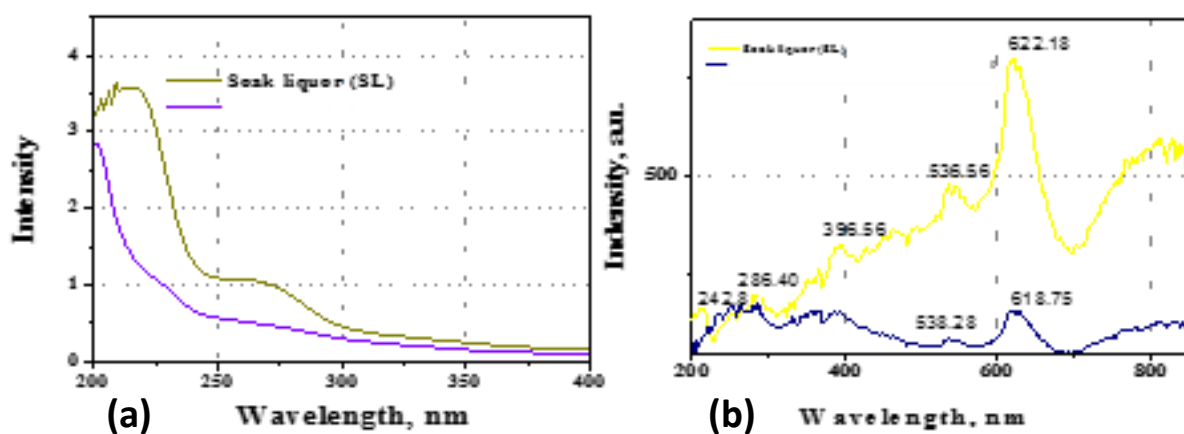### 3.5.1.Instrumental evidences for degradation

Fig 9 (a) UV-visible and (b) UV-fluorescence spectroscopic studies for protein degradation of soak liquor

UV-Visible spectroscopic studies were carried out for the confirmation of degradation of soak liquor in the presence of protease enzyme isolated from the halo-tolerant organism shown in the **Fig.9** The absorption intensity of the initial soak liquor was observed to be high before the treatment and the peak values were observed in the region of 210-230nm and 260-275nm which were denoted the presence of proteineous matter soak liquor. The above mentioned peaks were slightly disappeared after the treatment in the presence of protease at the optimized conditions. It was noted that the intensity difference between the absorption peaks appeared in the enzymatic and the microbial degradation of soak liquor and it was clearly shown in the **Fig. 9(a)**. Hence, the enzymatic degradation using the alkaline protease from the halo-tolerant organism would be the effective process compare with the pure organism for the degradation of soak liquor.UV-Fluorescence absorption studies were carried out for the confirmation of degradation of soak liquor in the presence protease enzyme and it was shown in the **Fig. 9(b)**. The spectrums show the difference in intensities between the soak liquor and the treated sample after the enzymatic degradation and microbial degradation at the optimized conditions.

4. Conclusions

The treatment of saline wastewater is feasible, through the bacterial extracellular protease. Generally, biological treatment is inhibited by high salt concentrations. However, this study has proved feasibility of degradation using salt-adapted micro-organisms capable of withstanding high salinities and at the same time of degrading the pollutants in wastewater. The organism isolated was identified by 16S rDNA sequencing as *Bacillus cereus.* The protease enzyme is isolated and extracted. The optimization studies were performed for the organism and the culture conditions were analyzed. Optimum growth conditions were analyzed. The degradation of soak liquor waste water was carried out. The enzyme was stable at pH 7, temperature at 30-40^{0}C at 48h.The degradation of the protein content in soak liquor by protease was confirmed with UV, Fluorescence, and FT-IR spectroscopy. From this study it is concluded that the biological origin of enzymes reduces their adverse impact on the environment thereby making enzymatic treatment of soak liquor was feasible. This would be the excellent pre- treatment of soak liquor and much favored for the further unit operations.

5.Acknowledgement

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THE EFFECT OF DIFFERENT PARAFFIN EMULSIONS ON THE THERMAL

STABILITY OF COLLAGEN

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Paraffin emulsions are an important lubricating agent in meeting needs for present-day leather properties. The fatliquoring process with paraffins penetrates deeply between fiber bundles and bonds physically or chemically with the reactive groups of collagen. In our study, paraffin emulsions containing ZnO nanoparticles were used to develop the stability of modified collagen. Zinc oxide paraffin emulsions were applied to the leather in the fatliquoring process. Shrinkage temperatures of leathers (T_s) were determined with a special test apparatus according to the IUP 16 standard method. Differential scanning calorimetry (DSC) measurements carried out on the fatliquored leather at a constant heating rate were used to determine denaturation temperatures (T_d) and melting temperatures (T_m) of the fatliquored leather. The results of the research show that the thermal stability of leathers was improved remarkably by fatliquoring with all paraffin emulsions.

Keywords: Paraffin emulsion, leather, fatliquoring, DSC, Shrinkage temperature, Denaturation temperature

1. Introduction

The chemical structure of collagen is important for its reactivity (Zhang and Wang, 2009; Żarłok et al., 2014). According to Covington (2009), this reactivity depends on its conformation to react with various chemical materials and on reactive groups. This reaction is one which has the effect of bringing the leather into a finished state.

Fatliquoring is a critical reaction in the leather-making process, wherein a lubricant is added to the leather to prevent the fibers from sticking together, thereby providing sufficient flexibility to the leather. This is accomplished by the utilization of lubricant emulsions with a small particle size capable of penetrating deeply into the hierarchy of the three-dimensional woven structure (Zhang and Wang, 2009; Żarłok et al., 2014). The use of synthetic fatliquors has become common in the leather industry because of their superiority to natural oil based fatliquor in terms of their high emulsifying power and their creation of mini emulsions.

Generally, synthetic fatliquors are prepared from paraffins obtained either by the Fischer-Tropsch technique of paraffin synthesis, or from the petroleum industry (Sivakumar et al., 2008). The saturated hydrocarbons in paraffins, which have a chain length ranging from C_{14} to C_{28}, are chlorinated with chlorine gas in the presence of catalysts and ultraviolet light (Randegger, 1998). Generally, chlorinated paraffins with low chlorine content and medium or long chain paraffins are used in leather applications. The fatliquoring activity of the sulfochlorinated alkanes and the chlorinated paraffins are clarified by the unipoint fixation mechanism and by the long chain lubricating the fibre (Sarkar, 2005). As well as the lubricating characteristics of the sulfochlorinated and chlorinated paraffins, it is known

that they confer such important characteristics as fullness to the leather and an increase in collagen stability (Zhang and Wang, 2009).

Natural leather products are susceptible to damage by heating during storage and use, and therefore knowledge of their thermal stability is of great importance (Budrugeac, 2015). The thermal stability of the collagen is characterised by the measurement of the shrinkage temperature in heated water, and this is one of the most important criteria for determining the overall hydrothermal stability of leathers. Differential scanning calorimetry (DSC) is another important tool for researching the stability of collagen. Determination of the thermal stability of leather by the DSC method can be achieved in two ways. The first is determination of the denaturation temperature (T_d) of the wet leather (Liu et al., 2014), and the second is determining phase changes in the collagen of the dry leather (Okamoto and Saeki, 1964; Cucos et al., 2013). The denaturation temperature of the leather can be determined by detecting onset temperature in the DSC curve (Wang et al., 2010). Phase changes in the collagen are interpreted as breakup of the helix structure of the collagen, breaking of the hydrogen bonds and other covalent bonds which maintain the helix structure, loss of free, bound and immobile water from the collagen, and change of the crystal structure in the collagen to a single chain amorphous structure (Okamoto and Saeki, 1964).

In leather production, the most important stage which increases thermal stability is the tanning stage. The shrinkage temperature for an untanned leather is 60-65°C, for collagens modified with vegetable tannins it is 80°C (Onem et al., 2015), and chrome tanned collagen can easily resist up to 100°C. This is explained by the high hydrothermal stability of the crosslinks formed in the collagen fibers (Covington, 2009). The formation of rigid crosslinks, making large cooperative units in the helix structure, enables high collagen stability in the leathers. Current trends have brought an expectation of higher thermal stability in leathers, and therefore R&D work has increased on strong crosslinks and on improving the functional characteristics of leather.

It has been reported in the literature that the paraffins used in the leather industry as lubricating material form covalent bonds with the amino groups of the leather, and that long hydrocarbon chains wrap around the fibers. Also, paraffins form hydrogen bonds with the hydroxyl groups of hydroxyproline amino acids, which have a great effect on thermal stability (Monti et al., 2013). It is thought that the stability of collagen is related to crosslinks in polypeptide chains, and that paraffins have a large effect on the stability of collagen. Understanding the stability of collagen can lead to the development of novel fatliquoring agents and new technologies, resulting in the production of leathers with high hydrothermal stability. Therefore, the basic aim of this study was to increase the thermal stability of collagen with the use of paraffin derivatives with various chain lengths at the lubrication stage of leather production, and to show this stability and the phase changes in the modified collagens by the DSC method.

2. Materials and Methods

2.1. Materials

Wet-blue American cattle leathers were used in the study. Nano ZnO provided by Sigma Aldrich and used in the emulsion was at ≤100 nm size, 99,9 % purity and was of hydrophilic character. Cloparten 330 was obtained from Harke Chemicals. Paroil 145, Paroil 1045, Paroil DO152, Paroil CW40AO, and Doversperse 3 NR were provided by Dover Chemical Corporation. The commercial lubricating material used on the control samples was obtained from Perfectol HBP Schill+ Seilacher.

2.2. Methods

2.2.1. Production of leathers

Leathers were finished according to the recipe used industrially; 30% more was added to the wet-blue weight and the chemicals were calculated from the weight of the hides. The leathers were left to dry for two days after the production recipe was finished. Paraffin emulsions of the optimized percentage were used for the fatliquoring experiment.

Table 1.
Recipe for the production of leathers.

Process	%	Chemicals	Temperature (^{o}C)	Time (min.)	Remarks
Material: Wet Blue American Cattle **Thickness**: 1.0-1.2 mm					
Pre-Soaking	200	Water	30		
	2	CH_3COOH		40	
Chrome Retanning	100	Water			
	3	Chrome		90	
	1	HCOONa		30	
Neutralization	100	Water	30		
	2	HCOONa		30	
	1	$NaHCO_3$		60	pH:4.5, drain
Washing	200	Water	30	10	Drain
Retanning-Dyeing	100	Water	40		
	5	Acyrilic syntan		30	
	3	Resin		30	
	3	Phenolic syntan		30	
	2	Chrome syntan		30	
	3	Dyeing auxiliary		30	
	2	Dye		30	
	5	Natural Oil	60		
	4.5	Sulphited oil			
	10	Paraffin Emulsion		60	
	3	HCOOH		40	pH:4.0, drain
Horsing up-Drying					

2.2.2. Determination of shrinkage temperature

Determination of the shrinkage temperature (T_s) of the leathers was achieved according to the IUP 16 standard test method. The basic principle in determining the shrinkage temperature depends on keeping the leather test sample in hot water and measuring the temperature at the moment when it starts to shrink.

2.2.3. DSC analysis

To investigate the phase transition of dry leather and the denaturation temperature of wet leather, each sample, typically weighting 3–4 mg, was placed in an aluminum pan, which was covered with an aluminum lid with three small holes. The reference had a similar empty crucible. The analysis was performed in nitrogen flow (purity 99.99 %; 20 ml min^{-1}). Leather samples were heated from 25 to 200

^{o}C at a heating rate of 5°C/min. The onset temperature of the DSC curve was taken as the denaturation temperature of leather.

3. Results and discussion

3.1. Results of shrinkage temperature (Ts) and denaturation temperature (Td)

Table 2 shows the shrinkage temperatures and denaturation temperatures of the leathers obtained with the different lubrication emulsions used in this study. As can be seen in the Table, the shrinkage temperature of leathers in the control group was measured as 106°C, and the denaturation temperature as 112.2 ^{o}C.

Table 2. Shrinkage and denaturation temperatures of leathers produced with different paraffin emulsions

Leather Samples	(T_s) (^{o}C)	(T_d) (^{o}C)
Control group samples	106	112,2
Samples produced with Paroil 140 emulsion	115	121,26
Samples produced with Paroil 1045 emulsion	109	115,1
Samples produced with Paroil DO152 emulsion	109	117
Samples produced with Cloparten 330 emulsion	121	130,8
Samples produced with Paroil CW40AO emulsion	118	122,5
Samples produced with Doversperse 3 NR emulsion	111	118,68

Larsen et al. (1993) determined that shrinkage temperature was an important property for determining collagen stability. The reason for the hydrothermal shrinkage of collagen is the weakening or dislocation of the triple helix configuration on heating, caused by the breakdown of intermolecular and intramolecular forces such as hydrogen bonding, hydrophobic bonding and crosslink bridges.

Examining the results, it can be seen that the cross-links formed by all of the paraffin emulsions used in the lubrication process produced significant increases in both shrinkage temperatures and denaturation temperatures. This result indicated that high hydrothermal stability is created in leather as a result of longer complexations with paraffin-collagen. Examining the relationship between denaturation temperatures and shrinkage temperatures, it is clear that there is a correlation between the results. The denaturation temperature and shrinkage temperature have the same increasing tendency. However, when it is considered that the same leather samples were used for both measurements, the denaturation temperatures obtained from the DSC analysis were 5-9 ^{o}C higher than the shrinkage temperatures.

The highest thermal stability was seen in leathers with long chain lengths and treated with sulfochlorinated Cloparten 330 emulsion. In particular, the shrinkage temperatures of leathers treated with sulfochlorinated paraffin emulsions were higher than those treated with chlorinated paraffin emulsions. This leads us to the conclusion that there is an increase in the hydrothermal stability of

leathers when more cross links are formed by the reactive groups of collagen in sulfochlorinated paraffins than in chlorinated paraffins in the emulsions. In a study by Monti et al. (2013), the interactions of sulfochlorinated paraffins with collagen were shown by FTIR and dynamic simulation. Examining the results, they reported that sulfochlorinated paraffins interact well with the amino acids of collagen, and also that the paraffins form covalent cross links with the peptide groups of collagen, and they form intermolecular hydrogen links with the hydroxyl groups of collagen. Gustavson (1956) stated that the shrinkage temperature of collagen varied according to the number of intermolecular and intramolecular cross links in its three-dimensional helix structure. Considering this statement, the greater hydrothermal stability of sulfochlorinated paraffins in comparison with chlorinated paraffins observed in the results of this study is to be expected. At the same time, it was determined when assessing the paraffins used in the study in terms of carbon chain lengths that as the lengths of the carbon chains increased, the hydrothermal stability of the leathers also increased. The lubrication mechanism of the sulfochlorinated or chlorinated paraffins can be explained by their very good penetration, homogeneous fixation and surrounding the fibers with a long chain structure connected to the mini-emulsions which they form. At the end of the study it was determined that the length of the carbon chains not only increased the lubricating effect but also significantly improved the thermal qualities of the leather.

3.2. Phase transition of leather

The determination of the phase transition of fatliquored leathers was performed by DSC analysis in nitrogen flow. This stability is characterized by melting temperature (T_m). The DSC curves of fatliquored leather obtained by analysis in nitrogen flow are shown in Fig. 3. Examining the results, similar DSC curves were obtained from the leathers produced with different paraffins. In the DSC curves obtained as the result of a $5^{o}C$ per minute increase, one endothermic peak can be seen. This peak is interpreted as the phase in which the absorbed water is separated.

The water content in the structure of collagen is an important factor in the stability of collagen's helix structure. Collagen's water retaining quality arises from the positive and negative charges (polarity) and the capillary effects in its structure. The provision of an unbound pair of electrons by the oxygen in the water molecule which binds to the collagen and its electronegativity give the water molecule its polarized character. In this way, hydrogen bonds are formed by the electrostatic attractive forces between the electrically charged reactive groups of the collagen and the polarized water molecule. It is by means of these hydrogen bonds that the water molecule is bound to the collagen (Ramachandran and Chandrasekharan, 1968). When heat is applied to the leather during DSC analysis, first of all water which is held by the collagen physically or by the force of gravity is separated, then immobile water which has a dipole-dipole interaction with other water molecules present is separated. Finally, water bound to the collagen by hydrogen bonds is also separated. Examining Figure 3, it can be seen that bound water is separated more slowly as the number of cross links in the collagen increases. It is thought that with increasing heat after the endothermic peak, the crystal structure of the collagen slowly changes to an amorphous state, and that after $200^{o}C$ the helix structure of the collagen breaks down and is converted to a completely amorphous phase. With the breakdown of the helix structure of collagen, breakage is observed in the bundles of fibers of collagen, and it loses its mechanical strength (Budrugeac and Miu, 2008).
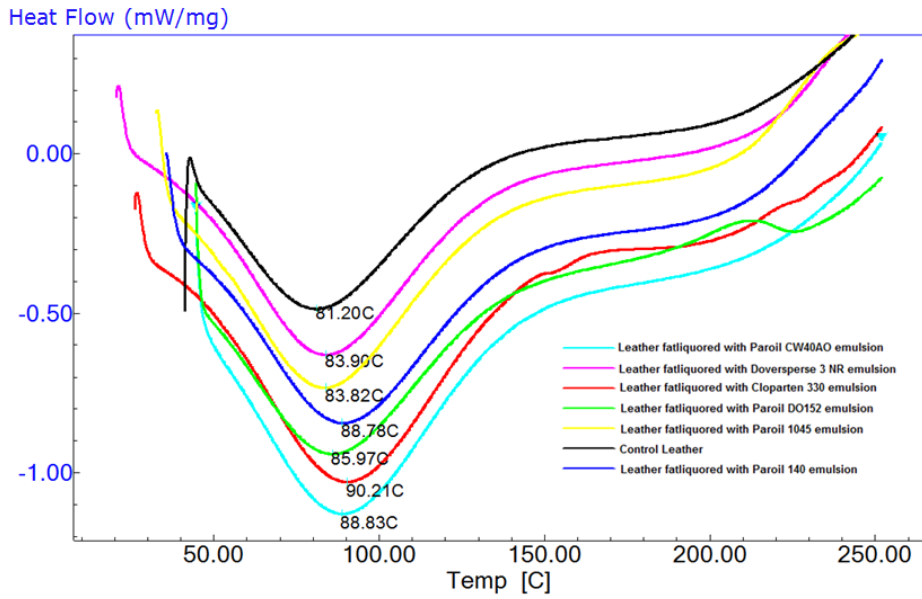
Figure 1. DSC curves of the fatliquored leather

Table 3. Melting temperatures of leathers produced with various paraffin emulsions

Leather samples	Melting Temperature (^{o}C)
Control group samples	81.20
Samples produced with Paroil 140 emulsion	88.78
Samples produced with Paroil 1045 emulsion	83.82
Samples produced with Paroil DO152 emulsion	85.97
Samples produced with Cloparten 330 emulsion	90.21
Samples produced with Paroil CW40AO emulsion	88.83
Samples produced with Doversperse 3 NR emulsion	83.90

Examining the melting temperatures given in Table 3, an increase can be seen in the melting temperatures of the leathers of all the paraffin emulsions used. The number of cross links in the collagen increased with the use of paraffin emulsions in the lubrication process, and the crystal phase of collagen remained more stable in comparison with the control samples. For this reason, the T_ms of the paraffin treated leathers were higher than those of the control samples. Okamoto and Saeki (1964) heated collagen from room temperature to 225°C in order to observe the phase change in collagen. In studies using the DTA method and X-ray diffraction, they determined that there were three different phases in the collagen: amorphous, an unstable crystal region and a stable crystal region. The first endothermic peak at between room temperature and 120°C was determined to be the evaporation of water adsorbed and absorbed in the collagen, while the second endothermic peak at 170°C and 215°C was the crystal phase in the collagen melting and changing to the amorphous phase. According to Petruchia (2005), this phase change was determined as group-dipole interactions with the acidic and basic amino acids in the collagen and the evaporation of the strongly linked bound water, and conformational changes in the helix structure.

4. Conclusions

In this study, leathers fatliquored with nano ZnO-paraffin emulsion were evaluated with the aim of developing leather with high hydrothermal stability. The shrinkage temperature results showed that paraffin emulsions can enhance the thermal stability of collagen. DSC analysis showed that paraffin fatliquored leathers have high denaturation and melting temperatures compared to control leathers. The study has great importance in determining that the number of cross links, which determine the thermal stability of collagen, can be increased by the use of paraffin emulsions in the lubrication process outside tanning.

5. Acknowledgement

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TREATMENT OF EFFLUENT FROM TANNERY WITH CONSORTIUM OF MICROALGAE

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The leather wet finishing and the final finishing are the end stages of production in tanneries which generate wastewater with different *types* of *chemical substances* classified in the groups of dyes, toxic metals, emulsifying agents, among others. Microalgae have been the subject of many studies in bioremediation due to their ability to assimilate various nutrients and organic matter. Wastewaters generated in tanneries for finished leather have essential elements for the growth of microalgae, but also some toxic compounds that may hinder or restrain the growth of microalgae in this medium. This work tested the growth of a microalgae consortium collected in a wastewater treatment plant of a beamhouse tannery for the treatment of wastewater streams of other tannery for finished leather (processing wet-blue to finished leather). The consortium of microalgae was incubated in wastewaters collected in the following stages of treatments: raw wastewater (RW), wastewater after primary coagulation/flocculation (PW), and wastewater after secondary biological treatment (BW). The wastewaters were characterized before and after incubation with the microalgae consortium to analyze the removal of Total Kjeldahl Nitrogen (TKN), Ammonia Nitrogen (N-NH$_3$), and Phosphorus (P-PO$_4$). It was verified the growth of the consortium of microalgae in the three wastewaters, with a maximum growth in the raw wastewater. The results with 50% of the concentration of the effluents presented after 16 days of cultivation, showed that the consortium had a decrease in the three effluents reaching maximum N-NH$_3$, TKN, and P-PO$_4$ removals of 99.9%, 82.88%, and 91.85%, respectively.
Keywords: microalgae consortium, tannery, wastewater treatment.

1. Introduction

The current scenario has shown ample interest on biotechnology research, focusing on the study of microorganisms such as microalgae, cyanobacteria and fungi, in several areas. Microalgae have a wide application range, the biomass being used as food supplements, agricultural biofertilizer, obtaining drugs, biofuel production, and applications in the treatment of wastewater (Derner et al. 2006; Angelis et al. 2012).

Known for converting solar energy into chemical energy, microalgae are autotrophic, heterotrophic and mixotrophic microorganisms, microscopic unicellular, which reproduce asexually, and may be prokaryotic or eukaryotic, can be defined and differentiated according to the natural habitat, in chemical composition and morphology. They offer several advantages such as easy and fast growing, in addition to having high storage capacity of substances, such as lipids, proteins and carbohydrates

that can generate biotechnological compounds with aggregate values (Wojciechowski et al. 2013; Lourenço, 2006).

An important step of the studies with microalgae is the isolation of the strains of interest. There are several techniques for obtaining a single strain described by several authors (González et al. 1995; Andersen and Kawachi, 2005; Lourenço, 2006). However, this is a laborious and slow step. The isolation process by pipetting and successive dilutions is used for microalgae larger than 10 µm. It consists of locating the microalga of interest by a stereoscopic microscope, collecting with a Pasteur pipette and transferring to appropriate culture media, the process must be done successively until obtainment a single cell.

The solid-medium isolation method is applied to small microalgae of nanoplankton and picoplankton in which streaks are made with a bacteriological in Petri dishes with agar medium using a culture sample. Another alternative is the serial dilution for isolation, where successive dilutions of the culture are carried out in culture medium; this method is most used when the desired species is abundant in the medium (Lourenço 2006).

In the context of wastewater treatment, microalgae have shown great potential for the treatment of several water pollution, since they are microorganisms that exhibit efficient removal of organic matter, nitrogen, phosphorus, and other nutrients present in the medium (Moreno-Garrido 2008).

In Brazil, many tanneries are player in the leather industry, according to data presented by the Brazilian Agency for Industrial Development (ABDI) in 2011 the country is the fourth largest producer of leather in the world. Together with the high production of leather, the tanneries have to care about the generation of large quantities of solid waste and wastewater. The wastewater generated in the beamhouse operations, as well as tanning and finishing, present relevant concentrations of organic matter, nutrients, heavy metals, among other contaminants, that need to be treated before being sent to the water bodies (Gutterres et al. 2010).

The wet finishing steps generate effluents with various chemical compounds such as residual chromium from the retanning stage, neutralization salts, leather dyes, fatliquoring oils, surfactants. The effluents from final finishing (leather surface treatments) have chemical compounds like polymers, pigments, solvents, resins, surfactants and other chemicals. The leather wet finishing and the final finishing are the end stages of production, when raw material receives the characteristics desired like physico-mechanical resistances, softness, color, durability, stamping and protective coating (Gutterres et al. 2010).

Some studies have been consolidated to carry out the treatment of wastewater from tannery industry with microalgae. Ajayan et al. (2015), different concentrations without pretreatment, using *Scenedesmus* sp. isolated from natural habitat. The authors found that the best growth species in the wastewater happened at a dilution of 50% and at 12 days of culture was removed 44.3% and 95% of NO_3-N and PO_4-P, respectively. There was also a removal of toxic metals Cr (81.2 to 96%), Cu (73.2 to 98%) Pb (75 to 98%) and Zn (65 to 98%) with varying dilution of the effluent, where results were more effective at 10% concentration wastewater.

Fontoura *et al.* (2015) cultivated the microalgae *Scenedesmus* sp. in raw tannery wastewater without previous treatment and without addition of nutrients in a 1:1 dilution in water. Cultures were carried

out in 5 liters bottles for 18 days. There were high removals of TKN (95.5%), Ammoniacal Nitrogen (97.9%), phosphorus (97.36%), COD (92.91%) and BOD (91.35%). Also in this same study, it was verified the ability of microalgae *Scenedesmus* sp. to assimilate the recalcitrant compound Nonylphenol ethoxylate 9.5 previously used as surfactant in the leather industry. The removal of 67.85% after 28 days of testing, for an initial compound concentration of 1000 mg L^{-1} was observed.

A recent study of Sundaramoorthy et al. (2016) with four microalgae species isolated from a tannery effluent (*Anabaena* VITMA1, *Oscillatoria acuminate* VITMA2, *Phormidium irriguum* VITMA3 and *Spirogyra maxima* VITMA4) examined the interaction of microalgae biomass with toxic metals. The results indicated that the isolates had the ability to tolerate stress to the chromium and therefore were grown in tannery effluents, in addition to the high biosorption capacity, reaching a 90% removal of chromium by *Oscillatoria acuminate.*

Another study was proposed by Meriç et al. (2005) in order to investigate the toxicity of the leather tanning wastewater by applying the *D. tertiolecta* microalgae in three treatment stages: raw wastewater after coagulation/flocculation and after biological treatment. Inhibition of microalgae growth was achieved when placed in crude or coagulated / flocculated effluent, but when the effluent used for analysis had undergone biological treatment resulted in a lower toxicity. Chemical oxygen demand (COD) and total soluble solids (TSS) removal were achieved in the coagulation / flocculation process of 62% and 84%, respectively. While the removal of COD, SST and NH_4-N in the final effluent from the treatment plant were 92%, 90% and 50%, respectively.

Speculation about the use of mixed cultures of microalgae have been carried out to obtain better results. Koreivienė et al. (2014) report that the microalgae consortium containing *Chlorella* sp. and *Scenedesmus* sp., was more efficient at removing nitrogen and phosphorus to treat the local municipal wastewater, as compared to individual culture of these microorganisms, obtained after three weeks of culture removals of 88.6 to 96.4% and from 99.7 to 99.9% of nitrogen and phosphorus, respectively. Studies with a consortium of ten strains of native microalgae of dairy farm wastewater showed good growth in these wastewater, removing 98% of the nutrients (PO4–P, NO3–N and NH4– N) present in 4 days of culture (Hena et al. 2015).

Thus, present study was aimed to investigate the treatment with a microalgae consortium of three types of wastewater collected in the treatment effluent plant of a tannery that processes leather from wet-blue to finished leather : raw wastewater (RW), wastewater after the physical-chemical treatment (primary coagulation/flocculation) (PW) and, wastewater after secondary biological treatment (BW).

2. Material and methods

2.1 Microalgae and tannery wastewater

The microalgae consortium was collected in a deactivated effluent treatment pond from a tannery located in Montenegro/RS. The culture was maintained in medium Tris-Acetate-Phosphate (TAP) in 250 mL Erlenmeyer flasks and adapted at room temperature, under constant aeration and continuous lighting. The growth until the exponential phase culture served as the inoculum (Gorman, D.S.; Levine, R.P. P. 1965).

Tannery wastewaters was obtained from a tannery that processes leather from wet-blue to finished leather located in Novo Hamburgo in the State of Rio Grande do Sul (Southern Brazil). Wastewater sample was collected from the effluent treatment plant of tannery, in the steps: raw wastewater - RW (without previous treatment), wastewater after the physical-chemical treatment - PW (primary coagulation/flocculation) and, wastewater after secondary biological treatment - BW.

2.2 Experimental setup and culture conditions

The cultivation of microalgae consortium was carried in raw wastewater (RW), wastewater after the physical-chemical treatment (primary coagulation/flocculation) (PW) and, wastewater after secondary biological treatment (BW) out in 500 mL erlenmeyers. The inoculum volume (from the exponential phase) was standardized for all experiments at volume corresponding to 10.0% (v/v) considering the final volume of 300 mL, with a final dilution of 1:1 (50% tannery wastewater and 50% water).

Initial pH of the dilutions was not adjusted prior to inoculation. The cultures were grown for 16 days at room temperature under constant aeration and continuous lighting**.**

2.3 Analysis of the biomass growth

The dry cell weight was measured by filtering the samples through 0.7 μm pre-weighed membranes, which were dried at 100°C for 24 h. The culture was also monitored daily by optical density (OD) measurement at 570 nm wavelength using a spectrophotometer (model T80+ UV/Vis, PG Instruments) after appropriate dilution.

2.4 Analytical methodology

The removal of nitrogen, ammonical nitrogen and phosphorus were evaluated before and after 16 days of cultivation. Ammonical nitrogen (NH_3) quantification was analyzed on the Metrohm Basic IC Plus Package Ion Chromatograph. Nitrogen NTK was quantified according to Standard Methods for the Examination of Water and Wastewater APHA (1998), and phosphorus by ABNT NBR 12772.

3.   Results and Discussion

The Figure 1 shows the concentration along the microalgae culture in the RW, PW and BW effluent. It is observed that the consortium was able to develop in all effluents analyzed, first undergoing an adaptation phase, and after showing a higher rate of growth after the 4th day of cultivation. The best growth was observed in the raw wastewater (RW). After the 12th day of cultivation, the growth began to have a decline in the wastewater after primary treatment (PW) and after biological treatment (BW), which may indicate that they were entering in the lag phase, because the curves begin to stabilize.
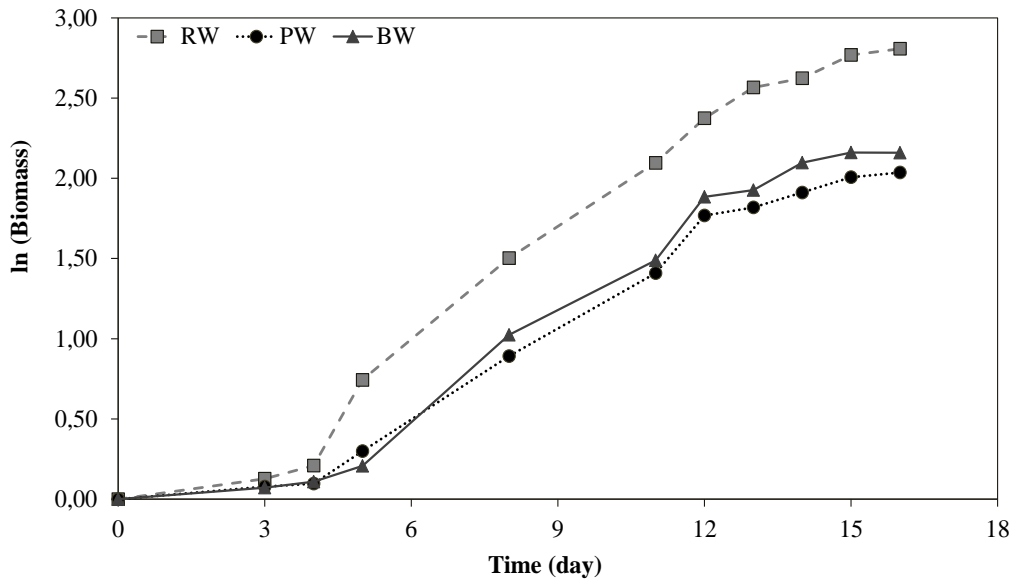
Figure 1. Growth of the microalgae consortium during 16 days of cultivation in tannery effluents: raw wastewater (RW) wastewater after the primary physical-chemical treatment (PW), and wastewater after secondary biological treatment (BW).

The pH values presented a discrete variation for all trials, throughout the treatment (Figure 2). The subtle elevation of pH in the first days of cultivation suggests an increase in photosynthetic yield.
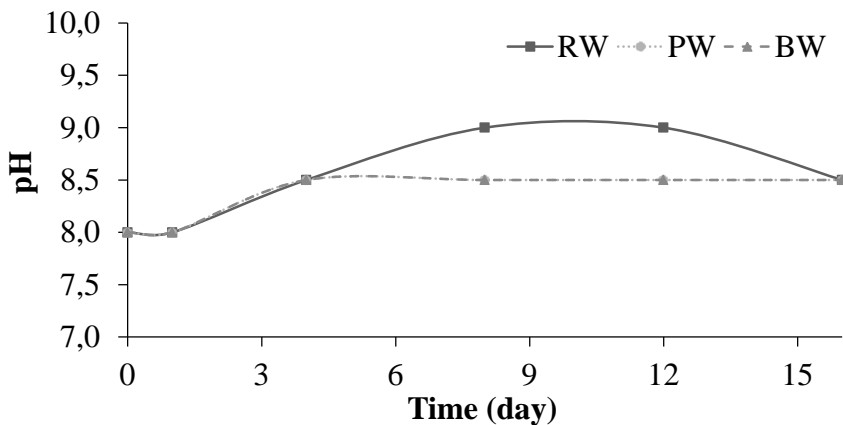

Figure 2. pH variation in the different culture conditions (RW, PW and BW) over time.

The results obtained for the phosphorus analysis are shown in Figure 3, where phosphorus concentrations, before inoculation with the microalgae and after 16 days of treatment are placed. The best result for this analysis was for the effluent after biological treatment (BW), reaching 91.85% of phosphorus removal, which started with 2.06 (mg L^{-1}) and was reduced to 0.17 (mg L^{-1}), followed by effluent after physical-chemical treatment (PW) with 88.59%, and treatment with crude effluent (RW) with 84.62% removal.

The assimilation of nitrogen by microalgae is carried out in the form of nitrates, nitrites and mainly ammonia, while phosphorus is consumed in its mainly inorganic form and with the help of enzymes in its organic form.

Concentrations of kjeldahl total nitrogen (NTK) before the test in the RW, PW and BW effluents were 155.4, 140.0 and 127.4 (mg L^{-1}), respectively, as shown in Figure 4, that were reduced to 26.6, 33.6 and 32.2 (mg L^{-1}) respectively, reaching the highest 82.88% removal value for the crude effluent.
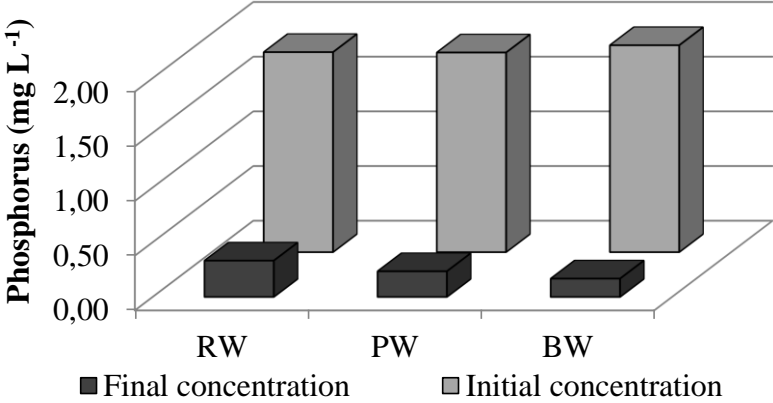

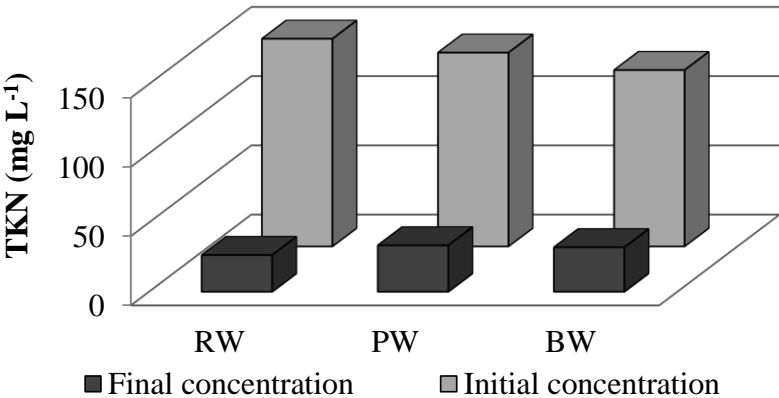**Figure 3.** Phosphorus in the effluents before and after the microalgae treatment.

Figure 4. Nitrogen in the effluents before and after the microalgae treatment.

According to the data presented in Figure 5, it can be observed that N-NH$_3$ concentrations, for all the final tests, are below the value detected by the ion chromatograph (0.05 mg L^{-1}), which indicates that the rate of removal was greater than 99.9% ammonia from the effluent. The fact that pH during the tests remains basic, around 8 to 9 may have facilitated the removal by volatilization of free ammonia (N-NH$_3$).
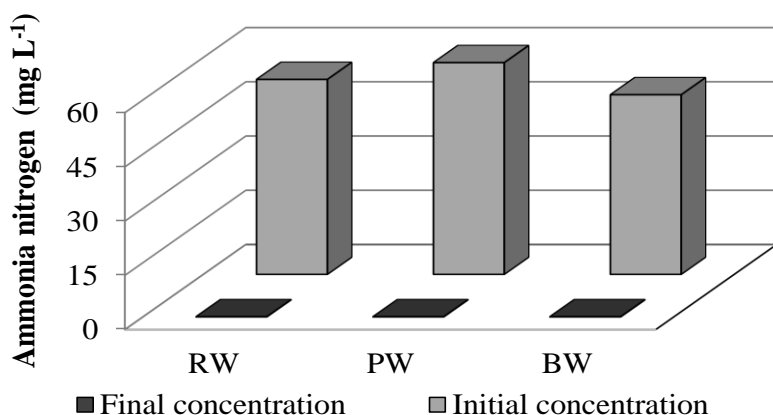
Figure 5. Ammonia in the effluents before and after the microalgae treatment.

These values are in agreement with the data found for NTK Nitrogen, since it is composed of free ammonia and organic nitrogen, in this way it can be stated that the remaining concentrations (26.6, 33.6 and 32.2 mg L^{-1} in the wastewater RW, PW and BW, respectively), are organic nitrogen.

In the crude effluent the results of effective removal of the nutrients, being above the values found by Meriç et. al. (2005), which indicates that the consortium of microalgae studied can be inoculated in a finishing effluent without any previous treatment.

4. Conclusion

The microalgae consortium showed to be able to grow in tannery effluents in the three different conditions, being efficient in the removal of nitrogen and phosphorus. The results obtained in the crude effluent obtained the best removal of nitrogen of 82.88%, while in the effluent after the physical-chemical treatment obtained the best removal of phosphorus of 91.85% and for the three effluents, there was removal of 99.9% of ammonia.

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TRIBAL INTEGRATION INTO LEATHER MANUFACTURING SYSTEMS (TRIMS): A CONCEPTUAL SOCIO-ECONOMIC BUSINESS MODEL FOR FASTER INCLUSION OF TRIBES INTO THE MAINSTREAM SOCIETY

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The Indian leather industry occupies a prominent place in the Indian economy in view of its massive potential for employment, growth and exports. The leather sector has been set a target of USD 27 billion to be achieved by 2020, which includes export target of USD 15 billion from the present turnover of USD 6.5 billion and domestic market turnover of USD 12 billion from the present turnover of USD 6 billion. The Indian leather industry comprises of major segments like footwear, finished leather, leather goods, leather garments, leather gloves, footwear components and harness and saddlery. All these segments have high growth potential. The tribes of India are among the poorest and most marginalized sections of Indian society, and most of them are living below the poverty line, illiterate and suffer from extremely poor physical health. To illustrate the poverty among scheduled tribes the people below poverty line were 47.1% in urban and 28.8% in rural for the year 2009-10. The scenario has been similar in the sphere of education and health. The literacy of tribes in 2011 was 58.96% as compared to 72.99% of the total population. Moreover as per the National Family Health Survey, (2005-06), the Infant Mortality rate was 62.1 per 1000 live births among tribes and Under-five mortality was as high as 95.7 per 1000 live births. The development of the tribal population in India has been a major concern of the government, voluntary agencies, NGOs, social reformers, social scientists, etc. The unemployment rate among urban scheduled tribe men and women were 3.4% and 4.8% in 2011-12 respectively, while in rural areas, unemployment rate for scheduled tribe men and women were 1.3% and 1.1% for the same period. Unemployment often leads to immense poverty. **TRIMS** (**Tr**ibal **I**ntegration into Leather **M**anufacturing **S**ystems) is a conceptual socio-economic business model which aims to elevate the tribes from the edges of the society, adorn them in the mainstream and create a state of equality. The feasibility of training the scheduled tribes in leather products manufacturing and integrating them into the leather product manufacturing system has been analyzed.

Keywords: Indian leather industry, tribes, poverty, training, integration, manufacturing system

1. Leather industry in India – the vanguard of inclusive development

The Indian leather industry is poised to reach an optimistic target of 27 billion USD turnover from the current level of 12 billion USD by 2020 [1]. Leather industry is among the top ten foreign exchange earners for the country. The leather industry is bestowed with an affluence of raw materials as India

is endowed with 21% of world cattle and buffalo and 11% of world goat and sheep population [2].The leather industry has undergone a dramatic transformation from being a mere exporter of raw materials in the nineteen sixties to being a producer of value added finished products in the nineteen nineties [3].

The leather industry in India is geographically well diversified, though Tamil Nadu, Uttar Pradesh and West Bengal account for bulk of the output. The major production centres for leather and leather products are located at Tamil Nadu (Chennai, Ambur, Ranipet, Vaniyambadi, Trichi, Dindigul and Erode), West Bengal (Calcutta), Uttar Pradesh (Kanpur, Agra, Noida and Saharanpur), Maharashtra (Mumbai), Punjab (Jalandhar), Karnataka (Bangalore), Andhra Pradesh (Hyderabad), Haryana (Ambala, Gurgaon, Panchkula, Karnal and Faridabad), Delhi, Madhya Pradesh (Dewas) and Kerala (Calicut and Cochin).The sector is dominated by micro and small units with bigger units accounting for just around 5 per cent of the total manufacturing units. The distribution of the units in this sector in terms of the broad classification of MSME and others is indicated below:

Table 1: Structure of Indian Leather Industry

	Large Units	Medium Units	Small Units	Micro Units	Merchant Units	Total
Finished leather	30	49	309	68	151	607
Leather Footwear	38	46	228	49	81	442
Non Leather Footwear	4	2	34	13	17	70
Footwear Component	29	32	182	28	22	293
Leather Goods	14	13	242	259	210	738
Leather Garments	8	8	132	49	72	269
Leather Gloves	4	3	38	36	24	105
Harness and Saddlery	3	9	74	69	26	181
Total	130	162	1239	571	603	2705

Source: Report of Working Group on Leather & Leather Products Twelfth Five Year Plan Period (2012-17)

The leather industry is spread in different segments, namely, tanning and finishing, footwear and footwear components, leather garments, leather goods including saddlery and harness, etc.

Table 2: The estimated production capacity in different segments

Product	Capacity
Leather hides	65 million pieces

Skins	170 million pieces
Footwear & footwear components	909 million pairs
Leather shoe uppers	100 million pairs
Non-leather footwear	1056 million pairs
Leather garments	16 million pieces
Leather goods	63 million pieces
Industrial gloves	52 million pairs
Saddlery & Harness	12.5 million pieces

The leather industry is an employment intensive sector, providing job to more than 2.5 million people, mostly from the weaker sections of the society. Women employment is predominant in leather products sector with about 30% share. The estimated workforce constitutes 1.1 million in finished leather, 1.1 million in footwear and components and 0.3 million in leather goods and garments. The expected employment by 2022 is 7.1 million, needing an additional 4.6 million workforce across various segments [4].

2. The tribes of India

The tribal communities in India are enormously diverse and heterogeneous. The census of 2011 enumerates the total population of Scheduled Tribes at 10.42 crore, constituting 8.6 per cent of the population of the country. There are wide ranging diversities among them in respect of languages spoken, size of population and mode of livelihood. The number of communities that find their place in the list of the Schedule of the Indian constitution is reflective of this diversity. As per the Census of India 2011, the number of individual groups notified as Scheduled Tribes is 705.

Scheduled Tribes communities live in about 15% of the country's area, in various ecological and geo-climatic conditions ranging from plains and forests to hills. A large proportion of Scheduled Tribes are collectors of forest produce, hunter-gatherers, shifting cultivators, pastoralists and nomadic herders, and artisans. Traditional occupations of tribal groups may range from honey-collection to hunting small animals to engaging in metal-work and rope-making. A majority of tribal groups work in the primary sector, and are heavily dependent on agriculture either as cultivators or as agricultural labourers. At the same time, a number of Scheduled Tribes no longer follow their traditional occupations and work as labourers on plantations or in mines and factories (in many cases, since the nineteenth-century). Displacement and enforced migration has also led to an increasing number of Scheduled Tribes working as contract labourers in the construction industry and as domestic workers in major cities. Over 80% of Scheduled Tribes work in the primary sector against 53% of the general population, primarily as cultivators.

Scheduled Tribes comprise 11.3 per cent of the Indian rural population and 2.8 per cent of the Indian urban population. The total male ST population according to the 2011 census is 5,24,09,823 of which 4,71,26,341 are residing in rural areas and 52,83,482 are in urban areas. The total female ST population is 5,18,71,211 with 4,66,92,821 in rural areas and 51,78,390 in urban areas. The Central-East Indian region (Andhra Pradesh, Bihar, Jharkhand, Madhya Pradesh, Chhattisgarh, Odisha and West Bengal) has the largest proportion of STs, about 52.51 per cent. The Western region (Rajasthan, Gujarat, Daman and Diu, Dadra and Nagar Haveli, Maharashtra and Goa) has 27.64 per

cent of STs. The Northeastern region (Sikkim, Arunachal Pradesh, Nagaland, Manipur, Mizoram, Tripura, Meghalaya and Assam) has 12.41 per cent of STs. The Southern region (Karnataka, Kerala and Tamil Nadu) has 5.31 per cent of STs. The Himalayan Region (Jammu and Kashmir, Himachal Pradesh, Uttarakhand and Uttar Pradesh) comprises 2.03 per cent of STs and 0.11 per cent of STs live in the island region of Andaman and Nicobar Islands and Lakshadweep [5,6].
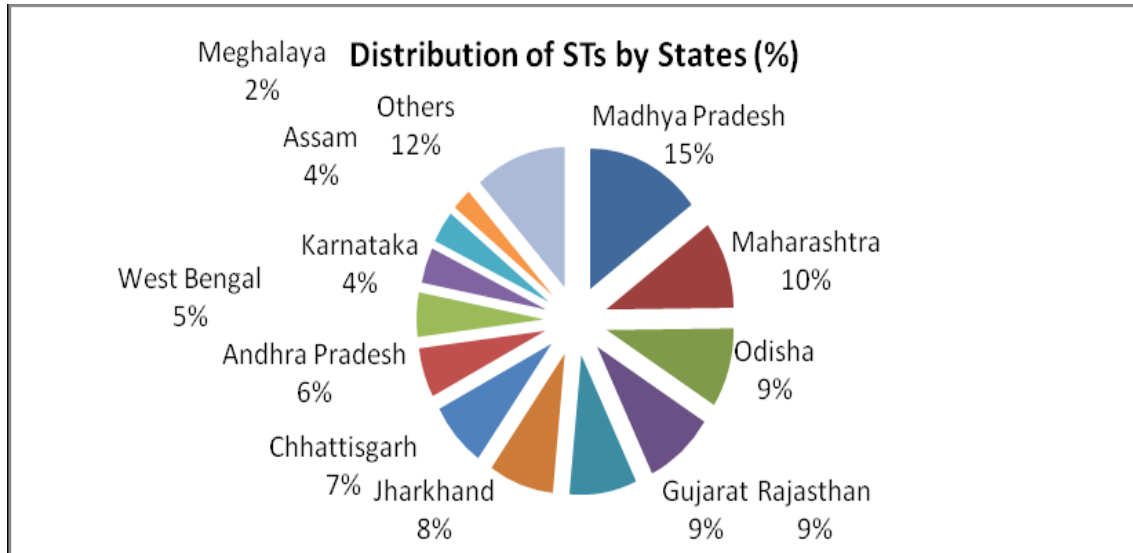

Figure 1: State-wise distribution of STs (as per the 2011 census)

3. Tribal Integration into Leather Manufacturing System

Tribal Integration into Leather Manufacturing System is a conceptual socio-economic business model for the faster inclusion of tribes into the mainstream society. It involves carrying out a comprehensive physical survey in the tribal region and identifying development opportunities for leather and allied sector. The tribal skills and artisanship like traditional weaving, block printing techniques, tribal embroidery, bead &jewellery making, rope making, painting etc possessed by the tribes need to be studied. Based on their native skills a customized training programme in leather product making is planned to be conducted by establishing training centres. The tribes will be trained in the training centres without losing their tribal uniqueness or identity. The tribes are then encouraged to start their own company or placed into the exisitng leather product manufacturing industries. The objective of this model is to conduct skill development programmes in leather product manufacture for the scheduled tribes and integrate the scheduled tribes into the leather product manufacturing system. The key achievements envisaged are development of skilled manpower for manufacture of leather products, creation of self employment for tribal people and enhancement of socio-economic status of the tribal people in the identified locations.

SWOT Analysis of Tribal Integration into Manufacturing System (TRIMS) model:

STRENGTHS

- Pan India distribution of leather industries
- Pan India distribution of tribes
- Tribal skills and artisanship – skills in traditional weaving, block printing techniques, tribal embroidery, bead & jewellery making, rope making , painting etc.

- Traditional knowledge of tribes in natural dyes and pigments which they use in their paintings and ethnic wears, ethnic crafts etc
- Possession of creative designs and applications by tribes that are known for their vitality, color and intricacy

WEAKNESSES

- Lack of tribal specific training centre in leather product manufacturing
- Infrastructure facilities like road, communication etc are poor in certain tribal areas
- Lack of teaching training learning materials in tribal language

OPPORTUNITIES

- Growing domestic market for footwear, fashion accessories like hand bags, wallets, purses etc.
- High growth potential and labor requirement of the leather sector
- Marketing of tribal products throughe-portals and government cooperative stores with the support of organizations like Tribal Cooperative Marketing Development Federation of India Limited (TRIFED)

THREATS

- Cultural resistance
- Growing shift towards automation

4. Conclusion

Tribal Integration into leather Manufacturing System (TRIMS) is a conceptual socio-economic model for the faster inclusion of tribes into the mainstream society. The model if adopted has the potential to eliminate unemployment, poverty and other socio-economic problems related to the tribes in India. As the tribes will be included in the leather product manufacturing sector, their income earning capacity will be increased. Tribes will be included in the holistic development of our country. Also it enhances the availability of trained workforce for the leather sector which is labour intensive.

5. Suggestions and recommendations

The government can provide financial support for establishing training centres and conducting specialized skill development programmes for tribes in leather product manufacturing.The government can also provide scholarships / stipends to the tribes undergoing the training programme in leather product manufacturing. Leather machineries at subsidized rates can be provided by the government for setting up of manufacturing units by tribes after they successfully complete their training programme in leather product manufacturing, to encourage entrepreneurship among them.

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LEATHER BIOCIDES: A REVIEW OF TECHNICAL AND REGULATORY REQUIREMENTS

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According to evolution microbes were the first living cells on earth, circa 3,5 billion years ago. Microorganisms are ubiquitous present in different amounts and different habitats. Micro-organisms can be useful, harmful, dangerous or material destroying.

In the leather industry raw hides and/or skins and as intermediates or even final leathers are constantly under attack, resulting in their down-grading or even destroyed by microbes.

So called "weapons" to fight against harmful micro-organisms can be classified as either physical approaches and/or chemistry based methods.

Nature has also developed so called "natural biocides" , derived from e.g. plants or even bacteria. The chemical industry has used these natural biocides as an example for the development of optimized and highly defined biocides to minimize environmental and human risk and to optimize efficacy. The main synthetic biocides used in the leather industry PCMC, OPP, OIT and TCMTB are roughly divided into two "modes of action", membrane and electrophilically actives.

Biocides are intended to kill living micro-organisms and could potentially pose risk to the environment and human health if not handled correctly. A risk assessment must be done where a combination of hazard and exposure is evaluated. The risk of a biocide in a special application, like leather manufacture or the risk of wearing leather shoes can be identified by a risk assessment. Using the example of the European Biocidal Product Regulation (BPR, Regulation (EU) 528/2012) it is intended to show how and why a risk assessment is not only useful but absolutely necessary.

Keywords: Biocides, mode of action, biocide regulation, risk assessment, PCMC, OPP, OIT; TCMTB

Microorganisms – The Good and The Evil

Life first emerged circa 3.5 billion years ago, approximately 750 million years after Earth first came into existence. Life is generally divided into two main classes, initially defined by whether they contain a nucleus or not. Prokaryotic cells lack a nuclear envelope. Eukaryotic cells have a nucleus where the DNA is separated from the cytoplasm. Microorganisms appeared as the starting point of the evolution of life on our planet. Microorganisms are ubiquitous and vary in their presence and preferred habitats. Wherever they find their ideal breeding conditions such as adequate available water and nutrient they reproduce exponentially. A gram of fertile arable soil contains up to 5,000,000,000 microorganisms per gram. Waste water and fluids used for technical purposes can have between 1,000,000 and 10,000,000 microorganisms per milliliter and even high-quality drinking water has up to 100 microorganisms per milliliter. Public opinion about microorganisms can be, in general, somewhat negative but microbes can also be useful for the human population. Alcohol fermentation, cheese production, baking of bread, penicillin and other anti-biotics, purification of waste water at biological treatment plants, biogas from waste, and in Germany even the production of "Sauerkraut"! are just a few examples of the many benefits. Nevertheless micro-organisms are

also responsible for many infectious diseases (tuberculosis, plague disease, pneumonia), forming poisonous or carcinogenic metabolites. Additionally, they are also responsible for microbiological decomposition and destruction, they attack valuable materials, disturb production processes and can influence the quality of final products.
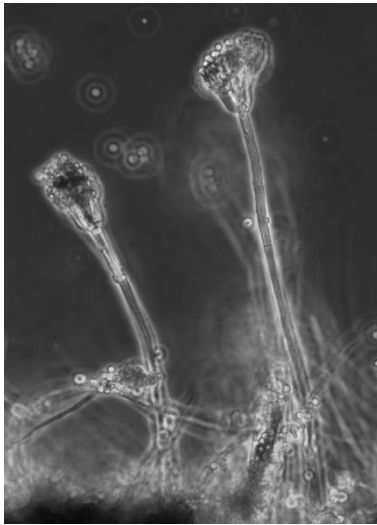

Figure 1: Microscopic picture of a mould found on leather samples. Picture from LANXESS Deutschland GmbH

The root causes of these effects and also the diseases and the decay of material and products was only investigated relatively late starting around the 19th century. Once it was discovered that microorganisms were the root cause, especially for the decay of materials and infectious diseases, physical weapons were the first methods relied upon in order to inactivate or kill them. Heat is a very effective physical treatment but in many areas of medicine and material protection not applicable. Chemicals and methods were developed relatively soon after and Joseph Lister made use of carbolic acid (phenol) for the first time to kill bacteria on medical instruments or even directly on wounds in 1867.

Influence on the leather industry

In the leather industry raw hides and/or skins and as intermediates or even final leathers are constantly under attack, resulting in their down-grading or even destroyed by microbes.

Once an intermediate (wet blue, wet white, vegetable tanned…) is affected by mold and the decay of the leather has started the damage cannot be reversed. This means that damage to the leather intermediates already has an effect on the final leather. A reduction in the quality of wet blue usually represents a significant economic loss for the tanneries.
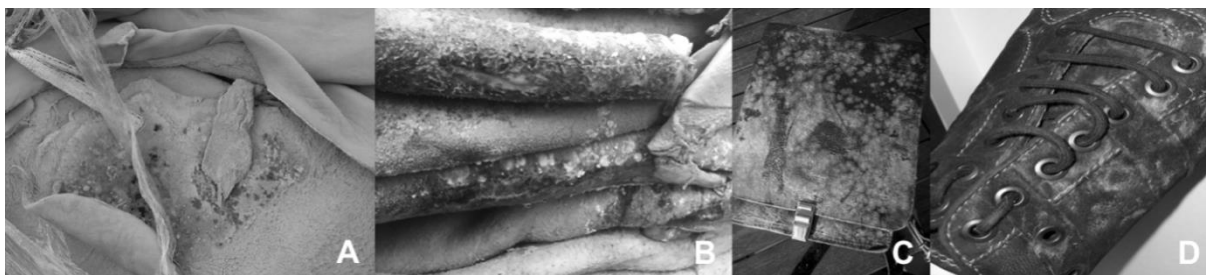
Figure 2: Examples of molded leathers and leather intermediates. A+B Wet blue; C Bag Leather; D Shoe Leather; Pictures from LANXESS Deutschland GmbH

Accordingly, tanneries in principle have a great interest in protecting their leather intermediates from microorganisms. Physical methods like heating are not applicable for tanneries as a sterilization process is usually done at >100 °C. A simple physical removal, such as washing off, of the microorganisms is also difficult in a tannery environment and not a permanent effect. To store and dispatch the wet intermediate leather goods in refrigerated units is a possible option but also here usually not easy and simply too expensive. So there is only currently the possibility for tanneries to protect their valuable goods by a chemical treatment. Which chemicals can be and should be used and what are the advantages of the origin of the chemicals? Nature has developed chemicals which are derived from e.g. plants or even bacteria itself (Harborne and Simmonds, 1964). The tannin compounds or polyphenols with carbohydrates are widely distributed in many species of plants, where they play a role in protection from predation, in plant growth regulation and also as pesticides. In general biocides derived from nature are from lower activity and stability. The minimal inhibitory concentration (MIC) for those chemicals is higher thus an increased amount must be used to have a protective effect which in combination with a complex production and high costs is not economical for the leather industry. Furthermore, usually these are combinations of different chemicals which are not clearly defined and sometimes even unknown which presents a risk for both tannery and leather workers and ultimately the consumer. Additionally these nature derived products often have variable tanning properties which is problematic and undesirable.

Chemical weapons for the leather industry

Tanners need to apply and are currently using synthetic chemicals to combat microbial leather decay. The chemical industry offers a compromise between costs and benefits and developed optimized and highly defined biocides to minimize environmental and human risk and to optimize efficacy. Several commercially biocides are available to choose from plus numerous formulations of these active ingredients. The main active ingredients used in leather biocide products are PCMC (4-chloro-3-methylphenol), OPP (2-phenylphenol), OIT (2-octyl-1,2-thiazol-3-one) and TCMTB [2-(Thiocyanomethylthio) benzothiazole]. These actives are the most commonly applied in the leather industry currently and are used in the latest developments in the world of preservative technology (Tysoe 2010). They can roughly be divided in two main categories ("mode of action"), firstly, membrane actives and secondly, electrophilically actives. Electrophilically actives (e.g. aldehydes or compounds with an activated N-S bond) are in search of substrates with a heightened electron density such as nucleophilic components of the microbial cell. This contact results in an electrophilic addition or substitution. Nucleophilic reaction partners on and in the cell are amino, thiol, amide groups and proteins. This results in an inhibition of cell components and cell death. Electrophilically actives are intrinsically non-persistent and do not accumulate which gives the disadvantage of stability and duration of activity. Membrane actives (e.g. alcohols or phenols) start relatively

unspecific by coating the microorganisms cell wall. This is a process which initially can be reversed by dilution especially to non-lethal concentrations. It causes changes in the outer membrane and the cell wall which then loses its integrity. Molecules can now pass the cytoplasmic membrane and can be effective within the cell. Disarrangements in the semi-permeable properties of the cytoplasmic membrane, inhibition of enzymes, escape of essential cell components and disintegration of the cells are the final result. The active ingredients remain intact can be effective again giving a long-term effect (Paulus 2005).

Defense of nature

Different microorganisms have variable individual sensitivity and resistance to biocides. This is very much dependent on the natural type and structure of the cell and the cell wall and is known as "intrinsic resistance". Whereas "acquired resistance" is a result of evolution and selection of a microorganisms permanently exposed to biocides. For the industry, its processes and also leather production, especially wet blue production, the acquired resistance plays a minor role and usually is a rare exception. The intrinsic resistance in contrast has very much to be taken into account when the optimum protection of leather and leather intermediates against microbiological decay has to be guaranteed. Thus, the mode of action, effectiveness and minimum inhibitory concentrations of the chemical weapon has to be considered. The interaction of membrane-active agents with the microbial cell is a process where the active ingredients remain intact and give a long-term protection when above an effective concentration. Electrophilically actives react with components and the stability and thus also the effectivity is very dependent on natural conditions like germ count. Membrane and electrophilically actives should be combined (Tysoe 2011) to cover most of the intrinsic resistance of different species and also to minimize the risk of acquired resistance.

Environment and health

The unavoidable use of chemicals in the leather industry and its production processes represents a certain environmental impact. As described above also the use of biocides is a must for the leather industry as there are so far no feasible alternatives which guarantee a long-term protection. Globalization of the leather market and the resulting international trade makes it absolutely necessary to protect the goods through the use of biocides. Biocides are designed specifically to kill microbes, however, they may also pose a risk to the environment and human health if not handled properly. Therefore, footwear, leather, fashion and textile brands have committed to protect consumers, workers and the environment. They have developed so called "Restricted Substances Lists" (or more commonly referred to as RSLs) to direct their suppliers in the production of safe and compliant products. Biocides are included on these RSL's and limits are set but some lists still contain outdated or even completely incorrect information or based on redundant data. This can unwittingly force tanneries to use problematic chemicals that are not on the "safe" lists which are not tested nor risk assessed. Such problematic chemicals can be dangerous for the consumer, leather workers and broader environment. Actually, the purpose of restricted substances lists is not only to minimize the risk to the consumer as far as possible but also make the production of leather, the one of the world's most sustainable materials, still feasible. Many brands and NGO`s have recognized this and already started to adjust the lists (Weckmann 2013). To evaluate the requirements of the leather industry regarding the safe use in terms of environmental and human health, the risk has to be assessed in every single application for every single active ingredient.

Risk assessment

In 2008, EPA (U.S. Environmental Protection Agency) completed a review of older pesticides to ensure scientific and regulatory standards. Scientific studies were reviewed and human health and

ecological effects assessed (https://www3.epa.gov/). A similar program was set-up in the European Union in 1998 which is now known as the Biocidal Product Regulation (BPR, Regulation (EU) 528/2012) and "concerns the placing on the market and use of biocidal products, which are used to protect humans, animals, materials or articles against harmful organisms" (https://echa.europa.eu/). After the first step of notification of the active substances (completed for all permissible actives) a registration of the active substance in the name of the supplier (in process) is needed, finally an authorization of the formulated product has to be started. In the course of this registration and authorizations, risk assessments are prepared. Risk is defined as combination of hazard identification and exposure evaluation. If a chemical has a high hazard classification but there is no expected exposure to human or environment in a special application the risk is acceptable. An example is the neurotoxin Botulinum toxin which is produced by the bacterium *Clostridium botulinum* and is the most acutely lethal toxin. But if there is no contact (exposure) there is no risk for humans or environment and therefore it is acceptable. More specifically, it has even been decided that small amounts are allowed in cosmetic surgery (Botox treatment). On the other hand when a chemical has a very low hazard classification but the exposure is high the overall risk for human and environment is also acceptable. An example is water (H_2O) which has no hazard classification and exposure to human and environment is high but the risk of water is acceptable. Unfortunately, the risk classification in chemistry is not always so clear. Normally, the chemicals have a medium hazard and the exposure must be determined and assessed. There are different areas of risk assessment under the E.U. Biocidal Product Regulation. Human health risk assessment which includes (I) primary exposure e.g. when manufacturing the pure chemical, preparing the formulations but also when applying the formulation to the drum in a tannery. The (II) indirect exposure is assessed when handling treated substrates (e.g. wet blue, or finished leathers) in industrial environment and consuming treated articles (e.g. wearing shoes). Additional to the human health risk assessment the environmental risk assessment includes a direct exposure emissions to water, air, soil, sediment, groundwater by the chemical industry and leather industry and indirect exposure like wearing treated substrates and the disposal of leather goods. Only acceptable risk can be tolerated. If a risk is unacceptable in a first step, risk mitigation measures (RMMs) may be introduced (e.g. use restrictions, concentration restrictions, personal protective equipment (PPE), disposal restrictions, etc.). Basic data, such as acute, long-term, genotoxicity, carcinogenicity, reprotoxicity, toxicity towards water organisms or biodegradability in water / soil are necessary. Also supporting data like air monitoring, migration tests, fogging tests, emission tests, influent / effluent monitoring etc., are the expensive basic data for all calculations and risk assessments and must be provided by the registrant. Risk assessments for human health and for the environment prove the safety for industrial workers and for the consumers (adult & children) coming into contact with treated leather articles.

Outlook

At present within the product type 9 (Fiber, leather, rubber and polymerized materials preservatives) 43 substances are listed, 2 approved, 3 not approved and the remaining are still under review. In fact, only a few of the listed chemicals are suitable and will likely be approved for leather production. Finally there will be a list of registered and approved substances. From 1 September 2013, the active substances contained in a biocidal product used in the treatment of the treated articles, have to be either already approved or under evaluation for the relevant product-type. For active substances which were not in the approval process, there was a transition period until 1 September 2016. After 1 March 2017 it will not be possible any more to place on the EU market articles treated with containing an active substance which is not already approved. Biocidal products may only be used

within Europe, if authorization has been granted to the supplier for this specific formulation. The registration and authorization costs for the active ingredients and the formulated products are high and with regard to a complete new substance the costs are even higher. With these regulations and associated costs and with even more regulations and registration requirements to come in future and in other countries or regions the hurdle to bring a complete new active substance on the market is very high and likely to get only higher. This should be carefully taken into account by shoe, leather and fashion brands before arbitrarily banning or limiting biocides unnecessarily. They should take into consideration that the risk assessment is already carefully completed for different product types and therefore RSL's should also distinguish between leather and e.g. textile or rubber. Maybe in future a global registry could be something to be considered even if there will be conflicts of interest.

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INK DYES FOR LEATHER INDUSTRY

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Dyes and pigments offer significant potential for functional and aesthetic design of leather materials. Search for viable alternative leather colorants, which have the potential to develop new creative designs and provide functionality are presently in focus. In this context application of alternative textile dye substances that can offer special effects to leather have increasingly gained importance. In this study, the potential application of ink dyes in leather industry was investigated as an alternative to conventional dyes for the production of leathers with high fastness properties. For this purpose, leather dyeing recipes were developed by using two different ink dyes including latex and dye based ink dyes and applied at dying process of metis type crust leathers in order to produce high performance leathers. The quality performance of dyed leathers were investigated in terms of colour measurements, to-and-fro and crockmeter rubbing fastness, before and after washing leathers in a washing machine under specified conditions according to ISO 15702 standard. The colour measurements prior and subsequent to washing process were determined with Minolta CM-3600A spectrophotometer. To-and-fro rubbing and crockmeter fastness properties were examined in accordance with TS EN ISO 11640 and ASTM D5053 standards respectively. The results of the study showed that latex and dye based ink dyes could be used as a newly adapted dye group and applied successfully in leather dyeing process for the production of leather goods with high fastness properties.

Keywords: ink dyes, colour fastness, leather dyeing, washing fastness, light fastness

1. Introduction

Inks are vended in liquid and paste form, which are used to color a surface to produce an image, text, or design. They are available in both pigment and dye-based formulations for commercial print and widely used in textile applications. Dyes and pigments provide a broad range of functional and unique aesthetic design options for textile industry, and they offer significant potential for leather materials. Pursuit of potential alternative dyes and colorants for leather industry, which provide both novel, creative designs and multifunctionality, has come into focus in recent years (Adiguzel Zengin et al. 2016)

In this study, the potential application of ink dyes in leather industry was investigated as an alternative to conventional dyes for the production of leathers with high fastness properties. For this purpose, two different types of ink dyes latex and dye based ink dyes are applied at leather dying process in order to produce high performance leathers.

## 2.	Materials and Methods

Leather dyeing recipes were developed by using latex and dye based ink dyes and applied at dying process of metis type crust leathers (Table 1). All the dyes were water based dyes and supplied from Akici Inkjet and Laser Technologies in Istanbul, Turkey.

Table 1. Dying recipe of latex and dye based inks

Re-wetting	Material	%	
	Water 35°C	500	60'
	Ammonia	1.5	pH:6-7 Ø
	Wetting agent	1.5	
Dying	Water 30 ^{o}C	130	
	Latex and Dye based inks	2	30'
Fatliquoring	Synthetic	5	
	Natural-synthetic	7	45'
Fixation	Formic 1:10	3	2x15'
	Formic 1:10	1	2x10' Ø
Drain and wash			

The quality performance of dyed leathers were investigated in terms of color measurements, to-and-fro rubbing fastness and crockmeter rubbing fastness characteristics before and after washing leathers in a washing machine under specified conditions according to ISO 15702 standard.

In order to assess the effect of dying process performed with ink dyes on the color of leathers, the colors of all leather samples and their color differences with the white standard were evaluated according to the CIE Lab color coordinate system (McLaren, 1983). The color measurements prior and subsequent to washing process were determined with Minolta CM- 3600A spectrophotometer. To-and-fro rubbing and crockmeter fastness properties of ink dyed samples were examined in accordance with ISO 11640 and ASTM D5053 standards respectively.

## 3.	Results and Discussion

CIE Lab color values of leather samples before and after washing was shown at Table 2 and 3 respectively. Higher values of lightness (L) indicate closeness to white (Mutlu et al., 2014). Considering the L values, grain samples of dye based ink leathers show lower lightness value. The second predominant color observed was green, which was detected by higher a values at dye based ink leather samples.

Table 2. Color measurement values of ink dyed leathers before washing

		L	a	b	dL	da	db	dE
Latex	grain	57.73	-0.03	2.06	-41.19	0.09	2.41	41.35
	suede	60.48	-0.787	2.12	-38.44	-0.67	2.47	38.80
Dye based	grain	65.12	17.98	-7.46	-35.36	19.01	-7.51	40.90
	suede	48.81	31.04	-8.36	-50.12	31.16	-8.00	59.63

The color measurement values of latex and dyed based ink leathers showed that there is a color difference between the grain and suede side of the leathers (ΔE; dE). In case of latex its clear that, there is difference between brightness of grain and suede side; so that, the suede side is brighter than grain. Both b and a values that corresponds to greenness and yellowness of suede are higher than grain. In addition, suede side of dye based ink is darker than grain, redness and yellowness of suede is higher than grain.

Table 3. Color measurement values of ink dyed leathers after washing

		L	a	b	dL	da	db	dE
Latex	grain	59.50	-0.03	2.66	-39.42	0.08	3.01	39.60
	suede	63.33	-0.1	2.45	-35.59	0.01	2.80	35.84
Dye	grain	70.62	10.71	-0.1	-28.30	10.82	-0.64	30.32
based	suede	55.56	27.01	-5.60	-43.36	27.11	-5.25	51.45

*L, a, b values of white color as a target given as respectively; 98.92 , -0.11, -0.35

After washing, the brightness of the ink dyed leathers was obtained higher than the brightness values of the leathers before washing. The color difference of the grain and suede side of the leathers was still found (ΔE).

In general, all the values were got a little bit through opposite direction; It means, grain and suede side were became lighter. The samples dyed with latex based ink dyes lost its greenness and became red; on the other hand the redness of the dye based samples, was reduced and turned into green. Both latex and dyed samples has become more yellowish. As a result, the washing behaivour of the latex dyed ink dye was found more stable than the dye based ink dye.

Table 4. To and fro rubbing fastness results of ink dyed leathers before washing

	Felt		**Leather**	
	Wet	**Dry**	**Wet**	**Dry**
Latex	4/5	4/5	3/4	3
Dye based	4	5	3/4	4

The to and fro rubbing results presented in Table 4 reveals that, in the case of felt, both dyes are satisfactory and the best performance is provided by dry-felt in dye based ink dye. However,the situation is not the same for the evaluation of leathers that the findings in leather rubbing are found lower than felts according to gray scale (Table 4).

After washing suprising results for the dye based ink dyed samples were obtained (Table 5). Its clear that, the results of felts are satisfying and no differences were found between before and after washing. On the other hand wet and dry leather samples have slightly increased their grades, in comparison to before washing process and the lowest grade was obtained from leather-dry specimen

Table 5. To and fro rubbing fastness results of ink dyed leathers after washing

	Felt		Leather	
	Wet	**Dry**	**Wet**	**Dry**
Latex	4/5	4/5	4	3/4
Dye based	4/5	5	4/5	4/5

Rubbing fastness performance of both dying agent are almost equal after the washing treatment. Hence, grade of wet and dry felt are practically same for both after and before washing process. But due to the removal of dye stuff in washing treatments, the results are pretended to be higher compared to results obtained before washing (Table 5).

Crockmeter fastness results before and after washing were given in Table 5 and 6.

Table 6. Crockmeter fastness results of ink dyed leathers before washing

	Fabric		Leather	
	Wet	**Dry**	**Wet**	**Dry**
Latex	4/5	4/5	2	1
Dye based	4	4	1	1

The obtained result of crockmeter before washing are quite satisfying, so that, the points which given to fabrics, in both wet and dry, are higher; in particular the latex one. In the case of leathers, it clearly shows that, grades are considerably high (Table 6).

Table 7. Crockmeter fastness results of ink dyed leathers after washing

	Fabric		Leather	
	Wet	**Dry**	**Wet**	**Dry**
Latex	5	4/5	2	1
Dye based	5	4/5	1	1

Similar to before washing results, the gray scale grades are acceptable. The comparison of "before and after washing" shows that there isn't any diferrence, which can be concluded that process of washing doesn't effect crockmeter fastness of ink dyed samples.

Washing fastness results indicate that, black dye has a satisfactory performance for this test (Table 8). It can be interpreted that the bonds beween latex based ink dye and leather fibers are so stable that dye molecules showed resistance and didn't washed off the leather during washing process. Dye based ink dye samples provided the lowest grade in fabric and lost their color intensity.

Table 8. Washing fastness results of ink dyed leathers

	Washing		
	Suede Side	**Grain Side**	**Fabric**
Latex	4	4	4
Dye based	3	3	1/2

4.   Conclusion

The preliminary results obtained from this study show that ink dyed samples provided satisfactory fastness performances before and after washing process. Latex and dye based ink dyes can be used as a newly adapted novel dye group and applied successfully in leather dyeing process for the production of leather goods with high fastness properties.

5.   Acknowledgments

6.   References

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PHOTOCATALYTIC DEGRADATION OF ACID BLUE 113 DYE USING ZnO/SiC NANOCOMPOSITES UNDER UV AND VISIBLE LIGHT IRRADIATIONS IN A SLURRY PHOTO REACTOR

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Removal of organic and inorganic toxic materials from wastewaters is being given   importance   in recent years due to the discharge of untreated industrial wastewater into the different environmental compartments. Acid Blue 113 is a most widely used di-azo compound in leather, textile, dying and food industry as a colouring substance,   hazardous and are to be treated before discharge into aquatic environment. Here, we are reporting the photocatalytic degradation of Acid Blue 113 dye using a ZnO/SiC nanocomposite as a photocatalyst under UV and visible light irradiations. The photocatalyst was prepared by a simple sol-gel method and   characterized by XRD, UV-DRS, FT-IR, AFM, EPR, FE-SEM, EDAX, HR-TEM and SAED analyses. The analytical results were proved that the prepared photo catalyst were highly crystalline, nanosized and possesses absorption in the UV and visible regions. The UV-Vis-DRS results suggested that the band gap energy was 3.019 eV for the prepared photo catalyst. ZnO/SiC photocatalyst produced OH radicals instantaneously within 60 s and 15 min for UV and visible irradiation respectively. The effect of various experimental parameters such as pH, catalyst dosage and variation of concentration were investigated in detail to optimize the efficient degradation of Acid Blue 113. It is observed that neutral pH was found to be optimum with maximum % of degradation of the Acid Blue 113 dye molecules. Catalyst dosage of 10mg/10ml resulted in higher percentage of degradation. The photo degradation process followed a pseudo first order kinetics and was continuously monitored by UV-Visible absorbance measurement and COD.

Keywords: Photocatalyst; nanocomposite; Acid blue 113; sol-gel; visible light

INTRODUCTION

Pollution has been found to be present widely in the environment. The major sources of water pollutants are derived from manufacturing processing industries, particularly chemical and textile industries in which organic and inorganic wastes including dyes, endocrine disruptors such as phenolics, heavy metals and phthalates are widely used. Although these dyes are an important part of textile industry but their discharge in water bodies is very complicated as these are highly toxic and have carcinogenic effects on human health (Zolinger, 1987). These dyes often have complex aromatic structure and do not degrade easily under natural conditions as these are highly photostable. Conventional methods such as solvent extraction, adsorption and chemical oxidation are suggested for dealing with the removal of organic molecules from water but they suffer from serious limitations and disadvantages (Curridal, 2003).Acid Blue 113 (AB113) is a blue colored dying

agent most widely employed in the tanning, textile and pharmaceutical industries. The industrial wastewater which contain these organics when let out into water streams cause harmful effects and are generally toxic to the environment (Valli Nachiyar et al., 2012). Hence, they must be treated and converted into less toxic substances to avoid complications. AOP (Advanced Oxidation Process) involving heterogeneous semiconductors like ZnO is one of the most promising techniques, which used for the removal of toxic effluents from water effectively, because of many advantages including high efficiency, photochemical stability, non-toxicity, low cost and possesses wide band gap of 3.2eV (Gouvea, et al., 2000, Dindar et al,. 2001, Yu et al, 2008) However, a major drawback of ZnO is that, it easily under-goes an electron-hole pair recombination and photo corrosion in the aqueous environment (Xin et al., 2009)which affects the photocatalytic activity of ZnO (Mohamed Barakat et al., 2008). The main frame work carried out in past decades has aimed at the modification of ZnO in order to enhance its photocatalytic activity under visible light. Efforts have been taken to enhance the activity of the ZnO in the visible region by creation of oxygen vacancies (Nakamura et al.,2000), semiconductor coupling (Jianhua et al., 2006) , and surface modification by incorporation of organic materials (Qiu et al., 2006), doping of metals and non-metals (Wang et al., 2016), rare earth metals (Xu et al., 2016), composite materials (Tu et al ., 2008), core-shell particles (Su et al ., 2007).

Nanocomposites materials are prepared by combination of a material such as SiO_2, Al_2O_3, glass, graphene, carbon nanotubes (Lim et al., 2010), etc along with ZnO is regarded as an efficient strategy to extend the photo absorption capacity of ZnO to the visible range and to reduce the tendency towards charge recombination (Zang et al ., 2008), It was observed that the desired properties could be achieved if the semiconductors were prepared as composites with suitable materials. Hence, the use of SiC as a composite material for the enhancement of photocatalytic activity of the semiconductor metal oxides would be highly promising. Silicon carbide (SiC) is an environmentally friendly narrow band gap semiconductor (~3 eV) and exhibits interesting properties that are quite similar to ZnO; combination of both these materials may show promising photocatalytic activity. In addition, the added advantage of SiC is that it is capable of injecting the photo generated electrons from its conduction band (CB) to the CB of the metal oxide via a charge transfer mechanism. This process would promoting the separation of electron-hole pair thereby facilitating the effective utilization of the separated electron and hole for the production of OH and $O_2^{\ddot{}}$ radicals which would be effectively used for the degradation of organic contaminants present in the aqueous phase (Wang et al ., 2012), Hence, the present work is focused on the preparation of Nanorod ZnO/SiC nanocomposite by simple sol-gel method and investigation on its catalytic activities were evaluated for the degradation for a model organic pollutant Acid Blue 113 (AB113) under UV and visible light irradiations. The prepared ZnO/SiC nanocomposite were characterized by XRD, UV-DRS, FT-IR, AFM, FE-SEM, XPS, EDAX, HR-TEM, SAED and EPR techniques. The photodegraded samples were analysed by UV-Visible spectroscopy and chemical oxygen demand (COD) analyses.

2 Experimental Sections
2.1 Preparation of nanorod ZnO/SiC Composites

ZnO/SiC nanocomposites were prepared by a simple sol-gel technique as mentioned below. Initially, Pristine ZnO (PZnO) was prepared from its acetate precursor. In a typical synthesis, 10g of Zn (CH$_3$COO) $_2$•2H$_2$O was dissolved in 100 ml of deionised water and 25% w/w of NH$_4$OH was added slowly until the solution attained pH 8.0. The white gel thus formed was separated from the

supernatant by filtration and further washed with double-distilled water repeatedly. The resulting Zn (OH) $_2$ gel was made into slurry by adding water. 1g of SiC surface was cleaned by using hydrofluoric acid and then soaked for 30 min finally it can be decanted and washed with acetone. Appropriate quantity of surface cleaned SiC was added into the previously prepared Zn (OH) $_2$ then the required volume of 25% NH_4OH was added until the aqueous phase reached pH 12.0 and the whole content was heated at 65°C until the excess ammonia evaporates completely. The formation of grey coloured ZnO/SiC precipitate was filtered and dried in a hot air oven at 150°C for 1 hour. The grey coloured ZnO/SiC powder was calcined at 300°C for 30 min in a tubular furnace under atmospheric conditions.

2.2 Photocatalytic study

Kinetic studies of the photocatalytic degradation of different initial concentrations of AB113 such as 5, 10, 15 and 25 ppm with prepared ZnO/SiC nanocomposites were measured by using a Annular batch type UV photoreactor equipped with eight lamp (each 8 W) at wavelength 254 nm supplied by Heber Scientific, India. Also, these studies were carried out in an annular type visible Photo reactor (Heber Scientific Company Ltd, Chennai, India) which possessed a 500 W power of tungsten filament lamp was used as an irradiation source.The preliminary experiments were conducted in 10 ml volume and 0 to 100 ppm concentration of AB113 solution and irradiated under UV light for 1 hr and under visible light for 6 hr. Similarly the kinetic studies were performed in 200 ml of the AB113 solution with appropriate concentration and its photocatalytic degradation was monitored by the withdrawal of 5 ml aliquots of sample at regular time intervals. The photodegraded samples were filtered and their absorbance was recorded at λ_{max} 568 nm (AB113) using UV-visible spectrophotometer (Shimadzu-2450). The progress of photocatalytic degradation was also monitored by COD analysis; employing the dichromate closed reflux method . The percentage of degradation (X) was also calculated by eq (1).

$$X= (C_o-C_e)/C_o \times 100 \qquad (1)$$

Where, C_0 and C_e are the initial and equilibrium concentration of AB113.

3. Results and Discussion

3.1. XRD Studies

The structure of ZnO/SiC nanocomposite was characterized by XRD studies and the corresponding patterns are shown in Fig.1 (i). The results revealed that the synthesized photocatalyst possessed ZnO/SiC of hexagonal structure. The XRD pattern exhibited strong diffraction peaks which indicate that no other impurity phases were present in the catalytic samples. These results clearly confirmed that the prepared ZnO/SiC composites are highly crystalline in nature. The average crystallite size of ZnO/SiC was calculated using Scherer equation eq.2:

$$d=0.94\lambda/\beta\cos\vartheta \qquad (2)$$

Where, λ denotes the wavelength of radiation equal to 0.154 nm, β is the full width at half maximum and θ half the diffraction angle. The results suggested the particle size of ZnO/SiC to be in the range of 36 to 59 nm. The lattice constants, the variation of aspect ratio (c/a), lattice volume (V), interplanar spacing (d) and the average crystallite size.

3.2 UV-Visible spectral studies

The band gap energy of photocatalyst was calculated using UV-DRS measurements and results are shown in Fig.1 (ii) and (iii). The results showed an increased absorbance in the UV region which is shift into the visible region, which suggests that, the prepared ZnO/SiC would be photocatalytically active under UV and visible irradiations. The band gap energy (Eg) for the synthesized photocatalyst could be calculated from the spectra by plotting $(Ahv)^2$ against hv as shown in Fig.1(iii). This narrow band gap (3.019 eV) indicates a shift in the spectrum of ZnO/SiC nanocomposite towards the visible region, which confirms that the material could act both in the UV as well as in the visible region.

3.3 FT-IR analysis

The FT-IR spectrums for ZnO/SiC photocatalyst were recorded in the range of 400 to 4000 cm^{-1} as shown in Fig.1 (iv). The FT-IR spectrum of ZnO/SiC photocatalyst shows the characteristic absorption peak at 600 cm^{-1} which confirmed the formation of Zn-O stretching. Fig.1 (iv) showed a peak at 860.50 cm^{-1} which is correspond to Si-C stretching vibration present in ZnO/SiC nanocomposite.
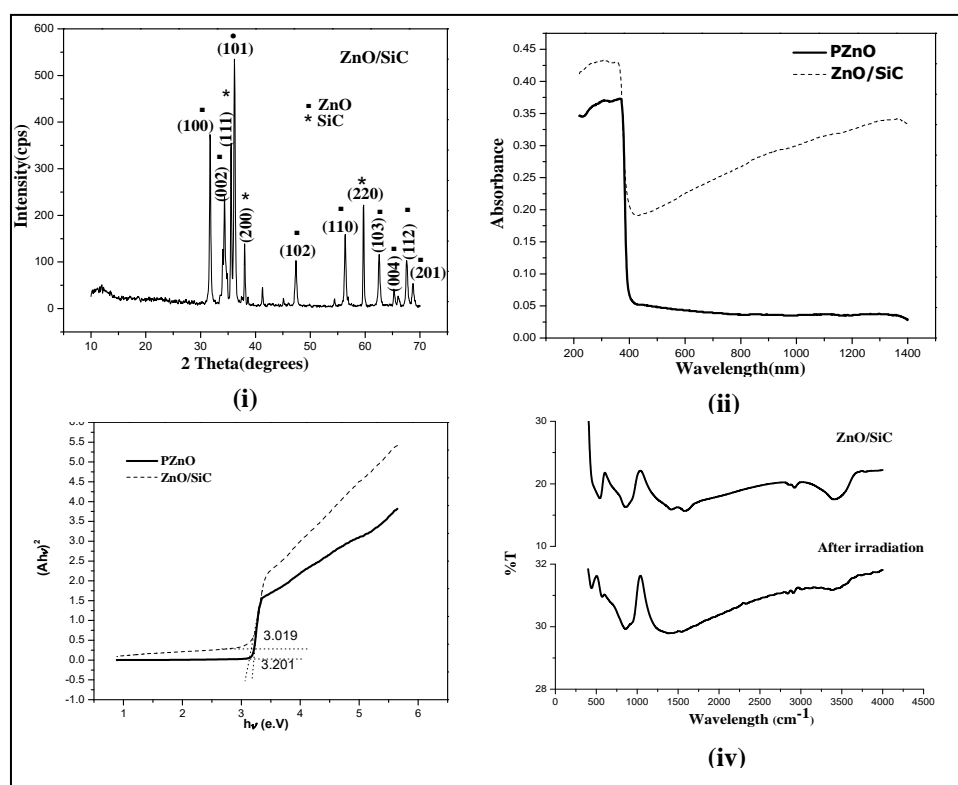
Fig.1. XRD patterns of (i) ZnO/SiC ((ii) UV-DRS spectrum of ZnO/SiC and (iii) shows a plot of $(Ahv)^2$ vs hv for the determination of band gap of ZnO/SiC (iv) FTIR spectrum of ZnO/SiC

3.4 XPS analysis

The chemical state of the elements, surface composition and oxidation states of ZnO/SiC (1:3) were confirmed by XPS analysis. The survey XPS spectrum of ZnO/SiC nanocomposite clearly revealed the presence of Zn (2p), Si (2p), O1s (530.2 eV)and C (1s) as shown in Fig.2 (i).The high resolution spectra for Zn, Si, O and C species are shown in Fig.2 (ii),(iii) (iv) and (v) respectively.
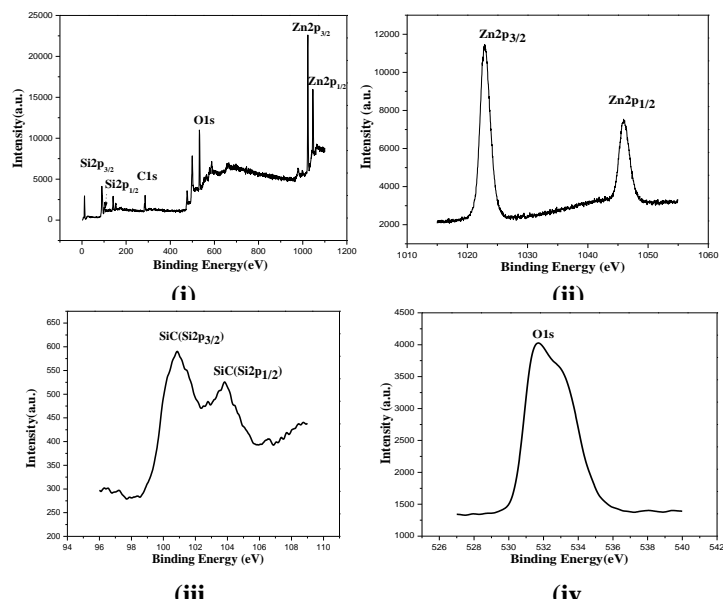
Fig.2.XPS spectrum of (i) wide scan spectrum (ii) Zn (iii) SiC (iv) Oxygen

The high resolution spectrum shown in Fig.4 (ii) and (iii) indicate the binding energies 1022.4 eV and 1045.2 eV for $2p_{3/2}$ and $2p_{1/2}$ states of Zn^{2+} and the binding energies 100.8eV and 103.4eV for $2p_{3/2}$ and $2p_{1/2}$ states of Si^{2+} in SiC respectively in the ZnO/SiC nanocomposite. The two peaks at (530.1 and 531.4 eV) indicate the two kinds of O elements in the sample are shown in Fig.2 (iv). The peak at 530.1 eV attributed to O1s of ZnO corresponds to the hydroxyl group.

3.5 Surface Morphology

The surface morphology of the prepared ZnO/SiC nanocomposite was studied with FE-SEM and the observed images were shown in Fig.3 (i). From this figure, it is inferred that the surface morphology of the prepared ZnO/SiC photocatalyst was nanorod in shape. The size of the ZnO nanorod in ZnO/SiC was in the range of 30 to 40 nm. The surface morphology of the catalysts were also analysed by HR-TEM and the results were shown in Fig.3 (ii). It is observed that the prepared ZnO/SiC photocatalyst is arranged as a rod like structure with particle size of 30 nm. The SAED pattern of ZnO/SiC is shown in the inset of Fig.3 (iii). The SAED pattern also suggested that the prepared ZnO/SiC catalyst is in polycrystalline nature. The EDAX spectrum together confirmed the elements present in the catalyst as illustrated in Fig.3 (iii). EDAX spectrum of ZnO/SiC catalyst clearly demonstrated the presence of silica in addition to zinc and oxygen.
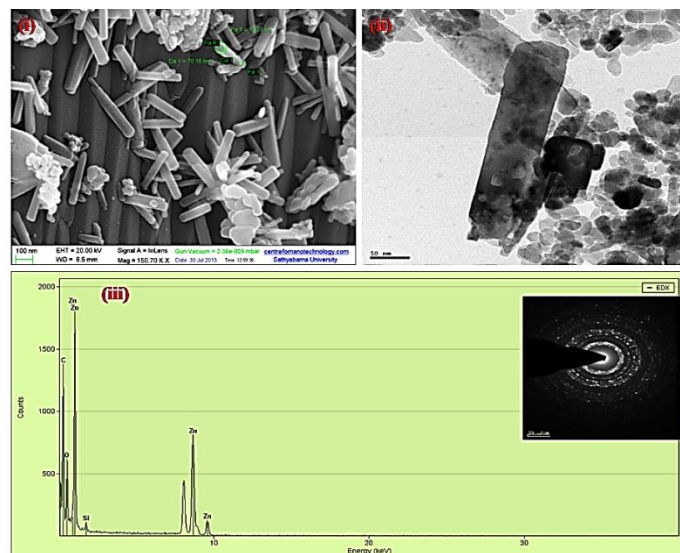
Fig.3. (i) and (ii) shows the FE-SEM and HR-TEM images of ZnO/SiC, (iii) shows the EDAX spectrum of ZnO/SiC. The inset of (iii) shows the SAED pattern of the prepared ZnO/SiC.
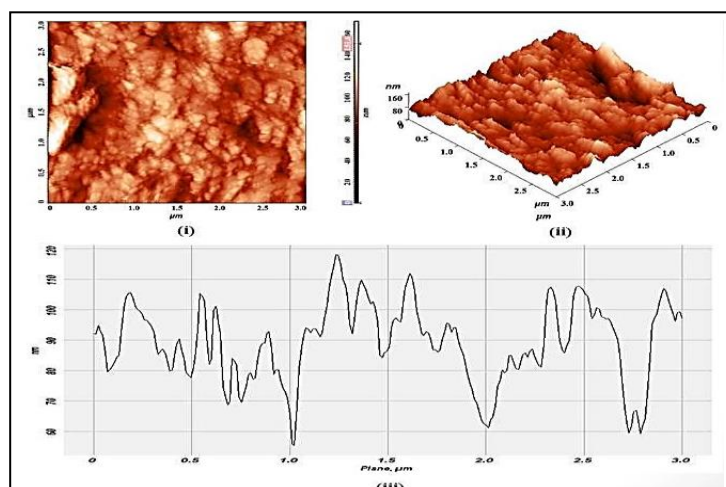

Fig.4. AFM images (i), (ii) and roughness profile (iii) of prepared ZnO/SiC

The porosity and roughness of both the prepared photocatalyst were analyzed by AFM analysis. The 2D and 3D AFM images shown in Fig.4 (i) and (ii) reveals that the prepared (1:3) ZnO/SiC photocatalyst had higher porosity and roughness. The result clearly demonstrates that the prepared photocatalysts were nanosized and possessed high surface roughness. The average roughness of ZnO/SiC photocatalyst was found to be 35 nm. The surface roughness profile of the photocatalyst is also shown in Fig.4 (iii). A number of peaks and troughs on the surface of the photocatalyst were observed which confirmed the high roughness of ZnO/SiC photocatalyst which increases the adsorption of organic moieties on the surface of photocatalyst and facilitates the enhancement of photocatalytic activity, thereby increasing the degradation efficiency.

3.6 EPR analysis of OH radicals

The in-situ formation of OH radicals by the photocatalyst under UV and visible irradiation were confirmed by the EPR spin trapping technique, utilizing DMPO (5,5-Dimethyl-1-pyrroline-N-Oxide) as the spin trapping agent. The prepared catalyst, water and DMPO were mixed and taken in a quartz reactor tube and glass tube, it was irradiated for 60 s for UV and 15 min for visible then the spectrum of EPR was recorded. The results are illustrated in Fig.5 (i) and (ii). It is observed that the characteristic 1:2:2:1 quartet at G values of 3413.90, 3428.80, 3443.67 and 3458.56 with a g factor of 2.0115 (UV irradiation) and G values of 3412.83, 3427.66, 3442.86, and 3457.83 with a g factor of 2.016 (Visible irradiation) showed relative EPR intensities for ZnO/SiC photocatalyst corresponding to that of DMPO-OH free radical. The experimental results evidenced the instantaneous production of OH radicals by ZnO/SiC within a short period of UV light and visible light irradiations which is proved to enhance the photodegradation of the organic molecules.
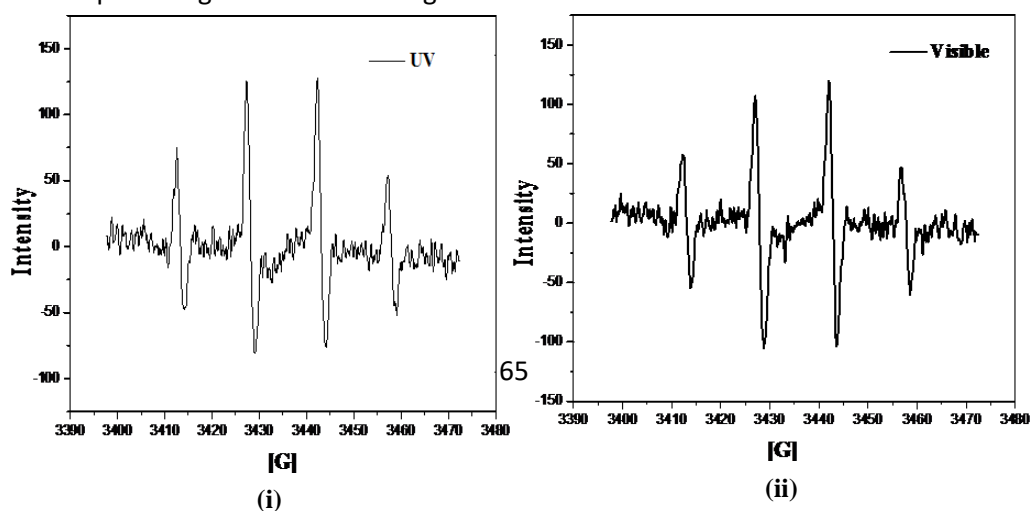
65

Fig.5. EPR Analysis of OH radical (i), (ii) for ZnO/SiC photocatalyst under UV and visible irradiation,

3.8. Photocatalytic activity

The effect of aqueous phase pH on the degradation efficiency of AB113 was studied in the pH range between pH 2 and pH 12 (10 ppm AB113 concentration and catalyst dosage is 500 mg/L for ZnO/SiC). The AB113 solutions were irradiated at 254 nm for 4 h and 400-800 nm for 10 h under UV light and visible lights respectively. The results are shown in Fig.6 (i). The experimental results clearly showed that the maximum degradation occurred at neutral pH for the prepared catalyst under UV and visible light irradiations. It was observed that more than 75% degradation occurred at pH 6.65 for ZnO/SiC photocatalysts at similar pH under both the irradiations. Hence, further experiments were conducted at neutral pH.

The reduction of AB113 molecules was performed by varying the catalyst amount as 3mg to 20mg keeping the other parameters constant under UV and visible irradiation for 1h, 6h respectively. The results are shown in Fig. 6 (ii). It is observed that under UV light, 5 mg of ZnO/SiC photocatalyst gave >80 % photocatalytic activity and for visible light irradiation 10 mg of ZnO/SiC photocatalyst showed >75 % photocatalytic activity. At higher catalyst dosage, the reduction in the degradation efficiency was observed due to light scattering and reduction in light penetration through the solution. Therefore, with minimum dosage of catalyst the photocatalytic efficiency is maximum in the case of ZnO/SiC. The effect of initial AB113 concentration was studied by varying the concentration from 10 to 25 ppm/10 mL at neutral pH with catalyst (5, 10 mg of ZnO/SiC under UV and visible lights respectively). As shown in Fig.6 (iii), it was observed that the photocatalytic degradation decreased as the AB113 concentration increased in the aqueous phase. For ZnO/SiC photocatalyst, the percentage of degradation decreased for 5 to 25 ppm of AB113 concentration under both the
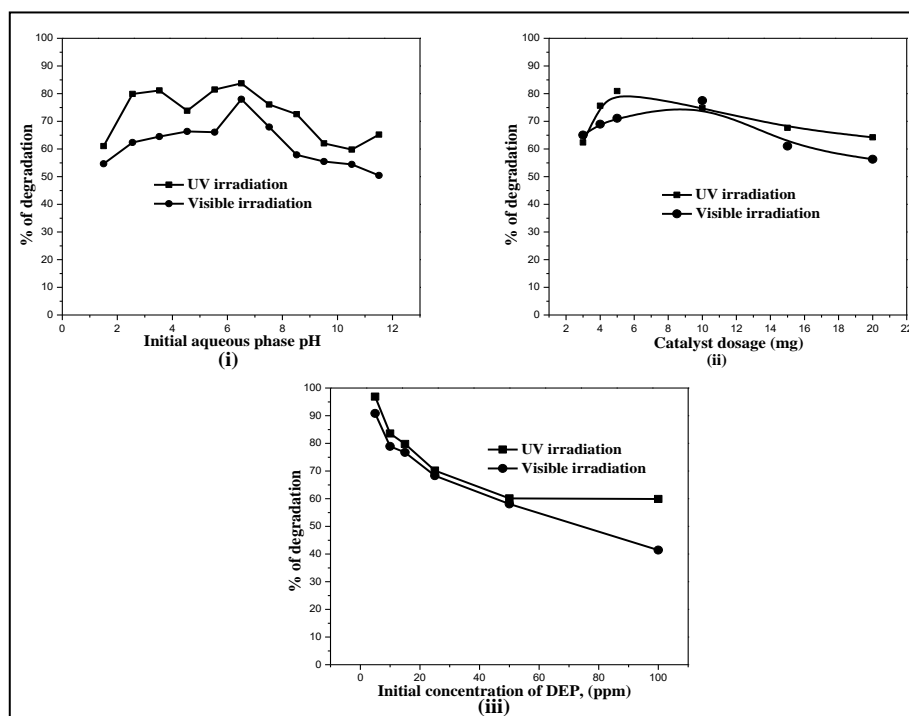

Fig.6. Photocatalytic studies on (i) effect of pH (ii) variation of catalyst dosage and (iii) variation of initial dye concentration for ZnO/SiC under UV and visible irradiation

irradiations respectively. The reason may be, at higher concentration the AB113 molecules act as a shield on the surface of the catalyst that affect the light penetration into the system thus leading to a decrease in the catalytic activities of the prepared photocatalysts .

3.8.1 Kinetics of Photodegradation

The kinetics of photodegradation was carried out with various initial concentration of AB113 (5, 10, 15 and 25 ppm) at constant catalyst dosage (50 mg/100 ml-UV, 100 mg/100 ml- visible) for ZnO/SiC catalysts at neutral pH under both light irradiations with standard experimental conditions. As shown in Fig.7 (i) and (ii), it was observed that the AB113 molecules degraded >80% within 120 min for the prepared ZnO/SiC photocatalyst under UV irradiation whereas, with visible light irradiation the time for maximum degradation was nearly 660 min. These results confirmed that the prepared ZnO/SiC photocatalyst showed better photocatalytic activity for both the light irradiations. The degradation of AB113 was also confirmed by COD analysis of the photodegraded samples collected at regular time intervals. From Fig.7 (iii) and (iv), it could be ascertained that the photodegradation of AB113 by UV light and visible light employing ZnO/SiC photocatalyst followed pseudo-first order kinetics (eq.3) with respect to the initial concentration of AB113.

$$ln(C_0/C_e) = k_{obs}\, t \qquad\qquad (3)$$

where, C_0 and C_e are the initial and final concentrations of AB113 molecules with respect to time "t", and k_{obs} is the observed pseudo-first order rate constant
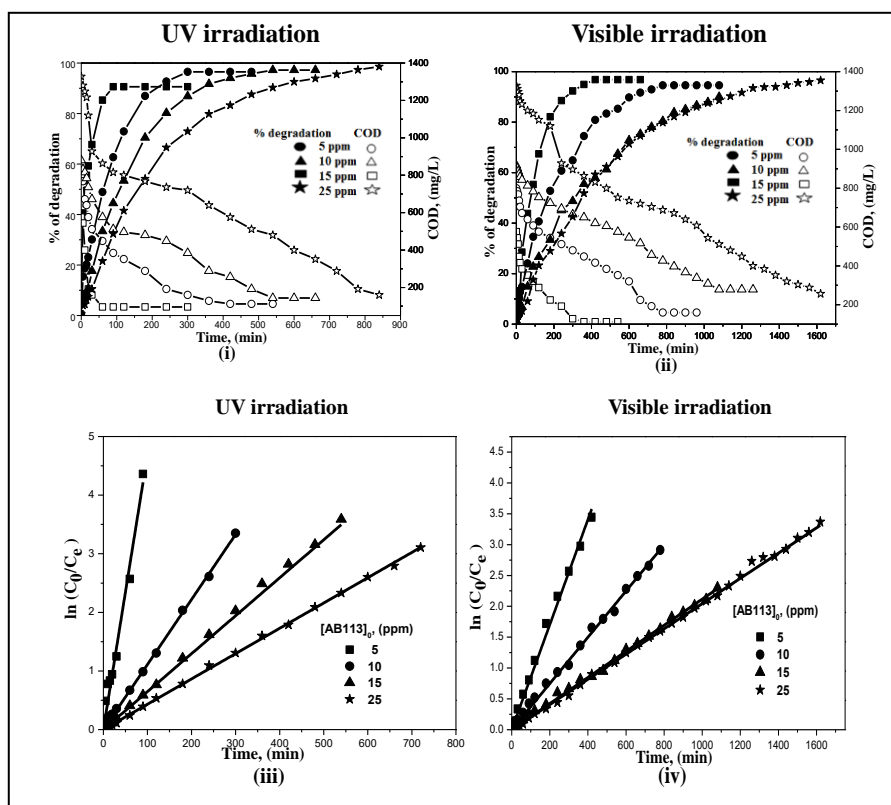

Fig.7. (i) and (ii) shows the kinetics and COD plot of ZnO/SiC under UV and visible irradiations, Fig.(iii) and (iv) shows the plot of rate constants for the prepared ZnO/SiC under UV and visible irradiations.

3.8.2 Effect of electrolyte addition

The effect of added electrolytes on photodegradation of AB113 molecules have also been studied in the presence of NaCl, Na_2CO_3, $NaHCO_3$, KCl and $MgSO_4$ for ZnO/SiC photocatalysts. The results are shown in Fig.8 (i) and (ii). It is observed that the percentage of AB113degradation was altered with an increase in the wt% of electrolytes in all photocatalytic experiments. In addition, all the percentage of $NaHCO_3$ and Na_2CO_3 as electrolytes with 500 mg/L of ZnO/SiC and 0.5 g/L of AB113 produced nearly 90% degradation which was a very high photocatalytic activity under UV and visible light irradiations. But, the presence of Na_2CO_3 and $NaHCO_3$ increased the percentage of degradation with an increase in the amount of electrolyte .
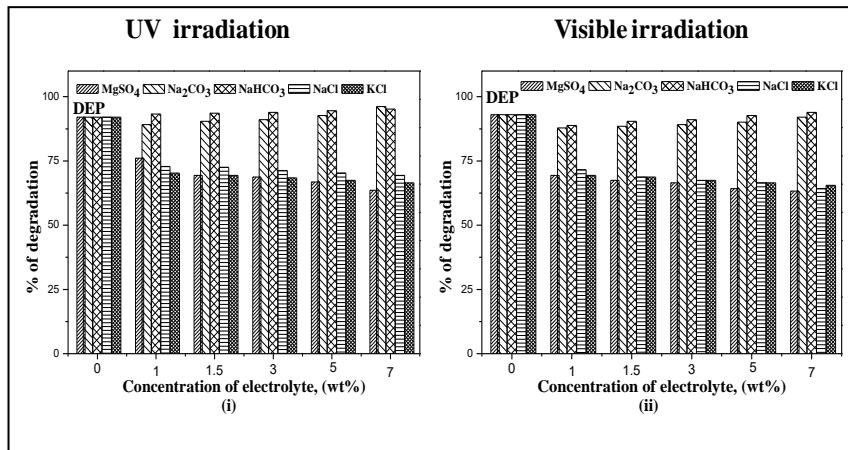
Fig.8 (i) and (ii). shows the effect of different electrolytes on the degradation of AB113 by the prepared photocatalyst under UV and visible irradiations.

On the other hand, the addition of $MgSO_4$ and NaCl retarded the photodegradation with an increase in the concentration of electrolytes. This is because of the presence of chlorides and sulphates ions in the aqueous phase which act as a radical scavenger thus leading to a decrease in the percentage of photodegradation.

3.9 Reusability

The reusability of the photocatalyst was examined to determine the cost effectiveness of the photodegradation process. For UV and visible light irradiation, the reusability of ZnO/SiC photocatalyst was evaluated upto three cycles. The results are shown in Fig.9 (i) and (ii).
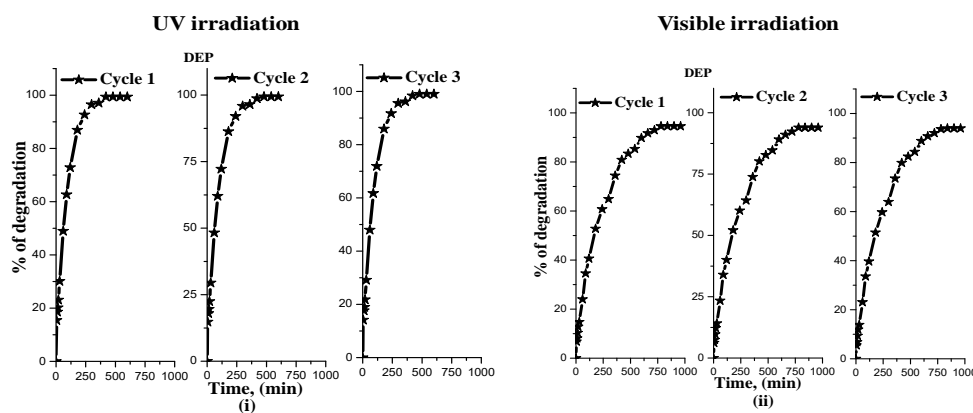

Fig.9 (i) and (ii) shows the reusability study for the prepared ZnO/SiC under UV and visible irradiations

The results revealed that ZnO/SiC in the 2nd and 3rd cycle were as active as observed in the 1st cycle of operation. The important advantage of the prepared catalyst is that, it is easy to separate from the reaction system when and was reusable even upto the third cycle without affecting the catalytic activities.

4. Conclusion

ZnO/SiC nanocomposite is a promising and efficient photocatalyst to degrade the AB113 (model pollutant) molecule. The photocatalyst was prepared by a simple sol-gel method and characterized by XRD, FT-IR, FE-SEM, EDAX, HR-TEM, SAED, AFM, XPS and EPR analysis. The ZnO/SiC (1:3) photocatalyst possessed charge transfer mechanism which retarded the recombination of electron-hole pairs and enhanced the production of OH radicals, which is used in the degradation of the AB113molecules. The in-situ formation of OH radicals was confirmed by EPR technique. The degraded samples were confirmed by COD and UV-visible spectroscopy. The kinetic studies of the photocatalytic degradation followed pseudo-first order kinetics with respect to AB113 concentration. The synthesized (1:3) ZnO/SiC composite showed reusable even upto the third cycle without affecting the catalytic activities. Therefore, it is concluded that the prepared ZnO/SiC photocatalyst is highly efficient and cost effective under UV and visible light irradiations.

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HETEROSTRUCTURED $(ZnO)_x(Bi_2O_3)_{1-2x}(Dy_2O_3)_x$: AN EFFICIENT UV ACTIVE PHOTOCATALYST FOR THE DEGRADATION OF DYES IN LEATHER BASED INDUSTRIAL EFFLUENTS

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We are here in reporting a heterostructured composite material with enhanced photocatalytic activity formed by combining Bi_2O_3 with a transition metal oxide (ZnO) and a rare earth oxide (Dy_2O_3) with a final overall composition of $(ZnO)_x(Bi_2O_3)_{1-2x}(Dy_2O_3)_x$. The highly nanocrystalline nature of the material was confirmed by XRD analysis. Peaks corresponding to α Bi_2O_3, ZnO and Dy_2O_3 phases are visible. The band gap energy as calculated from the UV-Vis-DRS spectrum is.. HRSEM imaging shows the micro-rod shaped α Bi_2O_3 to be distributed amidst the ball like clusters formed by spherical ZnO and Dy_2O_3 nanoparticles. Shape and morphology was further confirmed by HRTEM analysis. XPS and EDAX analysis confirm the presence of Bi, Zn, Dy and O in the material. The high surface roughness and porosity of the prepared composite was revelaled by AFM analysis. The photocatalytic efficiency of the composite material was evaluated by the degradation of a model pollutant-Orange G under UV light irradiation in an annular slurry type photoreactor. Orange G is a commonly employed mono-azo dye in the coloring of leather products. Preliminary studies showed the catalyst to exhibit at neutral pH with an optimum catalyst dosage of 0.5g/L. Kinetic studies carried out with various initial dye concentrations showed the photodegradation reaction to follow pseudo-first order kinetics. The presence of industrial effluents did not affect the photocatalytic efficiency of the material. Catalytic efficiency was retained even after the 3rd cycle of its reuse.

Keywords: Photocatalyst; UV irradiation; Orange G; Heterostructured composite; degradation

Introduction

The tanning industry is one of the oldest industries in the world (Chowdhury et al, 2013). The leather industry is characterized by its high consumption of both chemical and water resources in all the stages of the tanning process. Of all the stages of the tanning process, it is the dying step that discharges large quantities of organic pollutants (Lambert et al., 2013). In addition to the leather industry, dyes are also used widely in textile, paper, rubber and plastic industries (Azhar et al., 2005). It has been reported that over 7×10^5 tons of dye based waste is generated each year (Eren et al., 2006; Ozer et al., 2006) consisting of over 10,000 different commercial dyes and pigments. A large quantity of the dyestuff is discharged into natural water without being treated. (Crini et al., 2006) More than 60% of the discharged dyes are composed of azo dyes. These dyes are characterized by the presence of one or more azo groups (R_1-N=N-R_2) and complex conjugated and substituted aromatic systems that are responsible for their intense colour, high water solubility and their

recalcitrant nature (Ozdemir et al.). Even small quantities of these dyes, when present in water bodies is aesthetically displeasing, hindering the penetration of light through the water thereby adversely affecting photosynthesis and retarding the growth of aquatic flora and fauna (Carpenter et al., 2013). In addition to this, dyes are also known to be cytotoxic, mutagenic, carcinogenic and highly toxic in nature (Khehra et. al., 2006). Thus, it is of utmost importance to completely eliminate dyes from industrial effluents before they can be safely discharged into the aquatic environment.

Most of the organic constituents in industrial waste water are resistant to degradation. Moreover, the commonly employed water treatment techniques such as adsorption onto activated carbon, coagulation, precipitation, solvent extraction, filtration, etc., do not completely mineralize the pollutants. They are only successful in transferring them from one phase to another thereby requiring further treatment. (Phillipe et al., 1998; Slokar et al., 1998; Panduranga et al. 2001). It is thus necessary to employ an alternate efficient and clean means of treating the dyes and other organics present in industrial wastewater. In this regard, advanced oxidation processes (AOPs) have emerged as a promising means of treating the toxic pollutants present in water and waste water (Gao et al., 2007). AOPs rely on the in-situ generation of highly active radicals that are capable of reacting with and completely mineralizing the organics in the wastewater. (Kuo et al., 2001; Legriniet al., 1993) This method makes use of semiconductor metal oxides such as TiO_2, ZnO, Bi_2O_3, WO_3 etc as photocatalysts. When irradiated with light of a suitable wavelength, the electrons in the valance band of the metal oxides undergo photoexcitation to the conduction band leaving behind positively charged holes in the valance band. Water molecules adsorbed onto the surface of the catalyst react with these holes resulting in the formation of OH radicals which are extremely reactive and are powerful oxidizing agents. (Ma et al., 2013) However, the use of pristine semiconductor photocatalysts suffers from a major limitation in the possibility of recombination of the photogenerated electron-hole pairs thereby hindering the generation of the OH radicals (Jiang et al., 2007; Kuo et al., 2007; Yang et al., 2009). Thus it is of utmost importance to increase the lifetime of the charge carriers in order to increase the efficiency of the photocatalyst (Reddy et al., 2009).

In the present work, we have synthesized a heterostructured triple oxide material by combining Bi_2O_3 with a transition metal oxide (ZnO) and a rare earth oxide (Dy_2O_3) with a final overall composition of $(ZnO)_x(Bi_2O_3)_{1-2x}(Dy_2O_3)_x$. (ZBD). The material showed high photocatalytic activity in both the UV and Visible regions of the spectrum. The recombination of the photogenerated electron-hole pairs is overcome by the effective charge separation that occurs across the heterojunction formed by the two semiconductor metal oxides. The charge separation is further enhanced by the trapping of photoexcited electrons in the empty f orbitals of the rare earth oxide. This ensures that the holes are fully available for the generation of OH radicals in the system. The synthesized composite material was characterized by a variety of techniques such as XRD, FT-IR, UV-vis-DRS, XPS, HR-SEM, HR-TEM and AFM techniques. The photocatalytic activity of the semiconductor nanomaterial was evaluated by the degradation of Orange G- a monoazo dye under UV light irradiation.

Experimental

The ZBD composite was synthesized by a precipitation technique where the respective metal nitrates were employed as the metal oxide precursors. An aqueous solution of sodium hydroxide was employed as precipitating agent. Aqueous solutions of the metal oxide precursors and the alkali were alternately in a dropwise manner resulting in the precipitation of the metals as their hydroxides.

Throughout the addition process, the solution was kept under vigorous stirring. Addition of alkali was stopped once the pH of the solution reached 12. After 2 hours of stirring, the solution was filtered and washed thoroughly. The obtained solid was then dried overnight and sintered in a muffle furnace. The sintered powder was ground and stored in an airtight container.

Results and Discussion

X-Ray diffraction studies

The crystal structure of the prepared ZBD material was studied by using X-ray diffraction. The XRD pattern was measured between 10° and 70° and is shown in figure 1(a). Peaks of the monoclinic α bismuth oxide (JCPDS card no. 6-294) are visible at 27.5º and 35º and they correspond to the (120) and (031) planes respectively. In addition to this, tetragonal β bismuth oxide peak is also visible at 33º corresponding to the (220) plane (JCPDS card no 27-50) (Jhaa et al., 2005; Hou et al., 2013). The peak at 46.3º corresponds to the (102) phase of the hexagonal wurtzite structure of the ZnO crystal lattice (JCPDS 36-1451) (Zheng et al., 2010). A series of low intensity peaks between 50º and 60º can be attributed to various lattice planes of and Dy_2O_3 (JCPDS 01- 079-1722) (Karthikeyan et al., 2011). The sharpness of the peaks indicates the nanocrystalline nature of the material.

FT-IR

Functional group analysis of the prepared ZBD material was carried out by means of FT-IR spectroscopy. The spectrum was scanned in the range between $400cm^{-1}$ and $4000cm^{-1}$ and the graph is shown in figure 1(b). The peak at $521cm^{-1}$ is attributed to metal-oxygen stretching vibration (Zheng et al., 2010). The O-H stretching frequency of water molecules adsorbed on the surface of the catalyst results in the broad peak at around $3500cm^{-1}$.
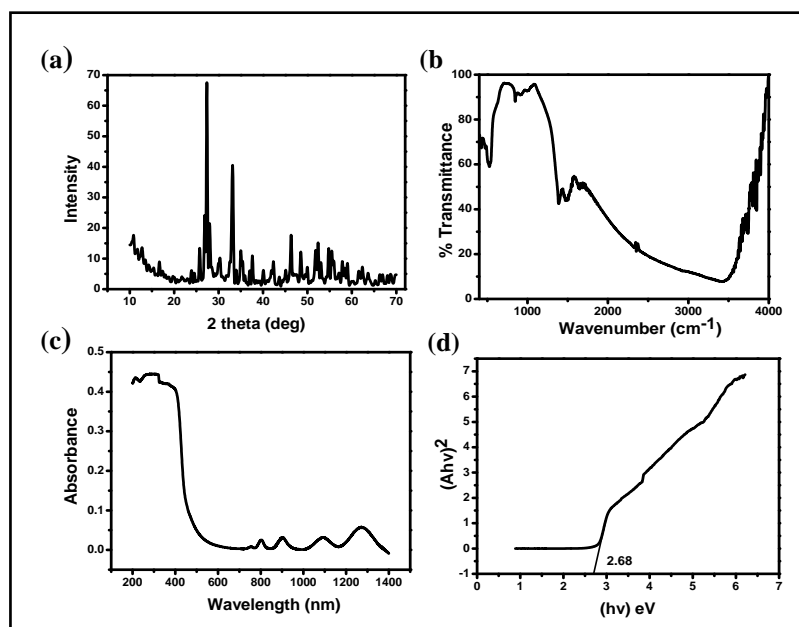

Figure1(a): XRD pattern, (b): FT-IR spectrum, (c): UV-Vis-DRS (d) Band gap energy calculation of ZBD

UV-vis-DRS Analysis

The band gap energy and the photo-absorption properties of the prepared ZBD material were investigated by the UV-Vis-DRS technique. The spectrum is shown in figure 1(c). Results indicate the material to absorb light in the visible region in addition to its strong absorption in the UV region. This implies that the material could be employed as a photocatalyst under both UV and visible light irradiation. The peaks between 800nm and 1400nm are attributed to the transitions in the f orbitals of the rare earth metal dysprosium present in the material. The band gap energy (E_g) of the material was calculated by plotting $(Ahv)^2$ vs hv. The graph is shown in figure 1(d) and the value of E_g was found to be 2.68eV. This reiterates the high photocatalytic activity of the material in the UV and Visible regions of the spectrum.

X-Ray Photoelectron Spectroscopy (XPS)

The presence of the various elements and their oxidation states was confirmed by XPS analysis. The survey spectrum (figure 2(a)) indicates the presence of Zn, Bi, Dy, and O. The peak at 530eV is due to the O1s state present in the metal oxides (figure 2(b)). The peaks at 1021.08eV and 1044.4eV are attributed to the Zn2p3/2 and Zn 2p1/2 states as shown in figure 2(c) (Bhirud et al., 2012). This also confirms that Zn is present in the 2+ state in the nanomaterial. The peaks at 158eV and 163.2eV are due to the Bi 4f7/2 and Bi 4f 5/2 states respectively (figure 2(d) (Xu et al., 2011). The peak at 1295eV is attributed to the Dy $3d_{5/2}$ state (Barreca et al., 2007).
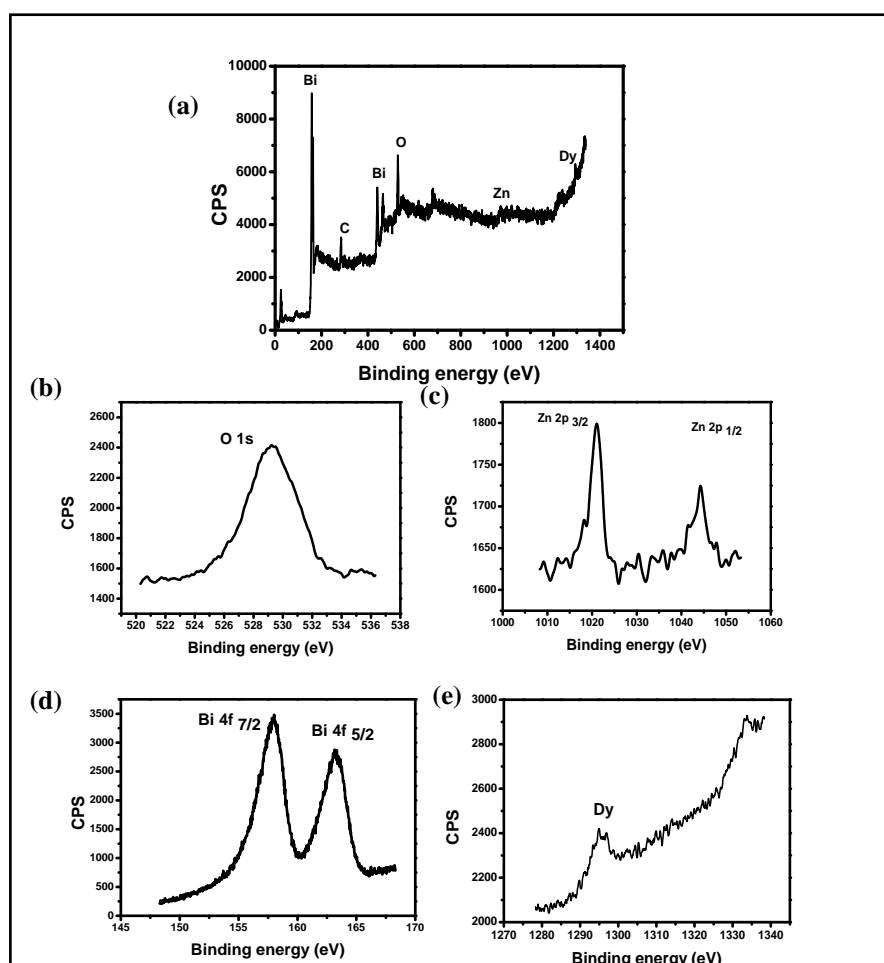
Figure 2(a) XPS spectrum of ZBD (b): O1s, (c) Zn2p, (d) Bi 4f and (e) Dy 3d$_{5/2}$ states

High Resolution Scanning Electron Microscopy (HRSEM)

The surface morphology of the prepared ZBD nanomaterial was studied by HR-SEM analysis. The obtained HRSEM image is shown in figure 3(a). The image reveals the presence of distinct micro-rod shaped α Bi$_2$O$_3$. The micro-rods are distributed amidst ball like clusters formed by the spherical ZnO and Dy$_2$O$_3$ nanoparticles. The individual paricles are not agglomerated and micro pores are seen between the ball like clusters. The pores would results in increased contact between the dye molecules and the surface of the catalyst resulting in faster photodegradation reactions. EDAX analysis (figure 3(b)) shows the presence of Zn, Bi, Dy and O in the material.

High Resolution Transmission Electron Microscopy (HRTEM)

The morphology and arrangement of the ZBD particles was further studied using HRSEM technique and the resulting image is shown in figure 3(c). Here once again microrods of αBi2O3 are seen to be distributed amongst the spherical ZnO and Dy2O3 particles. The HRTEM analysis also confirms the un-agglomerated nature of the individual particles. The SAED pattern reveals the highly crystalline nature of the material (figure 3(b)).
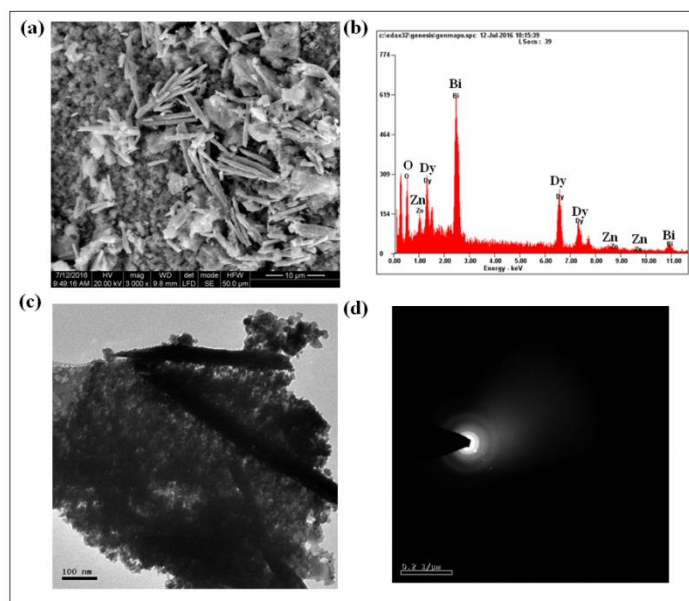

Figure 3(a): FE-SEM image (b): EDAX pattern, (c) HR-TEM image and (d) SAED pattern of ZBD

Atomic Force Microscopy (AFM)

The surface roughness and porosity of the material was investigated by AFM analysis. A 5µm x 5µm surface of the sample was analyzed and the image is shown in figure 4(a). The AFM image illustrates the highly rough nature of the material. The average roughness factor of the catalysts' surface was found to be 40.97nm. The vertical cross section of the ZBD material (figure 4(b)) shows the presence of numerous peaks and troughs once again indicating the uneven nature of the surface. This would be an important factor that accounts for the enhanced photocatalytic efficiency of the material.
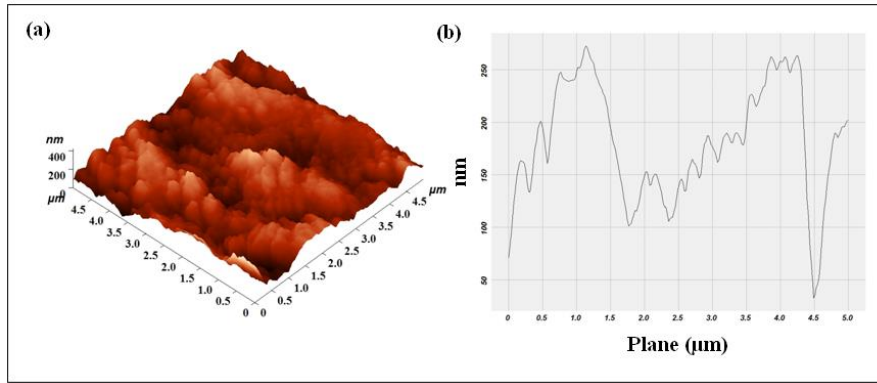
Figure 4(a): 3-dimensional AFM image and (b): surface cross section of the ZBD material

Preliminary photocatalytic reactions

The photocatalytic performance of the prepared and characterized ZBD material was tested for the degradation of a model pollutant – Orange G under UV light irradiation in a slurry type UV light photoreactor. Preliminary photocatalytic reactions were performed to study the effect of solution pH, catalyst amount and initial dye concentration on the photocatalytic degradation of Orange G. At the end of the reaction, the amount of undegraded due is estimated by measuring the absorbance of the solution at 483.5nm.The percentage of photodegradation is calculated using the following formula:

$$\% \, degradation = \frac{Co - Ce}{Co} X \, 100$$

where, C_o is the initial dye concentration and C_e is the dye concentration at the end of the reaction.

The effect of pH on the degradation of Orange G by the ZBD photocatalyst was studied by varying the solution pH between 2 and 12. The pH values were adjusted with the help of 0.1N solutions of HCl and NaOH. At each pH, a catalyst loading of 0.5g/L was employed to treat 10mg/L solutions of OgG under UV light irradiation. The obtained results are shown in figure 5(a). After an irradiation time of 90min, it is evident that the catalyst shows high activity across the whole pH range reaching a maximum of 97.3% degradation at neutral pH. Thus all further reactions were carried out at neutral pH.
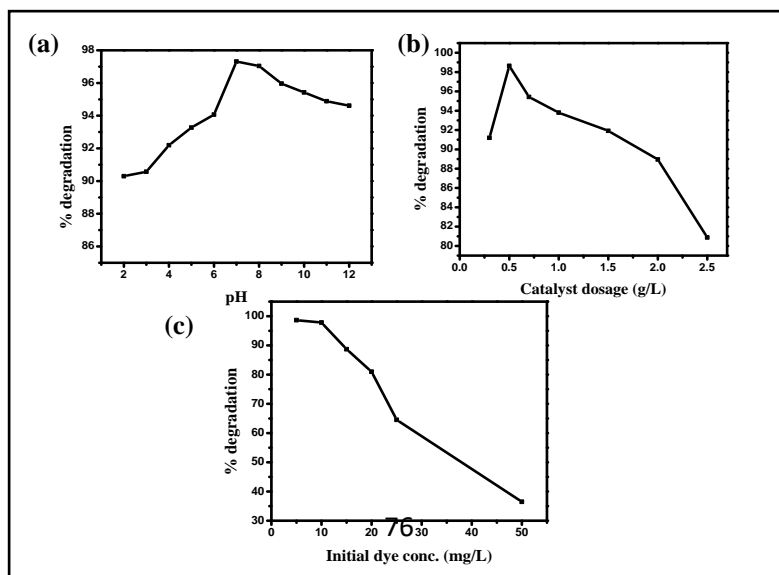
Figure 5(a): Effect of pH, (b): Effect of catalyst dosage, and (c): Effect of initial dye concentration

In order to determine the optimum catalyst loading required to ensure efficient degradation of OgG, the photocatalytic degradation reactions were carried out by varying the catalyst dosage from 0.3g/L to 2.5g/L. From figure 5(b) it is evident that an initial increase in percentage of degradation is seen as the catalyst dosage increases from 0.3g/L to 0.5g/L. Further increase in the dosage results in a decrease in the extent of dye degradation. This could be as a result of decreased light penetration on increasing the amount of catalyst in the solution. A maximum of 98.7% degradation was achieved with 0.5g/L catalyst dosage and thereafter all further photodegradation reactions were carried out with this dosage. Photodegradation reactions were carried out employing the previously fixed cata;yst dosage of 0.5g/L but varying the OgG concentration from 5mg/L to 50mg/L in order to study the effect of initial dye concentration on the photocatalytic efficiency of the ZBD nanocomposite material. The obtained results are shown in figure 5(c). With an increase in dye concentration from 5mg/L to 50mg/L a decrease in the extent of dye degradation from 98.9% to around 40% was observed.
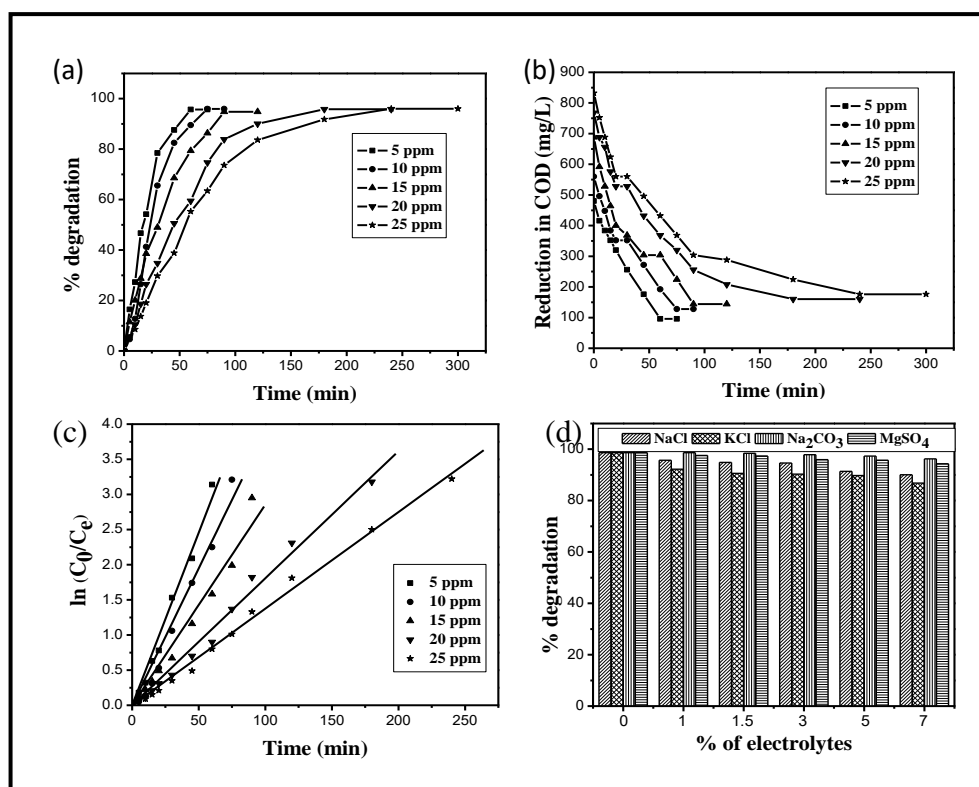

Figure 6(a): Kinetics, (b): Reduction in COD, (c): Pseudo-first order plot and (d): Effect of electrolytes on dye degradation

Kinetic studies

Kinetic studies were carried out for neutral OgG solutions with varying initial dye concentrations such as of 5mg/L, 10mg/L, 15mg/L, 20mg/L and 25mg/L concentrations under UV light irradiation. The corresponding results are shown in figure 6(a). In this case also the previously optimized catalyst dosage of 0.5g/L was employed. The advancement of the reaction was monitored by withdrawing 5mL aliquots of the reaction solution at regular intervals of time and measuring absorbance at 483.5 nm and by Chemical Oxygen Demand (COD) analysis. In all cases a decrease in the COD of the

solution to below 200mg/L was observed (figure 6(b)). From the kinetic data, the order of the reaction could be determined. The data was found to fit well with the pseudo first order rate equation give below

$$\ln\frac{C_0}{C_e} = kt$$

where Co is the initial dye concentration and Ce is the dye concentration at time t and k is the reaction rate constant. A plot of ln (Co/Ce) vs time is shown in figure 6(c) from which the pseudo-first order rate constant (k) for the photodegradation reaction was determined. A decrease in rate constant from 4.9 X 10^{-2} min^{-1} to 1.3 X 10^{-2} min^{-1} was noticed as the initial dye concentration increased from 5mg/L to 25mg/L thus indicating the reactions to follow pseudo first order kinetics.

Effect of electrolytes

The photocatalytic degradation reactions of OgG were carried out in the presence of electrolyte solution such as NaCl, KCl, Na_2CO_3 and $MgSO_4$ in order to determine their effect on the photocatalytic efficiency of the ZBD composite material. The previously optimized pH and catalyst dosage were maintained for these reactions. 10mg/L solutions of OgG were photocatalytically degraded in the presence of varying amounts (0% to 7%) of the electrolyte solutions and the results are shown in figure 6(d). It is observed from the results that the presence of the electrolytes did not hinder the performance of the ZBD photocatalyst. Thus the prepared nanocomposite would serve as an efficient catalyst to treat real house wastewater that could contain these electrolytes in addition to the organic pollutants.

Reusability studies

The economic viability of the system can be ascertained by carrying out reusability studies wherein the catalyst was used repeatedly to degrade 10mg/L solutions of OgG under UV light irradiation. Here too the progress of the reaction was monitored by measuring the absorbance of the reaction solutions withdrawn at regular intervals of time. From figure 7, it is evident that the catalyst shows a negligible decrease in efficiency from 95.7% in the first cycle to 92.2% in the third cycle.
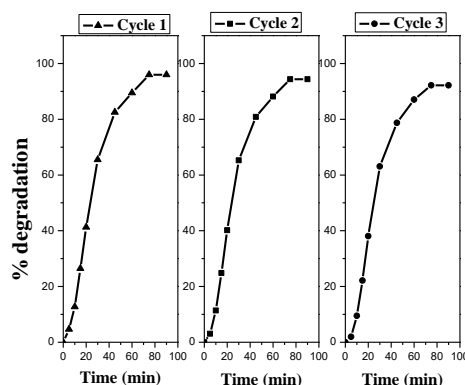

Figure 7: Reusability studies

Conclusion

A heterostructured composite material with enhanced photocatalytic activity was prepared by combining Bi_2O_3 with a transition metal oxide (ZnO) and a rare earth oxide (Dy_2O_3) with a final overall composition of $(ZnO)_x(Bi_2O_3)_{1-2x}(Dy_2O_3)_x$. XRD analysis confirmed the nanocrystalline nature of the material and peaks corresponding to α Bi_2O_3, ZnO and Dy_2O_3 phases are visible. UV-Vis-DRS analysis showed the band gap energy of the material to be 2.68eV. Morphological studies revealed the micro-rod shaped α Bi_2O_3 to be distributed amidst the ball like clusters formed by spherical ZnO and Dy_2O_3 nanoparticles. XPS and EDAX analysis confirm the presence of Bi, Zn, Dy and O in the material. The high surface roughness and porosity of the prepared composite was revealed by AFM analysis. The photocatalytic efficiency of the composite material was evaluated by the degradation of a model pollutant-Orange G under UV light irradiation. Preliminary studies showed the catalyst to exhibit at neutral pH with an optimum catalyst dosage of 0.5g/L. Kinetic studies confirmed the photodegradation reaction to follow pseudo-first order kinetics. The presence of industrial effluents did not decrease the photocatalytic efficiency of the material. Catalytic efficiency was retained even after the 3rd cycle of its reuse thereby validating the economic feasibility of the system.

Acknowledgements

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ADSORPTION OF AMIDO BLACK 10B DYE USING CHEMICAL ACTIVATED CARBON DERIVED FROM TANNERY WASTE BIOMASS: ADSORPTION DYNAMICS AND OPTIMIZATION THROUGH RESPONSE SURFACE METHODOLOGY

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In the present study, activated carbon is prepared from industrial waste biomass material by chemical activation method using sodium hydroxide. The prepared biomass chemical activated carbon (BCAC) is used for the adsorption of Amido Black 10B (AB) dye from aqueous solution. The preparation of activated carbon particles from industrial wastes has served two purposes: (1) Disposal of industrial wastes and (2) Generation of wealth from waste for a greener adsorption technology. The prepared adsorbent was characterized by SEM, FT-IR, TGA, zero-point charge and surface functionality before it was used. Response surface methodology was used to analyze and optimize the variables affecting the adsorption of AB dye onto BCAC by central composite design (CCD). Four factors were optimized in this method viz., pH (1.808), adsorbent dosage (64000 mg/L), dye concentration (376.87 mg/L) and contact time (6.15 h). The equilibrium data was investigated by Langmuir, Freundlich and Temkin adsorption isotherm models. The maximum monolayer adsorption capacity (q_m) was found to be 61.24 mg/g. The kinetic data was tested with pseudo-first-order and pseudo-second-order kinetic equations. The kinetics of the adsorption process was found to follow the pseudo-second-order kinetic model.
Keywords: Adsorption dynamics, Isotherm models, Response surface methodology, Optimization, Chemical activation, Amido Black 10B dye

1. Introduction

Amido Black 10B (AB) is a di-azo anionic dye which is widely used for dyeing in leather and textile industries. The application of AB also extends to the field of forensic science for tracing the victims by finger print. However it causes irritation to eyes, skin and also to mucous membrane. Hence, it becomes necessary to remove such a toxic dye from wastewater stream before it can be released into the aquatic environment. Various methods are available for the removal of dyes from wastewater treatment such as catalytic degradation, chemical oxidation technique (Liang et al 1996), coagulation, solvent extraction. Among these methods, adsorption is one of the best treatment methods. Because of its efficiency, cost effectiveness, eco-friendly nature and recovery (Crini 2005) Most of the industries are preferring adsorption technique using activated carbon for the removal of various water pollutants from effluents. This is due to its high adsorption control, high surface area and highly porous nature of the material. The activated carbon can be prepared using chemical

activating agents like NaOH, KOH, H_3PO_4, $ZnCl_2$ or H_2SO_4. Chemical activated carbon has been used for adsorption of H_2S and dyes (Malik 2004; Annadurai et al 2002).

Commercial activated carbons are also available in the market for the adsorption of dyes from various effluents but it is restricted due to its high cost (Onal et al 2006). Therefore, nonconventional adsorbent materials have been employed for the removal of wastewater treatment. In this method carbon was prepared from agriculture waste and industrial wastes. These are more economical adsorbents for treatment of wastewater.

Various agricultural waste and carbonaceous materials such as rice husk (Van et al 2014), sugarcane bagasse (Garg et al 2008), coconut shell (Din et al 2009), fruit stones (Valle et al 2005), orange peel (Khaled et al 2009), are used in the production of activated carbon. But the preparation of carbon from the above materials has some drawbacks as the preparation method is not cost effective as it needs higher temperature to carbonize. In modern years, number of industries is increasing, so the solid waste production is also increasing. Many of the industries are discarding their solid wastes into landfills, due to the industrial growth. This will lead to soil pollution on earth, so it must be controlled. The new way to reduce solid waste is by burning it into carbon material. The prepared carbon material is highly cost effective, eco-friendly and this method would minimize solid waste disposal problem in future and it is a renewable source for preparation of carbon material. In addition, the cost of the raw material also decreases so that the production of activated carbon can be carried out in large scale.

The adsorption efficiency depends on various experimental factors, such as pH, adsorbent dosage, initial dye concentration, time, and temperature. In order to determine the influence of each of the factors, the conventional experimental method involves varying one factor at a time and holding the values of all other factors as constant (Santos et al 2008). These methods are highly time consuming and expensive. These limitations could be overcome by applying Response Surface Methodology (RSM), which is an empirical and statistical technique used to calculate the relationship between a set of controlled experimental factors. RSM can effectively decrease the number of experimental runs and at the same time facilitate the construction of a response surface using which the effect of variables (on the response) can be studied. RSM would also be helpful to determine the optimal conditions required for better adsorption. In this study, RSM was used for the optimization of experimental parameters involved in the adsorption of AB onto the prepared activated carbon.

In the present study, waste biomass collected from an industrial effluent treatment plant was used for preparing activated carbon by simple carbonization process followed by chemical activation. The activated carbon was used for the adsorption of Amido Black 10B (AB) from aqueous phase. The efficiency of the prepared adsorbent was optimized using Response Surface Methodology (RSM).

2. Materials and Methods
2.1. Preparation of activated carbon

The waste biomass was collected from a tannery effluent treatment plant nearby Chennai, India. The collected waste biomass was washed and dried under sunlight for 7 days and then powdered. The powdered waste biomass was carbonized in a muffle furnace at 500-700°C for 3 hours under inert atmosphere. The carbonized carbon particles were impregnated with NaOH (in the ratio 1:2) and stirred for 2 h using magnetic stirrer. The slurry was dried at 110°C overnight in a hot air oven. The dried mixture was loaded in an alumina boat and activated in a tubular furnace under

N_2 flow (80 cm^3 min^{-1}). The mixture was heated to 700°C for 2 h at the rate of 5°C min^{-1}. Then the Biomass Chemical Activated Carbon (BCAC) was cooled to room temperature. The BCAC was washed and dried at 110°C for 12 h (Illa´n-Go´mez et al 1996). The prepared BCAC was stored in an air tight container for further use.

2.2. Experimental Design

In the present study, a four variable (with five level) second order central composite design (CCD) was followed. The factors and their levels are described in Table 1. The range considered for each factors are actual true limits and therefore inscribed type of central composite design (CCI) has been used. The full factorial CCD matrix was analysed using Minitab (version 17, PA, USA). The four independent variables considered were aqueous pH (X_1), adsorbent dosage (X_2), initial dye concentration (X_3) and time (X_4). Percentage adsorption of AB was taken as dependent output variable of the system. Generally, the CCD consists of a 2^k factorial runs with 2k axial runs and x_0 number of centre points. A total number of 31 experiments were used in the study, including $2^4 = 16$ cube points, 7 replications at the centre point and $2 \times 4 = 8$ axial points. The number of experimental runs was designed from equation (1).

$$N = 2^k + 2k + x_0 \qquad (1)$$

Where, N is the number of experimental runs required, k is the number of variables and x_0 is the number of centre points. Consequently for this design total number of experimental runs will be 31 (k=4, x_0 = 7).

For statistical calculations, the variable x_i was coded as X_i according to the following relationship. The coded values of the independent variables were determined by the following equation (2).

$$X_i = \frac{x_i - x_0}{\partial x} \qquad (2)$$

Where X_i is the coded value of the variable, x_i is the actual value of the variable, x_0 is the centre point value and δx is the step change between the levels. The quadratic model equation for the prediction of % adsorption (i.e., response function) is expressed using the second order polynomial equation (3)

$$\% \text{ Adsorption} = \beta_0 + \beta_1(X_1) + \beta_2(X_2) + \beta_3(X_3) + \beta_4(X_4) + \beta_{11}(X_1)^2 + \beta_{22}(X_2)^2 + \beta_{33}(X_3)^2 + \beta_{44}(X_4)^2 + \beta_{12}(X_1)(X_2) + \beta_{13}(X_1)(X_3) + \beta_{14}(X_1)(X_4) + \beta_{23}(X_2)(X_3) + \beta_{24}(X_2)(X_4) + \beta_{34}(X_3)(X_4) \qquad (3)$$

Where X_1, X_2, X_3, X_4 are independent variables; β_0 is constant; β_1, β_2, β_3, β_4 are coefficients of the polynomial for linear effects; β_{11}, β_{22}, β_{33}, β_{44} are coefficients of the polynomial for quadratic effects; β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, β_{34} are coefficients of polynomial for interaction effects. Optimum values of four parameters considered in this study were evaluated using OPTIMTOOL application of MATLAB (V 7.10). The same has been verified with the results obtained from Minitab.

Table 1

Independent variable	Factor	Coded levels		
		-1	0	+1
pH	X_1	1	2	3
Adsorbent dose (mg/L)	X_2	1000	50500	100000
Initial dye concentration (mg/L)	X_3	5	502.5	1000

Contact time (h)	X_4	0.5	5.25	10

3. RESULTS AND DISCUSSION

3.1. Characterization of BCAC

Fig. 1(a) shows the zero-point charge (ZPC) of the BCAC which was found to be pH_{ZPC} = 10.21. There were more basic functional groups (0.331 m_{eq}/g) than the acidic functional groups (0.063 m_{eq}/g) present on the surface of BCAC. The FT-IR spectrum Fig. 1(b) shows characteristic bands corresponding to the various functional groups in BCAC. Broad band at 3469 cm^{-1} corresponds to stretching vibration of hydroxyl group. The sharp C=C and C-C stretching vibration band appeared at 1640 cm^{-1} and 1428 cm^{-1}. Besides, the stretching vibration of C–O found at 1105 cm^{-1}. Fig. 1(c) illustrate the FE-SEM micrograph of BCAC. It is observed that BCAC surface is in porous and rough surface in nature. The TGA thermogram is shown in Fig.1 (d). The thermogram shows that 68% mass of BCAC remained as residue even after 800°C. Therefore, the prepared BCAC had good thermal stability. The nitrogen adsorption, surface area and pore volume distribution of BCAC was determined by BET theory model and is shown in Fig. 1(e), 1(f) and 1(g). The surface area of BCAC was found to be 67.11 m^2/g and the pore volume of the BCAC determined by BJH plot was 0.068 cm^3/g.
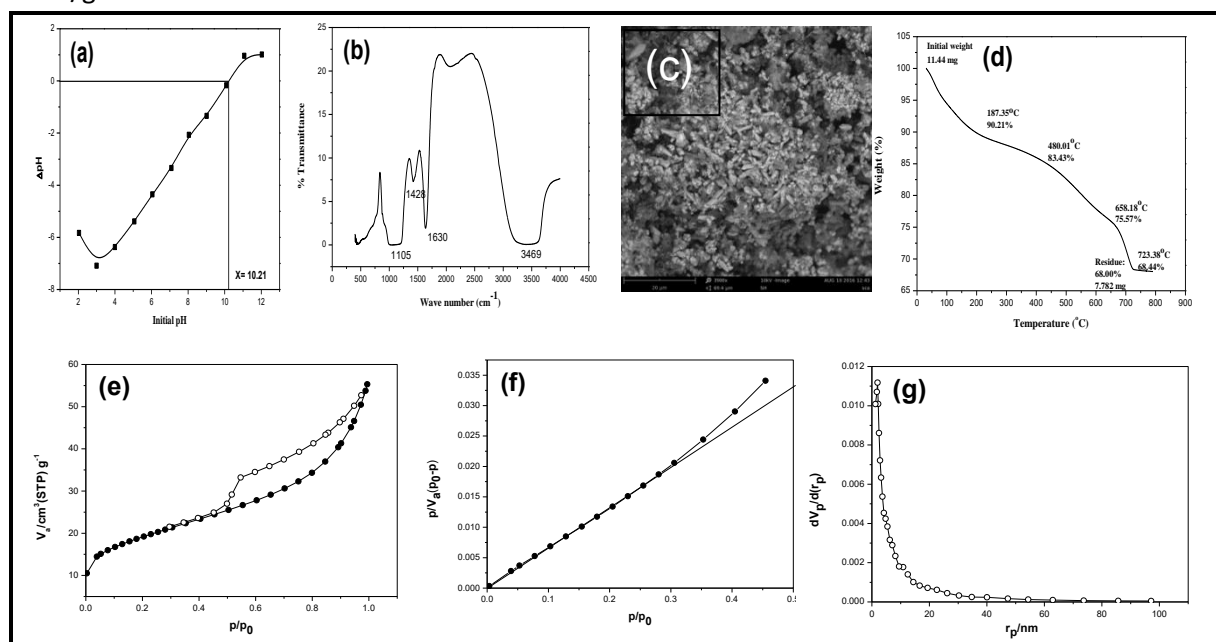

Fig. 1. (a) ZPC (b) FT-IR (c) SEM (d) TGA (e) N_2 adsorption (f) BET plot and (g) BJH plot of CAC

3.2. Batch adsorption experiments

Initial aqueous phase pH plays an important role in the liquid-phase adsorption process. Effect of aqueous pH was determined by varying the initial aqueous phase pH in the range of 2 to 12. From Fig. 5(a), it can be inferred that the maximum adsorption was observed at pH 2 (more than 98.16%). Since, ZPC of the prepared BCAC was found at pH_{ZPC} 10.21, the surface of BCAC would be negatively charged above pH_{ZPC} 10.21 and positively charged below it. Since AB ionizes into negative ions in the aqueous solution it is maximum adsorbed at positively charged surface of BCAC at pH 2. Hence, further experiments were carried out at pH 2. The effect of adsorbent dosage was studied by varying from 0.01 g/10 mL to 1.0 g/10mL. From the experimental results Fig. 5(b), it is clearly noticed that the % adsorption of dye increased with increase in the carbon dosage. The effect of initial dye

concentration was studied by varying the initial dye concentrations from 25 mg/L to 1000 mg/L. The results are plotted in Fig. 5(c). The % adsorption decreased with increase in the initial concentration of dye molecules. This study was further extended to calculate the equilibrium parameters using isotherm models such as Langmuir, Freundlich and Temkin isotherms.

3.3. Optimization of AB adsorption by RSM

The main effects plot showing the effect of each parameter is provided in Fig. 2. The plot clearly shows that adsorption dosage and dye concentration are highly significant parameters compared to pH and time. Using the experimental results, the regression model equation (second order polynomial) relating the % adsorption and process parameters was developed and is given in equation (4).
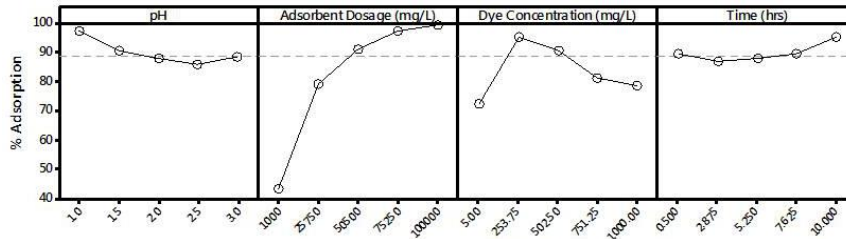

Fig. 2. Main effect plot for removal of AB dye

The RSM gives the linear, quadratic and interaction effect of the parameters for the dye removal. These analyses were made by Fisher's F-test and P-value. The parameters having an F-statistics probability value <0.05 indicated that the model is significant (Bagheri et al 2011). The P-values were also used to confirm the significance of each of the interaction between the variables. If the P- value is statistically significant (i.e., when P<0.05), model terms are at 95% confidence level (Hamsaveni et al 2001). The value of regression coefficient, F-value and P-value for all the linear, quadratic and interaction effects of AB are given in Table 3. The regression equation for AB is given below.

$$\% \text{ Adsorption} = 95.0290 - 4.4917(X_1) + 21.5341(X_2) - 8.3665(X_3) + 2.6558(X_4) + 0.4010(X_1)^2 - 20.3378(X_2)^2 - 16.0584(X_3)^2 + 0.9945(X_4)^2 + 4.9309(X_1)(X_2) - 0.9874(X_1)(X_3) - 0.1250(X_1)(X_4) + 20.3286(X_2)(X_3) - 3.9363(X_2)(X_4) + 0.5914(X_3)(X_4) \ (4)$$

The above equation (4) explains the effect of variables on the adsorption of AB onto BCAC. Equation (4) confirmed that the model is well fitted, considering the value of determination coefficient (R^2= 0.8739) which indicated that only 12.61% of total variation was not explained by the model. The relationships between experimental and predicted results are shown in Fig. 3.
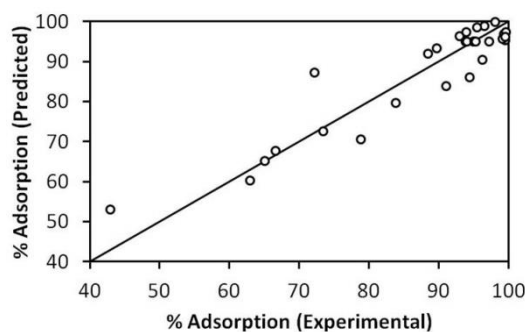

Fig. 3. Plot of the experimental and predicted AB removal value

The determined effects and coefficients for ANOVA model are listed in Table 3. In ANOVA model the coefficients of linear effect of adsorbent dosage (X_2) and dye concentration (X_3) (P=0.000, 0.016

85

respectively) for AB was most statistically significant and coefficient for the remaining two parameters, pH (X_1) and time (X_4) for the removal of dyes were least significant. The coefficient of interaction between the square of adsorbent dosage (X_2^2) and square of dye concentration(X_3^2) (P = 0.001, 0.006 respectively) for removal of AB were found to be most significant. However, the interaction effect between adsorbent dosage and dye concentration (X_2*X_3) (P = 0.008) of dye adsorption was also significant.

The model equations were optimized using OPTIMTOOL application of Matlab by employing *fmincon* solver through interior point algorithm. Reciprocal of the model equation was considered as the objective function for minimization. These results corroborated well with the response optimizer values of RSM as shown in Table 4.

Fig. 4 shows the surface and contour plots, for the three most significant variables namely adsorbent dosage, dye concentration and pH. Time being relatively insignificant, was not considered for analysis. While the responses due to the variation of two variables were considered, the other two variables were held at centre point values as shown in Fig. 4. The response surface clearly emphasizes the significance of adsorbent dosage and AB concentration on % adsorption.

Table 3

| Source	Degree of freedom (df)	Sum of squares (SS)	Mean square (MS)	F-Value	P-Value
Model	14	4982.51	355.89	7.92	0.000
Linear	4	3365.67	841.42	18.73	0.000
X_1	1	121.05	121.05	2.69	0.120
X_2	1	2782.31	2782.31	61.94	0.000
X_3	1	419.99	419.99	9.35	0.008
X_4	1	42.32	42.32	0.94	0.346
Square	4	1162.44	290.61	6.47	0.003
X_1*X_1	1	37.34	3.51	0.08	0.783
X_2*X_2	1	651.02	739.25	16.46	0.001
X_3*X_3	1	472.31	460.88	10.26	0.006
X_4*X_4	1	1.77	1.77	0.04	0.845
2-Way Interaction	6	454.4	75.73	1.69	0.189
X_1*X_2	1	24.31	24.31	0.54	0.473
X_1*X_3	1	0.97	0.97	0.02	0.885
X_1*X_4	1	0.02	0.02	0	0.985
X_2*X_3	1	413.25	413.25	9.2	0.008
X_2*X_4	1	15.49	15.49	0.34	0.565
X_3*X_4	1	0.35	0.35	0.01	0.931
Error	16	718.7	44.92		
Lack-of-Fit	10	711.36	71.14	58.13	0
Pure Error	6	7.34	1.22		
Total	30	5701.21			
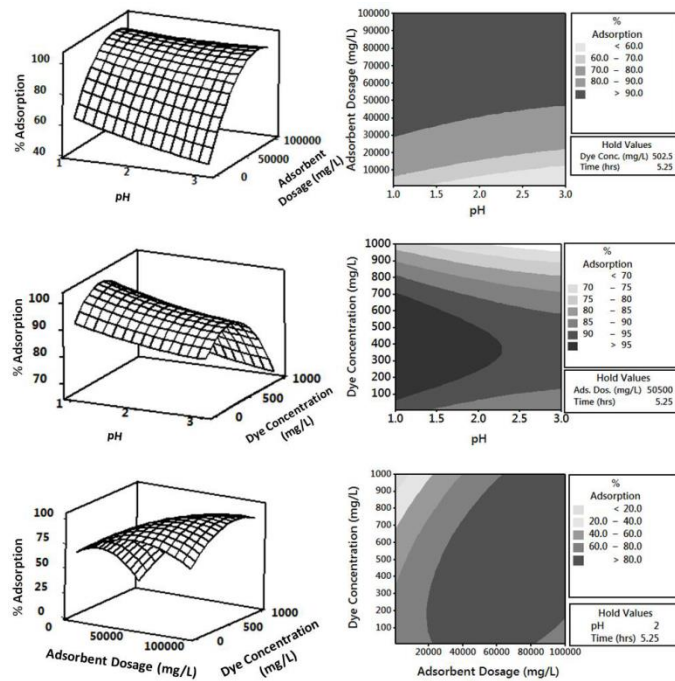
Fig. 4. Response surface plots and Contour plots for removal of AB

Table 4

Method	Efficiency (%)	X₁ (pH)	X₂ (ads.dosage, mg/L)	X₃ (dye concentration, mg/L)	X₄ (time, h)
MATLAB	100	1.81	61885	401.01	6.15
RSM	100	1.81	64000	376.87	6.15

3.4. Adsorption isotherms studies

3.4.1. Langmuir isotherm

The Langmuir equation (equation 5) is applicable to homogeneous adsorption where the adsorption of each adsorbate molecule on to the surface has equal sorption activation energy. The linear form of this isotherm is represented by the expression (Langmuir 1918):

$$\frac{1}{q_e} = \frac{1}{q_m K_L C_e} + \frac{1}{q_m} \qquad (5)$$

Where, q_e (mg/g) and C_e (mg/L) are the amount of adsorbed adsorbate per unit weight of adsorbent and unadsorbed adsorbate concentration in solution at equilibrium, respectively. The constant K_L (L/g) is the Langmuir equilibrium constant 0.022 and q_m is the theoretical monolayer saturation capacity value is 61.24 and the r^2 is 0.87507.

3.4.2. Freundlich isotherm

The most important multisite adsorption isotherm for heterogeneous surfaces is the Freundlich adsorption isotherm (Gerente et al 2007) and the linear form of this isotherm is expressed as equation (6).

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \qquad (6)$$

where, K_F (L/g) is the Freundlich constant 3.839 and n (g/L) is 1.904 the Freundlich exponent and the

r^2 is 0.9556. Therefore a plot of $logq_e$ versus $logC_e$ enables the constant and exponent n to be determined. Since the value of Freundlich exponent 'n' was between 1 and 10, the adsorption of AB onto BCAC was favourable.
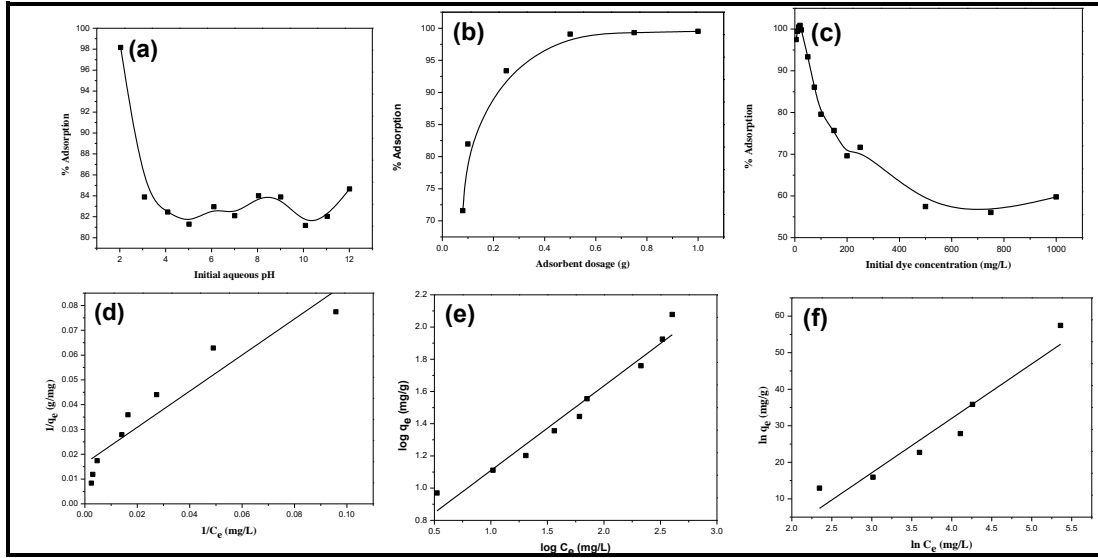

Fig. 5. (a) Initial aqueous pH (b) adsorbent dosage (c) Initial dye concentration (d) Langmuir isotherm (e) Freundlich isotherm and (f) Temkin isotherm model for adsorption of AB onto CAC

3.4.3. Temkin isotherm

Temkin isotherm model (Allen et al 2004) describes the behaviour of adsorption systems on heterogeneous surfaces. The linear form of this isotherm is expressed as equation (7).

$$q_e = B_1 lnK_T + B_1 lnC_e \qquad (7)$$

where, $B_1 = RT/b$, T is the absolute temperature (K) and R is the universal gas constant (J mol^{-1}K^{-1}), b is the Temkin constant related to the heat of adsorption is 14.90 and K_T is 0.157 the equilibrium binding constant (L/mg) corresponding to the maximum binding energy and the r^2 is 0.90218. The Langmuir, Freundlich and Temkin isotherm plotsare shown in Fig. 5(d), 5(e) and 5(f) respectively. While comparing the r^2 values of Langmuir, Freundlich and Temkin isotherms, it was observed that r^2 values of Freundlich isotherm were closer to unity and hence the surface of BCAC was heterogeneous in nature.

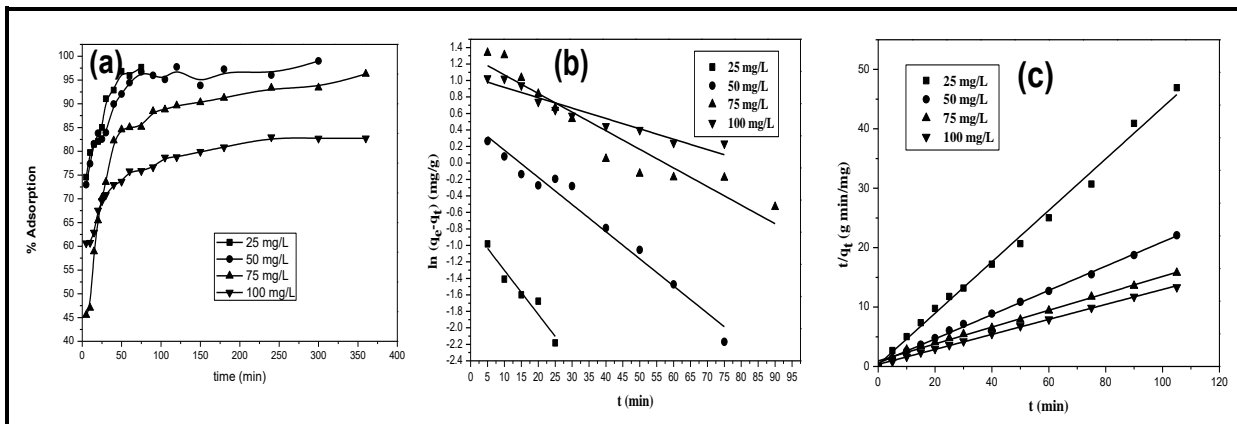### 3.5. Adsorption kinetic studies

Fig. 6. (a) Kinetics (b) Pseudo first-order and (c) pseudo second-order for removal of AB dye

3.5.1. Pseudo first-order model

The linear form of pseudo first-order equation of Lagergren (Li et al 2011) is generally expressed as equation (8).

$$\ln(q_e - q_t) = \ln q_e - k_1 t \qquad (8)$$

where, q_e(mg/g) and q_t (mg/g) are the amounts of adsorbed adsorbate at equilibrium and at time t, respectively. k_1 (min^{-1}) is the rate constant of pseudo first-order adsorption. The plot for $ln(q_e\text{-}q_t)$ versus t will be linear if the adsorption process followed the pseudo first-order kinetic model.

3.5.2. Pseudo second-order model

The linear form of pseudo second-order kinetic rate equation (Ho et al., 1998) is expressed as mentioned below (equation 9).

$$\frac{t}{q_t} = \frac{1}{k_2 q_2{}^2} + \frac{1}{q_e} t \qquad (9)$$

where k_2 (g/mg min) is the equilibrium rate constant of pseudo second-order adsorption process. If the pseudo second-order kinetic equation is applicable, the plot of t/q_t against t should give a linear relationship, from which q_e and k_2 can be determined from the slope and intercept of the plot.

The kinetics of the adsorption processes were carried out for the prepared BCAC for four different initial dye concentrations such as 25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L shown in Fig. 6(a). The kinetic experiments were carried out by withdrawing samples and analyzing their concentration at regular time intervals. The kinetic data of adsorption process were tested with the pseudo-first order and pseudo second-order models as shown in Fig.6(b) and 6(c). The parameters obtained from these plots are also shown in Table 5. The r^2 values of pseudo second-order model was closer to 1 when compared to the r2 values of pseudo first-order model. Therefore the kinetics for the adsorption of AB onto BCAC followed pseudo second-order model.

Table 5

C_0 (mg/L)	Pseudo first-order kinetic equation			Pseudo second-order kinetic equation		
	q_1 (mg/g)	K_1 (1/min) $\times 10^2$	$r_1{}^2$	q_2 (mg/g)	K_2 [g/(mg min)]$\times 10^3$	$r_2{}^2$
25	0.463	0.053	0.918	2.30	18.11	0.994
50	1.623	0.033	0.967	4.90	42.76	0.998
75	3.645	0.023	0.895	7.04	52.52	0.993
100	2.838	0.013	0.904	7.92	178.43	0.998

4. Conclusion

A new adsorbent namely, BCAC was prepared from the industrial waste biomass and used for the adsorption of AB dye. Preliminary batch adsorption studies were carried out and the maximum adsorption of dye molecules occurred at pH of around 2. Response surface methodology was used to optimize the adsorption process. According to the ANOVA, adsorbent dosage and dye concentration are found to be the more statistically significant parameters. The quadratic model adequately represents the determination coefficient (R^2= 0.8739).This R^2 value suggested that the model was a good fit. Freundlich isotherm was confirmed to best fit the equilibrium data. Adsorption of AB onto BCAC followed pseudo second-order kinetic model. Boyd plot suggested that the film diffusion is the rate-controlling step. Results of the present investigation showed that BCAC is a potential adsorbent for the removal of AB from aqueous solution over wide range of concentrations.

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CHARACTERIZATION OF BIOLOGICAL TREATMENT OF EFFLUENTS FROM TANNED SHEEPSKINS

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The tannery effluent is characterized by a high concentration of organic compounds (such as proteins, lipids), inorganic (as sulphide, chlorides, trivalent chrome) suspended solids (as degraded hair, not dissolved lime) , some anilines and other compounds that vary according to the raw hides processed. In this context the little tanneries are those that are more vulnerable as they have not enough means or sufficient space in their establishments. Taking into account these conditions, the aim of this work is to give solutions to the environmental problems for this kind of industries.

With the goal to establish the adequate methodology for testing at laboratory scale biological treatment in aerobic batch reactors was used the effluent obtained in the processes of tanning of sheepskins. This effluent, subjected to aeration, settling and precipitation through a physical-chemical process was employed for the experiences of secondary treatment using batch reactors aerobic type, with a culture of microorganisms as the inoculums from a sewage treatment plant effluent. The system was fed progressively up to 100% effluent; volatile suspended solids (VSS) and chemical oxygen demand (COD) were estimated for each step. After adaptation, the treatability assay was performed in order to obtain the kinetic parameters refer to the degradation rate of the effluent for to design biological systems at full scale. Furthermore, the evolution of the system was monitored through qualitative analysis related with macroscopic sludge sedimentation and microscopic observations.

The K (rate constant degradation) and Ks (coefficient of saturation) kinetic settings were obtained using the kinetic model of Monod, from them it was possible to calculate the residence time (60 hours) and the relation food / microorganisms (F / M = 0.47 / day) for an initial 5000mgO2 COD / l, an allowable overturning 250mgO2 / l and SSV 3500 mg / l. Microscopic evaluation of sludge allowed to establish the presence of the taxon Litonotus spp during the stages of development, maturation and aging, also once were identified and quantified crawlers and fixed ciliates, metazoans and amoebas.

Keywords: effluent, adaptation, sheep, tannery, microorganisms

Introduction

Leather industry significantly contributes to the economy of a state; however, effluents from this activity constitute one the most complex wastes because of their high levels of pollutants. In

Argentina, the 86% of the leather manufacturing is carried out by mineral tanning methods employing chrome salts, while the remaining 14 % is performed by plant tanning by using natural or synthetic tannins. Tanning industry generates important amounts of residues as it employs harmful chemical substances. Tanning effluents are characterized by a high concentration of organic compounds (such as proteins and lipids), inorganic ones (sulphide, trivalent chrome), suspended solids (degraded hair, not dissolved lime), high salinity, anilines and other compounds that vary according to the raw hide processed. Leather manufacturers must to follow quality water parameters as regards the generated effluents. The aim of this work is to offer a methodology to perform test of biological treatment for effluents from tanning sheepskins at Batch aerobic reactors. The treatment efficiency was characterized by determining physicochemical parameters and bioindicators. (Haydar et al. 2007)

Materials and methods

The effluent used was obtained from the pilot plant of tannery of the Center of Research and Technology on Leather employing sheep hides. The primary treatment was performed on the beamhouse and on the tanning line by separated to avoid toxic gas formation. Each effluent was sedimented and aerated to oxidize sulfides and remove suspended lipids, then physical-chemical processes were used to remove settled solids and colloidal solids. This treatment is intended to protect downstream processes. Before the biological treatment test, the treated effluents were mixed and the full effluent was characterized: BOD, COD, pH, NKT, conductivity and NaCl concentration were measured according to standard methods (APHA 1992)

The biological treatments were performed by duplicate using batch reactors of 1 liter, the sludge was provided by sewage treatment plant. In the first stage (adaptation) the value of the concentration of microorganisms present was determined (Volatile Suspended Solids) and the caudal (Q) was estimated for the industrial effluent and the maintenance´s substrate milk. In this stage, the biological reactors were fed with a progressive increase of the feed percentage (Q) of the tannery effluent. As the feed with the effluent increased, the feed with the substrate milk decreased. This was done to promote the adaptation of the microorganisms. The effluent was supplemented with phosphorus in the ratio of carbon: nitrogen: phosphorus 100:5:1.

Biological treatability test

A concentration of microorganisms of 3000 mg / l and an effluent volume of 160 ml were employed for the test start. For each time, a sample was taken out from the reactor under continuous agitation and VSS and COD of the sample were determined. From these results the parameters K (maximum rate of substrate removal) and Ks (average speed coefficient) were determined using the Monod and Eckenfelder kinetic models. With the kinetic model already defined and taking into account the allowable overturn value, the residence time that should be considered for the degradation of the effluent was calculated (Lateef et al. 2013).

Bioindicators of activated sludge

The active sludge status can be adequately characterized by observing the development of the sludge using microscopic techniques in conjunction with the physico-chemical parameters. Microscopic observation of active sludge is considered as a bioindicator of the state of operation of

the treatment plant. It provides an environmental quality assessment that marks the physical-chemical characteristics. This monitoring results in high sensitivity because any change is reflected in the composition of the species present in the sludge. Protist populations play a key role in the depredation of free bacteria and their contribution to bioflocculation. At low sludge age (< about 4 days) the simpler life forms are present. This includes amoebas and flagellates. As the sludge age increases (> about 4 days), more complex organisms such as the free swimming ciliates and stalked ciliates appear. At high sludge age multi-celled animals such as rotifers may be found. (Vázquez et al.1999).

For microscopic observation procedure homogeneous samples of the mixed liquor from the biological reactor were taken out in Falcon tubes, maintaining a sufficient air layer so that the oxygen would not be depleted until it was transferred to the microscopy laboratory. All determinations were under direct observation by light-field optical microscopy.

Macroscopy of the floc

The methodology consisted in taking samples of the mixed liquor from the aerobic reactor, for this it was gently shaken and the sample was introduced into a 1-liter beaker, allowed to settle for 30 minutes. After that the following observations were made: color, sedimentation volume (settleable solids), turbidity and presence of suspended flocs.

Results and Discussion

Rate of effluent degradation by microorganism biological oxidation was evaluated. The purpose was to obtain a kinetic model to use in a design for aerobic biological treatment systems. Aerobic system was chosen because residual sulphide in the effluent could affect the efficiency in an anaerobic treatment.

When VSS and COD obtained were estimated (table 1, graphic 1 and 2), the model that best adjusted was the Monod model´s which maximum substrate utilization rate (K) was 1.71/day and saturation coefficient (Ks) was 1090 mg/l. Then, these kinetic parameters were applied in the following linearized equation

$$X*t/(S_{in}-S)=K_s/k*1/S+1/k$$

where t is mean hydraulic residence time, Sin is initial COD=5049 mgO2/l, S is final COD=250 mgO2/l, X is VSS. The calculated hydraulic residence time was 60 hours with a relation food / microorganisms (F / M = 0.47 / day) (Metcalf & Eddy 2004).

Table 1 Data of samples for kinetic coefficient of Monod and Eckenfelder´s models

Time (min)	TSS (mg/l)	FSS (mg/l)	VSS (mg/l)	COD (mg/l)
1	7650	3470	4180	1157
5	8100	3650	4450	1052

10	7310	3290	4020	1157
15	7080	3190	3890	1262
20	8960	3980	4980	946
30	8200	3620	4580	1262
60	9280	4200	5080	946
120	8590	4020	4570	946
180	7300	3480	3820	946
240	9020	3990	5030	736
300	8430	3880	4550	736
360	6720	3070	3650	631
480	7570	3490	4080	631
1440	8170	3840	4330	526

Graphic 1: VSS mixed liquor at different hydraulic retention time
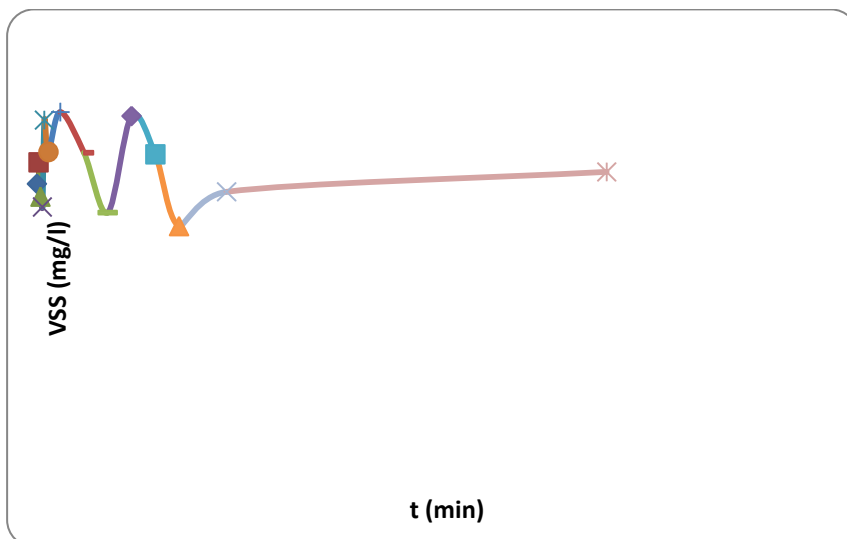
Graphic 2: removal of COD at different hydraulic retention time
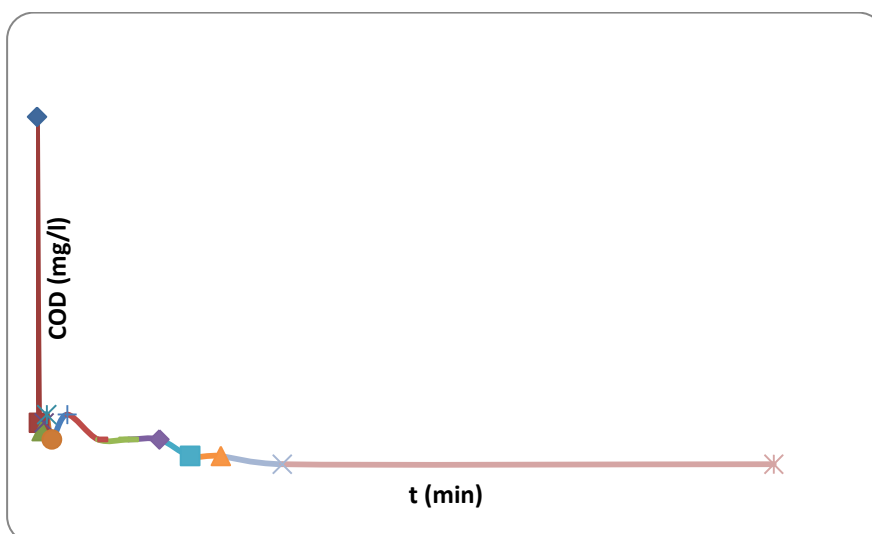
The macroscopic test showed a brown shade of the liquor mixture for the first minutes of sedimentation. This characteristic is associated with its maturity. After 30 minutes a low turbidity was observed, the presence of suspended flocs was low and the sedimentation rate was fast.

The main microscopic characteristics of the floc were observed: irregular shape, medium size, compact structure, weak texture, medium coverage (10-50%), protozoa division 4-7sp. This simplified study allowed to determine a good sludge index, being a preliminary, quick and simple assessment of the depuration yields (Salvadó et al. 1997).

In the analysis of the microbiota, rotifers from Philodinidae and Lecanidae families (Fig. 1) were detected at the beginning of the test but not at the end with 100% effluent. These communities, representatives of old sludge have been observed, since the test was initiated with a sludge of high age, with flocs that had excessive time of permanence in the reactor.

Further, naked amoebae of the family Amoebidae (Fig. 2) and different groups of protists alveolates have been detected. The ciliates are successive colonizing microorganisms that occupy diverse ecological niches, it is determinant the presence of them in the activated sludge processes since it improves the quality of the effluent as the turbidity by decrease the amount of free bacteria. The greatest diversity of protist ciliates of class Oligohymenophorea was found, which can be classified in three families. Bacterivorous free-swimming ciliates of the Cyclidiidae family, Cyclidium sp. (very active, never remains at rest, only when fed) (fig 3), swimming ciliates of family Parameciidae have been identified. During the last stages sessile ciliates appears, basically peritrichous from Vorticellidae family (Vorticella sp.) (Fig. 4); in this group the formation of mobile larvae have been identified. In adittion in this stage appears predator/omnivorous pleurostomates swimming of the family Litonotidae (Fig. 5) and bacterivorous spirotrichous crawling ciliates of the family Aspidiscidae (Fig. 6) (Kudo 1946; Perez-Uz et al. 2010).

The microscopic observation of the activated sludge constitutes a valuable contribution to determinate the evolutionary moment of the biological sludge as well as if it undergoes some alteration. One of the most efficient measures is to access to the composition and dynamics of the biological community since it indicates all time the state of operation, offering the possibility to resolve problems and improve yields.
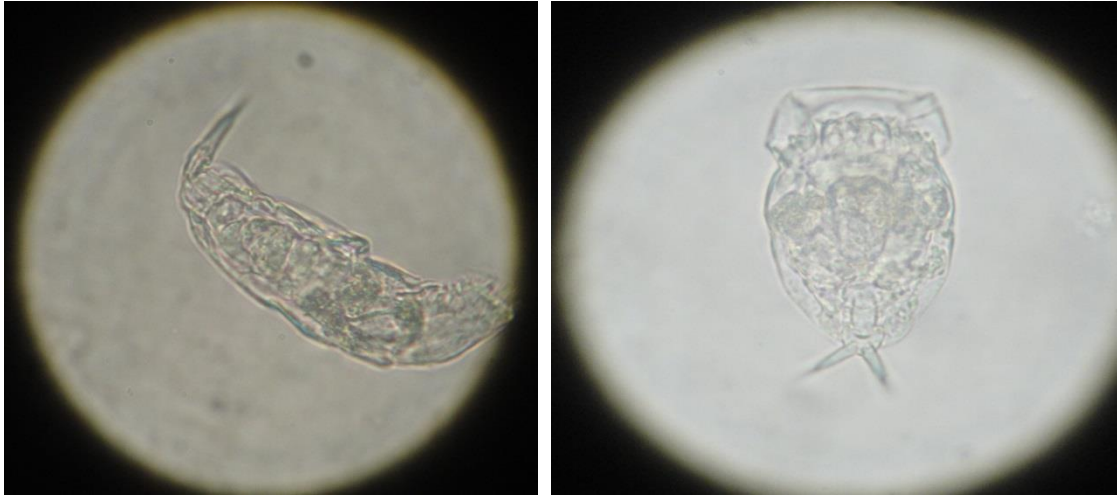
Fig. 1-Metazoa Family Lecanidae 40 x

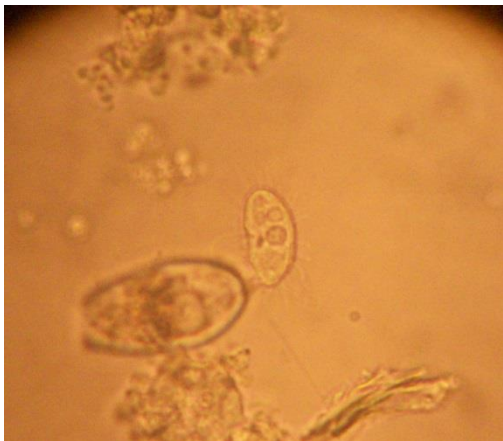Fig. 2-naked-amoebae 40 x

Fig. 3-Family Cyclidiidae 40x   Fig. 4-Sessile ciliate Vorticella sp 40x

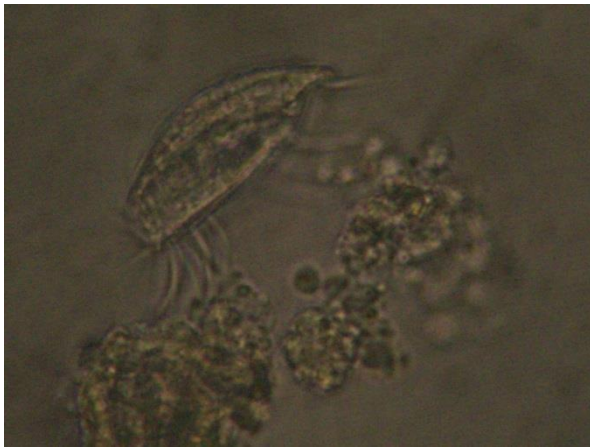Stalked ciliates attached to a floc 40x

Fig. 5-Family Litonotidae 40x

Fig. 6-Family Aspidiscidae 40x

Crawling ciliate-spirotrichous 40x

Conclusions

The characteristics of the treated effluent have affected the evolution of the microfauna that compose the activated sludge. As it has been observed, the number of live rotifers was decreasing whereas the group of litostomates was developed under all conditions. These individuals present a wide range of tolerance to the high concentration of salts in the medium, as mentioned this type of effluent presents a high concentration of NaCl. Free bacteria have been observed to proliferate freely in the interfloc solution, thereby increasing the turbidity of the liquor mixture. It has been detected in the last stages succession the presence of telotroch swimming larvae, they are formed when the conditions of the environment are changing and unfavorable being able to become encyst, instead they will emit a new stalk if the environment becomes propitious.

These preliminary results will be evaluated after the acquisition of more experimental data.

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CHARACTERIZATION OF CATTLE HAIR WASTE AS ADSORBENT FOR LEATHER DYES

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During the dyeing step in leather processing some chemicals are added to impart characteristics such as color and uniformity of surface appearance to the leather, consequently wastewaters are generated with colored substances that are not easily treated in conventional wastewater treatment plants. Among various treatment systems the adsorption occupies a prominent place for dye removal. The growing demand for efficient and low-cost treatment methods and the importance of adsorption has given rise to low-cost alternative adsorbents. In this work, surface of novel sorbent (cattle hair waste) was characterized and its dye removal ability was tested on aqueous medium of Acid Red 357 dye. The isoelectric point (pHpzc), functional groups and morphology were investigated. Sorbent surface and chemical composition were characterized using optical microscopy and energy dispersive X-ray spectroscopy (EDS). The functional group onto CHW was investigated using Fourier transform infra-red spectroscopy (FTIR). The specific surface area and the pore size distribution were determined by analyzes of BET/BJH that showed values considered low when compared to commercial activated carbon. By means of optical microscopy, EDS and FTIR was observed the presence of the dye in the adsorbent tested. The point of zero charge (pHpzc) was 6.5 and the adsorbent showed a good interaction with the AR-357 dye in acid pH range. The percentage of AR-357 dye removal on CHW was 92.94 % (pH 1.0) and 98.54 % (pH 2.0) with maximum amounts of AR-357 dye adsorbed of 9.81 (pH 2.0).

1. Introduction

Tanneries are among the oldest industries, transforming hides into leather through a cascade of several unit operations. The processing of hides involves three main phases, namely beamhouse, tanning, and finishing, which are further subdivided into several steps. Beamhouse operations are mainly responsible of cleaning the hide and preparing it for subsequent tanning steps (Mella et al. 2015a).

To date, the unhairing steps use sulfides and a large amount of chemical products. According to Jian et al. (2011), the conventional lime-sulfide process leads to the destruction of the hair, causing emissions with high chemical oxygen demand (COD), biological oxygen demand (BOD), and total suspended solid (TSS) loads in the effluent of tannery companies.

During beamhouse steps, basically, there are two types of hair removal process: one with hair destruction and another with total or partial hair preservation. The unhairing process use amount of chemical reducing agent to ensure the removal and subsequent dissolution of keratin are also known as hair burning process (Valeika et al. 2009). The most common reducing agent used is

sodium sulfide, because it is cheaper and efficient in the process, however the organic matter of the effluent is increased due to the hair that can no longer be removed by filtration (Souza, 2013).

During leather processes, in wet end steps, many dyes are used for dyeing in water system usually dissolved and remains are released in wastewater. Despite being a contaminant that significantly contributes to the elevation of chemical oxygen demand of wastewaters, at low concentrations they may change significantly the color of the water, causing aesthetic problems in water bodies polluted with industrial effluents. Moreover, in wastewater treatment plants the biological treatment systems may not be efficient for the removal of color, when the objective is the wastewater reuse, especially in tanneries that perform only the leather finishing, because in these cases the wastewater has higher dye concentrations (Piccin et al. 2013).

The use of adsorption processes, particularly those employing alternative adsorbents materials has drawn the interest of various researchers. Adsorption, from the operational perspective, has advantages over other processes for the treatment of soluble substances such as dyes, heavy metals and phenolic compounds in wastewaters. Among these advantages are the low initial investment requirement, the simplicity of the project and its operation, the reduction in energy use and its increased efficiency compared to conventional and other non-conventional processes and the reduction of contamination and toxicity of the treated effluent (Noroozi et al. 2007;Rafatullah et al. 2010; Piccin et al. 2013).

Natural materials or the wastes/by-products of industries orsynthetically prepared materials, which cost less and can be used assuch or after some minor treatment as adsorbents are generallycalled low-cost adsorbents (LCAs) (Gupta and Suhas 2009). Such as natural materials, or industrial wastes, whichcan reduce significantly process costs. Many researchers arefocusing their efforts in research alternative adsorbents suchas rice husk ash, palm-fruit bunch, treated sawdust, chitosan, leather waste, cattle hair waste and fungal biomass (Mane et al., 2007; Nassar and Magdy, 1997; Garg et al.,2003; Piccin et al., 2011; Mella et al. 2015b; Gomes et al. 2015; Puchana-Rosero et al. 2016).

In this context, the use of leather processing waste, especially cattle hair from unhairing step represents a new alternative adsorbent to remove leather dye in wastewater treatment. The objective of this study was to investigate the adsorption of Acid Blue 161 using the solid waste from tannery, the recovered hair (CHW). The adsorbent was characterized by optical microscopy, EDS, BET/BJH, FTIR and determination isoelectric point (pHpzc).

2. Material and methods

2.1. Adsorbent preparation

The cattle hair (CHW) was collected from a tannery that performs unhairing with hair saving way. Previously, the samples were extensive washing out with aqueous and then oven dried at 372 K for 12 h.

2.2. Adsorbent characterization

To verify the textural properties, thermal stability and morphology analyses were performed to characterize the CH:

Surface morphology was analyzed using optical microscopy (Olympus, model SZX16). Elementary analyses were performed with energy dispersive X-ray spectroscopy (EDS) (Oxford Instruments, model SwiftED3000).

The specific surface area was determined from the Brunauer, Emmett and Teller (BET) multipoint method and the pore size distribution were obtained using Barret, Joyner, and Halenda (BJH) method (Arenas et al. 2007).

The adsorbent was also characterized using Fourier transform infra-red spectroscopy (FTIR) (PerkinElmer, Spectrum Two model) to identify compounds present in the sample.

Following procedure was used for determination of the point of zero charge (pHpzc): 20.00 mL of 0.050 mol L^{-1} NaCl solution was added to various Falcon tubes containing 50.0 mg of the adsorbent. The pH (pHi) values of the solutions were adjusted from 1.0 to 10.0 using a 0.10 mol L^{-1} of HCl and 0.10 mol L^{-1} NaOH. The suspensions were agitated and equilibrated in a thermostatic shaker at 298 K for 48 h and centrifuged at 3500 rpm for 10 min. The pHi of the solutions without adsorbent and pHf of the supernatant after contact with the adsorbents were recorded. The value of pHpzc is the point where the plot of ΔpH (pHf - pHi) versus pHi crosses a line equal to zero (Prola et al. 2013).

2.3. Solutions and reagents

The leather dye, C.I. Acid Red 357 (AR-357; CAS: 57674-14-3; $C_{32}H_{20}CrN_{10}O_{14}S_{23}Na$, 956.7 g mol^{-1}, λ_{max} = 496 nm), was supplied by Lanxess (São Leopoldo, RS, Brazil). Fig. 1 shows its chemical structure and Fig. 2 shows the chromophores,responsible for producing the colour, and the auxochromes,which can not only supplement the chromophore but also renderthe molecule soluble inwater and give enhanced affinity (to attach)toward the fibers (Gupta and Suhas 2009).The preparation of stock solution of the dye (5.00 g L^{-1}) was done by accurately weighing a calculated amount of the dye and dissolving it in distilled water. The pH of the solutions was adjusted using the Digimed DM-22 pH meter with a 0.10 mol. L^{-1} NaOH and 0.10 mol. L^{-1} HCl.
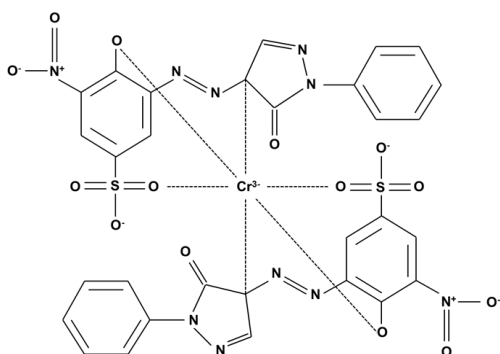

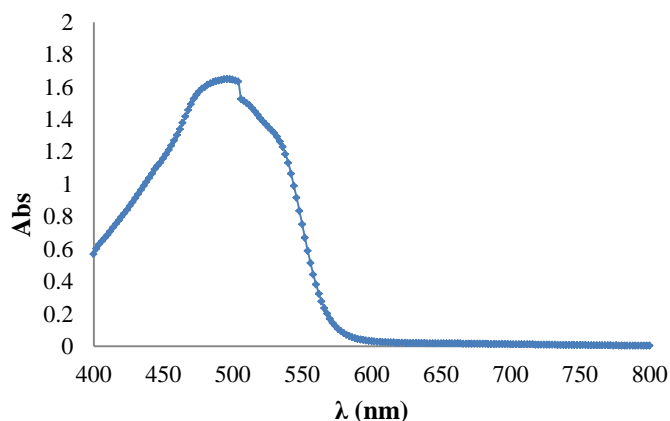**Fig. 1.** Chemical structure of Acid Red 357 Source: Lanxess, 2015.

Fig. 2. UV–vis spectra of aqueous solution of AR-357

2.4. Batch adsorption experiments

The adsorption trials for evaluation of CHW adsorbent for removal of AR-357 dye from aqueous solutions were carried-out in triplicate using the batch contact adsorption. In the trials fixed amounts of adsorbents (1.0 g) were placed in Schott flasks containing 100.0 mL of dye solutions (100 mg L^{-1}) that were prepared in pH from 1.0 to 10.0. The mixtures were agitated (150 rpm) by a suitable time (24 h) from 298 K to 323 K in a thermostatic shaker (NT 715, Novatecnica).

Subsequently, in order to separate the adsorbents from the aqueous solutions, the solutions were filtered with aid of a vacuum pump (Model 132, Prismatec).

UV/visible spectrophotometer (T80 UV–VIS spectrophotometer, PG Instruments, Leicester, LEC, UK) was used to quantify the residual of AR-357 dye in solution after adsorption at a maximum wavelength of 578 nm. When necessary, aliquots of the supernatant were diluted with distilled water before spectroscopic measurement.

The amount of the dye uptake and percentage of removal of dye by the adsorbents were calculated by applying Eqs. (1) and (2), respectively:

$$q = \frac{(C_o - C_f).V}{m} \qquad (1)$$

$$\%\,removal = \frac{(C_o - C_f)}{C_o}.100 \qquad (2)$$

where q is the amount of dye taken up by the adsorbents (mg g^{-1}), Co is the initial AR-357 concentration put in contact with the adsorbent (mg L^{-1}), Cf is the dye concentration (mg L^{-1}) after the batch adsorption procedure, V is the volume of dye solution (L) put in contact with the adsorbent and m is the mass (g) of adsorbent.

3. Results and Discussion

3.1. Point of zero charge (pH$_{pzc}$) and dependence of pH on sorption capacity

The interaction between sorbate and sorbent is affected by the pH of an aqueous medium in two ways: firstly, since dyes are complex aromatic organic compounds having different functional groups and unsaturated bonds, they have different ionization potentials with the pH, resulting in the pH-dependent net charge on dye molecules. Secondly, the surface of sorbent has many functional groups, so the net charge on sorbent, which could be measured in the form of zeta potential or isoelectric point, is also pH-dependent. Therefore, the interaction between dye molecules and sorbent is basically a combined result of charges on dye molecules and the surface of sorbent (Aksu et al. 2008).

The pH of the adsorbate solution is one of the influential factors by the adsorption of dye on an adsorbent (Silva et al., 2011; Cardoso et al., 2011, Cardoso et al., 2012). Different dyes have different ranges of suitable pH of adsorption depending on the type of adsorbent used (dos Santos et. al. 2014).
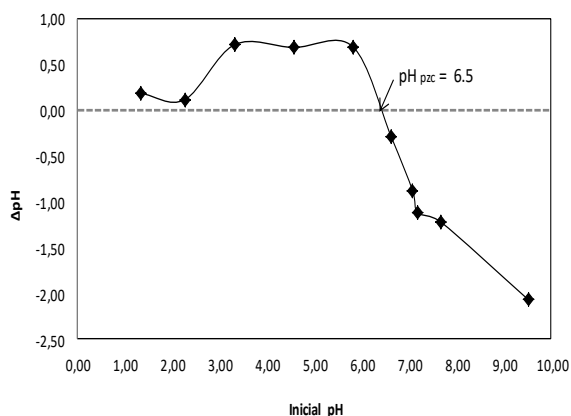
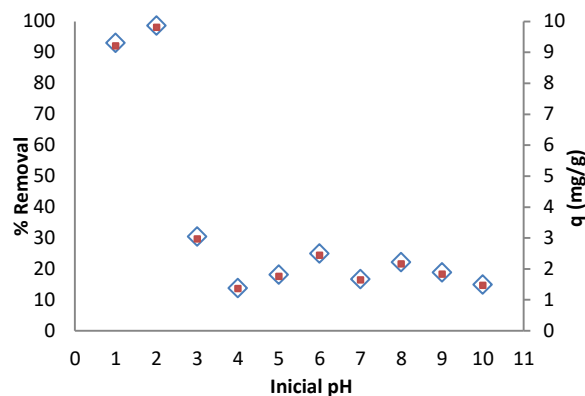**Fig. 3.** Point of zero charge for CHW

Fig. 4. pH effect on the adsorption of AR-357 from CHW

The pH$_{pzc}$ for CHW was 6.5 (Fig. 3). The effects of initial pH on percentage removal of AR-357 dye solutions (100 mg L−1) using CHW adsorbent were investigated within the pH range of 1 and 10 (see Fig. 4). For pH values lower than pH$_{pzc}$ the adsorbent presents a positive surface charge (Calvete et al. 2010). The dissolved AR-357 dye is negatively charged in water solutions, so the adsorption of the AR-357 dye takes place when the adsorbent present a positive surface charge, therefore, the electrostatic interaction occurs at pH < 6.5 for CHW. This behavior explains why the best removal efficiency in sorption process for CHW occurs at pH 2.

It is observed in Fig.3 that the best pH of dye removal was 2.0, achieving 98.54 % for dye removal CHW, with maximum amounts of AR-357 dye adsorbed of 9.81 mg g^{-1}.

3.2. Optical microscopy

Figure 4 shows optical microscopes of CHW sorbent before (a) and after (b) adsorption process. It is clear from Fig. 5 (a) that the surface texture of the sorbent before sorption was rough, uneven, and brown in the CHW structure. After sorption, the sorbent surface had color change (Fig. 5(b)). By this difference, it is possible to verify that occurred the coverage of surface by AR-357 dye.
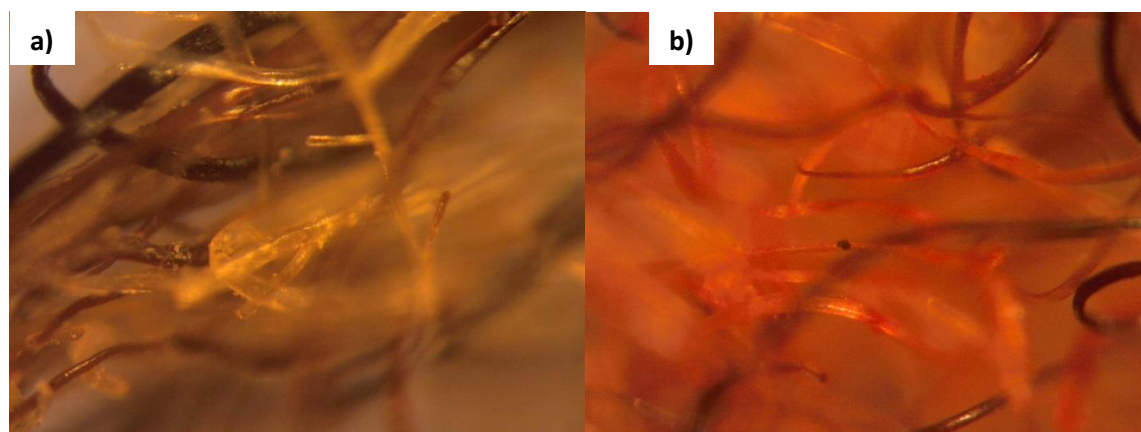

Fig. 5. Optical microscopy characterization of the sorbents: (a) CHW, (b) CHW + AR - 357.

3.3. Energy dispersive X-ray spectroscopy (EDS)

The hairs are keratinized structures and its main characteristic is the existence of high sulfur content due to the presence of cystine residues which provides chemical and physical stability to the keratin. Its structure is shown in Fig. 6.
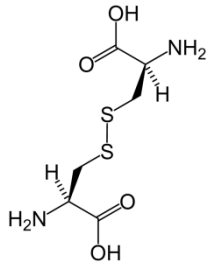

Fig. 6 Cystine structure
Source: Gutterres, 2004.

According to White et al. (1991), a typical analysis of the 'waste hair' after a "hair saving" unhairing process using sodium sulphide/lime supported by an unhairing amine/mercaptan agent in moisture free is: total solids: 25-30%, fat 2-4%, total nitrogen 11- 15%, Ca 2-3%, Na 1-2%, S 3-5%. These elements present in the structure of CHW were detected in the EDS (see Fig. 7 a)), and in presence of Cr and Na after the sorption process from the AR-357 dye (see Fig. 7. b)). The presence of Au is due to the metallization for the analyses.
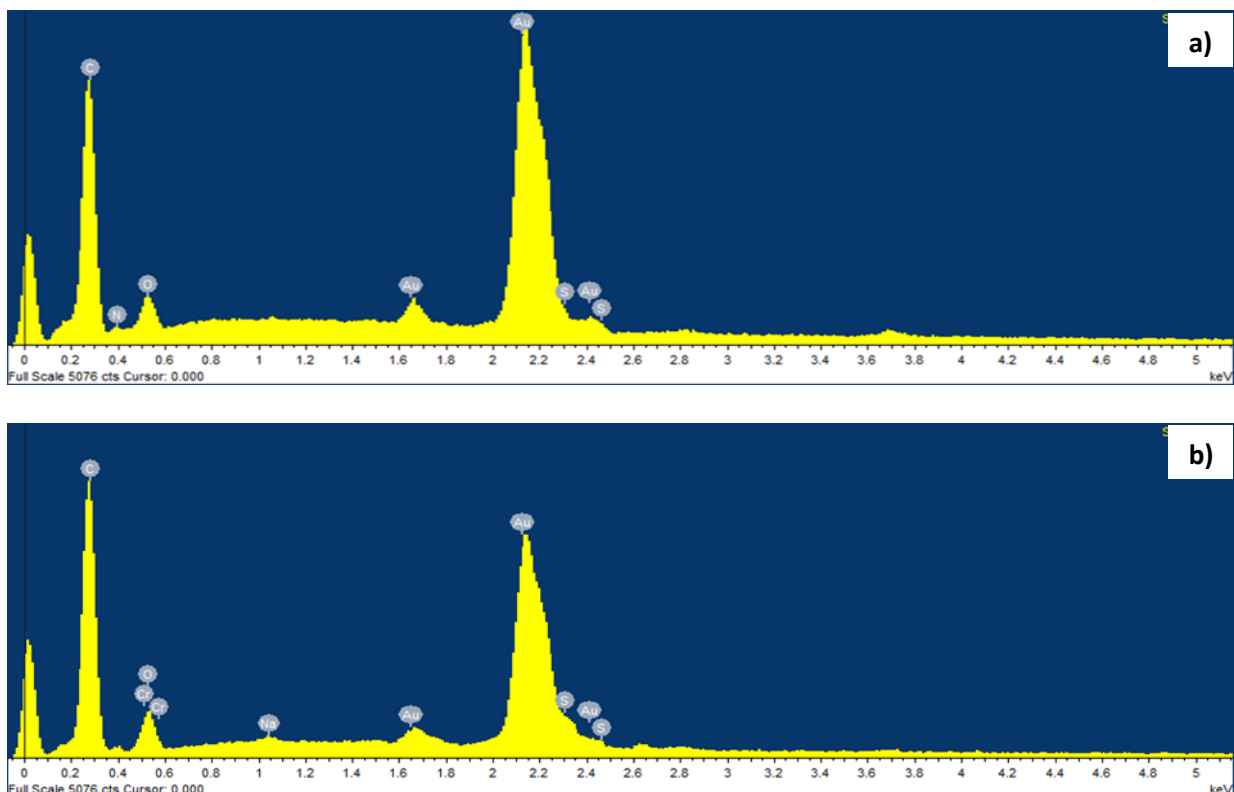

Fig. 7. EDS characterization: a) CHW and b) CHW + AR-357

3.4. Specific surface area and pore size distribution (BET/BJH)

The hair is very resistant to degradation because during the keratinization process, the cortex keratin is enhanced by disulphide bridges between adjacent cysteine residues leading to the cystine amino acid as final product and generating greater cross-linking. This process adds greater stability to the hair increasing its fibrous properties and consequently, its resistance to hydrolysis (Galarza et al. 2010). This resistance to degradation and high stability contributed for the CHW not to have significantly altered structure as can be seen in the Table 1, with low values of specific surface area, average pore volume and pore diameter if compared with commercially activated carbon (Cardoso et al. 2012).

Table 1. BET/BJH of the adsorbents

	CHW	CAC
Specific surface area - BET ($m^2\,g^{-1}$)	0.9491	652.5341
Average pore volume ($cm^3\,g^{-1}$)	0.0007	0.4991
BJH average pore diameter (nm)	2.9100	4.74

3.5. Fourier transform infra-red spectroscopy (FTIR)

Figure 7 shows FT-IR analysis of CHW adsorbent before and after adsorption of AR-357 dye. The spectra (Fig. 8.) taken from the two samples (CHW and CHW+AR-357) are very similar and are typical of hair, with the presence of the absorption bands of lipids, amides and cystine residues.

Spectra show the characteristic absorption bands of keratin proteins: (i) C–H stretching bands of amides A and B at about 3270,3 and 3050 cm^{-1}, (ii) υ(C=O) and υ(C–N) stretching, and δ(N–H) and υ (O=C=N) deformation bands of amides I, II and III at about 1630, 1520 and 1300 cm^{-1}, (iii) absorption bands due to cystine residues at 1042 and 1100 cm^{-1}, according to the oxidation state of the cystine (Cotte et al. 2004; Zhang et al. 2008; Wojciechowska et al. 2004)

Additionally, visible changes are observed in peaks of (1716 and 1730) cm^{-1}. Such behaviors are caused by changes in the molecular composition of the groups possibly by dye electrostatic interaction with the amino group of the leather sorbent, proving the chemical nature of adsorption.
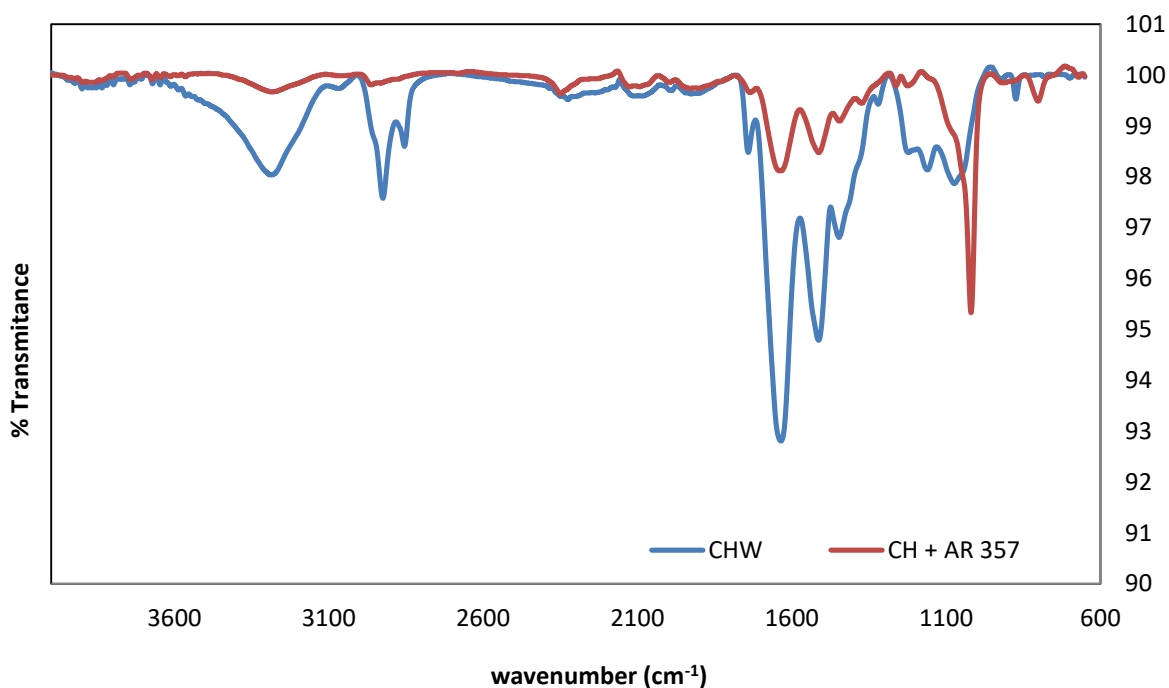
Fig. 8. FT-IR spectra of: (a) CHW; (b) CHW + AR-357.

4. Conclusion

Through optical microscopy analyses, EDS, BET/BJH, FTIR and pH_{pzc} analysis was possible to characterize the proposed adsorbent. Although the analyzes of BET/BJH showed values considered low of the new adsorbent purposed when compared to commercial activated carbon, through optical microscopy analysis, EDS and FTIR was observed the presence of the dye in the adsorbent tested.

The point of zero charge (pH_{pzc}) for CHW was 6.5 and the adsorbent showed a good interaction between the AR-357 dye in pH 2.0. The percentage of AR-357 dye removal on CHW was 98.54 % for dye removal CHW, with maximum amounts of AR-357 dye adsorbed of 9.81 mg g^{-1}. The obtained results open a promising via to use cattle hair (CHW) as efficient eco-friendly sorbent for the treatment of leather industrial effluents containing dyes.

5. Acknowledgements

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DEHAIRING PROTEASE PRODUCTION AND EVALUATION OF THE EFFICACY OF PROTEASE PRODUCTS OF RELEVANT DOWNSTREAM PROCESSING

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Globally as well as in Ethiopia the tanning industries are under a high pressure from strict legislation articulated for the purpose of protecting and preserving the environment. In the current situation of Ethiopia this industry is viewed from two perspectives; being an important economic activity and the most environment polluting industry. In order to operate within environmentally compatible limits and sustain the realization of Agriculture Development Led Industrialization (ADLI), the current development strategy of Ethiopia, with respect to the tanning industry, some sort of solutions should come out that mitigate the adverse effect of the conventional leather processing methods particularly that of lime-sulphide unhairing operation. This study deals with dehairing enzyme production as green technology alternative for the conventional unhairing practice.

The *B.subtilis* strain was obtained from Department of Biotechnology, CLRI, sub-cultured and characterized for its growth and dehairing protease production in terms of pH, temperature, incubation time and growth and production media composition. The combined effects of pH and temperature on protease production also investigated and they were found to have high interactive effect. Once the culture conditions for production were studied and known, the alkaline protease was produced in a pilot scale fermenter by submerged fermentation using soy bean flour media at optimal conditions of pH 6.5, temperature 310C and incubation period of 27 hours.

The final protease product was recovered, partially purified and stabilized by primary downstream processing such as crude enzyme formulation, ammonium sulphate precipitation, ultra-filtration and spray drying. The use of the protease products of each technique on sheep skins and cow hide resulted in a highly promising hair removal efficiency that can really compete with lime-sulphide chemical unhairing process.

1. Introduction

In Ethiopia the manufacturing of leather at industrial scale started in1920s by producing leather for horse seat back "koricha", which was required as one of the elements in fighting against the invaders, at formerly named "as kokoda" now called Addis Ababa tannery .Over this period the number of tanneries have increased and reached 31 and the industry has grown in terms of the type and quantity of leather it produces both for local and international market. Today the leather industry is generating about 110 million USD per year from exports made to different countries. However, this figure is believed to be very small and we need to do a lot to exhaustively exploit the sector.

In the five years Growth and Transformation Plan of Ethiopia the leather industry sector has been given high priority with the target of realizing 500 million USD at the end of the plan by transforming the industry through interventions in various areas. For this purpose some activities like benchmarking and twinning program are under taken and going on. However, it should be recognized that the work of creating conducive and competitive business environment for the sector should be more holistic in the sense that many of the inputs for manufacturing the leather need to be produced locally, local chemical producers and the leather industry should work jointly to represent Ethiopia in world leather market as one entity and due attention should be given to research to move the industry forward at fast pace. Although we are lucky in having comparative advantage of high livestock population, the past experiences show that the Ethiopia's leather sector has not registered significant performance for a number of reasons. Poor management practice, lack of state of the art technology in certain production segments, lack of labor force with qualification that exactly fits to the industry , very poor practice of relevant research work in assisting the industry for various challenges they are facing such as environmental constraints among other major factors.

One of the serious and environmentally ill nature of the leather manufacturing industry is evident if we look at the use of lime-sulphide method for dehairing. The adverse effects of this dehairing method on the environment and human health is high notably due to large amount of COD and BOD from dissolved protein and process chemicals, high pH, SS,TDS and sulphide. Experiences show that poor environmental performance results in the loss of competitive advantage at global market, loss of public relation, image and credibility and also leads to legal liabilities for the adverse impact it creates to the human health, safety and the environment. For these reasons bioprocessing of leather is becoming an essential part in mitigating the environmental pollutions of conventional leather making processes. Bioprocess operations make use of microbial, animal and plant cells and components of cells such as enzymes to manufacture new products and destroy harmful wastes [1].Industrially useful enzymes such as dehairing protease are commercial products of bioprocessing. In terms of providing environmental benefits in processing industries, enzymes are probably the most important compounds produced by microorganisms. Enzymes have got applications in soaking, unhairing, degreasing, bating and post-tanning of leather processing operations. As a class, they are extraordinarily efficient. Minute quantities can accomplish at a low temperature what, by ordinary means, would require a high temperature and greater amounts of strong chemical reagents [2].

In almost all tanneries in Ethiopia, the tendency to employ enzymatic dehairing method is very minimal because of the fact that high price of imported enzymes and enzymatic dehairing is disastrous if not carefully controlled as well as lack of access to ease of supply and technical services. In this research, as one means of encouraging the Ethiopian tanners in shifting from conventional to

bioprocessing of leather processing, the production of dehairing protease from *Bacillus subtilis* by submerged fermentation and the hair removal efficiency of the protease products of relevant downstream processing are extensively studied. And also the potential benefits of producing the enzyme locally and its contribution to the leather industry and the country at large in environmental and some other dimensions is presented. The chosen fermentation technique is best suited for microorganisms such as bacteria that require high moisture content [3].

2. Material and methods

2.1 Materials
a. Raw materials

- wet salted sheep skins and
- wet salted cow hides

b. Reagents and chemicals

Trichloroacetic acid(TCA)	Sodium benzoate
Agar	Bronopol
Ammonium sulphate	Tata salt
Buffer salts	Distilled water

Buffer salts
- Sodium carbonate
- Sodium bicarbonate

NaOH

Growth and production medium
- Source of carbon
- Source nitrogen (yeast extract, peptone, soya bean)
- Source of inorganic salts(NaCl,$MgSO_4$,$KH_2SO_4$and $CaCl_2$)

a. Machines, Equipment and consumables

Laminar air flow cabinet	Spectrophotometer
Test tubes	Centrifuge
Shake flasks	Magnetic stirrer
Shaker	Fermenter
Petri dish and paraffin film	Micro filtration
Graduated Measuring cylinders	Ultrafiltration
Micropipette and tips	Spray dryer
Refrigerator	Soaking liming pits/Drums

Water bath	pH paper
Auto clave	tyrosine filter paper
Incubator	Closures such as Cotton plugs, plastic foam, screw caps, metal caps and aluminum foil for closing end of culture vessels

b. Culture for fermentation

Bacillus subtilis bacterial species

c. Software

Design expert 7.0 aprobado

2.2 Methods

To carry out this study the methodology followed involved preparations of various reagents, preparation of pre-inoculums, characterization study of culture, production of dehairing protease using production media of soybean at pilot scale fermenter, carry out downstream processing and finally evaluating the efficacy of the protease products of each downstream processing employed.

2.2.1 Preparation of reagents

1.1% casein solution

1 g of Hammerstein casein in 100 ml of 0.05 M carbonate buffer, pH=9.5.

2. Carbonate buffer

A = 0.1 M Na_2CO_3 = 1.06 g / 100 ml distilled water

B = 0.1 M $NaHCO_3$ = 0.84 g / 100 ml distilled water

13 ml of A + 37 ml of B + 50 ml of distilled water

= 100 ml of buffer, 0.05 M, pH 9.5

3.5% TCA

5 g of TCA in 100 ml of distilled water

Procedure

- 1.9 ml of casein solution was added to all test-tubes marked as test and control.
- 3 ml of TCA was added to the tubes marked as control.
- All the tubes were pre-incubated for 10 minutes at 40°C.
- 0.1 ml (100µl) of suitably diluted enzyme was added to all the tubes and incubated at 40°C for 10 minutes for the enzyme to react with the substrate.
- After incubation 3 ml TCA was added to all tubes marked as test to stop the reaction.
- The precipitate was filtered through What man filter paper (Grade A, 110 mm)
- The absorbance of the filtrate was read at 280 nm using spectrophotometer
- The amount of tyrosine released was calculated from the standard graph of tyrosine.

2.2.2 Preparation of pre-inoculum

A preserved *B.subtilis* on agar slant obtained from a microbiologist was sub-cultured using LB(Luria-Bertani Broth medium) agar plate .Then the isolated individual colony with good microbial activity (i.e. large skim milk hydrolysis) was inoculated and cultivated in 100 ml standard shake flask using LB medium following standardized procedures. Periodically protease activity was measured by Kunitz method of enzyme assay.

Sub-culturing on LB Agar plates procedure

1. 4g of LB and 1g of agar was added to 75 ml distilled water in 250 ml shake flask and shaken well to ensure complete dissolve and uniform mixing
2. 1g of skim milk was dissolved in 25 ml distilled water in 100 ml shake flask
3. Autoclaved separately
4. Mixed them in laminar flow chamber which was already sterilized by UV for 10 minutes
5. It was poured to plates before it solidifies
6. The LB agar was allowed to solidify in laminar flow chamber
7. The plates were inoculated by streaking
8. The plates were sealed, labeled, dated, inverted and incubated at room temperature as shown in the following pictures.
9. The individual colony with good activity was also sub-cultured onto agar slant and preserved at 40Ctemperature for using it for further work.

Table2.1LB agar plate composition

Ingredients	Gram /liter
Casein enzymichydrolysate	10.00
Yeast extract	5.00
Sodium chloride	10.00
Agar	15.00

Pre-inoculums preparation in shake flask procedure

1. In each of two 100 ml shake flasks20 ml growth media was prepared
2. The media was autoclaved
3. Allowed to cool
4. A loopful of culture from agar plate that has shown good clear zone individual colony was inoculated into each flask
5. For each flask, enzyme activity was determined by Kunitz method.

2.2.3Characterization study of culture

Environmental or physical and chemical conditions allowing optimal growth and productivity of the culture were studied in terms of the following factors:

- pH
- temperature
- Time and
- Growth and production media: standard media vs soya flour.

pH, temperature and time effects were studied using lab grade standard media at flask level by applying 100 to 200_µl pre-inoculums. The formulation of the synthetic or designed LB media used for the characterization study is shown in table 2.2.

Table 2.2 LB growth and production media formulation for characterization study of *B.subtilis*

Ingredients	% (w/v)
Maltodextrine	0.5
Yeast extract	0.5
Skim milk	0.5
NaCl	0.12
KH_2PO_4	0.06
$MgSO_4$	0.06
$CaCl_2$	0.03

a. Effect of pH study

For the characterization study of pH a two replicates of experiments were conducted using 200 ml LB growth and production media in 250 ml shake flask.

Trial 1

In each of five 250 ml shake flasks 200 ml growth media was prepared and the pH of one of the flask was measured to be 5.5 and the other four flasks were adjusted to pH 6.0, 7.0, 8.0and 9.0 by flakes of sodium hydroxide. Then each was sterilized, inoculated and incubated. Activity was checked by Kunitz method of enzyme assay.

Trial 2

In each of five 250 ml shake flasks 200 ml growth media is prepared and the pH of one of the flask was measured to be 6.5 and the other four were adjusted to pH 7.0, 8.0, 9.0 and 10.0 as illustrated in Fig 3.6.Then each was sterilized, inoculated and incubated. Activity was determined by Kunitz method of enzyme assay.

b.Effect of temperature

For studying the effect of temperature on biomass growth two trials were carried out using LB standardized growth media. To do this, in each of three 250 ml shake flasks 200 ml growth media was prepared, sterilized, inoculated and incubated for 72 hours at 25, 30 and 33 degree Celsius. Activity was checked by Kunitz method of enzyme assay.

c. Effect of incubation period study

To get an idea of the effect of incubation period on the growth of culture and protease production, it was designed in such a way that it can be observed from the studies of pH, temperature and media.

a. Effect of growth and production media study

The effect of this culturing condition on the growth and protease activity was studied by comparing standard LB media against a media of soya flour. For this, 50 ml of standard LB media and 50 ml of soya flour media each in two 100 ml conical flask were taken.

All the flasks were autoclaved at 121^{0}C and 15 lb pressure for 20 minutes. After cooling the flasks were seeded with equal quantity of inoculums and incubated at 30^{0}C for 72 hours in shaker incubator. Protease activity was checked at time intervals of 24, 36, 48 and 72 hours by Kunitz method of enzyme assay.

b. The study of interaction effects of pH and temperature

An experiment designed by a Central Composite Design (CCD) was performed to investigate the effect of both the pH and temperature on protease production with time using standard medium. The levels of the variables were set based on the information obtained from the above characterization study of these two parameters.

Table 2.3 Process variables and levels used in the Full factorial Design

Variable (Factors)	Factor Coding	Unit	Levels	
			-1(low)	+1(high)
Temperature	A	^{o}C	25	35
pH	B	pH scale	5	9

The statistical analysis: analysis of variance (ANOVA), a regression analysis and the plotting of response surface was performed by Design expert 7.0 aprobado software.

2.2.4 Fermentation

a. Pre-inoculum preparation

In preparing the pre-inoculums, 200 ml of LB media in each of two 1000 ml shake flasks were taken. The flasks were autoclaved, cooled to room temperature, inoculated with one full slant of culture

and incubated for 18 hours in a shake incubator. OD (optical density) was measured and protease activity measured by Kunitz method.

b. Seed culture preparation

In going for pilot scale fermentation enough amount of inoculum was prepared. For this, in each of two 7 liters seed fermenters 4liters of production media of soya flour (formulation shown in the table 2.5) were prepared, pH was measured to be 6.0 and adjusted to 6.5by 1N NaOH, sterilized, cooled to process temperature of 30± 2^0c and each fermenter was inoculated with 200 ml pre-inoculum and fermentation was undertaken for 24 hours.

The OD was measured and the enzyme activity was determined at time intervals of 12, 18 and 24 hours. The one that has shown good activity was used for inoculating the main fermenter.

c. Pilot scale fermentation

In parallel to inoculum preparation, in pilot fermenter 40 liters of soya bean flour media was taken, sterilized and cooled to optimum operating temperature of 30± 2^0c. And also during this period, fermenter accessories like hoses that were used for transferring inoculum from flask to seed fermenter and from seed fermenter to pilot fermenter and needles, etc. were sterilized.

Table 2.5Media formulation for the seed and pilot fermenters

Ingredients	%(w/v)
Soya flour	1.1
NaCl	0.12
KH_2PO_4	0.06
$MgSO_4$	0.06
$CaCl_2$	0.03

After making sure that all the necessary equipment and conditions were ready and seed culture was prepared, pilot scale fermentation was commenced and undertaken for 27 hours. The protease activity was measured periodically during the fermentation process until it started decreasing. Then the broth was collected and centrifuged for recovering the protease in the supernatant and stored at 4^0C for further downstream processing.

During the fermentation process, the factors affecting it were closely monitored and controlled:

- Temperature:30± 2^0C
- PH:6.5-7.0
- Aeration:0.5-1vvm
- The RPM of agitator:100-150
- Foam formation: anti foam addition when required.

- Duration: until the growth reached stationary phase
- stationary phase

2.2.5 Downstream processing

After successful fermentation the enzyme was separated, purified and treated chemically to the required level of purity, stability and concentration to serve its ultimate purpose of effective and efficient dehairig activity. For this the primary downstream processing were employed:

a. Crude enzyme formulation

For this, 15 liters of fermentation broth was taken and 0.1% Bronopol, 0.2 % sodium Benzoate and 2% table salt were added to render it with enhanced stability. The formulated crude was kept in a refrigerator at a temperature of 4^0C until it was applied for dehairing.

b. Ammonium sulphate precipitation

The ammonium sulphate precipitation of the protease was done based on well-established principles and basic procedures [4,5]. In effecting this, 10 liters of crude enzyme was taken and the enzyme was precipitated from the crude extract by the gradual addition of solid ammonium sulphate with gentleand continuous overnight magnetic stirring to 80% saturation at a temperature of 4^0C.The protease pellet was collected by centrifugation at 10,000 rpm for 10 min and re-dissolved by bicarbonate buffer of pH 9.5 which should be1-2 times the volume of the precipitate, since that will be enough to reduce the salt concentration to well below the precipitation point of the proteins present [6].The collected pellet was kept in a refrigerator at a temperature of 4^0C until it was applied for dehairing.

c. Ultrafiltration (UF)

For this part of downstream processing, 8 liters of crude extract was taken and filtered through 0.2 μm pore size microfiltration membrane to remove the biomass that otherwise would clog the UF membrane. Then the extract was filtered and concentrated to about 20 timesby UF having a membrane of 10 kDa pore size. The filtrate was kept in a refrigerator at a temperature of 4^0C until it was applied for dehairing.

d. Spray drying

To get the protease in dry form, 5 liters of crude extract was taken and 20 % maltodextrinwas added into it to serve as a carrier. This preparation was processed in a spray drier and the powder was made into a paste using bicarbonate buffer of pH 9.5. The paste was kept in a refrigerator at a temperature of 4^0C until it was applied for dehairing.

2.2.6 Evaluations of the efficacy of the protease products of downstream processing

The enzyme prepared for use by ultrafiltration, spray drying and ammonium sulphate and crude enzyme were applied to pre-soaked raw cow hides and sheep skins to check hair removal efficiency by comparing against the conventional unhairing method.

a. Ammonium sulphate precipitate

As a rule of thumb, 3 to 4%offer of protease precipitate based on soaked hide or skin weight is accepted by most tanners for attaining virtually complete hair removal depending on the unit activity of the precipitate. So in exploring the efficacy of the precipitate in removing the hair, the upper limit i.e. 4% of the precipitate was taken. Then unit activity was measured andthe total activity which would serve as a base in determining the required quantities of protease products of other downstream processingwas determined. The protease precipitate was applied on the flesh side of soaked half piece of sheep skin and one side of cowhide. Then they were folded flesh to flesh and kept under cover for 5 hours. Hair loosening was rated every hour and finally hair removal efficiency was compared against the conventional lime-sulfide unhairing method.

b. Crude protease extract

For this, the unit activity of the crude extract was measured and the amount required was determined based on the weight of the material and the total activity of ammonium sulphate precipitate i.e. by dividing the total activity of protease precipitate by unit activity of the crude.Soaked half piece of sheep skin and one side of cow hide were soaked in protease crude for 5 hours. Hair loosening was rated every hour and finally hair removal efficiency was compared against the conventional lime-sulfide unhairing method.

a. Ultrafiltered concentrate

Similar to the case of the crude extract, first the unit activity of the protease filtrate was measured and the required amount was determined based on the total activity of ammonium sulphate precipitate. The filtrate was applied on the flesh side of soaked a half piece of sheep skin and one side of cow hide. Then they were folded flesh to flesh and kept under cover for 5 hours. Hair loosening was rated every hour and finally hair removal efficiency was compared against the conventional lime-sulfide unhairing method.

b. Spray dried formulation

The unit activity of the protease paste was measured and the required amount was determined in similar fashion to the other methods. The paste was applied on the flesh side of soakeda half piece of sheep skin and one side of cow hide. Then they were folded flesh to flesh and kept under cover for 5 hours. Hair loosening was rated every hour and finally hair removal efficiency was compared against the conventional lime-sulfide unhairing method.

3. Results and Discussion

3.1 Preparation of pre-inoculum at shake flask level

In preparing pre-inoculum for the study, a preserved *B. subtlis* was obtained from Department of Biotechnology, CLRI and sub-cultured on LB agar plate. Subsequently the isolated individual colonies that have shown large clear zone (i.e. large skim milk hydrolysis) were inoculated into and cultivated in two 100 ml standard shake flasks by taking 20ml LB medium in each of them. The cultivation of the culture was done at a measured pH of 5.0 (flask A) and 5.5(flask B) and a temperature of 30^0C.Protease activity was determined for the samples taken from each flask within 12 to 72 hours by Kuntz method of enzyme assay.

3.2 Characterization of the culture

By performing a range of experiments under applicable different operating conditions i.e. by varying medium constituents, pH, temperature and incubation time, optimum growth/production conditions have been established.

a. Effect of pH study

To accomplish this two trials or runs were carried out. For the case of the first trial, in each of five 250 ml shake flask 200 ml growth media was prepared and the pH of one of the flasks was measured to be 5.5 and the other fourwere adjusted to pH 6.0, 7.0, 8.0 and9.0 by flakes of sodium hydroxide. In similar fashion the effect of pH for the second trial was studied; the media of one of the flask was measured to be 6.5 and the rest were adjusted to pH of 7.0, 8.0, 9.0 and 10.0.

b. Temperature effect study

In doing this, 200 ml of standard media was taken in each of three 250 ml shake flasks for two replicates. The flasks were then sterilized, inoculated witha full loop of inoculum and incubated at temperatures of 25, 30 and 35 degree Celsius. The response for this independent variable effect was protease activity which was determined by Kunitz method of enzyme assay.

c. Media

The impact of standard or designed LB and soy bean flour media on the growth and protease production by *B.subtilis*was investigated.

d. Time

The effect of time can be observed from the studies of pH, temperature and media. Running the fermentation process up to the time range in which a higher and an increasing protease activity was observed (27 to 38 hours), could be logical and feasible.

e.The study of interaction effect of pH and temperature

In this part of the study, the relationship between controllable experimental factors(pH and temperature) and the response (protease production) was investigated by Response Surface Methodology (RSM), which is the most accepted statistical technique for bioprocess optimization.

3.3 Fermentation

a. Seed culture preparation

In going for pilot scale fermentation enough amount of inoculum was prepared. For this, in each of two 7 liters seed fermenters 4liters of production media of soya flour were prepared, pH was measured to be 6.0 and adjusted to 6.5 by 1N NaOH, sterilized, cooled to process temperature of 30 to 31^{0}Cand each fermenter was inoculated with 200 ml pre-inoculum and fermentation was undertaken for 24 hours.

b.The pilot scale Fermentation

The pilot plant provides the cultured broths needed for downstream processing and can generate information to determine the optimal cost structure in manufacturing and energy consumption as well as the testing of various raw materials in the medium.

3.4 Evaluations of the efficacy of the products of downstream processing

The enzyme formulations were prepared for use by ultrafiltration, spray drying and ammonium sulphate and crude enzyme. Then protease product of each downstream processing technique was applied to pre-soaked raw cow hides and sheep skins to check hair removal efficiency by comparing against the conventional unhairing method.

4. Conclusion

In an attempt mase in producing the dehairing protease as green technology option for the conventional lime-sulphide method of unhairing following the dictated methodology ,very encouraging results were obtained.The sub-culturing of the B.subtilis was done so effectively and it resulted in with good skim milk hydrolyses.The characterization studies of chemical and physical conditions in the cultvation of the culture revealed that an interesting optimization work was done as protease with promising activity was produced both at flask and pilot scale fermentations.The pH ranges of 6-8,temperature ranges of 30-32^{0}C,a media composition of soy bean flour and a fermentation period of 27-38 hours were found to be the optimal physical and chemical environments for the production of the enzyme. In addition, the existence of high degree interaction effects between pH and temperature on protease production was explored by RSM (Response Surface Methodology).

After successful production of protease at pilot scale fermentation,the partial purification of the broth was done by employing the primary downstream processing.In the downstream processings of crude enzyme formulation,ammonium sulphate precipetation,UF and spray drying,the difference in ease and simplicity techniques of processing and difference in the final protease product activity were observed.UF and spray drying are technicaly laborious and energy intensive and need higher capital investments than crude formulation and ammonium sulphate precipetation methods of downstream processings. The final protease product activities were 20 U/ml for crude extract, 360 U/ml for ammonium sulphate precipitate, 50 U/ml for Spray dried and 90 U/ml for UF.

Under the investigation of the efficacy of the protease products of the employed recovering and partial purification methods,use of the proease resulted in about 96% unhairing efficiency.Such a higher level of hair removal efficiency is a good indication for the protease products of each method in competing with the conventional chemical unhairing process with out taking other technical and economic factors into account.In this experiment the use of sodium sulphide was totally eliminated and the remaining hair was removed completely in the fibre opening up process of liming by 8 % offer of lime powder.

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STUDIES ON USE OF SODIUM POLY ACRYLATE (SPA) FOR LOW SALT ANIMAL SKIN PRESERVATION

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In this study, commercial sodium poly acrylate (SPA) is used along with sodium chloride for low salt skin preservation. SPA is a super-absorbent polymer which can absorb water many times of its own weight. The SPA used in this study was characterized using NMR, IR and its water absorption characteristics were determined. Fresh goat skins were taken for experimental and conventional preservation studies. Control skins were applied with 40% salt and kept under ambient condition. Preliminary studies were carried out on optimization of SPA contact time, quantity and amount of salt for preservation. The experimental skins were applied with 5% SPA on flesh side and kept for 4 hours for moisture removal and then, SPA was removed from the skin by gentle scrapping and taken for drying and reuse for subsequent batch of raw skins. The SPA recovery was found to be 95%. Then 15% salt was applied on experimental skins and both experimental and control skins were stored for 21 days. The skins were observed periodically for hair slip and foul smell, which are indications for onset of putrification and microbial growth as per the conventional method. After preservation period, both control and experimental skins were processed into chrome tanned leathers and tested for their strength and other properties. The results suggest that SPA aided moisture removal along with minimal salt has adequate curing efficiency similar to conventional salt preservation and has comparable physical and organoleptic properties with a substantial reduction in TDS and chlorides in effluent. This SPA aided low salt skin preservation if implemented on commercial scale pollution caused due to sodium chloride would be significantly minimized.

Keywords: Pollution reduction, Preservation, Raw skin, Sodium Poly Acrylate, TDS

1. Introduction

Animal skin, fibrous protein materials essentially consist of collagen fibers (Kanagaraj et al., 2004) apart from fat and sweat glands and other materials. They are more susceptible for bacterial attack (Birbir and Ilgaz, 1995) if not preserved properly leading to disintegration of skin matrix because of enzymatic degradation (Pankaj Kumar et al., 2012). Hence, it is important to preserve the skin from bacterial contamination (Covington, 2009) properly before converting into leather in tanneries. Conventional skin/hide preservation involves application of common salt to an extent of 30-70% by weight of raw hide/skin (Kanagaraj 2004). Skin preservation using salt is the most effective method which is in practice for decades because of its low cost. While processing the cured hides/skins into leather, mechanical de-salting and soaking steps are carried out during which process salt gets removed. Soaking of hides and skins generates large amount of Total Dissolved Solids (TDS) and chlorides in tannery effluent which is of serious environmental concern (Kanagaraj, 1999). In view of this, salt free or low salt skin preservation methods are attempted using eco-friendly chemicals or

bio-based compounds. Many of these methods have few advantages and have some constraints as well.

There are many alternate tried in the past such as irradiation (Ross 1997), electron beam (Bailey et al. 2001), cooling and chilling (Chandra Babu 2012), vacuum drying (Gudro et al. 2014) and chemical methods such as aryl alcohols (Water et al. 1997), potassium chloride (Bailey and Gosselin 1996), silica Gel (Kanagaraj et al. 2001), sulphites (Vankar and Dwovedi 2009), benzoic acid (Valeika et al. 2013) and crude glycerol with polyethylene glycol polymers (Aldema-Ramos et al. 2015). These methods are either expensive or potentially hazardous. Few environmentally-friendly method of phyto based preservation were attempted using different plant materials such as neem oil (Krishnamoorthy et al. 1982), and phytochemicals from *Aristolochia indica* (Vaghasiya and Chanda, 2007), *Wedelia chinensis*, *Cassia alatta*, *Clerodendrum phlomidis*, *Solanum trilobotum*, *Calotropis procera* (Sivabalan and Jayanthi, 2009), *Acalypha indica* (Vijayalakshmi et al., 2009) and deoiled-neem cake (Vedaraman 2016) for skin preservation however there are some problems in commercial exploitation.

2.Material and methods

Materials

Skin

Freshly flayed goat skin of average weight 1 kg was used for the study.

Chemicals

Sodium chloride (commercial grade), lime, sodium sulphide, ammonium chloride, formic acid, sulphuric acid, sodium bicarbonate, sodium formate, fat-liquors and syntans purchased from leather chemical manufacturers, Chennai, India. Sodium polyacrylate (SPA) of industrial purchased from large scale trader, Chennai, India.

Methods

SPA Characterization

To characterize the SPA used, IR, NMR techniques were used. The equilibrium water uptake (EWU) was also measured as per the method given below.

The super absorbent polymer was studied for its water retention capacity. A known weight of SPA was taken in a beaker and large excess of distilled water was added, till water is free flowing. The SPA was allowed to swell to equilibrium for 24 hrs. The excess water was removed by filtration under slight vacuum. The EWU of SPA was measured using the following equation:

$$\mathrm{EWU} = \frac{Ws - Wd}{Wd}$$

W_s : weight of equilibrium swollen SPA; W_d: weight of dried SPA

NMR analysis

Nuclear Magnetic Resonance spectroscopy study of SPA was done in D_2O as solvent. The SPA sample was prepared by addition of small amount of NaCl so as to make SPA solubilise in D_2O. A proton spectrum was recorded on a 200 MHz Brucker instrument.

Infra red spectroscopy

The infrared spectroscopic analysis was done in the diffused reflectance mode in Perkin-Elmer IR-instrument.

Method

Freshly flayed goat skin brought from a local slaughter house was cut into equal sizes, weighed and noted as green weight. The experimental skins were washed to remove dirt and blood stains. The washed skins were allowed to drain the excess amount of water for 1h in a slanting position and then weighed.

Preliminary studies

Optimization of SPA-skin contact time

Five experimental goat pieces was taken for this experiment. To fix the contacting time required for the SPA to absorb the maximum moisture from the skin without affecting the skin for subsequent processing. 5% of SPA based on the green weight of the skin was applied thoroughly on the flesh side of different skins. SPA was recovered after 1, 2, 3, 4, and 5 hours from the corresponding skins and from increase in weight of SPA the moisture absorbed was calculated.

Optimization of SPA quantity

After finalizing the contact time required through earlier experiment, and to fix the amount of SPA required for maximum moisture removal, different quantity of SPA (2, 5, 8 %) were applied on the flesh side of the goat skinspieces.

Optimization of salt quantity

After estimating the contact time and amount of SPA required for moisture removal, to fix the minimal amount of salt required for the experiment to avoid putrefaction the following experiments was conducted. Varying amount of salt i.e. 12%, 15%, 18% salt by weight was applied on the flesh side of skin after moisture removal with specific quantity of SPA for fixed time period. The salt applied skins were stored for 21 days to evaluate the efficacy of this preservation method. The purtification onset parameters such as hair slip, bad odor were noted during this storage period.

After the above preliminary experiments freshly flayed five goat skins were taken for this experiment. One skin was cut into two halves. The left half was taken for the SPA curing and the right half's was taken for conventional salt curing, which serve as control for comparison. With other four full skins two skins were marked for control experiments and two skins for SPA aided low salt curing experiments. All experimental skins (two full skins and one left half skin) were first applied with specific quantity of SPA based on preliminary experiment and stored for specific duration. Then optimized salt quantity was applied and for control experiments two and half skins were applied with 40% salt. The skins were piled and stored at the ambient temperature of 32-35°C. The skins were

monitored periodically for physical changes like odor and hair slip, which are indications for putrefaction till 21st day of curing.

Determination of moisture content in SPA

The moisture content of the SPA was determined based on the total moisture absorbed by the SPA after the experiment. The mass of the SPA before the application and the mass of the SPA after the application was noted to determine the percentage of moisture absorbed by the SPA from the goat skin.

Determination of bacterial load

Samples (5 g) were cut from the preserved skins during the experimental period and determined for the bacterial growth. The samples were soaked in sterile distilled water, ten times of its weight and kept in orbital shaker for 30 min. The soak liquor was serial diluted till 10^{-3} dilution using sterile normal saline. From the serial dilution, 1 ml was taken which was poured and spread on a sterile nutrient agar plate, incubated for 24 h at 25°C. The number of colonies appearing on the plate was counted in CFU/ml.

Leather processing

After the storage period of 21 days, the cured skins were processed into leather by conventional chrome tanning process as given in Annexture 1.

Pollution load generated in soaking process

The experimental and the control skins were soaked before processing into leather. The soak liquor was collected and analyzed for Total Dissolved Solids (TDS) and chlorides using standard procedures (Eaton et al. 1995).

Physical strength of leather

The preserved skins were processed into white crust leather and tested for physical strength properties. After conditioning the crust leather at 20$\pm$2^{o}C and 65$\pm$2% of relative humidity over a period of 48 h, the properties such as tensile strength, tear strength and lastometer strength load were assessed in comparison of experimental skins with conventional salted cured leathers.

Organo-leptic properties.

Organo-leptic properties of white crust leathers were evaluated by three leathers technologists.

3. Results and Discussion

EWU was found to be 148g/g of SPA which shows SPA can absorb significant quantity of water.

NMR results

The spectra is as given in Figure-1 The peaks at 1.54 ppm is for the CH_2 protons and the peak at 2.14 ppm is for the CH protons of both acrylic acid and acrylamide
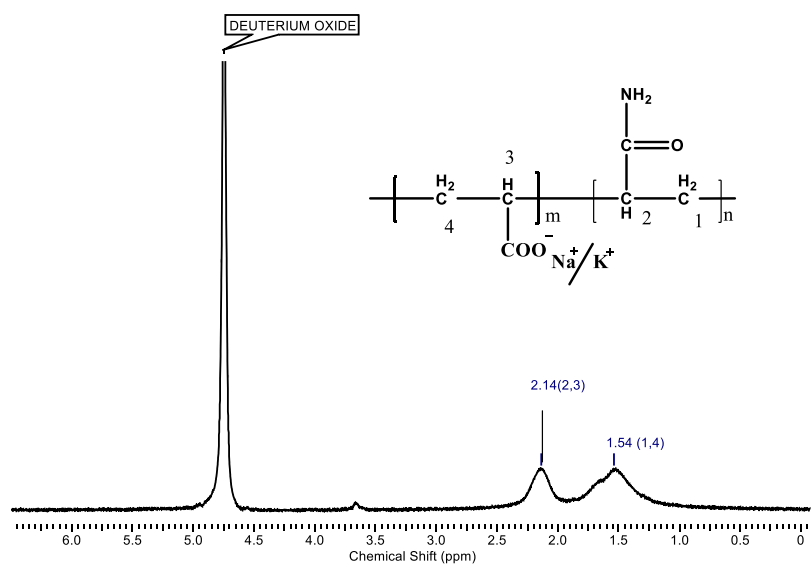
Fig-1 Proton NMR of SPA
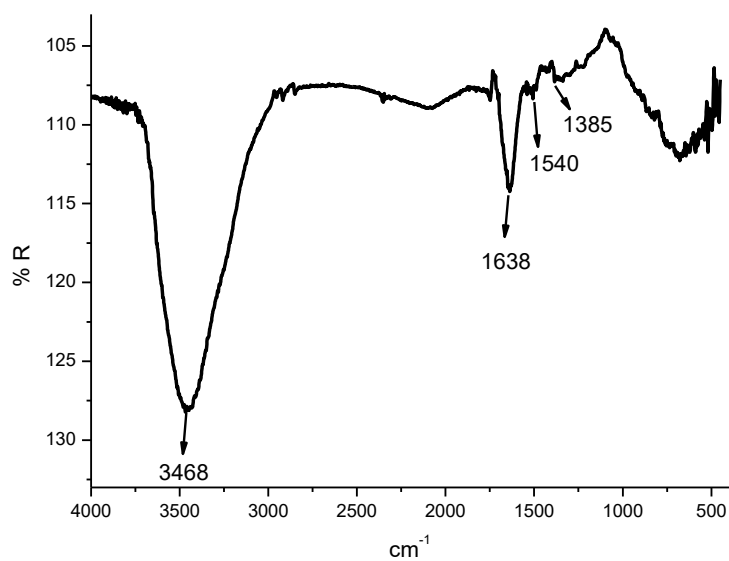
The IR spectra is shown in figure-2

Figure-2 IR-spectra of SPA

The peak at 3468 and 1638 Cm^{-1} is for the C=O and peak N-H stretching of amide in acrylamide and peaks at 1540 and 1385 Cm^{-1} are for the acrylic acid carboxylate stretching.

The preliminary studies on moisture absorption of SPA up to five hours when in contact with raw skin as shown in Table 1 reveals the following. As the contact time increases the amount of water absorbed increases from one hour to four hours and there is a marginal increase at the end of 5 hour. Hence the contact time was fixed as 4 hours for further experiments.

Table-1.Effect of SPA contact time on moisture removal from raw skin.

Particulars	SPA contact time on skin (h)				
	1	2	3	4	5
Moisture absorbed from skin (%)	121	231	255	261	263

Three levels of SPA (2, 5, 8 %) was taken for this study and the results are shown in Table 2. As the amount of SPA increases from 2% to 5% the moisture absorbed increases but a further increase in SPA quantity to 8% the increase is not high. Hence the amount of SPA was fixed as 5% for subsequent experiments.

Table-2.Effect of quantity of SPA on moisture removal from raw skin.

Particulars	Amount of SPA used (%)		
	2	5	8
Moisture absorbed from skin (%)	113	218	248
Cured skin curing characteristics Hair slip, putrefaction odour	Present	Absent	Absent

From Table 3 it is evident that curing with 15 % salt with 5% and 8 % SPA respectively showed curing properties for 21 days. Goat skins preserved with less amount of SPA (2 %) showed hair slip, putrefaction odour and got decayed. Therefore 5% of SPA with 15 % salt with contact time of 4 h is the optimized condition for curing experiment. Water is an important factor for bacterial growth. 65-70 % of moisture in the skin support the favourable condition for the growth of bacteria. Reduction in this moisture content using SPA reduces the moisture level thereby creating a favourable environment and the salt applied resisting the growth of bacteria for a successful curing process.

The amount of salt required for animal skin preservation was studied at 10%, 15%, 20% level and the results are shown in Table 3.

Table-3.Effect of salt quantity on skin preservation and SPA application.

Particulars	Amount of salt used for skin preservation		
	10%	15%	20%
Amount of moisture absorbed by 5% SPA applied (%)	225	231	235
Duration of skin storage (days), and Skin condition	10, further storage leads to purtification	21, good	21, good

In all the above three cases 5% of SPA was applied and the moisture removal was almost same after 4 hours. When salt is applied at different quantities 10%, 15%, 20% after 4 hours and kept for storage between32-35°C, the 10% salt cured skins starts decaying at the end of 10th day hence discarded. The skins preserved with 15% salt and 20% salt were good at the end of 21st day.

To confirm the SPA aided low salt skin preservation process experiments were conducted with five full skins (two and half skin for experiment and two and half for control) as follows. Control skins

were applied with 40% salt and experimental skins were treated with 5% SPA for 4 hours then the SPA was removed totally. Then 15% salt was applied on the skin and preserved for 21 days.The details of the bacterial count obtained during the preservation period are mentioned in the Table 4.

Table – 4. Bacterial counts on 7th and 14th day of preserved goat skins

% of salt used	% of SPA used	Bacterial count of skins (CFU/ml) 10^{-3}	
		14th day	21th day
40	--	4	NG
15	5	6	NG

NG- No growth, Average value of two experiments

The bacterial load was estimated for the conventional salt cured skin and 5 % SPA + 15 % salt and tabulated in Table 4. There was no growth of bacterial colonies observed on the 21th day, this shows that 5 % SPA + 15 % salt method of goat skin preservation was effective.   Thus showing the curing process was satisfactory. The SPA recovered was dried and the recovery was 95%.

The cured skins were soaked and the collected liquor was tested for Pollution load such as TDS and chlorides are given in Table 5. The tabulated values show that there is 67.5% reduction in TDS and 71.3% chlorides value.

Table – 5. Pollution load generated in the soak liquor of preserved skins.

Amount of SPA used%	% of salt used	Pollution load (mg/l)	
		TDS	Chlorides
--	40	280	192
5	15	91	55

Average value of two experiments

The experimental skins were processed into white crust leather. The white crust leather was tested for their physical strength such as tear strength and tensile strength are given in Table 6.The experimental leather and conventional leather showed similar strength properties.

Table –6. Physical properties of leather

Amount of SPA used	% of salt used	Tensile strength (N/mm^2)	Tear strength (N)	Lastometer Load (kg), Distance (mm)
--	40	23.85	48.26	38, 10.66
5	15	21.20	43.64	36, 9.91

Average value of two experiments

Table 7 shows experts evaluation of the organo-leptic properties of leathers produced from SPA aided low salt skin preservation along with conventional salt preservation. The experimental leathers have almost similar properties with respect to grain smoothness, fullness and softness.

Table-7.Comparision of organo-leptic properties of SPA aided low salt skin preservation in with conventional salt preservation

White crust leathers	Grain smoothness	Fullness	Softness
Control	8	9	8
Experimental	7	8	8

Average value of the evaluation of three experts

Conclusion

As an initiative for raw goat skin preservation, an attempt was made to use SPA primarily as dewatering agent along with reduced amount of salt was tried and compared to conventional salting process. The efficacy of this SPA aided low salt skin preservation was checked by analysing the bacterial count of the preserved skin, physical tests and organoleptic properties of the processed leathers. The results reveal that the SPA aided low salt skin preservation has produced leathers with similar characteristics to conventional salt preserved leathers. TDS, chlorides levels in the soak liquor effluent significantly low compared to conventional method. This study shows that 5% SPA dewatered and preserved with15% of salt effectively preserved the skin for a minimum period of 21 days.

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Annexture – 1. Process description for processing cured goat skins into leather

Process	MaterialQuantity	Duration	Remarks
Soaking I	Water-300 % Wetting agent-0.5 %	30min	Float was drained and aliquot was tested for TDS
Soaking II	Water-300%Preservative-0.1%Wetting agent-0.5%	Left overnight	Float was drained and aliquot was tested for TDS
Next day washing			
Liming	Water 25 % Lime 10 % Sodium sulfide 3 %Wetting agent 0.5 %	Left overnight	Paint prepared and applied on flesh side.
Unhairing			
Reliming	Water 200 % Lime 5 %	Left for 2 days	
Fleshing			The limed pelts were fleshed and taken for washing
Washing	Water200 %	10 min	
Deliming	Water150% Ammonium chloride 1 % Bating agent 1 %	Run for 1 h	Washed and drained pH 8-8.5
Washing		10 min	
Pickling	Water 80 % Sodium chloride 8 %Formic acid 1 % Sulphuric acid 0.5 %	Run for 15 min 3 x 10 min, Run for 10 min 4 x 15 min, Run for 1 h	pH 2.8-3
Next day the pelts drummed for 30 min pH at cross section adjusted to 2.8- 3.0. Then 50% of pickle bath drained			
Chrome tanning	Basic chromium sulphate (BCS)8 %Sodium formate 0.5 % Sodium bicarbonate 1%	2 x 30 min 10 min 3 x 20 min, run for 1 h	Check for penetration in cross section. Check the pH to be 3.8 to 4. Drain the bath and pile overnight.
Next day sammed and shaved to 1.0 mm. The shaved weight noted.			
Washing	Water200 %	10 min	Drain
Neutralization	Water150 % Sodium formate 0.5 % + Sodium bicarbonate	10 min 3 x 10 min + 1 h	pH 5-5.5

	0.5%		
Washing	Water 100 %	10 min.	Drain
Dying and Fatliquoring	Water 100 %	40 min.	pH 3.5
	Fatliquor 10 %	1 h	Drain /
	Syntan 8 %	45 min	wash/Drain
	Formic acid 1 %	3 x 5 min + 40 min	
Pile over night. Next day hook to dry.			

POLYACRYLATE/HOLLOW ZINC OXIDE COMPOSITE EMULSION AND ITS APPLICATION IN LEATHER FINISHING

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Polyacrylate/hollow ZnO composite emulsion were prepared by physical blending of polyacrylate emulsion and hollow ZnO. The appearance, dilution stability, centrifugal stability and chemistry stability of as-obtained polyacrylate/hollow ZnO composite emulsion were measured. At last, the composite emulsion was applied in leather finishing. The results showed that polyacrylate/hollow ZnO composite emulsion has a clear bluish tinge and exhibits excellent dilution, centrifugal and chemistry stability. The comprehensive performance of the leather samples sprayed with polyacrylate/hollow ZnO composite emulsion is superior to that with pure polyacrylate emulsion. Among them, the tensile strength, water vapor permeability and air permeability are increased by 103.33%, 27.17% and 50.46%, respectively. It is mainly interpreted by the fact that the introduction of hollow ZnO into polyacrylate matrix would significantly increase the free volume in the polyacrylate film and generate interfacial pores between ZnO microstructures and polymer chains, which provide many channels for transmission of water vapor and air.

Keyword: Polyacrylate; Hollow ZnO; Leather finishing; water vapor permeability; air permeability

1 Introduction

Polyacrylate is an excellent film-forming material with superior cohesiveness, flexibility, low-cost, transparent, brightness and many potential outstanding performances, that is widely used in leather finishing, coating fabrics, textile adhesive and so on [1]. However, when dense polyacrylate emulsion formed on the coatings for leather or textile finishing, the poor hygienic properties (air permeability and water vapor permeability) affect significantly the wearing comfort of their products, and often restrict its applications. It can be caused by blocking the water vapor and air molecular diffusion channels to the outside. Therefore, a large number of efforts based on designing and modifying of polyacrylate film to fabricate the high-performance polymeric film have been investigated, including modification of epoxy resin, polyurethane, organic silicone/ fluorine, and inorganic nano-materials principally et al [2-3].

Recently, organic-inorganic composite systems have attracted considerable attention due to their unique functional behavior arising form the synergy between polymeric and inorganic phase as well as their interfaces promising extensive potential applications in various fields of material science [4]. There are several studies reporting on the synthesis of polymer/inorganic composites with different inorganic materials, such as SiO_2, TiO_2, and ZnO [5]. Among all inorganic materials, ZnO exhibits considerable chemical and physical properties, high stability, excellent antibacterial and environmental friendliness behaviour due to its direct wide band gap of 3.37 eV and large exaction

binding energy of 60 meV at room temperature [6]. Hollow ZnO, which have special hollow structure, low density, large specific surface area, good permeability and high amounts of hydroxyl groups on the surface, are promising inorganic fillers in improving the comprehensive performances of polyacrylate film.

In this paper, Polyacrylate/hollow ZnO composite emulsion is obtained by physical blending of polyacrylate emulsion and hollow ZnO. The results demonstrated that the introduction of hollow ZnO can significantly improve the water vapor permeability, air permeability, water resistance and mechanical properties of polyacrylate film. It is mainly interpreted by the fact that the introduction of hollow ZnO into polyacrylate matrix would significantly increase the free volume in the polyacrylate film and generate interfacial pores between ZnO microstructures and polymer chains, which provide many channels for transmission of water vapor and air.

2 Chemicals and Methods

2.1. Chemicals

All chemical reagents were of analytical grade without any further purification. Deionized water was used throughout this study.

2.2 Preparation of polyacrylate/hollow ZnO composite film

Polyacrylate latex of core-shell structure was synthesized by ourselves via a seeded emulsion polymerization technique. Polyacrylate/hollow ZnO composite emulsion was prepared by physical blending method. To obtain homogeneous and stable hollow ZnO suspension, self-regulating hollow ZnO microspheres (0.06 g) were slowly added into deionized water (30 g) in a 50 mL beaker using an ultrasoniccelldisruptor. The resulting suspension was then transferred into three-necked flask with refluxing device under 300 rpm at 80℃ for 5 h, and also 40 g polyacrylate latex was added to it. The resulting composite (35 g) was coated on polyfluortetraethylene plate with the side length of 130 mm, then it allowed to stand overnight to remove water vapor and any trapped solvent for natural exposures. Uniform polyacrylate/hollow ZnO composite films were obtained at room temperature. The polymer film without hollow ZnO microspheres was taken as the control to compare the effect of filler addition on the physical and chemical properties of polyacrylate. The amounts of hollow ZnO were varied from 0% to 1.5%.

3 Results and Discussion

The content of hollow ZnO is an important factor influencing the comprehensive performance of polyacrylate/ hollow ZnO composite films, such as water vapor permeability, water uptake, tensile strength, and elongation at break. The effect of the hollow ZnO content on water vapor permeability of composite film is shown Figure 1. Compared with pure polyacrylate film, the water vapor permeability of the polyacrylate composite film prepared by different dosage of hollow ZnO, is improved to some extent. This is explained by the fact that the introduction of hollow ZnO into polyacrylate matrix increased the free volume in the composite film and interfacial pores between hollow ZnO and polymer chains. It is worth noting that the water vapor permeability of composite film is optimal with the 0.25 wt% dosage of hollow ZnO relatively. However, when the content of hollow ZnO is more than 0.25 wt%, the water vapor permeability of composite film is decreased graually. That could be assigned to the agglomeration of hollow ZnO occurs at a higher content,

which prevents the transportation of water vapor molecules of composite film. As expected, It become apparent that the water vapor permeability is relatively increased by 27.17%.
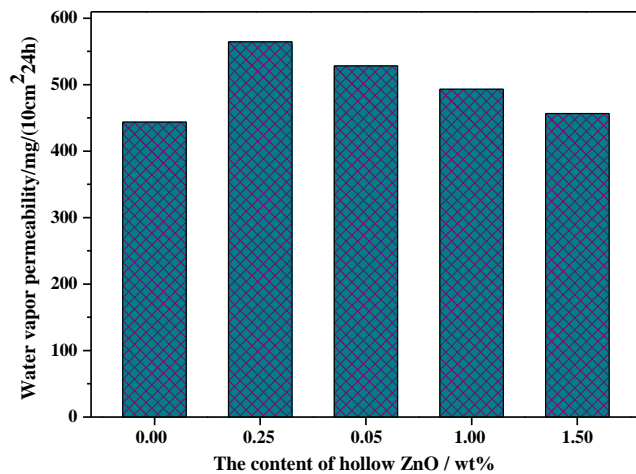

Figure 1. Effect of hollow ZnO content on water vapor permeability
of polyacrylate/ hollow ZnO composite film.

Figure 2 indicates that the air permeability of polyacrylate/hollow ZnO composite film were much higher than that of pure polyacrylate film. When the content of hollow ZnO amount is about 0.25 wt%, the air permeability of composite film is optimal, and it was relatively increased by 50.46%. This is explained by the fact that the introduction of hollow ZnO into polyacrylate would significantly increase the free volume in the composite film and interfacial pores between hollow ZnO and polymer chains. However, when the content of hollowZnO spheres was more than 1.0 wt%, the air permeability of composite film decreased slightly.
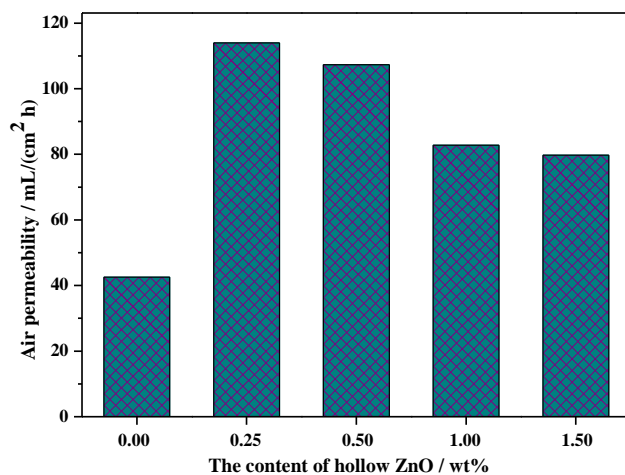
Figure 2. Effect of hollow ZnO content on air permeability of
polyacrylate/ hollow ZnO composite film.

The effect of hollow ZnO content on water uptake of composite films is shown in Figure 3. The water uptake of the composite film increases significantly when 0.25 wt% dosage of hollow ZnO are introduced into polyacrylate. It is attributed to hollow ZnO of 0.25 wt% integrated with polyacrylate chains more tightly via hydrogen-bond interaction and van der waals force, which reduces the number of hydrophilic groups of polyacrylate matrix. However, when the content of hollow ZnO is more than 1.0 wt%, the water uptake of composite film is decreased dramatically.
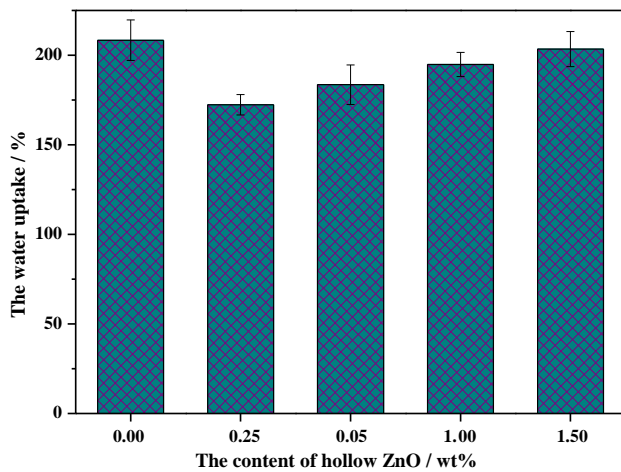
Figure 3. Effect of hollow ZnO content on water uptake of polyacrylate/ hollow ZnO composite film.

Figure 4 illustrates the effect of hollow ZnO content on tensile strength, elongation at break, bursting strength and tearing strength of composite film. Compared with pure polyacrylate film, the tensile strength and elongation at break of the polyacrylate composite film prepared by different dosage of hollow ZnO (Figure 4a), is improved to some extent. However, when the content of hollow ZnO is about 1.0 wt%, the elongation at break of composite film is decreased dramatically. As shown in Figure 4b, the bursting strength and tearing strength of the polyacrylate composite film prepared by different dosage of hollow ZnO, is improved significantly.
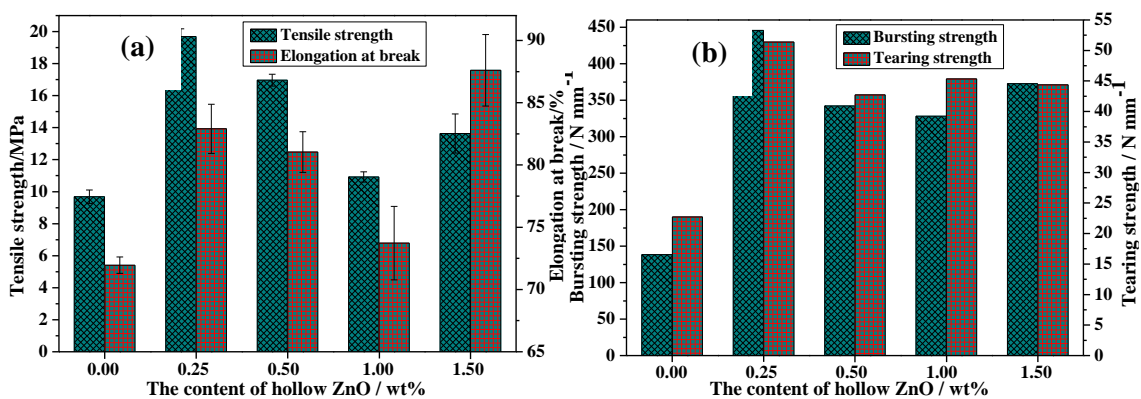

Figure 4. Effect of hollow ZnO content on
(a) tensile strength and elongation at break; (b) bursting strength and tearing strength
of polyacrylate/ hollow ZnO composite film.

4 Conclusion

Polyacrylate/hollow ZnO composite emulsion were prepared by physical blending of polyacrylate emulsion and hollow ZnO. The comprehensive performance of the leather samples sprayed with polyacrylate/hollow ZnO composite emulsion is superior to that with pure polyacrylate emulsion. Among them, the tensile strength, water vapor permeability and air permeability are increased by 103.33%, 27.17% and 50.46%, respectively.

5 Acknowledgements

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UNCERTAINTY AND SENSITIVITY IN THE CARBON FOOTPRINT OF LEATHER PRODUCT

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Carbon Footprint (CF) was used to evaluate potential environmental impacts of leather products must be taken into account considering its whole life cycle. In this paper, the bag made of leather was take as case study, uncertainty and sensitivity in the carbon footprint of leather product was given the major concern on to analyze. There were many parameters and scenarios used in the whole life cycle of leather products. It was concluded that the key of leather product CF is the emission factor of main surface material (leather), and leather thickness could not be ignored. Energy consumption scenario may be the second ranked supplier of the final value of CF, because that the CO_2 equivalent (kgCO2e) varied with energy material. At the meantime, the choice of waste treatment scenario had a greater effect on the ranking of leather product than scenarios uncertainty in all stages. Therefore, in the early stages of ecological design of leather products and ecological control of industrial clusters, the impacts of various materials and stages on the environment should be considered and balanced to accomplish a fairest evaluation.

Key words: leather product, Carbon Footprint, Uncertainty

1. Introduction

Leather product as consumer goods can be seen around us everyday. With the development of leather technology, it has been deeply welcomed by consumers owing to its unique nature property and irreplaceable conformability. China as leather products manufacturing powerhouse plays a decisive role in the world, which has more than 20,000 bag production enterprises mainly concentrated in the eight industrial clusters as the representative to Shiling town (CLIA, 2015). Their production has accounted for more than 70% of the global output (CIR, 2016). China is officially promoting the industrial system geared toward green, low-carbon and circular development, and the local government is responsible for the Environmental protection. So, the environmental pressure of local government is to be reckoned with. At the meantime, China is big exporter, which exported 4.1 billion bags in 2015. And it mainly exported to USA, EC countries and Asia. A survey conducted by Eurobarometer presented that a product's environmental impact and energy-efficient are perceived as being more important in purchasing decisions by slim majority respondents (EC, 2009).Therefore,

environmental-friendly property has become a key indicator to assess an industry and a product, which is the trend of the market an marking healthy concept.

Ecodesign is an approach to design of a product with special consideration for the environmental impacts of the product at all stages of the product life cycle, starting at the designing and development phases. The objective is to create sustainable producing and consuming system (Karlsson, 2006). The identification and appraisal of the environmental burdens require the application of evaluation tools. The different available indicators offer complementary visions of the studied scenario; therefore, they cannot be replaced by each other and, in most cases, more than one should be applied at a time (Byggeth, 2006). In this work, an assessment methodology, given the major concern on carbon footprint (CF), was used for quantitative analyzing the ecological property of leather products on the basis of Life Cycle Assessment (LCA). The CF accounted for the total sets of greenhouse gas emissions caused by leather products. So it could support product promotion and green purchasing, also be quantitative basis on the product analysis and the industrial cluster ecological control scheme.

At present, a growing number of industries and enterprises is to assess and certify CF, such as agricultural product (Matheswarappa, 2011), food (Jensen, 2014), chemical industries (Sharma, 2012) and so on(Wang, 2015). However, due to the universal guidance, the diversity and complexity of manufacturing and other characteristics, several issues need to be discussed when calculating the industrial carbon footprint, including the issues that are carbon emission factors and calculation methodology of energy and materials are not unified and the impact assessment is absent (Li, 2014). So does leather. The boundary defined of leather CF is discussion in leather industry all the time, the primary focus on the livestock raising in the upper phase. There are more specific works about the CF or carbon label of the tannery (Rivela, 2004)), finished leather (SGS, 2014) and footwear (Mila, 1998) on the basis of LCA was investigated by some experts from German, Indian and Spain, etc.. Because the diversity of research perspective, specific products, boundary defined and carbon emission factors, their result was different. Thus, in this study we try to identify the most problematic phases of a leather product, such as leather bag, from an environmental point of view, in order to determine in which one an important would more efficiently reduce the associated environmental impact.

2. Materials and methods

First, the case study is described, as well as the steps followed for inventory data collection associated to life cycle assessment. Then, the section introduce CF applied in the analysis. The life cycle of leather product is defined to design, purchasing of raw materials, manufacture, package, use and disposal.

2.1 case study

The bags made of leather, fastest growth rate of leather products, were taken as case study to be evaluated. 1000 leather bags (specification 200mm-400mm, volume 2L-9L), include 800 medium size of women's handbags and 200 briefcases, are sampling from bag manufacturers in shilling town, Chinese capital of leather products according to retail sale statistics.

The manufacture is the main influence process in life cycle of leather product. A wide number of operations is required for making a bag and they are generally performed by a separate machine.

After the design is finished, the first stage of the bagmaking process is the pattern-making. This operation is directly concerned whether can make bag well, each component match or not. Only if the pattern would fit the bill, the bag could be taken to cutting of pieces. This operation needs a high level of skill, especially when the material is leather, to minimize the generation of waste and to avoid the likely defects on the surface that cannot be part of the bag. Gluing and oiling edge are to decide amounts of glue and edge paint. The combination of raw materials, such as sewing and binding, determines the power consumption. And package is the main source of solid waste before using.

2.2 Inventory data collection

The principles of data collection and data quality control are as follows: ① data collection based on the full lifecycle model. ②CF calculation based on the description and data of raw materials in the production process and finished products. All carbon factors are taken from Ecoinvent 2.0 database, official figures, references and background database of some relevant product in multinational company. ③ For the process inside the system boundary, foreground data for representing actual production scene, which provided by factories, is taken precedence. ④ the key process is identified according to the contribution, in addition, the contribution was less than 1% was ruled out of the system boundary.

The type, content and amount of main materials in the manufacture of the leather bag, were provided by factory to calculate CF. the choice of carbon emission coefficients were based on type and content of materials. Further, their transformation into mass units (or square) was required for the application of CF. Given that samples of the entire bag were provided, they were weighted and measured directly. For the main parts of the leather bag, the bagmaking factory provided average consumption values of materials for the same size of bag, as well as included wastage in the manufacture. These data were expressed in square (or length), units usually handled in the leather product industry. To convert these consumption values into mass units it was necessary to determine the apparent density of the materials. Thus, the mass of samples with known dimensions was determined in the laboratory using a precision balance and then the apparent densities were estimated in compliance with ISO 2420-2002 Leather - Physical and mechanical tests - Determination of apparent density. For special elements like hardware, the mass of a single piece was measured and then multiplied by the number of pieces in the leather bag.

The carbon emission in the process of production is taken from electricity consumption, as well as the solid waste produced. Bagmaking factories provided average consumption values of electricity for different kinds of bag, include wastage in the manufacture. Solid waste is main surface material, lining, hardware and reinforcement. These data were expressed in percentage usually handled in bag manufacturer. It was necessary to convert these consumption values into mass units.

The carbon emission in the process of transport is not considered, because that transport where many different hypotheses can be made. All the trade was done between factories in China, not including importations nor exportations of final or intermediate products. It can represent a big difference to consider only road transport. Again, the manifold possibilities make it difficult to choose a standard.

In most cases there are not CO_2 emissions in the usage phase. But a lot of packaging materials were junked by consumers before usage, which lead to the carbon emission. These data was based on the data in the manufacture stage.

The end of bag was disposed. Leather bag is generally treated thermally along with household garbage as city solid wastes in the disposal stage, so it was calculated in accordance with city solid wastes on mass site of entire bag. So primary process data were collected from the Chinese manufacturers.

2.3 Carbon footprint

As defined by ISO/TS14067:2013, carbon footprint of a product is "sum of greenhouse gas emissions and removals throughout the life cycle of a product(i.e. cradle-to-grave) from raw material acquisition through production, use and end-of-life treatment", expressed as CO_2 equivalent (kgCO_2e).

The parameters for CF uncertainty analysis were chosen on the basis of two criteria: the calculated variability and the observed uncertainty during the life cycle inventory (LCI) collection (Mattila, 2011).

3 Results and discussion

3.1 Inventory parameter

The inventory parameter ranges (based on a functional unit of a leather bag) in purchasing of raw materials, manufacturer, usage and disposal stages regarding the consumption of materials were presented to estimate CF and uncertainty in Tables 1,Tables 2 andTables 3. A lognormal distribution was selected for all parameters from 1000 leather bags chosen, because it remains always positive and fits skewed and highly variable experimental data well. In addition, parameters for lognormal uncertainty distributions are readily available from some LCI databases. We parameterized the lognormal distributions by using observed or estimated minimum and maximum valued as the upper and lower ends of the 95 confidence interval.

Table1 Inventory parameter ranges to estimate the CF of raw material and uncertainty

Element	Material	Consumption [a] /m^2	Thickness [b] /cm	Apparent density [b] /g/ cm^3	Mass [b] /g
Surface material	Bovine leather	0.59-1.18	0.12-0.16	0.55-0.70	560-1190
Lining	Polyester	0.47-0.94	0.012-0.017	0.6-0.7	66-133
Reinforcement	Scrap rubber	0.022-0.037	0.15-0.31	0.1-0.25	10.5-31.5
ent	Tissue paper	----	-----	----	200-500
Package material					
Hardware	Material	Consumption [a]	Weight [b] /g/ piece	Mass [b] /g	

		/No. pieces		
Zipper	Zinc alloy	2	3-8	6-16
Buckle	Zinc alloy	2-4	8-13	16-52
Rivet	Zinc alloy	4	1-3	4-12
Chemical material	Material	Mass[a]/g		
Edge paint	Mixed polyurethane with acrylic resin (7:3)	400-600		
Glue	(water-solubility, Polyvinyl alcohol, plasticizer and etc.	150-300		

[a] Provided by factory. [b] Experimentally measured and calculated.

Table2 Inventory parameter ranges to estimate the CF and uncertainty in the manufacture

Variable	Material	Range(Uncertainty)
electricity	Coal, etc.	0.6 kwh -1 kwh [a]
Weight of waste leather	Bovine leather	120g-340g [b] (30%-40%) [a]
Weight of waste textile	Polyester	4.8g-12g [b] (8%-10%) [a]
Weight of waste reinforcement	Scrap rubber	0.3g-1.5g [b] (3%-5%) [a]

[a] Provided by factory. [b] Experimentally measured and calculated.

Table 3 Table2 Inventory parameter ranges to estimate the CF and uncertainty in the usage and disposal stages

Variable	Material	Range(Uncertainty) [b]
Weight of waste package	Tissue paper	200g-500g
Weight of waste bag	Leather, textile and metal hardware	700g-1000g
		-----
	Leather	400g-850g
	Textile	60g-120g
	hardware	30g-80g

[a] Provided by factory. [b] Experimentally measured and calculated.

3.2 CF results

3.2.1 CF of raw material

The CF of raw materials is the sum of each material CF, which the weight of each material multiplied by its carbon emission coefficient. Bovine leather, polyester and tissue paper accounted for the largest proportion in these raw materials in Table1.

The carbon emission coefficient of each material is up to its production attribute. Because their property of chemical synthesis, the carbon emission coefficients of reinforcement, glue and edge paint are lower. And their dosage are not enough high. It was concluded that their contribution was less than 1% compared to the other compounds.

The CF boundary defined of bovine leather as biomass material, is argued all the time, the primary focus on the livestock raising in the upper phase. Livestock raising represented the greater CF and EF, which would produce more CO_2, and require the land area to feed. This is also a reason why natural materials obtain higher CF than synthetic materials (Cherrett, 2005). Co-products have economic value and would be allocated according to the PAS2050:2011, skin accounts for a part of earnings in whole cattle. Some enterprises, certification institutes and research institutes thought that leather should be beard a part of carbon emissions of animal farming, estimating that about 6%-9%of a cow is destined to produce leather (Mila, 2011 and SGS, 2014). But some industry institutes considered that cattle are primary produce for milk or meat production but not specifically for leather production, the hide is considered as by-product. So agriculture and animal farming for all tanning production should be excluded from system boundaries (Helton, 2006 and Mattila, 2011).The system boundaries of leather CF are considered to be starting in the Slaughterhouse, where activities and treatments are carried out in order to prepare the hides to be used for tanning (e.g.: conservation of the hides and skins by way of cooling systems or salting) by the UNIDO Leather and Leather Products Industry Panel in sep.2012. In this case, it was chosen as carbon emission coefficient of leather that 56.83 kgco$_2$eq/ square meter bovine leather (1.2-1.4mm), which was based on the CF data of leather (PrimeAsiaComfort E1.2-1.4mm) manufactured by PrimeAsia leather corporation in China (PrimeAsia, 2012) from agriculture to the finished leather. It is calculated that the CF of surface material (bovine leather) is 33.53kgco$_2$-67.06 kgco$_2$.

The carbon emission coefficient of textile (polyester) is less than bovine leather. There is not clear used square discrepancy between textile and bovine leather. But polyester is thin, as a result that its weight is lighter. It is calculated that the CF of lining (polyester) is 1.70kgco$_2$-3.42 kgco$_2$.

The CF boundary defined of packaging materials (tissue paper) covers plants growing (Ma, 2012).carbon storage in tissue paper could offset some carbon emissions in its production. Therefore, carbon emission coefficient of packaging materials is not high. It is calculated that the CF of packaging materials (tissue paper) is 0.56kgco$_2$-1.4 kgco$_2$.

3.2.2 CF in manufacturer

（1）Electricity

Electricity was the only source of energy used in bagmaking factory, consequently, effluent and exhaust gas (GHG) were not released in the manufacturing process of leather product.

The power consumption in table 2 was used in the manufacture and office, exclude dormitory. There is controversial whether dormitory electricity was calculated during CF assessment of factory. The electricity consumption depended on the technique, staff size and workshop facilities, etc. the frequency of large machine and ventilation facilities need spend more electricity. However, the staff size grew in inverse proportion to the power consumption.

The energy ratio of countries has great influence on the carbon emission coefficient of electricity power. For example, according to the documents issued by NDRC in 2014, the emission intensity of net purchased electricity ranged from 0.5276 $kgCO_2$/KWh to 0.8843 $kgCO_2$/KWh in six regional power grids of mainland China, which are Northeast China (NE), North China (NC), Northwest China (NW), East China (EC), Central China (CC) and South China (SC) respectively (NDRC, 2015). The energy percentage varies greatly by country, due to diverse national structures. Guangdong province as the main production base of bags in China, accounted for 26.48% of China's sales. Its CO_2 emission intensity of net purchased electricity was only 0.449$kgCO_2$/KWh in 2014, which has decreased by 37.1% in the past nine years because of the growth of non-fossil energy (such as nuclear, hydropower and other renewable sources) (Tian, 2016). It was (Joseph, 2009) presented that the usage of clean energy could reduce the carbon emissions in the case of "material flows in the life cycle of leather". Coal accounted for 80% of electricity supply, followed by hydroelectricity in China. The coal and gas is main source of electricity production in the other Asian counties (IEA, 2016).

It is calculated that the CF of electricity consumption about a leather is 0.27 $kgCO_2$-0.88 $kgCO_2$ in China.

（2）Solid waste produced in manufacturer

The dosage and weight of waste have great relationships with process control, technician skill and the types of materials. Main solid waste produced in manufacturer is surface material (bovine leather). Its attrition rate is higher than other materials because of some leather properties, such as the location difference, uneven, and some market reasons. Different types of leather are used in a leather product, mainly bovine leather (for raw material). The attrition rate of bovine leather is lower than goat and sheep leather (even reached 100%-150%). At the meantime, the attrition rate of lining (polyester) is at much lower levels, which depends on cutting level. In fact, solid wastes were bargained away to obtain some economic values in factory. The remaining cannot sale were treated as waste disposal.

The waste materials produced in manufacture are generally treated as city solid wastes, so it was calculated in accordance with city solid wastes on site. The CF of waste materials produced in manufacture is 0.03$kgCO_2$-0.41$kgCO_2$ in China.

3.2.3 CF in usage and disposal stages

Waste materials are packaging material and the whole leather bag before and after usage. The weight of waste materials mainly depended on the size of entire bag. Apart from the leather bags themselves and their packaging, these wastes are generally treated thermally along with household garbage as Urban Solid Waste (USW), but their carbon emissions coefficients varied with methods to treat the USW in different countries. Now USW treatment/disposal methods includes simple landfill, sanitary landfill, incineration and composting. Different countries and regions have greater different treatment due to the economic development level, natural geographical conditions and household habits. For example, sanitary landfill with a high proportion in USA, Italy and Britain, incineration gave priority in Japan, Denmark, the Netherlands, and a large proportion of composting in Switzerland, in Finland and Belgium (Hua, 2014).In China most USW was landfilled, even simple landfilled. The carbon emission from MSW in China were calculated from 2003 to 2012 (Geng, 2015),

sanitary landfill (1.16 tCO_2/t), simple landfill (0.79tCO_2/t), incineration (0.51tCO_2/t) and composting (0.30tCO_2/t). So MSW treatment/disposal methods directly decided the value of the CF of leather bag. Leather bag as organic waste, which had high heat, was generally incinerated for power generation. References vales for incineration of solid waste was 0.26tco_2/t according to the documents issued by Guangdong provincial development and Reform Commission in 2014. It is calculated that the CF in usage and disposal stages is 0.23 kgCO_2-1.74 kgCO_2.

In fact, treatment method of solid waste plays an important role in reducing carbon emissions on the basis of 3R principle of circular economy (Reducing, Reusing and Recycling)(Han, 2016). Degradable organic carbon content of each material in leather product was not low, for example, plastic (leather) is 360 kg/t, paper is 190kg/t, textile is 230kg/t, hardware is almost 0kg/t. The carbon content of waste leather on an energy basis is 6.00gc/MJ (GDDRC, 2014). It would be assumed that these materials were mixed landfilling not be sorted, and consequently follow greenhouse gas (GHG) produced could not be ignored. If there materials were sorted, the recyclable could be reused, and different disposal methods were adopted, such as incineration or composting according to their property. Consequently the CF of leather product would be reduced effectively. But so far waste leather was rarely recycled and reused. However, some research in this area have been studied, for example, waste leather was digested and pretreated for composting after recycling process (Lei, 2015).

3.2.4 CF uncertainty analysis

Uncertainty analysis is used to judge the uncertainty and identify the most influential factors based on the data accumulation and variability. Uncertainty can be classified into parameter (data), model (relations) and scenario (choices) uncertainty, whereas variability can be spatial, temporal or technological (Huijbregts, 2001 and Lloyd, 2007).

The source and influence of CF uncertainty about leather product were analyzed in this study. It was found that the carbon emission factor of leather is key factor in all parameters, which is much higher than the other material. Therefore, to reduce leather carbon emission factor, is crucial to win in the sustainability and carbon labeling of leather products. It was presented in the Handbook with basic information for the Calculation of a Corporate Carbon Footprint (CCF) for a leather factory with evaluation of internal energy consumption in comparison to the BEET energy benchmark (Best Energy Efficiency for Tanning) by Verband der Deutschen Lederindustrie, energy distribution in production process was 74% (ECO$_2$L-Energy Controlled Leather, 2011). The thickness of finished bovine leather had significant impacts to the CF (Chen, 2014). The usage of clean energy could reduce the carbon emissions in the case of "material flows in the life cycle of leather" (Joseph, 2009).

Followed by the waste disposal in all scenario. If each waste in leather product, include leather, textile and packaging, could be recycled and reused on the basis of 3R principle of circular economy (Reducing, Reusing and Recycling), CF of waste derived from leather product would be reduced effectively. Actually these material are capable to reuse technically after recycling process, in particular to strengthen the research of waste leather. It was shown from the CF of a single leather product, the dosage and carbon emission factor of waste are of equal importance. China is a big manufacturer and consumer of leather product, which exported 4.1 billion bags, imported 19.85million bags. The pollution of waste produced by leather cannot be ignored. To a certain extent,

improving technical and skilled worker level could reduce waste produced in the manufacturing stage. In addition, the high attrition rate of leather is conditioned by the property of leather itself. It is important to reduce the attrition rate of leather for economic value and carbon footprint.

Finally is energy consumption. With regard to the leather product production part, energy consumption is seemed to be lesser. But for a leather product industrial cluster, it has turned to be almost the second most impacting phase after the waste. In recent years, we have seen a fundamental shift in the way governments around the world approach energy-related environmental issues. Although the target for the reduction of CO_2 emission intensity in Guangdong (GD) has not been released by the central government, GD has set a goal for increasing the share of non-fossil energy in total consumption to 25% in the provincial 13th Five-Year Plan (Tian, 2016).

4 Conclusions

The eco-design as the first step in the production, is crucial for finished leather. Technical, functional, aesthetic, economic and ecological should be taken into account and balanced to accomplish a fairest evaluation in eco-design of leather product. Therefore, environmental-friendly property need to be focused on for the leather product industrial cluster and a socially responsible manufacturer. In this work, the most problematic phases was identified according to the uncertainty and variability of leather product CF, which refining process control and material chosen, minimizing the value of CF.

(1) The carbon emission factor of main surface material (leather) is the most important factor in all parameters. At present, the database of leather CF is in progress) by the UNIDO Leather and Leather Products Industry Panel.

(2) The CF value of a leather bag was appraised, yielding electricity consumption 0.27-0.88 $kgCO_2$, waste consumption 0.26-1.29 $kgCO_2$, which therein 0.03$kgCO_2$-0.41$kgCO_2$ in the manufacturer, 0.23 $kgCO_2$-1.74 $kgCO_2$ after the sale.

(3) For industrial cluster, the carbon emission factor of electricity and waste disposal methods are key to reduce local CF. It is urgent to explore recycling and reusing of waste leather extensively, and to promote the industrialization of this technology. For the leather bag manufacture stage, the best advice is to advancing process efficiency and refining production to reduce the waste of materials.

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MECHANISM OF HIGH BASICITY CHROME AGENT PREPARATION AND ITS APPLICATION

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Basicity, representing the molecular shape of Cr-complexes, is an important indicator of chrome tanning agent. The chrome tanning agent mainly produced by traditional reduction methods such as sulfur dioxide reduction method and glucose reduction method. The sulfur dioxide method produces chrome tanning agent with 33.3% basicity and the agent can havedifferent basicity by glucose method

In this paper, the difference and specificity of the two methods were discussed. Moreover, the effect of high basicity chrome tanning agent is analyzed and the preparation process is optimized by adjusting the adding quantity of sulfuric acid. The influence factors in the process of reduction were studied, The factors affecting the reduction of dichromate in sulfuric acid dosage, carbohydrate reductantnd and water consumption were summarized. The reducing content of hydroxyl groups in the basic chromium lead to decreasing of the basicity, Meantime, increasing alkalinity will result in poor reducibility. Besides, Carbohydrate as the reducing agent will lead to the masking characteristic of chrome agent. Especially for high basicity chrome tanning agent, the masking effect is obvious. The structure of the chrome complexes in the high basicity chrome agent was analyzed in this paper, and the application in chromium hydroxide sludge disposal and the utilization of the Cr-sludge were explored. The application of high basicity chromitan has the important significance in Cr-wastewater recycle process.

Key Words: high basicity；chrome tanning agents; carbohydrate reductant; chrome sludge; resource utilization

1. Introduction

The production of high quality leather depends on the tanning agents and correct tanning method. What the common concern of scientists are the development and the proper usage of superior quality leather tanning agents. Although there are a wide range of tanning agents, the chrome tanning agent is the most widely used agent in the present. Chrome tanned leather is the most popular leather product because of its practical performance such as high thermal stability and good mechanical strength. Therefore, chrome tanning method has been the core of leather technology while there are a variety of leather chemical materials to match it.

Basicity is an important indicator to evaluate the quality of chrome tanning agent, which representing the molecular shape of complexes in chromitan. The higher the basicity, the higher the proportion of the multinuclear complex in chromitan. It leads to better physical and mechanical

properties of finished leather, the internal causes is the multi point coordination in collagen fiber bundles crosslinking become more. Therefore, it is significant to develop high basicity chromitan for producing high-quality leather.

Traditional industrial production of chrome tanning agent mainly rely on two basic reduction methods, the one is sulfur dioxide reduction, which can produce inorganic chrome tanning agent with basicity of 33.3%; the other is that glucose is used as the reducing agent under acidic conditions,which can prepare chrome tanning agent with different basicity, and the tanning effect of this kind of basicity chromitan with some organic acid is better than the one.

In this paper, how to reduce of potassium dichromate by carbohydrate was summarized; the preparation of chrome tanning agent with different basicity by adjusting the adding quantity of sulfuric acid was mentioned; expecting to get high basicity chrome tanning solution; And the effect of high basicity chrome tanning agent and the Instructions were discussed.

2. Experimental

2.1 Main materials

Chemicals used for experiments are all of chemical grade, and those used for analysis are of analytical grade(Jiangtian Chem. Co., Ltd, Tianjin, China).

2.2 Experiment content

2.2.1 Preparation and analysis of chrome tanning agent

Chrome tanning agent was made by glucose reduction method. 100g potassium dichromate was dissolved in water with the certain amount of glucose and sulfuric acid. The diphenylcarbazide was used to evaluate the reduction degree of finished agent. The basicity of the solution was determined by sodium hydroxide titration.

2.2.2 Adjustment of basicity of chrome tanning agent

The alkalinity of high basicity chrome tanning agent was adjusted by adding acid such as acetic acid, succinic acid, phthalic acid and sodium citrate. Then chrome tanning agent with appropriate alkalinity can be successfully prepared.

2.2.3 Application of chrome tanning agent

The chrome tanning liquor with different alkalinity was used to re-tan the cattle wet blue leather. And chromium absorption rate and shrinkage temperature of retanned leather were determined.Then the physical and mechanical properties of finished leather were assessed.

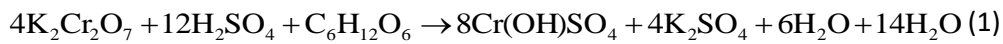#### 2.2.4 Application of high basicity chrome tanning agent in chromium hydroxide precipitation treatment

Chromium hydroxide precipitate extracted from chromium retanning waste was dissolved in sulfuric acid. The basicity was adjusted to 33% through 66% basicity of chrome tanning agent. Then retanning process was conducted with the adjusted agent while compared to the commercial chrome retanning agent.

3. Results and discussion

3.1 Preparation principle of chrome tanning agent and the influencing factors

3.1.1 Prepare the chromium tanning solution by carbohydrate reduction

Dichromate in acidic conditions can be reduced to trivalent basic chromium sulphate by carbohydrates, which are oxidized to carbon dioxide and water in this process. The principle was based on equation below:

$$4K_2Cr_2O_7 + 12H_2SO_4 + C_6H_{12}O_6 \rightarrow 8Cr(OH)SO_4 + 4K_2SO_4 + 6H_2O + 14H_2O \text{ (1)}$$

$$4K_2Cr_2O_7 + 8H_2SO_4 + C_6H_{12}O_6 \rightarrow 8Cr(OH)_2(SO_4)_{1/2} + 4K_2SO_4 + 6CO_2 + 6H_2O \text{ (2)}$$

Basicity(B) is defined as the hydroxyl groups in chromium sulfate(3).:

$$B = \frac{N_{(OH^- \text{ binding with chromium})}}{N_{(total chromium)}} \quad (3)$$

It can also be expressed in acidity (A) is defined as equation (4) :

$$A = \frac{N_{(SO_4^{2-} \text{ binding with chromium})}}{N_{(total chromium)}} \quad (4):$$

A+B= 100%　　　　　(5)

The basicity of chrome tanning liquor prepared by reaction formula (1) and (2) was 33.3% and 66.7% respectively. By adjusting the dosage of sulfuric acid, different basicity of the chrome tanning agent can be acquired.

As can be seen from the equation (1)-(2), the chrome tanning solution with different basicity can be prepared by use of carbohydrate as a reducing agent. Therefore

3.1.2 Influencing factors of carbohydrate reducing dichromate

When prepare the chromium tanning solution by carbohydrate reducing dichromate, the reduction rate was influenced by sulfuric acid, carbohydrate reductant and water. Increasing sulfuric acid can make, the reduction reaction more quickly and completely. Meanwhile, carbohydrate plays an indispensable role in reducing dichromate.Theoretically, it reduces Cr^{6+} to Cr^{3+} and produces the final oxidation as CO_2 and H_2O. However, the reaction is often stay in the intermediate stage of organic acids, which makes the chrome tanning agent achieve the masking characteristic. Besides, the amount of water also directly affects the reduction degree. The addition of water determines the

concentration of H^+ in the reaction system, which is particularly evident in the preparation of high basicity chrome tanning agents.

3.2 Application of High Alkalinity Chrome Tanning Agent

3.2.1 Preparation of High Alkalinity Chrome Tanning Agent

Retanning is an supplement of chrome tanning effectively, as well as giving leather the main sensory properties. Traditionally, the basic chrome sulfate with 33.3% basicity is used as the retanning agent. But the low basicity agent has some drawbacks. There is a lack of flexibility and fullness of finished leather due to the smaller molecules Cr-complexes. Moreover, the smaller molecules Cr-complexes in the re-tanning waste water make it difficult to disposal the waste water.

In the preparing process, organic acid is used to adjust and its equivalent formula is represented as R-COONa. The ratio of the organic acid to the basic chromium sulfate is setted as X, so the structure of the chromium complex is $[Cr(H_2O)_4(OH)_{2-X}](SO_4^{2-})_{1/2}R-COO_X^-$. The basicity of the chromium complex is setted as B_1. It can be calculated by equation (6)

$$B_1 = (2-X) \div 3 \times 100\% \qquad (6)$$

The high alkalinity chrome tanning agent can not only reduce the defects of traditional retanning agent, but also give them the better performance. In this study, the basicity of high alkalinity chrome tanning agents is prepared as 58% and its structure can be seen as the formula $[Cr(H_2O)_4(OH)_{0.26}](SO_4^{2-})_{1/2}R-COO_{0.58}^-$

3.2.2 Application of High Alkalinity Chrome Tanning Agent in tanning wastewater disposal

Currently, the absorption rate of the chrome tanning agent in tanning process is generally low , which results in a large number of chromium ions existence in tanning wastewater. So, deal with chrome tanning waste and re-use of the chromium have become major technical issues facing with leather industry challenge.

Until now, alkaline precipitation method is the most widely used in Cr-containing wastewater disposal. It means that Cr^{3+} ions are converted to $Cr(OH)_3$ precipitates by adding alkali substances such as sodium hydroxide. The precipitates were made to chrome mud-cake through the plate and frame filter process.. In the chrome mud-cake, it contains a large number of crumbs, soluble collagen peptides and oils, which limiting the re-use effect. Add sulfuric acid to dissolve chrome mud-cake is a good method to turn mud-cake to chrome solution for re-tanning. However, when $Cr(OH)_3$ precipitates were reduced to chromium complexes, an excessive amount of sulfuric acid has to be used in the process., It results in the basicity of recycle Cr-containing liquor is too low to achieve the required performances.

In our study, the recycle chrome tanning solution can be adjusted with high basicity chrome tanning liquor to become a moderate alkalinity, and tanning agent with good effect can be get(Fig 1.).

In the process, the basicity of recycle chrome solution is -5-5%, The alkalinity was adjusted by the high basicity chrome tannin with alkalinity of 58% so that the alkalinity is adjusted to 33-40%, which has been successfully used in re-tanning.
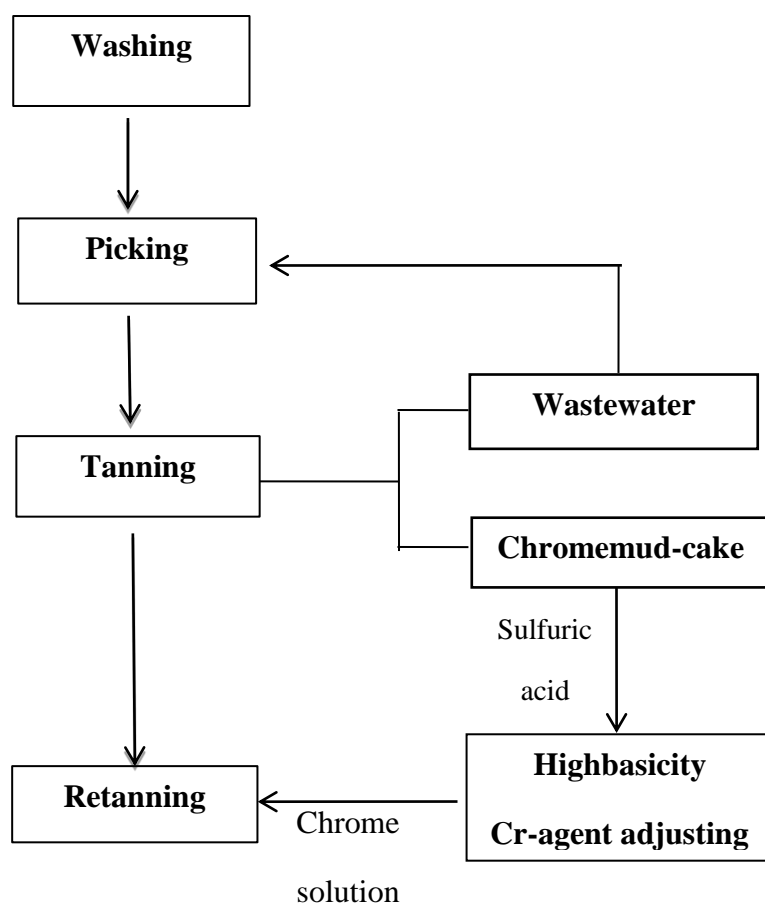
Figure1: Recycle-reuse process

4. Conclusion

The development of high basicity chrome tanning agent can be effectively used in the following two aspects. First, it can be effectively used in retanning process with different basicities by adjusting with organic acid. Second, high basicity chrome tanning agent can be helpful to the treatment and utilization of chrome tanning waste water. It can reuse the chromium mud-cake and achieve the required reuse effect..

In addition, the research also reflects the guidance of alkalinity in chrome tanning, enriches the theory of chromium coordination compounds greatly. It has important significance for tanning theory.

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DEGRADATION OF THE MICROBICIDE 2-(THIOCYANOMETHYLTHIO) BENZOTHIAZOLE BY DIRECT PHOTOLYSIS

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Microbicides in the leather industries are employed to avoid the biodeterioration of the hides by microorganisms. This problem may lead to harmful characteristics to the end product such as stains, roughness and decrease mechanical properties of leather. Therefore, microbicides like the 2-(thiocyanomethylthio) benzothiazole (TMCTB) are applied in distinct steps of the tanning process as alternative to the chlorinated phenols compounds. The microbicides have low mineralization through usual treatments in the leather industries effluents, furthermore it prejudices efficiency of biological treatment if present in high concentration. Thus, the main purpose of this research is to degrade TCMTB by direct photolysis and monitor the degradation by high performance liquid phase chromatography (HPLC) in TCMTB standards solutions. Photolysis tests were performed with a reactor equipped with 250 W mercury lamp. For the experiments were used standards solutions of 20, 40, 60 and 80 mg L^{-1} TCMTB concentration. The photolysis tests were performed during 7 h and the degradation was monitored through sampling collection at predetermined periods of time. The monitoring of the TCMTB degradation by HPLC analysis has proved to be appropriate, since it was possible to note that all samples showed total degradation of the microbicide in the first 30 min of the photolysis treatment. Also, it was noted the formation of photoproducts along the photolysis tests, which were reduced to low concentrations after 6 h of treatment.

Key words: TCMTB, photolysis, high performance liquid chromatography, degradation.

1. Introduction

One problem of the tanning industry is the biodeterioration of the hide due to the microorganism development. This microorganism development leads to undesired features of the leather, such as pigmented stains, defects, roughness surface and decrease of the physical-mechanical resistance, which affect the final properties of the product (Tolfo et al. 2015). To avoid this problem, during the storage and the transport, microbicides are employed in some steps of the process, such as soaking, pickling and tanning (Bryant, Hurlow, Whittemore 2011).

Several preservatives are employed to avoid the hide or leather biodeterioration during the process, these substances are classified in the chemical groups, phenolic or heterocyclic. The 2-(thiocyanomethylthio) benzothiazole (TCMTB) belongs to the heterocyclic group, and this compound is the most used as preservatives in industries of leather (Tolfo et al. 2015). The TMCTB is a chemical

compound derived of the benzothiazole group, widely used as a fungicide for wood (Hinojosa et al. 2002), paper (Meneses et al. 2005) and leather preservation (Fiehn et al. 1998). In the leather industry, this fungicide is applied as substitute for the chlorinated phenols compounds, namely pentachlorophenol (PCP), banished due to it high toxicity and to its difficult removal from the environmental system (Kennedy 1986; Fiehn et al. 1994;Nawrocki et al. 2005). The TCMTB is formulated in mixtures with commercial emulsifiers (nonylphenol ethoxylate, and calcium alkyl benzene sulphate) and organic solvents (dimethyl sulfoxide, dimethyl formamide, xylene), because of its poor solubility in water (Hinojosa et al. 2002).

The characterization and identification of TCMTB is not possible by Gas Chromatography (GC) because of it is instability at high temperatures (Brownlee et al. 1992; Daniels 1987), therefore High Performance Liquid Chromatography (HPLC) has been used to analyze TCMTB (Parbery and Taylor 1989; Kloepfer et al. 2004; Font et al. 2013). Another method has been studied for quantification of TCMTB by spectrophotometry UV-vis, although, this method needs the derivation of this compound to the degraded product, the 2-(mercaptobenzothiazole) (2-MBT), becoming an expensive technique(Hinojosa et al. 2002).

Several compounds classes of the benzothiazole group have been found in the environmental, in rivers, drinking water and groundwater (Kirounani-Harani 2003; Reemtsma et al. 2006; Chen et al. 2012). Furthermore, these compounds removals are difficult by conventional process in wastewater treatment plants (Bahnmüller et al. 2015), because of low concentration and the resistance due to the chemical nature (Kloepfer et al. 2005).

Recent researches have named the compounds of benzothiazole group as emerging contaminants (Jover et al. 2009;Felis et al. 2016). These compounds impart a significant risk to the ecosystem and they have no guidelines and legislative intervention to regulate its presence in the environmental (Dimpe and Nomngongo 2016). Also, because of its poor degradation through conventional treatment, that leads to the increase of the concentration in the environmental system and consequently, becomes a hazard to living organisms (Tremblay et al. 2011). The emerging contaminants are mainly considered as synthetic (man-made compounds) or natural chemicals (hormones secreted by invertebrates and vertebrates) (Janna 2011), including pharmaceuticals, personal care products, pesticides, fungicides, surfactants and endocrine disrupting compounds (Koumaki et al. 2015; Dimpe and Nomngongo 2016; Bila and Dezotti 2006).

In the leather industry a portion of the chemicals products applied in the process do not fix in the hide or leather. As result, these substances are discarded in the effluents, and its presence causes hazard to the environment, beside of hinder the treatment. To improve the efficiency of the effluent treatment plants in the industry the use of Oxidative Advanced Processes (OAPs) has been raised (Brienza et al. 2016) to be employed as previous treatment. This process favors the degradation of the substrates and facilitates the elimination of toxic compounds even in low concentration.

Among the OAPs, the direct photolysis is a method based in the light irradiation into the aqueous matrix containing the pollutant, covering the spectrum of the wave length from 200 to 400 nm (Wright and Cairns 1998). Direct photolysis is possible when the compound can absorb the light at wavelengths present in the light irradiated, what is contrary by the indirect photolysis that occurs when molecules of chemical compounds in the environmental system absorb light and produce

reactant substances which subsequently react and transform the organic molecules (Koumaki et al. 2015).

Studies have been development to apply photolysis process to degrade pollutants in standards solutions, water and wastewater (Russo et al. 2016; Yang et al. 2016; Carlson et al. 2015). These studies describe that the photolysis process has the influence of many factors, such as concentration, pH and temperature (Jin et al. 2017;Zhang et al. 2016).

Therefore, the main objective of the present study was to investigate the degradation process of the commercial microbicide TCMTB in different concentrations by the direct photolysis technique, whose technique is efficient to eliminate group derivatives of benzothiazole (Brownlee et al. 1992;Kirounani-Harani 2003; Bahnmüller et al. 2015). The TCMTB degradation is important because the presence of this microbicide in the biologic treatment decrease the efficiency of the process of wastewater treatment.

2. Materials and Methods

2.1 Chromatographic separation

High Performance Liquid Chromatograph (Agilent 1260 system; *Agilent Technologies*, USA) coupled to a detector *Photo Diode Array* (PDA) was used to determinate the TCMTB degradation in the photolysis process. The chromatographic separation was performed using *Zorbax Eclipse Plus* C18 column (4.6 mm x 250 mm, 5 µm particle size) (*Agilent Technologies*, USA). The mobile phase was a mixture of ultrapure water (solvent A) and acetonitrile (solvent B), both acidified with phosphoric acid in pH 4.5 – 5.0. During the analysis the gradient conditions were 60% solvent B for 6 min and increased linearly to 95% solvent B during 9 min at a flow rate of 1.0 mL min^{-1} and column temperature of 30°C. Sample volumes of 20 µL were injected. Before of the injection the samples in the chromatographic system they were filtered with PVDF membrane 45 µm. These conditions were selected after experimental studies. Firstly, to evaluate the HPLC technique were injected two standards samples of TCMTB in 40 and 80 mg L^{-1} concentration, these concentration are found in the tanning effluents (Font et al. 2013). After this evaluation, the HPLC technique was applied to follow the degradation of TCMTB in photolysis essay. The degradation of TCMTB after irradiation was obtained comparing peak retention time in pure sample to those treated sample.

2.2 TCMTB photolysis

The irradiation experiments with solutions of TCMTB (commercial microbicide) were performed in a reactor coupled with exhaust fans and mercury lamp of 250W in. Throughout the photolysis experiments, samples were taken at different time intervals during 7 h. Concentrations of 20, 40, 60 e 80 mg L^{-1} of TCMTB were employed in this irradiation essays. In the end at the photolysis experiment were obtained thirteen samples for each concentration to analyze the degradation with HPLC.

3. Results and Discussion

3.1 High Performance Liquid Chromatography – HPLC

The efficacy of monitoring the degradation of TCMTB using the HPLC technique can be evaluated from the results obtained in Figure 1. A high resolution peak formation between 8 and 9 minutes is observed for both 40 mg L^{-1} solution and 80 mg L^{-1} solution, evidencing a separation of the TCMTB by the proposed chromatographic method. Thus, these results corroborate the use of this technique in the monitoring of TCMTB degradation in the further photolysis assays.
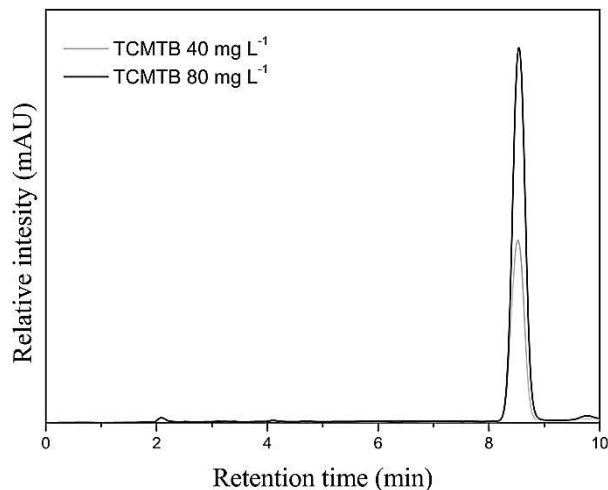

Figure 1. Chromatogram obtained for 40 and 80 mg L^{-1}TCMTB solutions.

3.2 Photolysis essays

Results of photolysis assays performed at concentrations of 20, 40, 60 and 80 mg L^{-1} respectively before the treatment at 0 min and after 15 and 30 min treatment are shown in **Figure 2**. At time null (0min) it is possible to identify the presence of TCMTB, referring to the peaks between 8 and 9 min. This peak decreases in the time of 15 min and is almost absent in the time of 30 min. Therefore, in general these results demonstrated that the degradation of the compounds was found to be efficient at 30 min, excepted for 60 mg L^{-1} concentration. Furthermore, the results demonstrated that the degradation by direct photolysis had similar behavior for all concentration.

Numerous researches reported emergent pollutant degradation by direct photolysis, with UV lamp or sunlight radiations. This treatment can be applied for several contaminants such as biocides, pesticides, insecticides, pharmaceuticals and personal care products, and endocrine disrupting compounds (Hossain et al. 2013; Koumaki et al. 2015; De La Cruz et al. 2012). Nevertheless, there is little information about TCMTB degradation by direct photolysis (Felis et al. 2016)(Brownlee et al. 1992).
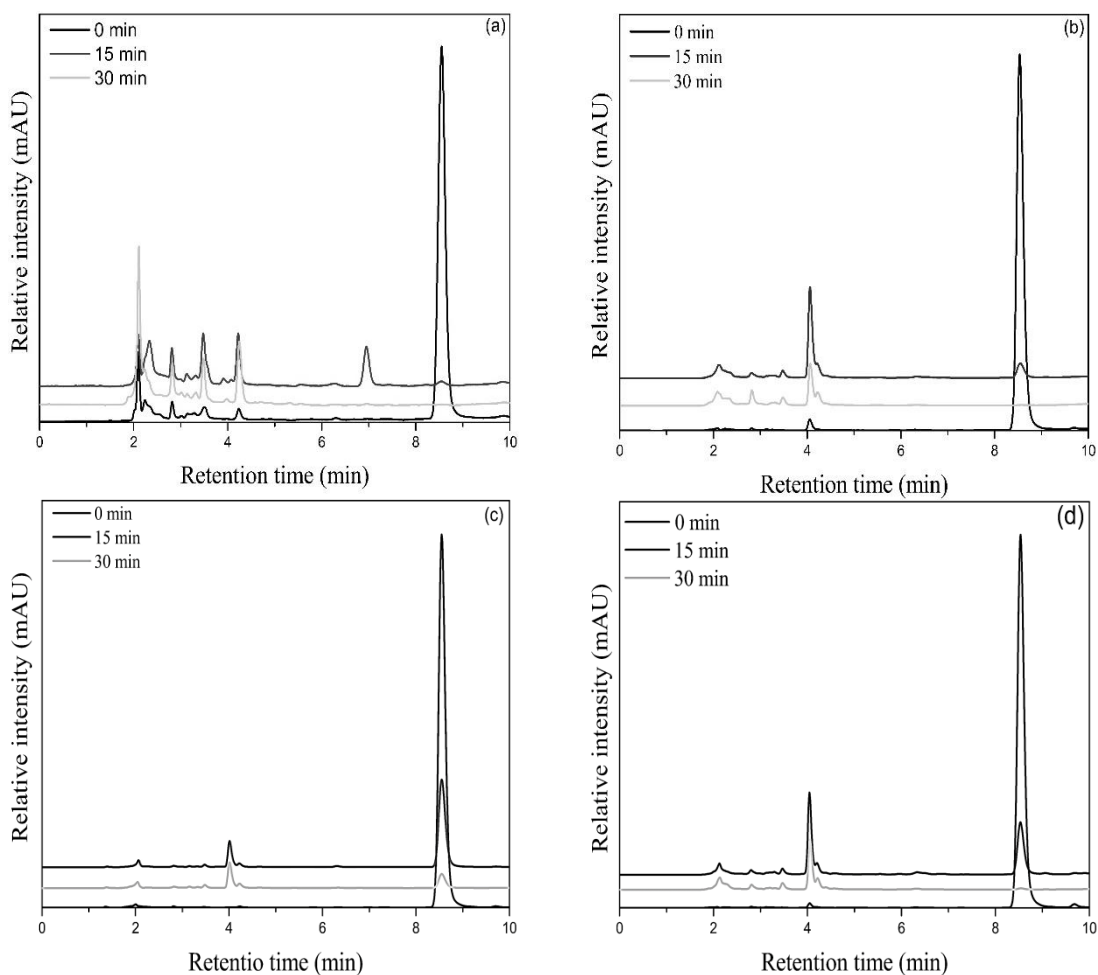
Figure 2. Degradation of TCMTB in concentrations (a) 20 mg L⁻1, (b) 40 mg L^{-1}, (c) 60 mg L^{-1}, (d) 80 mg L^{-1}.

With the obtained results it was possible to calculate the degradation of the TCMTB throughout the experiment, obtaining values of 98, 96, 76 and 96% for the concentrations of 20, 40, 60 and 80 mg L⁻¹, respectively, in the initial 15 minutes of treatments (Figure 3). These results are in agreement with the studies conducted by Brownlee et al. (1992), who verified the rapid photolytic degradation of TCMTB throughout the irradiation of sunlight.

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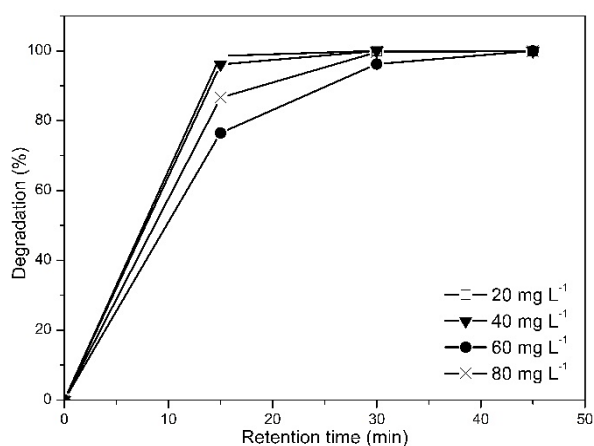
Figure 3. Degradation of TCMTB solutions in photolysis essays along the time.

In addition to the degradation of TCMTB it is possible to observe a formation of photoproducts during a photolysis. In the Figure 4, is demonstrated the formation of compounds with high intensity in retention times around 2 to 4 min for the 60 mg L^{-1} concentration of TCMTB. However, it can be observed that after 6 h of treatment these by-products are degraded at low concentrations. The same by-products were identified for 7 h of treatment. The same behaviors were found for the other concentrations studied.
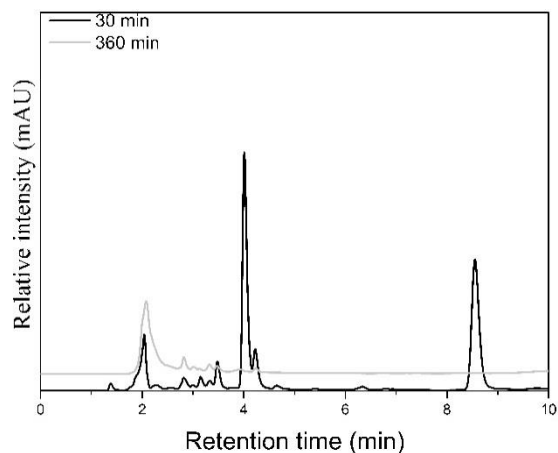
Figure 4. Formation of photoproducts during photolysis in the concentration of 60 mg L^{-1} TCMTB solution.

Among the compounds generated during the photolytic treatment, it is reported in the literature that the main compound resulting from the photolysis and hydrolysis routes of TCMTB is 2 (mercaptobenzothiazole) (2-MBT) (Brownlee et al. 1992). This fact, highlight the possible 2-MBT formation in the process. These compounds formed during the process may also be due to the degradation of the surfactants by which is produced the TCMTB.

4. Conclusion

The use of HPLC was shown to be effective and essential to investigate the behavior of degradation of TCMTB by photolysis. During the photolysis assays, it was possible to observe the total degradation of TCMTB in standard solutions of 20, 40, 60 and 80 mg L-1. In addition, it was also visualized the formation of photoproducts, due to the degradation of the TCMTB, throughout the photolytic process. However, these compounds are mineralized at low concentrations at the end of treatment. Thus, it is important to investigate and identify the nature of these compounds, as well as their toxicity. Further investigations should be done to identify the compounds of the transformation. This study purposes the potential of using the photolysis for the treatment or pre-treatment of recalcitrant substances such as TCMTB (emerging contaminant) in real effluents.

5. Acknowledgements

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WASTE TO WEALTH APPROACH: DERIVING HIGH VALUE PRODUCTS FROM RAW TRIMMING WASTE OF HIDES AND SKINS

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Leather industry plays a notable role in today's global economy. Raw hides and skins are the by-products of the meat industry and serve as raw material to the leather industry. During the process of leather manufacturing, the huge amounts of hide and skin trimmings are generated. These trimmings account for a minimum of 5% of the total quantity of raw material processed in tannery. Raw skin or hide trimmings contain proteins especially collagen and keratin. These trimmings currently find use in very low value application. 50-60% of the total solids associated with trimmings are collagen. Hence skin trimmings are either dumped or used for making identical glue. Collagen, its pure form or denatured form could derive high value which is untapped in current practices. The paper will focus to highlight the utilization of the raw hide/skin trimmings from the tannery for making products of high value such as collagen, gelatin, protein hydrolysate, biodegradable packing materials etc.

Keywords: Trimmings, Collagen, Gelatin, Protein hydrolysate

1. Introduction

Meat and meat product industries offer by-products such as hides and skin which are used as raw material for manufacturing leather and leather products. Thus, the leather industry could have easily been considered as an eco-friendly industry because it processes waste products from meat production (Langmaier et al. 1999). Leather industry plays a significant role in today's global economy, transforming hides/skins into a physically and chemically stable material by subjecting them to chemical and mechanical sequential processes to obtain amenable leather products for meeting various needs of people (Ozgunay et al. 2007). Transformation of hides and skins into leathers involves a complex combination of mechanical and chemical processes which are divided into fundamental sub-processes such as preparatory stages, tanning and crusting. For every ton of raw hides, nearly 730 kg is generated as solid waste in leather processing. Only 270 kg of the raw material is converted into usable product. Solid wastes generated in leather industry are mainly skin trimmings, fleshings, and shavings, buffing dust and keratin waste (Taylor et al. 1998; Alexander et al. 1998). Among these wastes, skins or hide trimming wastes generate massive quantity of proteins and are also less contaminated by chemicals than tanned leather or finished leather wastes. For every 1000 kg of raw material processed, 50 kg of raw trimmings are generated. The raw trimming also contains chiefly collagen and is used as a raw material for production of various products such as glue, gelatine, proteins sheath, feed, fertilizer and even acts as a substrate for biogas production. Collagen and its pure form or denatured form could be used to derive high value products which are untapped in current practices. This paper will focus on the

utilization of the raw hide/skin trimmings from the tannery for making products of high value such as collagen, gelatin, protein hydrolysate, biodegradable packing materials etc. and also on the reduction of environmental concerns.

2. Methods for recovering collagenous product from trimmings

Globally on an average, 6,500,000 tons of hides and skins are processed into leather (FAO 2013). At 5%, about 325,000 tons of raw trimmings wastes are generated and considering minimum amount of 20% collagen content (% based on wet salted raw material), potentially 65,000 tons of collagen is available annually. These waste materials are a rich source of proteins, lipids, carbohydrates, minerals, salts, and water. Among several classes of proteins (collagen, elastin, keratin, albumins and globulins), collagen is present in the largest amount and is responsible for the formation of leather by combination with tanning agent (Kanagaraj et al. 2006). Raw trimmings of skin/ hide contain proteins especially collagen and keratin. These trimmings are currently used in very low value applications. Hence skin trimmings are either dumped or used for making identical glue. In this article, we are mainly focusing on the methods available for converting collagenous waste in to high valuable products such as Collagen, Gelatin, protein hydrolysate, and biodegradable packaging material.

2.1. Collagen:

In animal hides and skins, Type I is the predominant of all types of collagen and it is also the major structural component of tendon, bone and connective tissue (Ricard et al. 2005). Till date, 29 types of collagens have been identified (named types I-XXIX) of which type I is the most abundant (Chi et al. 2014) The common structural motif of all collagens is the triple helix structure in which each of the three parallel polypeptide strands with left-handed polyproline II (PPII)-type helical conformation coil around each other to form a right-handed triple helix. The tight packing of PPII helices within the triple helix mandates that every third residue be glycine (Gly), resulting in a repeating Xaa-Yaa-Gly sequence, where Xaa and Yaa can be any amino acid. This repetition pattern occurs in all types of collagen, although it is disrupted at certain locations within the triple-helical domain of non-fibrillar collagen (Brazel et al. 1987). The amino acids in the Xaa and Yaa positions of collagen are often occupied by Proline and hydroxyproline, respectively. Pro-Hyp-Gly is thus the most common triplet (10.5%) in collagen (Ramshaw et al. 1998). Collagen is the material that gives skin its properties of toughness and elasticity (Sundar et al. 2011).

2.1.1. Methods for extracting the collagen:

Several methods are available for extraction of collagen from the different sources such as skin, tendon, bone etc. Before extraction of collagen from the skin or bone, pre-treatment is performed using an acid, alkaline process to remove non collagenous protein and also increase yield of the process. The most commonly used extraction methods are based on the solubility of collagen in neutral saline solutions, acidic solutions, and acidic solutions with added enzymes. Summary of the procedures employed in the extraction of collagen from animal by-products are represented in the table 1.

Table 1: Methods for extraction of collagen

Source	Pre-treatment	Extraction procedure	Reference
Goat skin Trimmings	The skin trimmings were soaked in water to increase the moisture	Collagen was solubilised from skin matrix using 1:250 w/v	Masilamani et al. 2016

	content to 60-65%. Un-hairing of rehydrated trimmings was carried out using 10% lime and 3% Na_2S and the percentage of chemicals was based on the weight of trimmings. After un-hairing, the trimming matrix is opened up by treatment with 3% lime for 24 h. Then the trimmings pieces were subjected to deliming using 2% NH_4Cl for 120 min.	organic acids (acetic acid, propionic acid). Then the collagen solution was centrifuged at 12,000 g for 15 min. Salt precipitation was carried out using 5% NaCl and then re-dissolved in 0.5 M acetic/propionic acid. Then the solution was dialysed against 0.1 M acid followed by 20 mM disodium hydrogen phosphate for 72 hr and centrifuged with 12000 g for 20 min. and re-dissolved in 0.1 M organic acid.	
Emu skin	Homogenization with 10% ethanol for days. Extraction with 0.1M NaOH for 2 days. Washing with distilled warer.	Extraction of collagen with 0.5 M acetic acid for 48h, 0.9 M NaCl in 0.5Macetic acid and pepsin (10%) in 0.5M acetic acid for 4 days.	Wang et al. 2014
Bovine Pericardium	Immersion in 0.1M NaOH for 48h at 4°C	Extraction of collagen with pepsin in 10mM HCL (1:20 w/v) for 12 h at 4°C.	Santos et al. 2013
Achilles tendon	Washing with 0.15 M Nacl and acetone.	Enzymatic hydrolysis with pepsin in 0.5M acetic acid for 2 days at 20C with ultrasound (40kHz, 120W, pulsed 30 for 30min.)	Li et al. 2009
Bovine Limed split	Bovine limed split waste pieces were fleshed and then delimed with 2% NH4Cl and 0.5% HCl for 60 min. The delimed pieces were neutralized to pH6.0-7.0 with 0.5% HCl followed by rinsing with distilled water.	30 volumes of 0.5 M acetic acid containing 1% pepsin treated at 4°C for 48 hrs for extraction of collagen. After solubilisation, solution was centrifuged at 10,000g for 15 min at 4°C, and then salted out by adding NaCl to a final concentration of 3 M. The precipitate was again dissolved in 0.5 M acetic acid, and then dialyzed against 0.1 M acetic acid.	Zhang et al. 2006

2.1.2. Applications:

Collagen, apart from their utility in leather manufacture, can be utilized for a wide range of applications viz., cosmetics, biomedical materials, food and agriculture which is represented in the schematic diagram shown in figure 1.
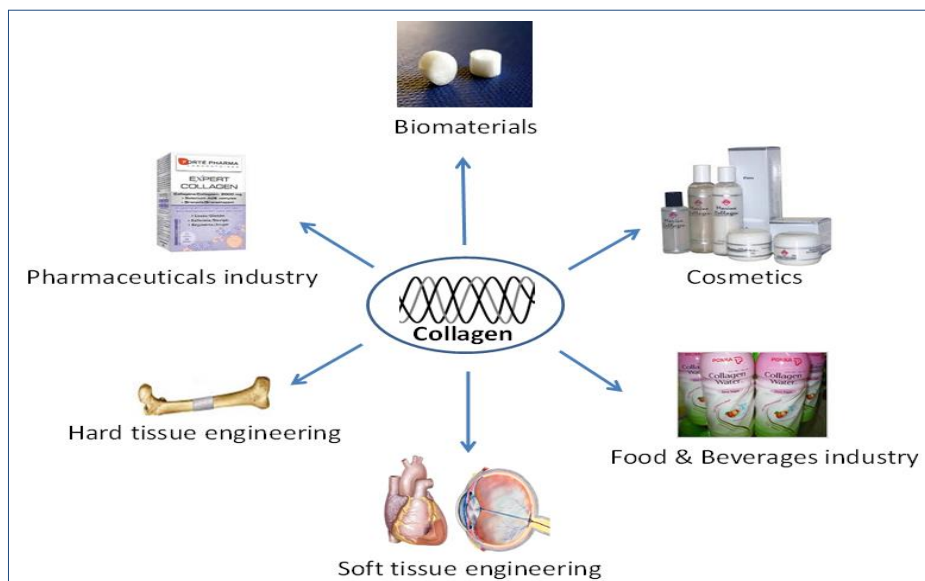
Figure 1: Schematic diagram of collagen and its application

Collagen is used in pharmaceutical industries, tissue engineering, and biomedical industry as micro-particles, injectable dispersions, shields in ophthalmology, sponges, drug delivery system. Its application in the pharmaceutical as well as biomedical field is due to its characteristics such as weak antigenicity, cell attachment ability, biodegradability and biocompatibility. In modern medicine, collagen based scaffolds play a vital role. It helps in cartilage and bone reconstruction. Collagen based dressing in the form of sponge for wounds or burns in the form of collagen films and powders, surgical suture. It is also used in urogenital disorders, corneal defects, study of neural migration, dental purpose, and bone grafting, arthritis and obesity (Silvipriya et al. 2015).

2.2. Gelatin:

Gelatine is a valuable protein derived from animal by-products obtained through partial hydrolysis of collagen originated from cartilages, bones, tendons and skins of animals. It is a translucent brittle solid substance, colourless or slightly yellow, nearly tasteless and odourless. Most of the commercial gelatin is currently sourced from beef bone, hide, pigskin and, more recently, pig bone and fish. It was reported that 41% of the gelatin produced in the world is sourced from pig skin, 28.5% from bovine hides and 29.5% from bovine bones. At neutral pH, about 7.5% of the gelatin is positively charged (lysine and arginine), 12% is negatively charged (glutamic and aspartic acid), and 6% of the chain is hydrophobic (leucine, isoleucine, methionine, and valine) in nature leaving about 58% of the chain comprising of glycine, proline and hydroxyproline to be neutral gelatin is used in conjunction with various surfactants to provide emulsification capacity or to control interfacial tension (Jaswir et. 2009).

2.2.1. Methods for extracting the gelatin:

Gelatin is a protein compound derived from denatured collagen. In order to obtain gelatin, a pre-treatment process is required to convert the tissue collagen into a suitable form for extraction. This

means the loss of the ordered structure of the native insoluble collagen, which results in a swollen, but still insoluble collagen. Subsequent heating cleaves hydrogen and covalent bonds, leading to the conversion into gelatin by α helix-to-coil transition. Based on the pre-treatment of raw materials, gelatin can be divided into type A and type B. Type A, with iso-ionic point of 7 to 9, is derived using exclusively acid pre-treatment. Type B, with iso-ionic point of 4 to 5, is the result of an alkaline pre-treatment. In aqueous solutions, gelatin is a mixture of different polypeptide chains including α-chains, β (dimers of α-chain) and γ (trimers of α-chain). The quality of gelatin is largely determined by its gelling strength and thermal stability. This is dependent on the amino acid composition which is species specific and molecular weight distribution as influenced by processing conditions. (Gomezet al. 2005) (Karim and Bhat 2009). There are several types of methodology that are followed by researchers for extraction of gelatin (Table 2).

Table 2: Methodology for extraction of gelatin from various sources:

Source	Pre-treatment	Extraction method	References
Skin	The skin was mixed with 0.1 mol L^{-1} NaOH at a sample to solution ratio of 1:10 (w/v) for 2 h at 15–20 °C with the changes of solution every 40 min. Then de-mineralized using 1 mol L–1 HCl at a solid to solution ratio of 1:10 (w/v) for 1 h at 15–20 °C. Thereafter, the de-mineralized skin was swollen by mixing the skin with 0.2 mol L–1 acetic acid at a sample-to-solution ratio of 1:10 (w/v) for 15 min at room temperature (26–28 °C).	To extract the gelatin, the swollen skin was mixed with distilled water at different temperatures (45, 60, and 75 °C) using a sample-to-water ratio of 1:2 (w/v). The mixtures were continuously stirred for 6 h. The mixtures were then filtered. Then, the filtrates were freeze-dried using a freeze-dryer	Kittiphattanabawon et al. 2010
Bone	Bones were degreased by tumbling in warm (35°C) water and then demineralised using 3% HCl, at ambient temperature (20–25 °C) until the bones did not have any hard cores. The acidulation solution was changed at 3 day intervals. The time required for complete demineralisation was 9–12 days. The leached bones (ossein) were washed with water for 7-7 times until the	The pre-treated materials were transferred to beakers, covered with water and gelatin extracted in water baths by three sequential 5 h extractions at 50, 60 and 70 °C, followed by boiling for 5 h. The remainder of gelatin extracts were filtered through compressed cotton wool and passed through a column of activated carbon.	Muyonga et al. 2004

	wash water pH was greater than 4.		
Skin	Skin samples were treated with 0.55 N NaOH solution for 67.5 min at ambient temperature and then with 0.1N HCl solution (both 5:1, v/w) for 45 min at ambient temperature. After each alkali and acid treatment, skin samples were washed with distilled water (5 : 1, v/w) 3 times at ambient temperature	Water extraction was done in a water bath at 50 °C at 4: 1 (v/w) water/skin ratios for 3 h.	Boran et al. 2010
Skin splits	Bovine limed split wastes were relimed with 3-4% lime and 0.5% NaOH for two weeks. The relimed split wastes were washed and neutralized to pH5.5-6 with 1.5% HCl.	Gelatin was extracted from the pre-treated split at the temperature of arround70-90 °C for 5 sequential extractions.	Zhang et al. 2006

2.2.2. Application of gelatin:

Gelatin is one of the most consumed colloids in food, pharmaceutical and other industries, and is produced in four grades pharmaceutical, edible, photography and industrial. It is used for various applications in the food industries such as jelly production confectionary, edible films, encapsulation, fruit juice clarification, dairy processing, soups and other applications.(Tavakolipour 2011).The following figure 2 represents the application of gelatin in the various fields. The unique hydrocolloidal nature of gelatin has enabled it to find numerous applications in the food industry. These can be divided into four main groups, namely confectionery (mainly for providing chewiness, texture, and foam stabilization) and jelly deserts (to provide creaminess, fat reduction, and mouth feel), dairy products (to provide stabilization and texturization), meat products (to provide water-binding), and hydrolyzed gelatin applications (Nishimoto et al. 2005; Karim and Bhat 2009). Generally, gelatine is used in foods as a beverage and juice clarifier, desserts and yogurt thickener. Further uses include fruit toppings for pastry, instant gravy, instant sauces and soups, edible films for confectionery products, as a stabilizer in ice cream, cream cheese, and cottage cheese, as well as in food foams and fruit salads [McWilliams 2001]. Gelatin is also used in coating meat products to reduce colour deterioration and gelatin coat is equally effective during light and dark storage (Antoniewski et al. 2007; Tyburcy 2010).
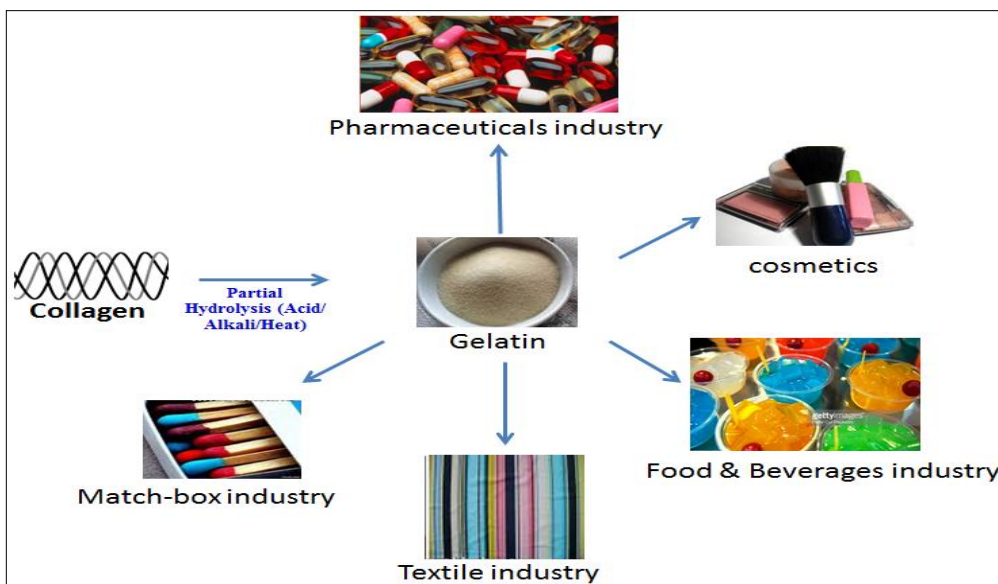
Figure 2: Schematic diagram of gelatin and its application

The largest proportion of gelatin procured by the pharmaceutical industry is used mainly for hard and soft gelatin capsules (Softgels) and for tableting, tablet coating, granulation, encapsulation and microencapsulation. Gelatin capsules (gel-caps) are commonly used to encapsulate various foods, nutritional supplements, and medicines. Applications for this technique have increased in the food industry since the encapsulated materials can be protected from moisture, heat or other extreme conditions, thus enhancing their stability and maintaining viability. Gelatin is used as excipients in pharmaceutical formulations, including vaccines, and is used as a binder for tablets. Gelatin is still the best medium known for making photographic emulsions and also enhances the ability of the developer to distinguish between the exposed and the unexposed crystals (Mariod et al. 2013).

2.3. Protein hydrolysate and its applications:

The trimmings wastes can be used as raw materials to make protein hydrolysates. We have carried out efforts in our lab prepare Protein hydrolysate (PH) from trimming waste therough enzymatic hydrolysis and alkaline hydrolysis. Based on the nutritional value and biological activity, it has been used in various industrial sectors such as the food and feed industry, agriculture, biotechnology, cosmetics and biomedical sectors. The utilization of PH as foods or feeds is primarily related with their nutritional value. They have an excellent amino acid balance, good digestibility and rapid uptake. They also exhibit generally good functional properties, particularly high water solubility (Tahiri and Guardia 2009). Protein hydrolysate is a valuable protein resource, used as soil fertilizers, biodegradable polymers and additives for cosmetic industry, building materials etc. According to Tonkova et al. (2007), hydrolysate product obtained from calf skin waste acts as an important ingredient in bacterial growth media and it may act as alternative to the peptone. Alkaline hydrolysis of trimming waste is converted into biodegradable end product at low cost with high end application such as broad care soil conditioner (Gousterova et al. 2008). Chemical and enzymatic hydrolysis of trimmings can be used as plant growth enhancers and bio-stimulators for fruit and vegetable crops (Lacatus et al. 2009).

2.4. Biodegradable packaging material and its applications:

Development and modernization have brought about a huge increase in the production of all kinds of plastic commodities, which directly or indirectly generate waste due to their wide range of applications coupled with their versatility of types and relatively low cost. Non-biodegradable plastic is a huge environmental concern. Now people are looking for biodegradable packing material from the biodegradable polymers. Biodegradable polymers are derived from replenishable agricultural feedstock, animal sources, marine food processing industry wastes, or microbial sources. In addition to renewable raw ingredients, biodegradable materials break down to produce environmentally friendly products such as carbon dioxide, water, and quality compost (Marsh and Bugusu 2007). The trimming wastes are not contaminated with hazardous chemicals and highly proteinaceous product and it can provide biodegradable polymer (BDP) and value added products through process biotechnology. The improvement in biodegradability of plastic materials used in packaging may be obtained using gelatin as a biodegradable additive (Deselnicu et al. 1999). Enzymatic hydrolysates of trimming waste can be reacted with dialdehyde starch to produce biodegradable (or even edible) packaging materials for food, cosmetic and pharmaceutical products (Langmaier et al. 2008). A wide range of value added products are making integrated business with value creation opportunities in leather industries. (Mukhopadhyay et al. 2004). We have successfully made packaging material using gelatin extracted from trimming waste in combination with biodegradable polymers.
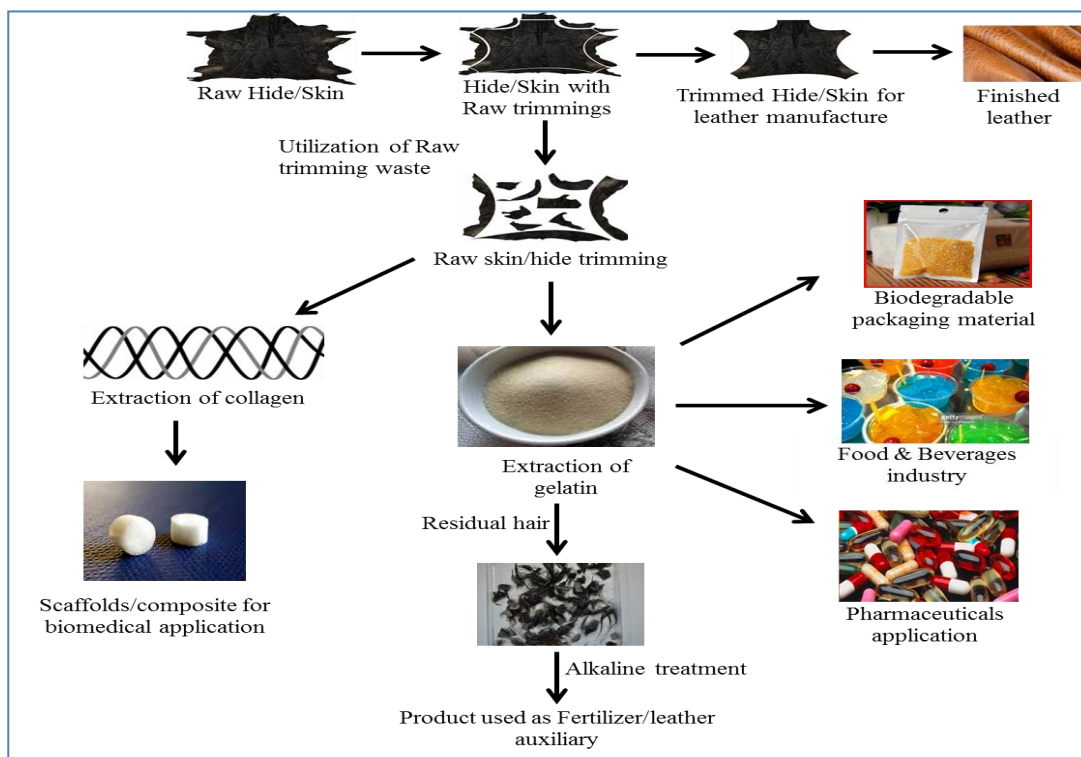
Figure: 3. Utilization of Raw trimmings into high valuable products

3. Conclusion

Leather industry, especially in developed and emerging economies, has come under severe threat mainly due to the exorbitant costs associated with the management of wastes. There exist opportunities in tanneries to utilize many of the solid wastes and maximise them as resources. Raw trimmings wastes are a very good source to derive significant value to offset the environment cost and make leather in a

sustainable manner. In this paper, we have highlighted the options of making high value products such collagen, gelatin, protein hydrolysate and bio-packaging material from raw trimming wastes. The value addition by effective utilization of trimmings could auger well for the sustained growth of leather sector (Figure 3), which is important for inclusive growth of economies.

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GREEN BIOPOLYMERS FOR ECOFRIENDLY LEATHER

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1   Introduction

Leather processing is one of the earliest industrial activities taken up by mankind. Today the Leather Industry significantly contributes to economic development, and is facing global environmental challenges in order to reduce pollution level and chemical hazard. Close monitoring by pollution control authorities and growing awareness in the society is increasing pressure on industry to adopt cleaner processes.. The leather making process involves a set of unit operations, most of which are performed in water, such as:;pre-tanning, tanning, post-tanning and finishing.

Consequently ,chemical concentration and pollution levels of waste water are one of the major concerns in tanneries. The characteristics of non treated wastewater are a high chemical and biochemical oxygen demand, and a high salt and process chemical content.

Leather making is highly water consuming posing a very important from environmental point of view. In fact, leather manufacturing needs 30-50 L of water for processing 1 kg of raw hide (John Sundar et al., 2001)Statistics show that the global leather industry generates 548 billion liters of wastewater per year (Rajamani, 2013).

The environmental effects that have to be taken into account in any tannery comprise not merely the load and concentration of the classic pollutants, but also the use of certain chemicals, e.g., biocides, surfactants, and organic solvents. The chemicals normally used in leather making process are based on petrochemical chemistry, due to the easy fossil raw materials availability and to their high chemical stability.

In order to protect environment and laborers, EU has compiled BREF (Best Available Techniques Reference document) and IPPC UE 2008 Directive, highly recommending the reduction of water consumption in the leather making process and pushing to an identification of efficient and sustainable alternatives concerning the use of Non Hazardous Substances and more Environmental Friendly products, that can also guaranty a reduction in the Carbon Foot Print.

LIFE BIOPOL, project co-financed by European Union as part of the LIFE 2014-2020 program, takes place in this context. For instance its main target is the synthesis of a new class of biopolymers that represent an innovative, eco-friendly and suitable alternative to traditional products based on petrochemicals, currently used in leather making process. Biopolymers are produced using as raw material some industrial by-products like animal and vegetable biomasses from leather and agrochemical industry.

The project intends to re-use hydrolyzed animal proteins, that can be found in the leather scraps and shavings, and vegetable proteins coming from soya, sugar beet and corn by-products, modifying these secondary resources into raw material for the production of biopolymers with very high tanning, retanning and fat liquoring characteristics.

After a preliminary research activity, biopolymers will be produced in an industrial prototype plant specifically designed and built up.LIFE BIOPOL main target is to produce biomass derived biopolymers for leather making. Life Cycle Analyses of the new Biopolymers will be assessed and compared to conventional products. LIFE BIOPOL intends to verify that:

 - the environmental impact and the total water consumption of the leather making process by using the new biopolymers will highly reduced compared to conventional processes..

- the Product Environmental Footprint (PEF) of the new Plant and of the Biopolymers produced will be highly inferior compared to conventional leather making auxiliaries.

Scope of LIFE BIOPOL is to produce biopolymers in order to reduce:

- 20-30% COD in waste water,
- 50-60% of inorganic salts (Sulphates and Chlorides),
- 90% of Cr(III) salts,
- 20% of water used in the leather process.

Other important impact will be demonstrated as:

- reduction 70-90% of hazardous substances normally found in conventional chemicals (for example. inorganic salts, free formaldehyde, Cr(III) salts, heavy metals),
- reactivity enhancement of 30-40% of the new biopolymers compared to other current leather technologies,
- reduction of 70-80% of the Product Environmental Footprint of the new biopolymers related to the state of the art.

The present work concerns the synthesis of biopolymers starting from animal biomass, glycerol and a condensation agent, and involving several reactions such as polymerization, esterification and sulphitation. The multi-functional reactivity of these kind of biopolymers allows their use in different leather making steps (Scheme 1). Biopolymers performances have been tested in comparison with traditional chemicals through leather making process.

Biopolymers characterization using SDS-PAGE, NMR and FT-IR have rationalized the synthetic strategy and practical application of the products giving important parameters such as molecular weight and chemical composition.
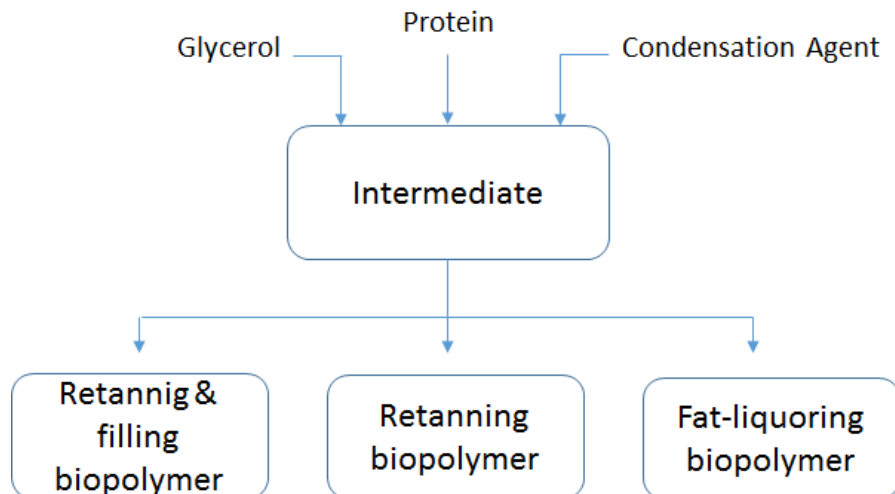
Scheme 1: synthesized biopolymers used in different leather making steps

2   Experimental

2.1   *Materials:* materials used for the synthesis were obtained from Codyeco's conventional raw chemicals.

2.2   *Reaction procedure:* a glass reactor fitted with stirrer, heating mantle and condenser has been used in the synthesis of novel biopolymers. The reaction was carried out between 50 °C-100 °C.

Initially glycerol was heated into the reactor and animal biomass was added. Once an homogeneous dispersion is achieved, the condensation agent was added.

The temperature was raised slowly and steadily in about 30 minutes. The reaction was continued   for several hours till the desired molecular weight was achieved. At the end of this period, the   reaction was stopped and the prepared polymer was ready for packing. The synthesized Intermediate could be used in order to produce a range of biopolymers for different leather making steps:

- *Biopolymer A: Filling and fat-liquoring biopolymer*: Intermediate could be used for re-tanning and greasing purpose thanks to its carbonyl groups able to react with the tanned leather.
- *Biopolymer B: Retanning biopolymer:* Intermediate was involved in polymerization reaction using acrylic acid as monomer. The resulting co-polymer has the capacity to react with chrome leather through coordinated bonds, thanks to its carbonyl groups. The possibility of reactions with collagen amide groups is under investigation.
- *Biopolymer C*: Retanning and Fat-liquoring biopolymer: Intermediate was sulphited using sodium bisulfite. The reaction involved conjugated bonds located into the intermediate chemical structure and introduced during the condensation step with acrylic acid. Theretanning capacity depends on the polymerization grade and the fat-liquoring properties are linked to the sulphonic groups.

BIOPOLYMER SYNTHESIS
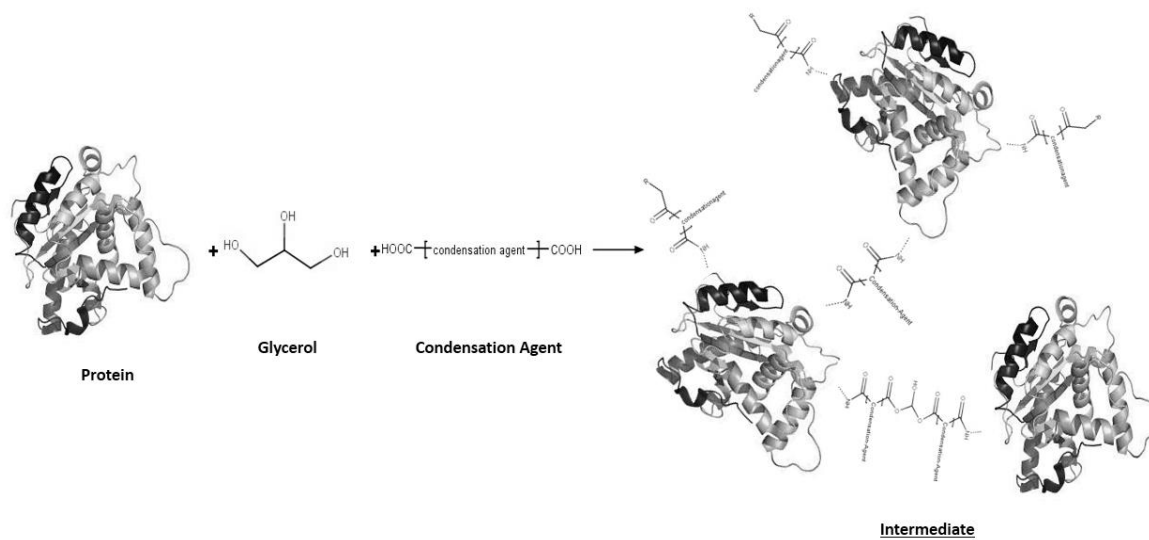
Scheme 2: synthesis of the Intermediate (filling and fat-liquoring biopolymer)

BIOPOLYMER CO-POLYMERIZATION
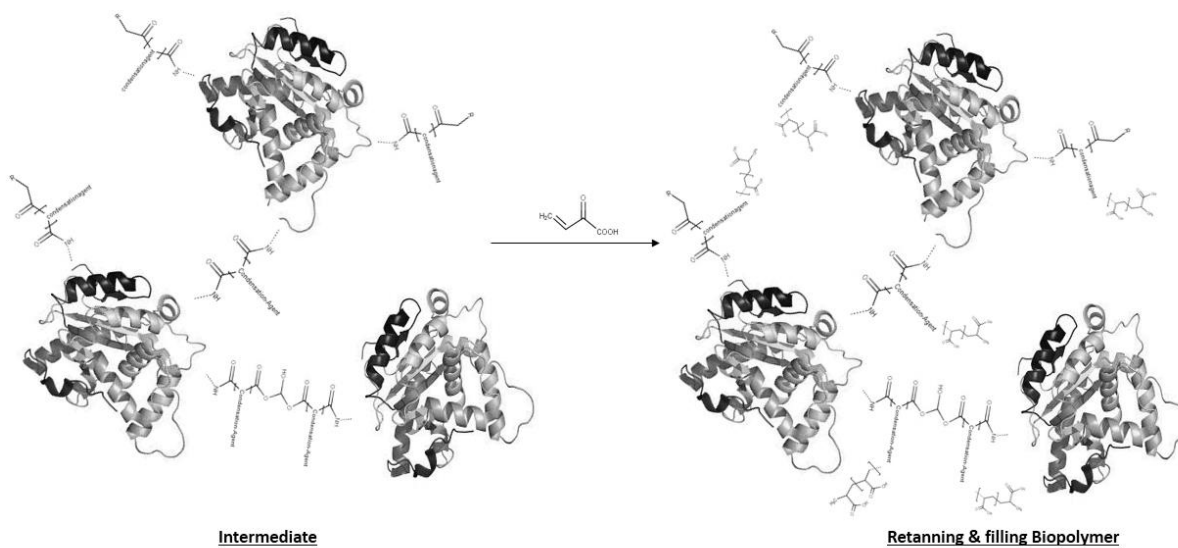
Scheme 3: Intermediate co-polymerization with acrylic acid (retanning biopolymer)

BIOPOLYMER SULPHITATION
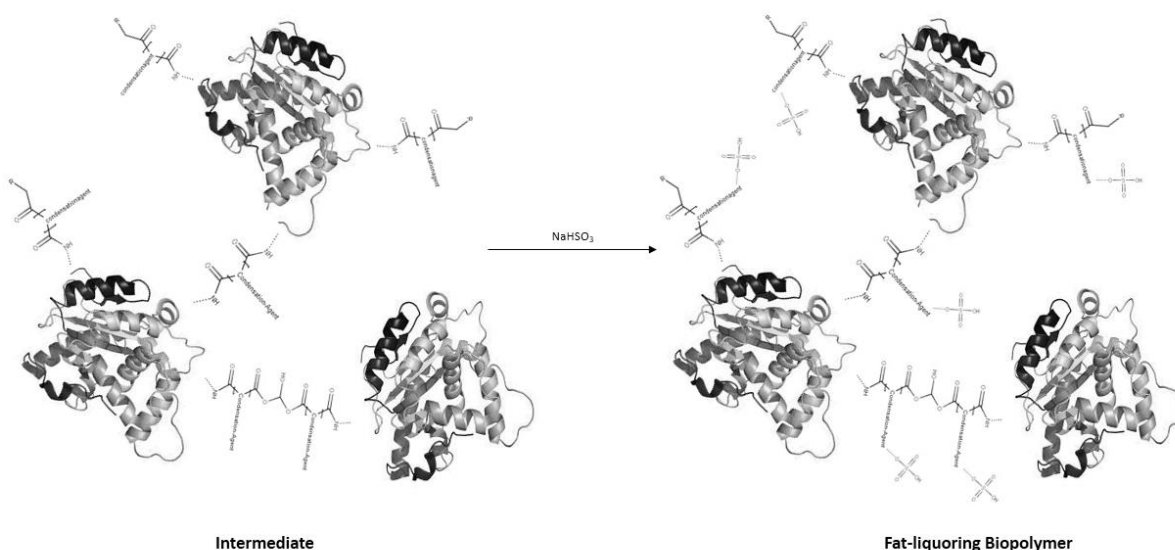
Scheme 4: Intermediate sulphitation (retanning and fat-liquoring biopolymer)

2.3   Biopolymers application in leather making lab test

Chrome tanned bovine leathers of a thickness of 1,1-1,2 mm were treated by the Biopolymers A, B, and C following the retanning formulation as reported below:

% Based on wet blue weight

Wetting Back: Ethoxylated surfactant
Retanning: 10% Biopolymer A or B or C or Standard (= 5% Acrylic resin + 5% Phenol syntan)
Neutralization: at pH 5.0-5.2
Fatliquoring :   3 % of Sulphited oil
Dyeing :   5 % of Acid Brown 425

2.4   Trials results

The leathers treated with Biopolymers showed very good performance in comparison with the standard chemicals (Acrylic resin + Phenol syntan).

Biopolymer A led to very full crust, with a fine grain and pleasant feel.
Biopolymer B led to a very tight and firm crust.
Biopolymer C led to a round and full leather with an excellent fiber lubrication effect.

All of the leathers showed a good dye ability: very even and more intense than the standard crust

Some physical tests were performed on the obtained crust, as reported in Table 1

Table 1. **Physical tests on leather crust**

	EN ISO	**A**	**B**	**C**	**Strd**
Tear Load (N/mm^2)	3377-1	93	85	110	89
Grain Strenght(mm)	3379	10	11	12	9
Light Fastness (blue scale)	105-B02	4	3/4	3/4	3/4
Softness degree *		3+	2	4+	3

A: Biopolymer A, B: Biopolymer B, C: Biopolymer C, Strd: Standard treatment.

Leather thickness: 1.2 mm.

*Values between 1-5.

3 Biopolymers Characterization

Proteins are complex natural organic compounds which may be characterized by different analytical and spectroscopic techniques (see below). In this work we will combine the information achieved by different methods in order to collect important data such as molecular weight and chemical structure.

The characterization of the proteins is necessary to provide an interpretation about the synthesis of the various tanning, retanning and fat liquoring agents. Moreover, the data collected are required to standardize the production and modification of the protein batches and provide a product as possible standardized from bath to batch.

Spectroscopic magnetic resonance ^{1}H NMR and FT-IR spectroscopy have been used so far to gather understand preliminary information on the chemical composition of starting materials, intermediates and final bio-polymer, deriving from animal biomass and synthesized by Codyeco S.p.A. A brief outline of the different technologies used for the characterization is given below.

3.1 Characterization by Nuclear Magnetic Resonance

NMR can probe protein dynamics, kinetics, and thermodynamics all at atomic resolution. This technique is greatly helpful for the characterization of proteins smaller than 25 kDa. (Dominique P. Frueh at al., 2013). NMR spectra were recorded by using a spectrometer Bruker Avance 300 working at a frequency of 300.13 MHz for the proton spectrum in deuterium oxide as deuterated solvent.

3.2 Characterization by Infrared Spectroscopy

Infrared spectroscopy is one of the classical methods for structure determination of small molecules. This is due to its sensitivity to chemical composition and architecture of molecules which makes it a useful technique also for highly complex biological systems such as proteins. (A.Barth, 2007)

FT-IR spectra were recorded by using a spectrometer Perkin-Elmer Spectrum One, in a range frequency 4000 a 400 cm-1. All the samples have been prepared in KBr.

3.3   Characterization by SDS-PAGE Electrophoresis

Molecular weight of the samples has been estimate by using a Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis (SDS – PAGE). This technique is widely used to separate biological macromolecules, usually proteins or nucleic acids, but it can be helpful for our purposes since it can provide a first estimate of the molecular weights of the starting protein and other processed samples and, at the same time, it can furnish detailed information for the selection of other techniques to be used in the future (GPC) to get closer and closer to the real molecular weight value.

^{1}H NMR spectra were obtained for all the starting materials involved in the production cycle in order to observe all possible kind of interaction among the reagents and the protein. In Figure 1 and 2below are reported the ^{1}H spectrum acquired for the animal biomass and glycerol in D_2O.

As expected for complex molecules the ^{1}H-NMR of the protein shows numerous unresolved multiplets. These signals between 0.7 and 4.7 ppm may be attributed of different CH_2 moieties due to the various amino acid present in the bio-polymer. A relevant signal is the singlet at 8.5 ppm, which is zoomed in the upper left corner of Figure 1 and is supposed to correspond to -NH amide protons of the protein from animal biomass. The intensity of the signal is relatively modest, which can be in accordance with the relatively low molecular weight of the protein. This signal is present also in the spectra of the intermediates, even if at different chemical shift.

This signal is important and characteristic for amide bonds which are present in animal biomass; it can be easily identified and usually is between 8.0 and 9.0 ppm; no superimposition with other signals is observed. A very weak unresolved multiplet is present between 7 and 8 ppm, characteristic of the aromatic part of the protein.
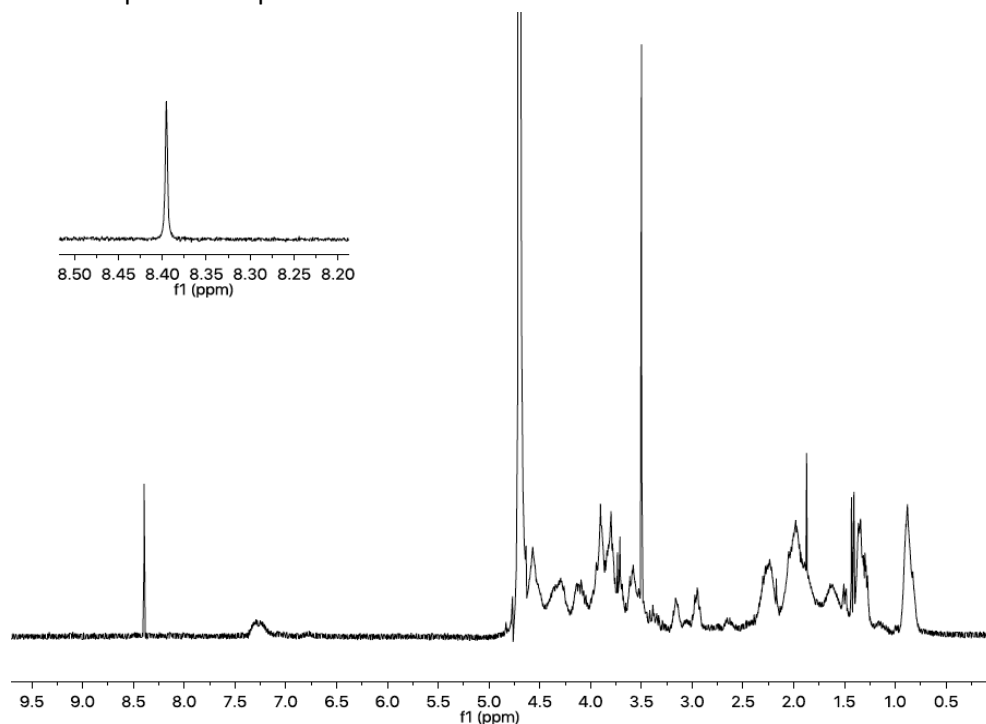
Figure 1: ^{1}H NMR spectrum of animal biomass in D_2O

The ^{1}H NMR spectrum of glycerol, reported in Figure **2,** shows very strong and sharp signals which make this compound easily detectable.
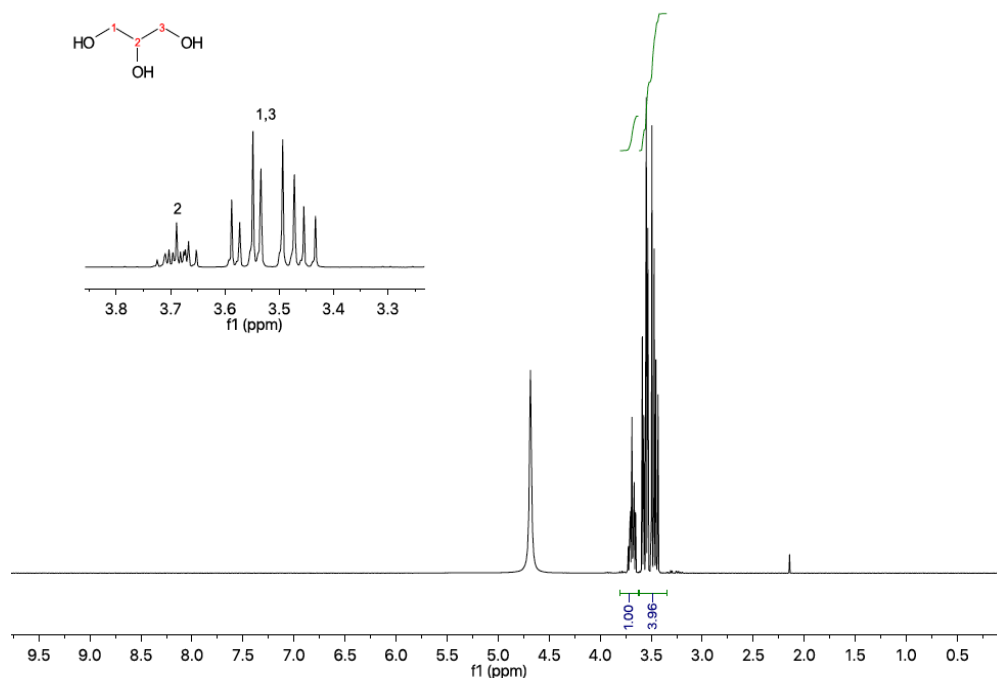
Figure 2: ^{1}H NMR spectrum of glycerol in D_2O

So far samples characterized by ^{1}H NMR are (see Scheme 1):

- the intermediate obtained by reaction of the protein with glycerol;

- the intermediate obtained by reaction of the protein with glycerol and a condensation agent;

According to the changes observed between the starting materials and the intermediate, it is possible to hypothesize that the reaction leads to the synthesis of stable covalent bonds and consequently stable Bio-polymers.

Data collected using ^{1}H NMR spectroscopy were confirmed by FT-IR spectroscopy. As a reference the FT-IR of the protein used as starting material is reported (Figure 3)**.**

This spectra is in accordance with data reported by D. Castiello et al. (2009).

In the intermediate obtained by reaction of the protein with glycerol and a condensation agentit is possible to recognize the detection of a strong band in the zone of esters C=O bond (1640-1690 cm^{-1}) which correspond to the interaction between glycerol and the condensation agent; it is possible to

observe a strong band in the region of amide N-H bond as well (1550-1640 cm^{-1}), which should correspond to the interaction between the animal biomass proteins and the condensation agent.

The estimation of molecular weight of the biopolymer has been obtained by using SDS-PAGE protocols for low-molecular weight compounds. The protocol differs for the concentration of both bis-acrylamide and the cross-linkers used for the preparation of the hand cast gels. The first estimate of molecular weight of the biopolymer provided by SDS-PAGE is a value below 35 kDa.
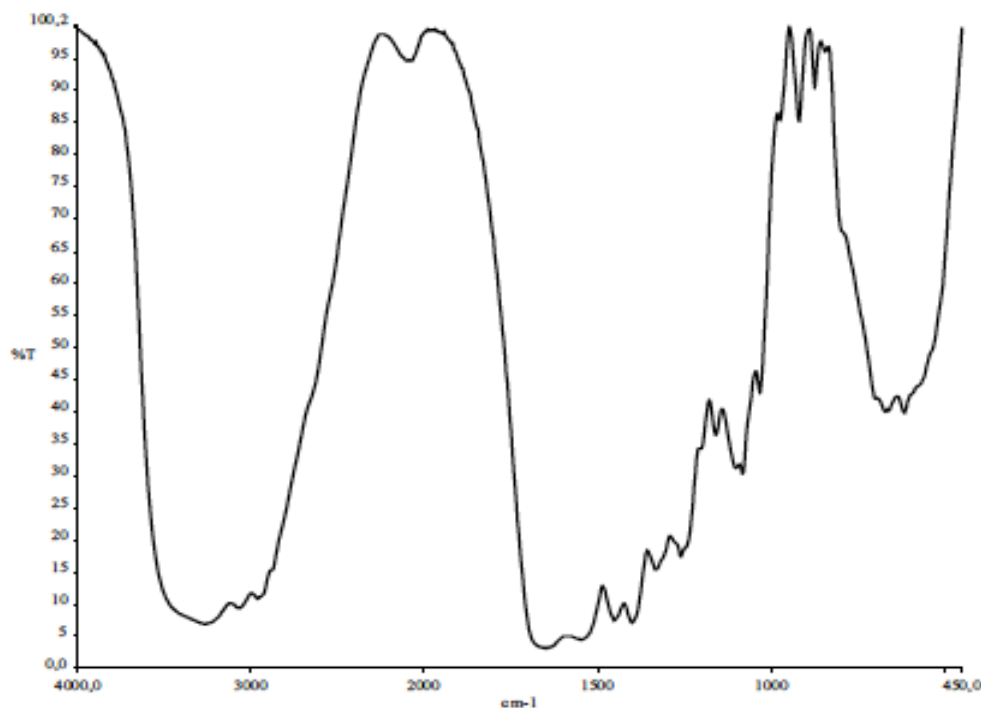
Figure 3: starting material protein FT-IR spectrum in KBr

4 Conclusions

Green and eco-friendly tanning agents based on Biopolymers will be developed within the LIFE BIOPOL project co-financed by EU. The new biopolymers are non hazardous chemicals and do not show any danger in manipulation and use in tannery process.

Several protocols involving polymerization, esterification, and sulphitation led to chemicals able to replace the traditional re-tanning and fat-liquoring auxiliaries. Macromolecular characterization of the biopolymers gave important indication on the synthesis procedure strategy and practical application of the products. As previously described, all laboratory trials conducted until now showed promising and encouraging results .

5 Acknowledgements

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SYNTHESIS AND PROPERTIES OF GRAPHENE OXIDE / HYPERBRANCHED WATERBORNE POLYURETHANE

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Hyperbranched waterborne polyurethane (HWPU) dispersion was synthesized by using acetone method based isophorone diisocyanate(IPDI), polyether polyols(GE-220), dimethylol propionic acid(DMPA) and terminal hydroxyl hyperbranched polymers as raw materials. A series of GO/HWPU composites were prepared by adding various amounts of graphene oxide (GO) through in-situ grafting polymerization process. The structure of the product was characterized by FT-IR, SEM, XRD, TG and AFM. And the mechanical properties of film formation, water absorption, hydrolysis resistance and conductivity were detected. The results were as follows: GO was grafted onto HWPU molecular. Crystalline resin was increased with the addition of GO. When 0.6% of GO was added, the thermal decomposition temperature of the pure HWPU film increased by 48.67 ℃ and tensile strength and elongation at break increased by 79.31% and 28.7% respectively. When the addition of GO was 1.0%, the volume resistivity of the composite film reached 4.75×10Ω·cm. Compared with the pure HWPU film, the four orders of magnitude reduced showing that the addition of Go can improve the polyurethane electrical insulation. As a result, the antistatic effect of composite materials was improved.

Key words：graphene oxide，hyperbranched waterborne polyurethane，in-situ grafting

1. Introduction

Graphene has 2×10^5cm^2/(V.S) electron mobility and 5000W/(m.K) heat conductivity coefficient. What's more, the highest bearable force of its nano unit reaches 2.9N, and its strength reaches up to 110~130GPa, and its specific surface area is also huge. In recent years, as a two-dimensional nanometer material, and due to its unique structure and prominent physicochemical property, graphene appears to be the most potential one among the synthesis of numerous functional materials（Stankovich et al. 2006; Novoselov K S et al. 2012;Wang et al.2012）. (Nariman Yousefi et al. 2012) adopted solution mixing method by mixing GO and PU and then adding a bit of hydrazinium and heat. In such a way, the Hydrogen bond was formed by the reduction-oxidation of graphene oxygen-containing functional group and amide group of PU chain terminal, making GO achieve molecule dispersion. By means of sol-gel method,Xin Wang (Wang et al. 2012) recombined GO modified by KH-550 and WPU. （Mingkai Liu et al.2013） dealt with CNT and graphene in the process of hybridization to form the three-dimensional structure. In such a structure, CNT played a supporting role on the layer graphene, thus avoiding the overlapping and agglomeration of different layers. After recombining with thermoplastic PU, the

electrical conductivity of the new materials has remarkably increased. （Mukesh Kumar et al.2013）synthesized the graphene-PU nanocomposites using graphene as a pseudo cross-linking agent.（S.H.Yoon et al.2011）prepared UV curing WPU/iGO nanocomposites.

Among many polymers applied into the functional grapheme, more attention was paid to hyper branched polymer for its unique structure and excellent physicochemical property. (Sivakumar C et al.2009) has prepared 4 kinds of AB_2 type monomers from four commonly used monomers with aside method and then used solution copolymerization and melt copolymerization methodto make two hyperbranched water PUs, hydroxy-terminated and amine-terminated. (Kumar et al.1996) utilize 3,5-Dimethyl benzoic acid anhydride to prepare AB2 type monomer by azide method and then using this AB2 azide, aromatic hyper branched PUs was amde through a series of reaction.(Xu Jicheng et al 2013)prepared steady HBPU using multifunctional polyester diol as soft segment, HBP as modified cross-linking agent, IPDI and DMPA as hard segment with in situ polymer method and then analyzed its structure and properties. Using melamine as core, DMPA, IPDI and diethanol amine et al. as main raw materials, Ning Sun (2011) prepared HWPU. (Dai et al.2014) prepared graphene/water PU-polyacrylate oligomer by using IPDI, polyether polyol, GO, DMPA and ethoxy MA as raw materials and then through UV-curing, UV-light cured type GO/water PU-PA composites was prepared. The composites present good mechanical properties and wide spread application value.

The graphene's micro-structure leads to the intensive overlapped trend between graphene layers resulting in the aggregation in polymers. In this paper, GO was grafted onto PU prepolymer by means of in situ polymer method. Then using hydroxy-terminated hyperbranched polymers as the modified crosslinking agent, with the consideration of its three dimensional ball structure, we prepared a series of GO/HBPU. The study not only enriches the theory of hyperbrached polymer/graphene composite and achieves the mutual complementary of combined materials, but also solves the problems of hard dissolution and easy agglomeration of GO. Hence we can make graphene uniformly disperse into polymer and improve the mechanical properties and dielectric properties of WPU materials.

2. Material and methods

GO was prepared by improved Hummers method in this paper. A certain amount of GE-220 and IPDI was added to a three-necked flask equipped with a mechanical stirrer, thermometer and a reflux condenser. And then catalyst (organobismuth) was added when the temperature reached 60℃. When the mixture was heated up to 85℃, GO was added and the temperature of the flask was kept at 85℃for 3 h and then cooled to 75℃. After Moderate DMPA was added, the mixture was heated up to 85℃and kept this temperature for 1 h. When the mixture was cooled to 60℃, a certain amount of hydroxy-terminated hyper branched polymer was added and reacted for 30 min. When the mixture was cooled to room temperature, certain amount of TEA was added reaction for 30 min. Finally, after adding some deionized water and stirring the mixture at high speed for 30 min, GO/HWPU composite was obtained.

The TEM analysis was carried out with a Hitachi H800 transmission electron microscope (TEM) at an accelerating voltage of 120kV. All samples were placed on copper grids and then dried by incandescent lamp irradiation for the observations. The FT-IR analysis was carried out with a BRUKER VERTE70 Fourier

transform infrared spectrometry (FT-IR) with wave length from 4000 to 400cm^{-1}. The X-ray Diffraction (XRD) patterns were scanned by using a D/Max 2200PC diffractometer (Rigaku, Japan) at room temperature. Conditions are as follows: θ accuracy is ±0.002°. Scanning range is from 5° to 80°. Heat stability of GO/WPU composites was measured by a STA449F3 differential thermal analysis (DTA) (NETZSCH, Germany) in nitrogen atmosphere. Measuring conditions: Nitrogen airflow rate is 50 ml/min. The sample was heated from room temperature at 10℃/min. Measuring range was from 30 to 600℃.After the brittle fracture of GO/WPU complex film in the liquid nitrogen, the sample was coated with gold. And the fracture morphologies of GO/WPU complex film were examined in a scanning electron microscope (SEM) at an accelerating voltage of 2kV. Also the product mechanics, surface resistivity and heat stability were carried out measurement.

3 Results and Discussion

3.1 GO TEM

As shown in Figure 3-1, GO appears to transparent yarn-shaped. The transparency differs due to its thickness of lamella, indicating not all the GO obtained in this experiment are in different forms, some are single, some are overlapping. The distinct foldings on the surface of the GO is probably caused by the weakening acting force between layers from functional groups containing oxygen after oxidation.
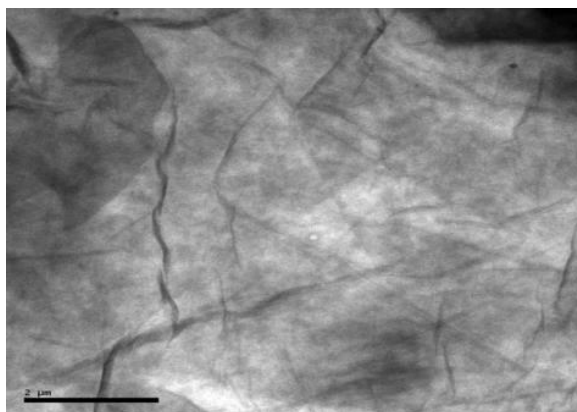
Figure 3-1 TEM micrograph of GO

3.2 FT-IR spectrum

From Figure 3-2 (curve a and b) , the FT-IT spectrum of graphene is relatively flat. The C=C stretching vibration band appears at 1628 cm^{-1}. Compared to the traditional graphene, the GO prepared by improved Hummers method has more absorption bands. The absorption bands appearing at 1021cm^{-1} , 1382cm^{-1}, 1628cm^{-1} and 1723cm^{-1} are stretching vibration of C-O-C bond, deformation vibration of O-H bond from C-OH, stretching vibration of C=C and C=O respectively. And the wide range of absorption band at 3380cm^{-1} is the absorption vibration frequency of –OH group or partly absorbed water molecules. From Figure 3-2（curve c and d）, at 1730 cm^{-1},it can be seen that the strong stretching vibration characteristic absorption peak of isocyanate C=O appeared, indicating the existence of PU chain segment. The band at 2945 cm^{-1} is C-H strong characteristic absorption peak from carbamic acid ester. The -OH absorption band at 3340cm^{-1} disappeared. However, at 3315cm^{-1} hydrogen bonded N-H

stretching vibration characteristic absorption peak appeared. And N-H bending vibration absorption peak appears at 1533cm^{-1}. Such results show that –NCO and –OH have completely reacted and has produced urethane group. The band at 1727cm^{-1} is the characteristic absorption peak of ester carbonyl because the hydrogen bond formed from ester carbonyl and urethane group makes the characteristic absorption peak shifted. The band at 1242 cm^{-1} is C-O stretching vibration characteristic absorption peak of carbamic acid ester and ester bond. The band at 1457cm^{-1} is deformation vibration characteristic absorbing peak of methylene. But there also existed absorption peak at 1730 cm^{-1} due to the existence of GO carbonyl. And –OH peak at 3340cm^{-1} disappears, indicating that hyperbrached polymer has been through a full reaction. Hydrogen bond of N-H vibration characteristic absorbing peak appears at 3315 cm^{-1}, indicating that –OH has reacted with WPU. And all these above–mentioned results showed that GO/HWPU composite can be obtained successfully.
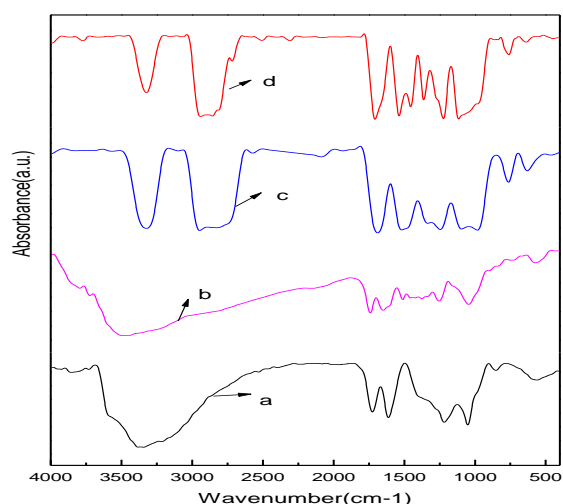
a-GO, b-graphene, c-HWPU, d-GO/HEPU-0.6%
Figure 3-2 FT-IR spectra of graphene, HWPU and GO-HWPU-0.8%
3.3 SEM characterization

Microscopic morphology of dispersion system film of GO, HWPU and GO-HWPU is shown in the Figure 3-3. In Figure 3-3(a), it can be seen that the layer and keratin structure, made after strongly oxidation of the graphene, has been removed. Compared with Figure 3-3(b, c and d), HWPU surface is smooth and almost free of cracking. GO alters the HWPU structures and makes some creases in the HWPU layer surface. From figure3-3(c and d), GO creases and the layer surface of HWPU interweave with each other, indicating the stronger interaction between GO and PU. When GO content was 0.6%, a small agglomeration appeared and the agglomeration became heavier and heavier (see Figure 3-3d).
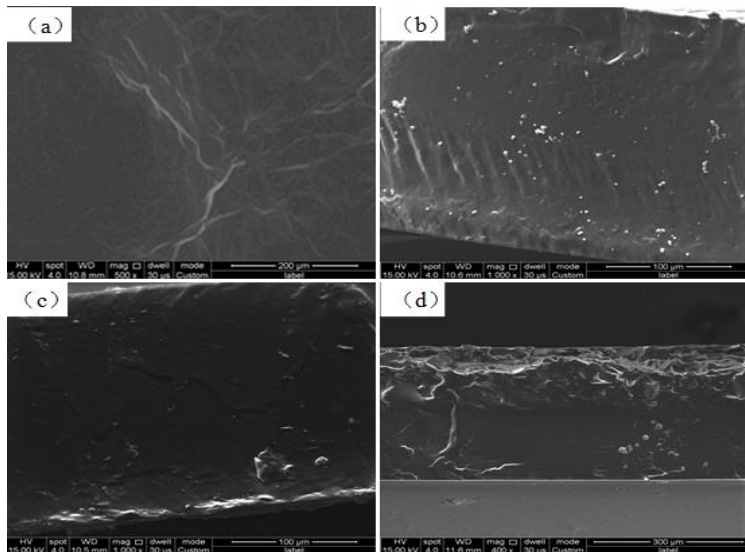
Figure 3-3 SEM micrographs of GO(a), HWPU(b), GO-HWPU-0.6%(c) and GO-HWPU-0.8%(d)

3.4 XRD analysis

XRD patterns of GO, HWPU and GO-HWPU 0.8% are shown in Figure 3-4. GO diffraction peak appeared in $2\theta=10.5°$ corresponding to the crystal face of GO (001). According to Bragg equation and the diffraction peak conversion, GO interlayer space was calculated as d=0.84nm. As for the pure HWPU film, broad diffraction peak appears in $2\theta=18.4°$ according to figure 3-4(curve b), But the diffraction peak of GO-HWPU-0.8% film appears in $2\theta=20°$. Compared with the pure HWPU film, its diffraction peak shifts toward right. These phenomena showed that GO addition can increase the resin crystalinity.
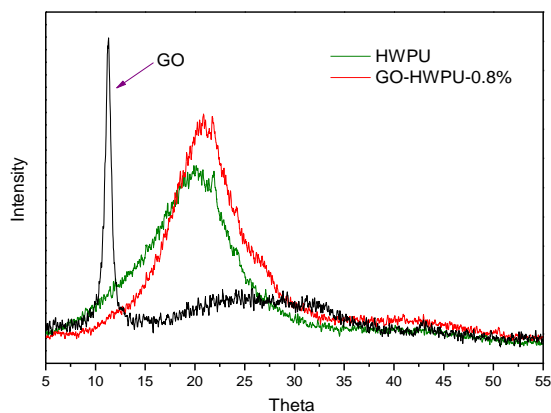
Figure 3-4 XRD patterns of HWPU and GO-HWPU-0.8%

3.5 TG analysis

TG curves of the GO-HWPU composite films are shown in Figure 3-5. Curves A,B,C,D are the TG results of composites when GO additive is 0.4%, 0.6%, 0.8% and 1.0% respectively. Compared with pure HWPU film, GO/HWPU composite film has higher heat stability. When GO content is 0.6 %(curve B), the

thermal decomposition temperature of composites enhances 48.67℃ than pure HWPU film and all the films' thermal decomposition temperatures remains above 250℃. And when residual solid is 5%, the thermal decomposition temperatures are even above 400℃. Therefore, GO additive obviously improves HWPU' heat stability because GO has excellent heat-conducting property, big specific surface area, small particle, and lots of active groups on the surface, which increase physical and chemical combination between PU and GO and limit the chain segment movement, hence improving the heat resistance of GO/HWPU composite material.
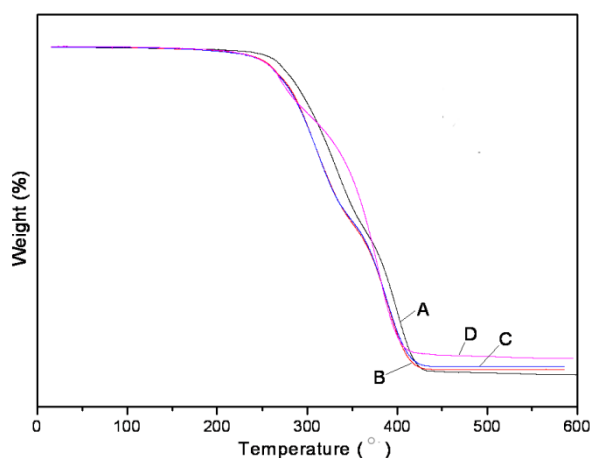
GO dosage: A-0.2%；B-0.4%；C-0.6%；D-1.0%

Figure 3-5 TG curves of GO-HWPU composite films

3.5 Effect of different GO dosage on GO/HWPU composite properties

Table 3-1 shows the effect of different GO dosage to the properties of GO/HWPU composite material. With the addition of GO, the apparent viscosity of GO/HWPU composite emulsion had no change. While its solid content increased with the addition of GO content. When GO content was 0.6%, the material tensile strength and elongation at break of composite increased by 79.31% and 28.7% respectively than pure HWPU film . This indicated that moderate GO additive is conducive to improve mechanical properties of HWPU film but has little influence on water-absorbing quality of GO/HWPU composite material. When GO content is 1.0%, the electrical resistivity reduced four orders of magnitude than pure HWPU materials, showing that GO/HWPU composites presented better anti-static property with the increase of GO.

Table 3-1 Influence of different GO content on GO/HWPU composite properties

GO Content/%	apparent viscosity /mPa·s	solid content /%	tensile strength /MPa	elongation at break /%	water absorption /%	suface resistance /Ω·cm	hydrolysis resistance /24h
	37.5	23.97	0.87	573.38	27.72	8.01×10^{12}	no change

0.2	40.4	24.13	0.96	602.32	44.02	4.21×10^{12}	a little bubble
0.4	39.5	25.44	1.51	639.78	46.02	3.63×10^{11}	no change
0.6	41.5	26.4	1.56	737.68	48.46	7.76×10^{10}	a little crack
0.8	42.4	25.62	1.52	701.25	48.26	9.68×10^{9}	color becomes lighter, a little crack
1.0	40.2	25.52	1.48	689.25	46.39	4.75×10^{8}	crack increases

4. Conclusions

(1) GO with 0.84nm interlayer space was prepared by improved Hummers method. At room temperature, the homogeneousdispersion GO liquid was obtained from acetone, which was used as dispersion solvent and taken ultrasound for 15min (600W).

(2) FT-IR analysis showed that GO was successfully grafted onto HWPU molecule by in situ polymerization method to produce GO/HWPU composite materials. XRD analysis indicated that GO additive can increase resin crystalinity.

(3) With 0.6% GO content, the thermal properties of composite materials increased. Its thermal decomposition temperature increased by 48.67℃ than that of pure HWPU films.What's more, its tensile strength and elongation at break increased by 79.31% and 28.7% respectively than that of pure HWPU film. All these showed that moderate GO additive was conducive to improve the mechanical properties of pure HWPU film, but had little influence on the water-absorbing property of GO/HWPU composite materials.

(4) With 1.0% GO content, the electrical resistivity of the composite materials reduced four orders of magnitude than that of pure HWPU materials, indicating that GO/HWPU composite presented better anti-static property with increase of GO content.

5. Acknowledgements

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INNOVATIVE AND BUILT-IN SURFACE TECHNIQUES ON LEATHER FOR VALUE ADDED PRODUCTS

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With growing trends in fashion and smart production the essence of craftsmanship on a leather product is neglected beyond its "classics". This paperwork examines the past techniques of surface embellishments and finds a way in exploring directional usage of the surface in the product lines. Inspired from the zero-waste pattern making of Mark Liu, where the darts are inverted and are further cut along the fold to act as a decorative element throughout in his collections. Driven by this thought, the base concept of inverted darts is subtracted to pin tucks and where the explorations are made from the folds of the pin tucks. The research report follows this innovation towards embellishing the surface of leather through explorative and modelling research methodology. Keeping the robust aspects of built-in embellishment, the researchers have brought innovations in the traditional techniques of tessellations using motifs that are repetitive and progressive are depicted through the pin tucks that results with both tactile and visual textures. Furthermore the techniques that involved the flesh side of leather reveal through the pin tucks accentuated the latter side of leather. Leveraging such innovative techniques would change the perception of leather as an embellishment material. Though the fabrication may seem slightly exorbitant; but with increasing innovations in material developments and the desires of the consumer to gape for newer looks, it is the responsibility of the manufacturers to deliver proficiencies in their trade. Thus, the techniques dealt in this research work ease out these responsibilities of the manufacturers to offer a range of innovative and value added products to the fashion consumers. The leather jacket with one of the techniques used, momentarily directs to the surface embellishment appreciating the essence of the same. This brings us down to the discrete analysis to the paralysed thoughts for the "classic" product lines in leather could be changed with innovative surface techniques. As the technique is pristine, the scope for future work ensures in greater extent for innovations in fabricating the concepts.

Keywords: Built-in-techniques, Leather embellishments, Leather, innovation, Pin-tucks, Surface design, Value added products

INTRODUCTION

Traditionally leather products are used the way it is with minimal embellishments on the surface or none at all, the classics later accompanied with variations with the trims used and further more the surface is cut to strips and altogether woven, and the other techniques practiced on leather include

appliqué, patchwork, cutwork, embossing and etching. Although these techniques have been vaguely marketed the high end brands often cripple between the classics and basics thereby fixing an image for leather products.

This paper discusses the essence of leather being a versatile material and its ability to adapt to in built surface techniques using the combination of grain side and flesh side.

LITERATURE REVIEW

Picken (1922), had offered this book with an in depth knowledge to woman in the areas of dressmaking. It contains the pattern making of garments and the construction of the same where more detail is given to the process of constructing the garment. The author introduces various techniques in sewing of everyday garments along with a detailed description on its pattern making. They include close fit garments, corsets, lingerie and also draped garments to name a few.

Kathy and Paoletti (1987) examine the popularity of crazy patchwork quilts waned at the end of the nineteenth century, the decorative features which characterized them also declined in quantity and quality. The purpose of this study was to test this hypothesis as means of understanding how needlework styles may change throughout the duration of their popularity. In order to determine the characteristics of the crazy quilt fad, a sample of 37 dated Victorian crazy quilts was examined. A method was developed to quantify the features found on the sample quilts, such as average number of patches and different embroidery stitches for a given area on the quilt. The distribution of the number of quilts by year of origin supported documentary evidence of the fad's appearance, duration and decline. Deviations from the popular form of the crazy quilt did not occur until after the fad had lost its mass appeal. The historic approach that led to the early crazy quilts, with no. of stitches, colors, fabrics and pictorial motifs were so much higher that the samples required for systematic form analysis and other research areas were limited placed on samples as the geographical limits encountered by researchers with little money for travel, joint research on a national level should be undertaken.

Lawrence & Marie (2002), have determined if the aesthetic qualities associated with the traditional hand surface design techniques of batik and discharge could be retained using graphics programs and digital printing technology. The digital batik and discharge fabrics were created and evaluated by both non-experts and experts. At first, the literature was reviewed to gain expertise in traditional surface design, batik, discharge, printing methods, industrial production of batik and discharge, digital printing, and evaluation of aesthetics. Considering these techniques, new method was developed to achieve the handcrafted aesthetic qualities digitally for industries. The aesthetic qualities of batik and discharge were defined through hands-on exploration of the techniques in conjunction with critical evaluations from fellow artists. Evaluation tools were developed for batik and discharge based on the defined aesthetic qualities. Handcrafted and digital batik and discharge fabric samples were evaluated by non-expert and expert subjects. The digital samples were successfully created through graphic programs and digital printing. The article shows that the digital batik and digital discharge samples were successful in achieving a majority of the aesthetic qualities associated with handcrafted batik and handcrafted discharge.Hence, the use of digital fabric printing in textile and apparel companies is increasing. Continuing research on increasing color quality, speeding up production rates, development of new inks,

and increasing the number of print-heads will facilitate adoption of this technology. Thus giving directions for new and innovative techniques.

Borman et al, (2004) had examined the ways for digital ink-jet printing on textiles and the advantages it offers to textiles industry and consumers in comparison with conventional printing. The paper also reports on some of the problems associated with this technique. One of the important issues associated with digital ink-jet printing on textiles is speed and reliability, as this has commercial implications for the industry. Further research has been carried out to establish the issues surrounding the print quality and implications of designs. The paper will concludes with the quality of the ink-jeet printing on textile based on its assessment. The project has brought together a range of technologies and has been successful in most aspects. The designs and concepts that were proposed have effectively been tested and a number of companies have expressed interest in developing the prototypes further. Prototyping of the vision system has been done online using paper substrates and tightly woven textiles. Work so far has been very successful in these areas and there is optimism for taking the ideas forward to a wider range of textiles substrates.

Pabst et al (2008), begin with the study of the significant parameter that attributes to the accurate bending behavior of cloth simulation with respect to the bending stiffness of a given textile. Past research work has proved that fast and authentic reproduction of the effect of bending in cloth simulation systems treat the textiles as consisting of a single, homogeneous material. The effects of seams, interlining and multilayer materials have not been addressed so far with respect to cloth simulations. Recent work showed that the bending stiffness of a textile is greatly influenced by the presence of seams and that a good cloth simulation system needs to consider these effects. This paper work shows how accurate modeling of bending and seams can be achieved in a state-of-the-art cloth simulation system so as the current system can make use of measured bending stiffness data, and also allowing intuitiveusercontrol, if desired. The data were verified using virtual draping tests and garments in the simulation and comparing the results to their real-world counterparts. Furthermore the results provide heuristics derived from measurements that can be used to approximate the influence of several common types of seams. The method is indeed capable of capturing the essence of the non-linear bending behavior of fabrics. The performance impact is minimal. Even experimental evidence and data demonstrate that the approach is able to capture key aspects of the effects that the presence of seams in textiles causes. However, seams and fabric bending are far from being fully understood, and thus a number of promising directions for future work remains open to the article.

Laura Curran (2009), analyses the visual culture during the years 1450-1550 at the Lune Nunnery, concludes that nuns were living lives outside the dominant norms and traditions of the Catholic Church's male patriarchal cosmology. The paper discusses the embroideries as living histories--rather than as art objects in order to come to this conclusion. The embroideries show the nuns at Lune have constructed a cosmology that unites the realms of spirit with matter, heaven with earth, and body with soul. The importance of studying the embroideries in this way will change how we experience the recording of medieval women's history. Because of the vast amount of textile work produced by women, this field of study contributes significantly to our limited understanding of both the production of the nun's needlework and the lives of the women who created it. The needle art of convents will provide a distinct

framework to view the role of female monasticism in the middle Ages. This paperwork evaluates productivity of these techniques and their active meanings and doesn't evaluate the artistic merits of the embroidery by measuring its forms, color or style.

Gibbons, (2009) had studied the eye and hand movement of the people associated with cloth sorting task. Both eye fixations and hand movements were tracked during the experiments. The eye and hand movements were categorized and present alone a time line. The observations are consistence with a model of task driven covert global processing of the scene followed by overt sequential processing of fixated areas of interest for grasping. A computational model is presented.The analysis of eye movements led to the development of a general computational model for the selection of grasping points during a sorting task. A number of details of this model need to be developed or investigated further. In particular the global image-processing for the selection of grasping regions of interest and also the processing to shape. An interesting side effect of the implementation of such a model, which is the generation of robot eye movements quite similar to that of humans performing a similar task. For the observer this may make the robot "understandable".

Liam Revell (2010), implores the necessity of decoration and decorating on a fashion design practice, exploring the transformative effects of both in relation to surface and structure.

The oblivion fact of decoration being considered only an after-effect, an element that can be differentiated from the object it adheres to is counter parted by in this project where the author considers decoration as having its own function – the ability, through its link to the 'exterior' world, to transform the shape, appearance and materials of forms by modifying their façade. Decoration can be adjunct to the surface or structure of garments but it need not be an arbitrary consideration, and ultimately that can be the catalyst in the design process either by representing an idea or through analyzing its methods of manufacture to inform ways in which disparate elements can be combined to create a singular outcome.

Nilsson (2011) had briefed the research areas on developing smart fabrics have successfully surpassed the initial stages, exploration and testing should be done to raise the bar. In this paper, the understanding of the base material is done to further take it to the levels of exploring the abstract material that can enter traditional design practices and what role smart textile can play in the design of our environment. This paper describes a process of the maturing of a research field from the initial explorations designed to give a basic understanding of what is at play, to formulating more specific questions and designing more focused explorations. The research program proposed here is still, however, a sign of an early stage in a research field. It is a program formulated to find ways for the new materials possibilities to reach a greater audience in parallel with studying in what this could mean for the design of textile products and environments. The research program not only demarcates between the maturing of the research within smart textiles, but also to formulate an invitation for others to participate.

Baba (2012), has discussed the hands on experiential practice of textile printing by using the principles of repetition and, also how unique surfaces could be created using visual cultural identity combined with it. A group of students with art, fashion and textiles background developed patterns inspired from visual

identities of their culture using Computer Aided Design (CAD) and subsequently using silkscreen with other printing and dyeing techniques to create artistic printed fabrics that reflect their cultural identity. In doing so, a more simpler and sophisticated method adapted to create the patterns resulted in more variations in the surface style and lot of explorations of similar concepts were witnessed and it also helped them to apply the skills they have learned to produce a printed fabric. The examination of this study case indicated that the incorporation of different textile printing processes could therefore facilitate a novel creative approach to textile printing design. At the same time the multicultural origin of this group of undergraduate designers created a unique contextual basis for intercultural dialog during the creation process. The paper outlines this practice-based method as a constructive educational approach to expand the students' skills by developing the creative fusion of traditional craft techniques with artistic practice, and the significance of creating value and emotion by expressing their cultural identities in printed fabrics through the practice

Ruth Singer (2013), has delivered the book with an in-depth exploration of a wide range of fabric manipulation techniques used for decorative effects which can be applied to fashion or interior projects, or incorporated into embroidery, quilting and other decorative textile projects. Many of the techniques are inspired by historical and contemporary techniques.The book is divided into three sections; Pleat and Fold, Stitch and Gather & Apply and Layer. All of these techniques explores the extent through its base concept.

A.K.Choudhary and Amit Goel, (2013), address the effects of some fabric and their sewing condition while constructing an apparel. Underlining the demands on the aesthetics of fabric while keeping the quality on a low note, the study explains the necessity to focus on the quality of the fabric that contributes to the sewing conditions in the construction of the garment. As the fabric quality influences not only the quality of the garment but also the ease with which a shell structure can be produced out with flat fabric. The specifications of fabrics for apparel manufacturing are laid down in terms of primary and secondary quality characteristics. The primary quality characteristics are static physical dimensions and secondary characteristics are the reactions of the fabric to an applied dynamic force. The production of high quality fabrics not only gives comfort to the wearer but also helps working with ease during the manufacturing processes and brings to almost defect-free garments. With these finding, awareness for wearing the right quality fabric should not only hit the minds consumers of but also the manufacturers as the selection of right fabric contributes to the quality for sewing conditions itself.

Arora et al. (2014) revive the craft, Chamba embroidery, & its traditional techniques also the changes that had taken place in order to revive the craft. The craft was characterized by spontaneity and rhythm rooted in the soil of Himachal Pradesh. It was believed that Chamba
region witnessed the tradition of embroidery from early times. Probably the tradition of embroidery started with basic line work, simply human figures and limited subjects known as folk style. Later on, the nice composition, soothing colours, fine stitch work was carried out.
With time the addition of varied subjects made it so popular that by the mid of 18th century the art of embroidery was patronized by the rulers of the Himalayan region. Once royalty started taking an interest in Chamba *rumal*, it's popularity reached new heights. In terms of the difference in selection of subjects, line drawing, use of soft colours with good composition and variety of themes was evident in

these classical style coverlets[1]. In fact such coverlets were often reminiscent of the *pahari* style of miniature painting.

The distinctiveness of Chamba embroidery lies in its double satin stitch which was *dorukha*. The fabric used was unbleached muslin and threads used were untwisted silk dyed in myriad and mellow colours. The most fascinating aspect of classical style *rumals* was the depiction of vast subject theme which was based on miniature paintings. In folk style the simplest of designs purely according to the imagination of embroiderer were seen. Chamba embroidery was not only confined to *rumal*s but was also seen on religious textiles, apparel and other utilitarian household objects.

The study identified the different types of stitches used, analysis of direction of stitches as well as outline stitches used in earlier times from museum pieces. The scope of work also included various factors resulting in stitch variations and intervention with artisans. The detailed analysis of museum material revealed the traditional ways of practicing the craft. The following features were identified : i. Execution of the craft, ii. Identification of stitches used traditionally, iii. Analysis of direction of stitches, iv. Types of outline stitches, v. Factors resulting in stitch variations, vi. Intervention with artisans.

Sandhya(2014), Associated the current trends where going eco-friendly has become mainstream, but taking them into design practice has always been besmirched. As the authenticity of using eco- friendly products gains more importance, the focuse through developing the same in turn increases. The growing concerns about environmental issues are playing an increasingly important role in the textile industry. The use of eco-friendly dyes, as one of the means to create hand painted silks to protect the environment. The silk craft is a very noble art, exalts the rich and helps the poor. The technique of decorating cloth with a free hand application of color or using a tool which does not of itself produce a repeat image has been practiced mainly in the east. "Painted silks" evoke all the mystery and magic of the orient or the eastern cultures. As a Garment or fabrics, silk drapes beautifully, and the weaves and weights of the different texture will affect the flow and line of each piece. The study aims at Hand painting silks with eco-friendly dyes with different solvents/buffers with limitless variety of exciting designs. The techniques used are easy, simple and can be finished at home which do not cause harm to the environment and ecology. Hand paintings express a rarer fineness in which the forms radiate a warm and gentleness through the techniques used. The colors are exuberant and bright and speak volumes for the depth, the fine skill and passion which reflect ethereal beauty. The richness of the ancient art, showcased through the contemporary designs developed in this study brings in the freshness of intricate designs, which is seen through art practiced on silk and the colors used. A preliminary pilot study helped in gathering the trends associated with it. The finished samples were evaluated for mechanical properties, subjective assessment, Eco parameters of the selected dyes Spectro-photometric assessment to find out the change in color properties. The seven techniques of Hand painted fabrics were used for designing and development of Crepe silk and silk chiffon Kurtis, Sarees, blouses and Accessories. Both the textile and non-textile respondents were surveyed to study the acceptance of crepe silk and silk chiffon Kurtis, sarees, blouses and accessories. The developed products with special eco-friendly characteristics when introduced into the market will have bright future of using unique hand painted products.

Prendergast, (2014) explains about various sewing techniques that help in the design process through the ability to turn a two-dimensional design into its three-dimensional shape where realization comes from having mastered basic sewing techniques. Sewing Techniques simplifies the often complex techniques that lie behind this process by arming designers with the precise information and skills needed to undertake each task. Designers who develop a basic understanding of the processes involved in sample and garment construction often produce successful outcomes based on a more experimental and creative approach applied during the product development process. This book explores the essential equipment needed - from fabric types to diagrams identifying component parts of a sewing machine, to machine operation. Each section concludes with a project, progressing from easy to challenging. Fabrics for each project give designers the opportunity to handle different fabric types and understand the complexities of sourcing and selecting.

Mihyun et al, (2014), examined the process of developing a carpet prototype using innovative and sustainable sampling techniques. A sample carpet tile was developed abiding the sustainable sampling process. Since only a sample of the carpet tile was developed, the implementation stage was not applicable and was replaced with a deliberation stage. The deliberation process for sustainable sampling was evaluated based on the five major performance categories of the sustainable carpet assessment standard, Public Health and Environment; Energy and Energy Efficiency; Bio-based Content, Recycled Content, and Environmentally Preferable Materials; Manufacturing; and Reclamation and End of Life Management. This study fills a gap in the current literature since it incorporates a case study of the design of a sustainable interior product. Previously, few studies have tracked the inception and development of a carpet product through its design phases but no evidence is supported for sustainable sampling method of color imparted carpet tiles. Utilizing sustainable sampling in a custom colored carpet tile is anticipated to reduce environmental impacts related to the performance categories of public health and the environment; energy and energy efficiency; bio-based content, recycled content, and environmentally preferable materials; and reclamation and end of life management. More studies about examining custom color application and sustainable prototyping of textile and non-textile products will be necessary to fill a gap in existing design prototype research and to reduce waste throughout the design industry. This study fills a gap in the current literature since it incorporates a case study of the design of a sustainable interior product.

Meenu & Neha (2014) have addressed the changing trends in Mojari craft in Rajasthan. A comparative study is made across the regions to find the difference in materials and ornamentations used on the traditional footwear. As Rajasthan has a rich and long tradition of leather crafts. Each village had its families of leather workers. The region influence on the footwear gives various trends within the same style. In Rajasthan there are about a hundred thousand households engaged in the production of this traditional hand stitched shoes made out of coarse vegetable –tanned leather, which are known locally as Mojari. The best varieties are found in Jaipur, Jodhpur, Barmer, Bikaner, Jaisalmer and Dausa. In Jaipur the embroidery on the Mojari is done on velvet while in Jodhpur on leather with proficient golden ornamentation speak of gorgeousness and high skill of artisans. The distinctive design variations is found in the pattern making itself where the previously non-directional mojari is now being made with a left-right distinction although still using the three-piece last integral to indigenous footwear construction technology. – Conforming patterns to impart more accuracy and enhanced comforts – Contemporary

styles and designs detailed with the existing skills. Variety of embellishment techniques are used for the uppers. Appropriate mechanization in the processes of stitching and finishing that improves the production speed and quality and also use of alternative cheap materials to add variety and reduce the cost. The customized versions of these mojaris would help the tradition to always be in trend and win bread for the artisans.

Mona Verma*, Seema and Khambra K, (2015) have appreciate the ancient art of embroidery where the basic sewing was done through needle and thread through adapting various methods for surface techniques. With the evolution of numerous techniques in the past the usage of the same has faded over the crisis of mass production and the adaptationof styles that have merely any embellished surface.It focuses on reviving the art in upcycled textiles while balancing the colors and material subtly. The data collection was done through survey and descriptive methodology to collect information of the practices followed to reuse discarded textile and the embellishment that are been used. The data collection helped in the preferences of the breakdown of various surface techniques being practiced. And it is evident that the techniques haven't variated far much from the basics. The research demands that new techniques could be experimented and put to use in having scope for future. In doing so the cost of the upgraded textile with the surface embellishment would be more commercially viable and accepted for the increased price.

SUMMARY

The study made had given a direction in which the leather products could be embellished using different techniques. Unlike the explorations in textiles that have been immensely practiced and appreciated when the same was applied on leather, Also the research over the recent years explains that how the development of new techniques have headed towards the idea of mass production while still most of them revived few craft techniques and their advancements in manufacturing.

RESEARCH GAPS

Leather being a versatile material has been least explored in developing surface techniques in reality to its sophisticated classic style lines. A new technique that could be implemented on leather products would open to new areas of research and implementation thereby creating greater demands in inclining the buyer behaviour towards innovations in the value added products.

RESEARCH OBJECTIVE AND METHODOLOGY

For the exploration of the techniques, modelling research methodology followed by design research and prototype development is applied in this paper.

The explored techniques include the base concept of developing the shapes from pin tucks, which include:

1. Cut and open

In this technique the shapes are cut along the edges so as the resulting panel opens to with the alternate surface. Here in the following explorations the technique is overlooked with its ability to adapt to different styles and shapes.

2. Cut, open and paste
   In this technique the shapes are cut like the previous one along the edges that reveals the latter side of leather and the same is further pasted on to base to give a close contradictory in the surface's color and texture. In doing so, the visual texture could be developed along all the directions to the resulting patterns.

3. Cut, fold and paste.
   The pin tucks are a deeply slit leaving 1mm from the stitch line at desired intervals so as the individual shape is folded across with the help of these splits.

4. Cut, open, sew and fold
   This technique brings 3D forms, when the stitch line is close and shapes are smaller on the freshly opened pin tucks. The immediate difference in the surfaces on the small shapes captures the surface visual texture and tactile texture instantly.

5. Cut, open, smock
   The concept of smocking is interpreted by joining the edges of each shape that are cut along the pin tucks. This gives a detailed 3D form along the linear lines of pin tucks where the variations could be made along.

6. Cut, open, sew and open
   The technique explores ways in which a shape can be molded through construction. The concept of layering is illuminated through the shapes cut along the edges.

7. Cut, open, sew, smock
   This technique explores ways in which the entire swatch could be acting to change the direction of the patterns irrespective of the linearity of the base concept used.

DESIGN RESEARCH AND PROTOTYPE DEVELOPMENT

Prototypes bearing these techniques were executed through following the base concept of pin-tucks.

TECHNIQUE: CUT AND OPEN
STEP 1 : Pin tucks
STEP 2 : Cut along the edges
Step 3 : Open along the cut edges.
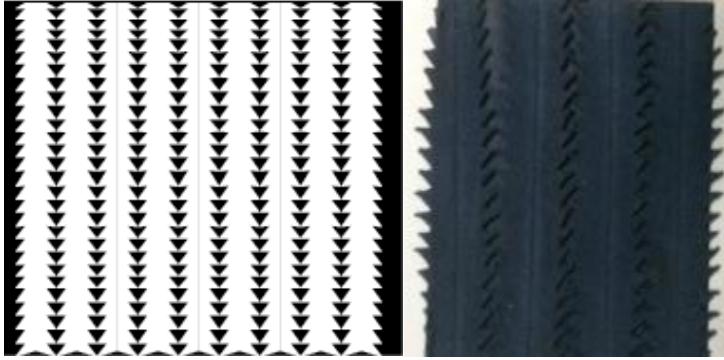
Figure 2 : Swatch on cut and open technique
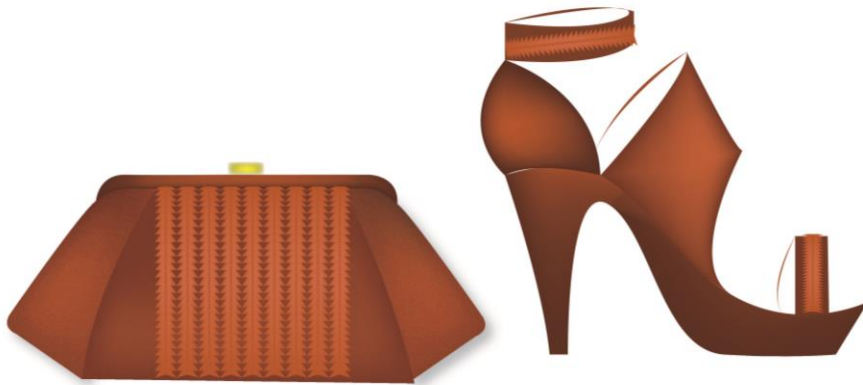
Figure 3 : Prototype on Cut Open

TECHNIQUE: CUT, OPEN, CUT AND PASTE

STEP 1 : Pin tucks
STEP 2 : Cut a straight line along the edges and open
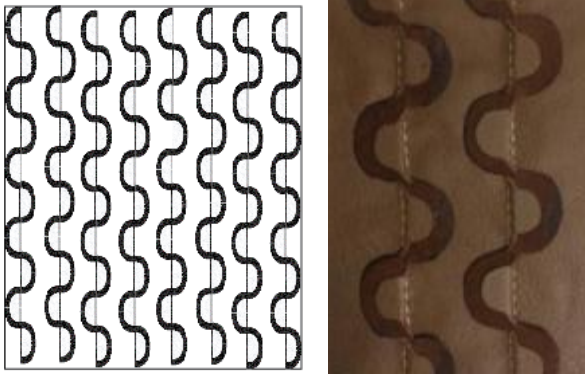Step 3 : Cut along the edges individually for the each of the strip and paste
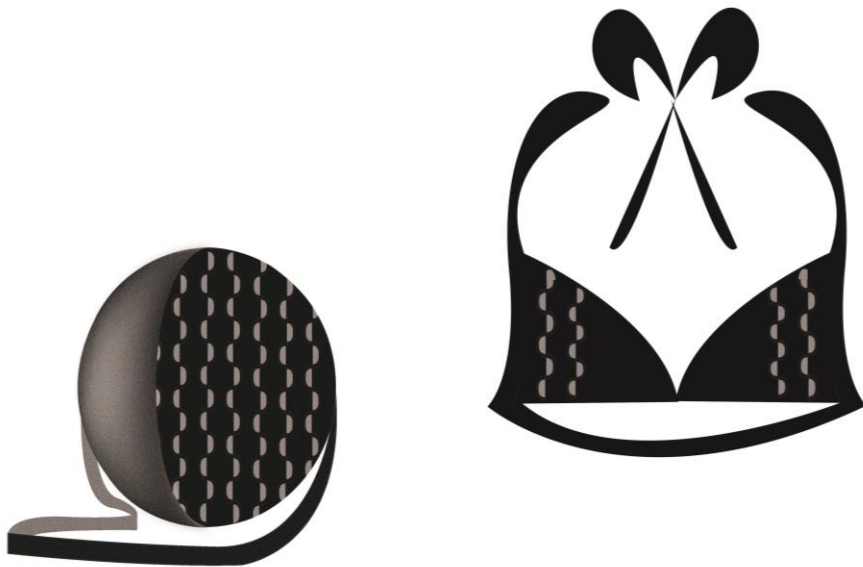
Figure 4 : Swatch on Cut, open and paste

Figure 5 : An Example Of Cut, Open Paste

TECHNIQUE: CUT AND OPEN

STEP 1 : Pin tucks
STEP 2 : Cut along the edges
Step 3 : Open along the cut edges.

Figure 6 : Swatch on cut and open technique

Figure 7 : Prototype Of Cut Open

TECHNIQUE: COMBINATION OF CUT, OPEN AND CUT, OPEN, PASTE

STEP 1 : Pin tucks
STEP 2 : Cut along the edges
Step 3 : Open along the cut edges.
Step 4 : On the adjacent pin tucks cut, open and paste
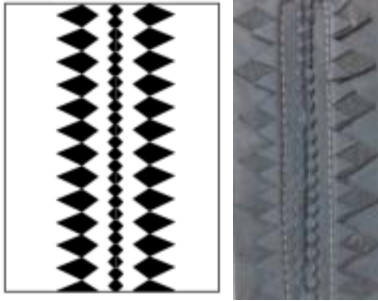
Figure 8 : Swatch on the combination of cut, open and cut, open, paste
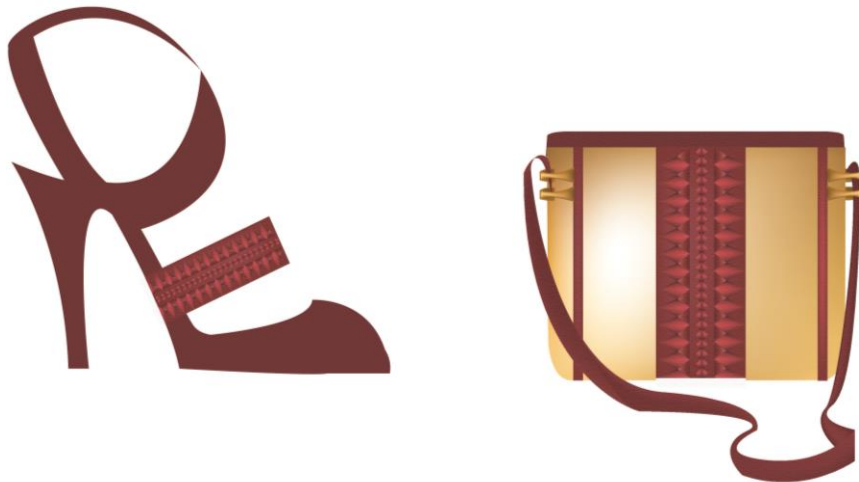
Figure 9 : Prototype of the Combination Of Cut, Open & Cut, Open And Paste

TECHNIQUE: CUT, FOLD AND PASTE

STEP 1 : Pin tucks

STEP 2 : Cut along the edges

Step 3 : Fold along the cut edges and sew

Figure 10 : Swatch on cut, paste and sew

Figure 11 : An Example Of Cut, Paste And Sew

Figure 12 : A Prototype Development Of Leather Jacket Having Cut, Paste And Sew
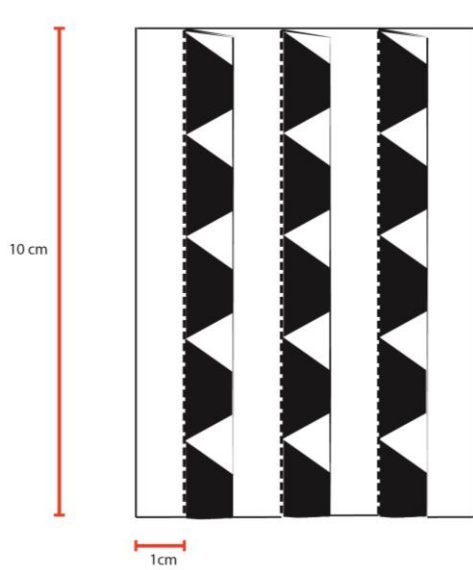
Figure 13 : Cut Along The Edges　　　　　　**Figure 14 : Resulting Pattern With Negative Space Containing　　The Hexagonal Shape**
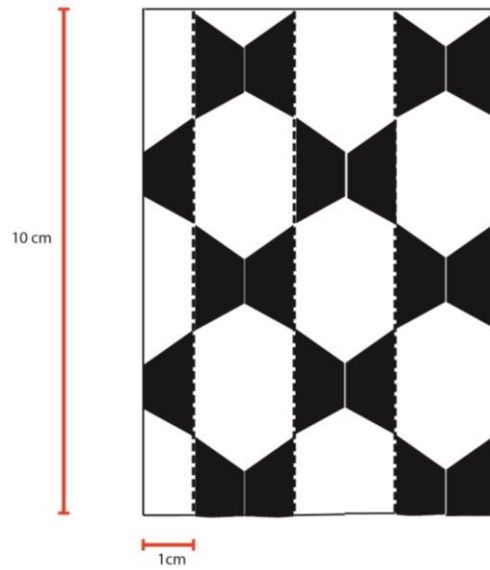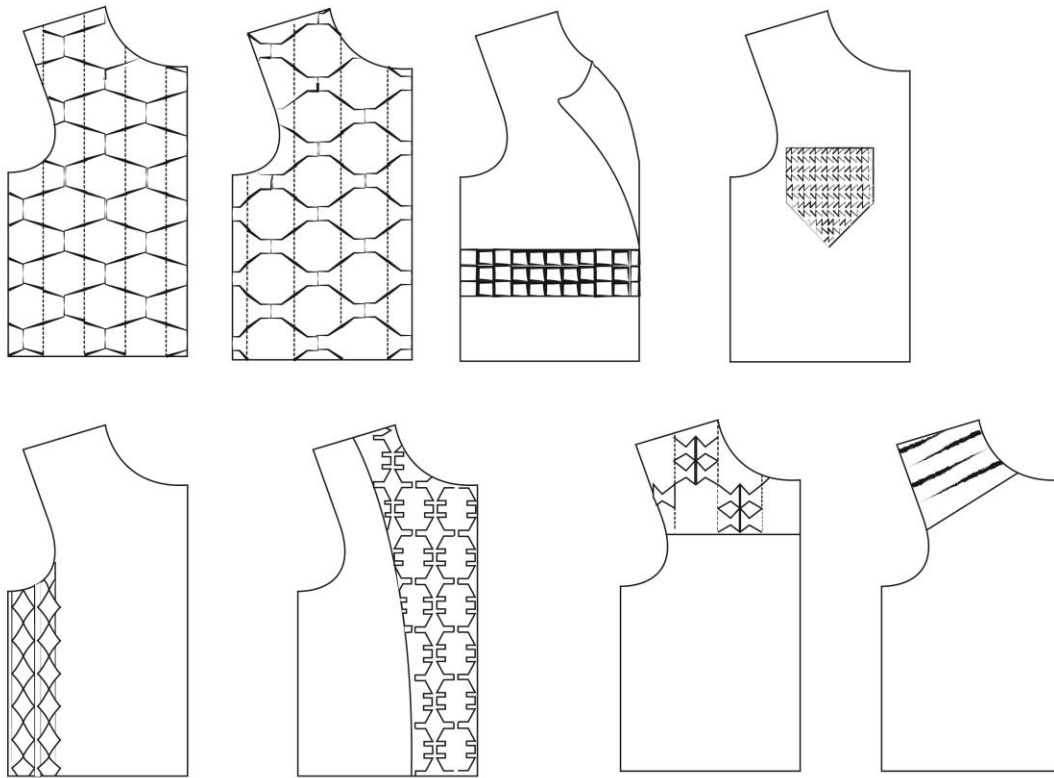
Figure 15 : Samples Explored On The Jacket Panels

FUTURE DIRECTIONS

As the technique has outwitted the usage of leather in terms of appreciating the base material through in-built techniques that sustains the traditional craftsmanship throughout the swatches abiding to the principle concepts of repetition, tessellation, smocking, layering and more. Also the advantage of these techniques' application on different product lines would enhance the value and a different perspective in terms of frozen fixation on "Classic" leather products. Since the technique is newly discovered the future explorations in these techniques will ensure greater extent of innovations.

CONCLUSION

The forethought of this paper to eradicate the consumers' and brands' fixation on classic and basic product lines have been successfully accomplished by developing innovative surface techniques. The understanding of the explorations made over years on surface techniques irrespective of the material/medium helped in developing possibilities of leather by its natural ability to act as an in built surface embellishment material. Since it's an out and out least researched material in terms of embellishments, leveraging such techniques would further add value to the product itself also the explored techniques will significantly contribute to various product lines in leather giving a wider perspective on value added leather products and their finishes.

ACKNOWLEDGEMENT

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DESIGN AROUND SHOES

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Shoes are available in various designs. The cost components of the shoes are so high that unlike garments and dresses they are not brought in large numbers by individual users. Other reason being they do not appeal to the eyes and therefore consumers give less preference to them.

In order to make the shoes attractive, various designs are applied on the shoes. The designs applied on the shoes are two dimensional and three dimensional. There is an increased trend of applying 3D designs over the 2D designs, as commonly applied in children and sports shoes. Three dimensional designs are increasingly being brought out with innovative ideas.

Though there are number of varieties, most of the 3D shoe designs are "applied" on the shoes during the manufacturing. As a result, they remain intact for the whole life time of the shoes. User has no option to alter the design. Therefore, the design remains with manufacturers' choice.

Technological development has resulted improved durability of soles of the shoes. However, any damage, wriggle or tear on the surface leads to disposal of shoes. With increased technological advancement & complication, it is almost impractical to repair the damaged shoes.

So far, the 3D designs on the shoes applied either above the quarter and counter or collar or in some case towards to toe areas. The rear half of the shoes, the quarter, vamp and counter or collar, in most cases, are bare of any three dimensional designs. Only two dimensional designs are applied on these parts.

The new design is based on removable and reusable three dimensional designs, which can have instinct appeal, if so desired, and applied to the shoes with minimum addition of functional aspects during the manufacturing process of the shoes. The replaceable designs applied on the shoes will enable the users to change the design as and when s/he wanted to do with other designs that too fit into the shoes.

The new designs are matching pieces of clothes or other materials attached to the top edge lining of the counter, quarter and/or collar at the one end and welt or sole portion of the shoe where counter and vamp join on the other end. The clothes or other such matching material is attached with the shoes either by zip, Velcro or other sticking materials.

It can be applied to regular shoes, ankle shoes and boots. The reusable fitting designs can be brought in large numbers and can replace one another whenever the user desires without causing any damage or alteration to the shoes.

Its advantages include (i) removable design – with ease, (ii) replaceable design – comfort, (iii) reusable design – cheap, (iv) washable material – bright and (v) ease of change in design like changing cloths after wash.

Shoe design for all

Shoe design for kids   Plan view
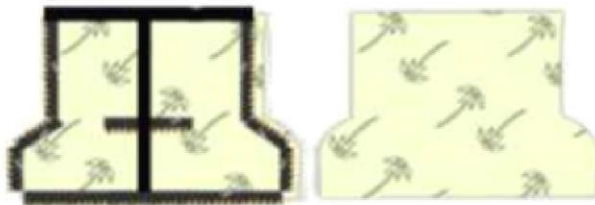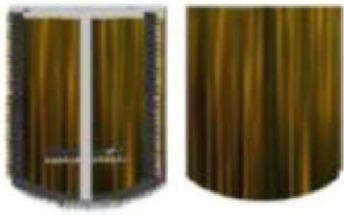
Design for Ladies and Girls

Inside view

Outer look

Design applied to shoes

Design for men, women and uniform person.

Inside view

Outer look

Design applied to Shoes