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GCMS analysis of tannery effluent by using Halophilic Bacterial Strain Pseudomonas aeruginosa sthc002 and Keratinase Enzyme

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Abstract
The discharge of untreated effluents during the starting of new industries and development of the industries results in water, air and soil pollution. During this period industrial waste effluent considered as a pollution causer because of the disposal of untreated effluent. Water pollution is the major pollution that effects the environment much and one of the sources of this pollution is tannery industrial effluents. Effluents are so toxic that fishes cannot survive in it even for two hours and also effects the drinking water and hence it should be treated effectively before release. Bioremediation is the only way to tackle these xenobiotics and reduce the pollutants which is eco-friendly. Keratinase enzymes and halophilic microorganisms are ubiquitous in nature and usually found in all living organisms. This paper based on the characterization and GCMS analysis of tannery effluent by using halophilic bacterial strain Pseudomonas aeruginosa sthc002 and keratinase enzyme. An experiment were conducted to isolate keratinase producing microbial strain from tannery effluent from Puthalam salt pan, Kanyakumari District, Tamil Nadu. Biochemical tests were done to identify the microorganisms.
Key words: Keratinase,*P. aeruginosa sthc002*, tannery waste, GCMS, Waste degradation

Introduction

Tannery effluents are the major reason for the pollution in environment[1]. In leather industry Chromium sulphate is the pollution causing agent[2,3]. Indian progress and elevated life styles of individuals are the indicators of industrialization and economic development[4,5]. However, industrialization brought many environmental hazards like land, water, soil and soil solid pollution[6,7]. All the sectors like industry, agriculture, construction, transportation, mining and consumers of our society generate waste materials[8]. Industries have been regarded as a major source of pollution due to inappropriate treatment of waste disposal methods[9]. The environmental pollutions results in the discharge of untreated effluents from the industries which harmfully effect the assimilation capability of the agriculture[10]. The quantity and the toxic materials in the waste effluent changes with the process in the industries[11]. Water pollution is a major pollution that effects the environment much and one of the main sources is industrial effluents[12]. The inherent property of the industrial technique is such that heavy quantities of water is consumes[13]. Tannery waste is a major hazard that affects the drinking water and hence it should be treated effectively beforedisposing.Chrome tanning is the major pollution by leather industry[14]. *Bacillus subtilis* are a great interest for the biotechnology. *Bacillus subtilis* is a gram positive organism found in soil and GIT of ruminants and humans[15,16]. These were

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popular worldwide before the introduction of antibiotics[17]. By using the *Bacillus subtilis*, we are converting the harmful tannery effluents to less harmful[18].

The enzyme keratinaseis one of the all rounder, because it involves in the different biological reactions like cellular and organism level and also in nitrogen circulation naturally.Exoprotease (cleaves at the end of the polypeptide chain) and endoprotease (cleaves inside the chain) are the two classes of protease enzyme, based on the site where cleavage happens (hydrolytic enzyme). This enzyme may divided into 7 groups; ser, cys, thr, aspartic keratinases., glutamic keratinases, metallo proteases and asparagine peptide lyases depends on the sequence of amino acid at active sites of enzyme. On the basis of evolutionary relationshipsbetween the proteolytic enzymesera of sequences are introduced for upgrading system of classification. Establishment of MEROPS database, in that peptidases are arranged into clans of 62 and families of 268, according tothe classification based on structur[19]. Bacillus genus includes the strains that produce serine keratinases, industrially. For various reasons the genus has major part in white biotechnology– well established, non-pathogenicspecies (for example *Bacillus cereus*, *Bacillusstearothermophilus*, *Bacillus licheniformis*, *Bacillus subtilis*) and into the culture medium multiple extracellular proteins are released. Years ago, interest improving, with culturing methodology, manipulation and genetic engineering were the factors supporting the adaptation mechanism of halophilic microbes to environment. Highsalinity adaptation can be acquired byvarious methods for controlling the osmotic stress. Salt-in process include the accumulation of KCl=NaCl in environment utilized by haloarchea, and studied about that, its enzymesneed 4-5M salt. Salt-out technique is another one, which accumulate or produce de novo solutes excluding salts. It is mainly utilized by halophilic bacteria and eukarya. Some halophiles associated with adaptive mechanisms may also work[20].

The analytical method GC/MS analysis, it identifies different substances within a liquid sample. Gas chromatography mass spectrometry analysis which identifies the unknown samples in the tannery effluent. Representation of industrial catalysts share can be done by the enzymes secreted by microorganisms. Several applications of keratinases makes them as major enzyme types. Here in this study we analyzed both raw and treated tannery effluent and find out the known and unknown components in the sample[21,22].

Methodology

Collection of Brine Sample from the Salt Pan

The experimental brine was collected from the reservoir and condenser pond from Puthalam salt pan, Kanyakumari District, Tamil Nadu, India. The brine sample was collected aseptically and transported to the laboratory for the isolation of enzyme producing microorganisms.The pH and salinity were recorded.

Isolation of Bacterial Strains

Bacterial strains were isolated by serial dilution technique. For stock solution 1000 microliter of sample was diluted to 10ml with deaminized water. Six test tubes each with 9ml of distilled water were set, marked as 1-6. 1 milliliter from stock solution transfer into first test tube without contamination making the dilution 10^{-1},the tube were shaken well to mix the contents properly. 1ml from 10^{-1}tube was transferred to next test tube marked it as 10^{-2}. The process were continued

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till to get 10^{-6} dilution. From each test tube 100 µl samples was poured to sterile petri plates having halophilic agar and spread properly. Then it kept for incubation at thirty seven °C for 24hours.The bacterial strains were maintained by sub culturing every 3 weeks on slants containing Nutrient Agar. After incubation the culture plate was examined to confirm the presence of microbes on the plate. The isolated colony on the plate were subcultured to get pure culture of the specific microorganism.

Identification of Bacteria

Various tests were conducted to identify the bacterial strains. Enzymatic activities of microorganisms are widely used to differentiate and characterize bacteria. Related bacteria can be separated into distinct species by using biochemical tests. The isolated organisms were subjected to the following biochemical tests and were identified by comparing with the standard data provided in Bergey's manual of Systematic Bacteriology (2000).

Colony Morphology

Colony morphology was the initial step in identifying a bacterium. The colony morphology of the bacterial culture grown on the halophilic agar was examined for their size, colour, shape, margin and elevation.

Screening of Extracellular Enzyme Producing Bacteria

The extracellular enzymes protease and keratinase producing bacteria were screened with the help of suitable medium.

KeratinaseEnzyme Producing Bacteria

For assessing the keratinase activity, the bacterial strains were streaked on skim milk agar plates and incubated at 37 ^{o}C for 24 hours for production. The milk consist of casein, which converted to small particles by keratinase enzyme. The cells producing keratinase would give a clear transparent zone around them in an otherwise opaquemilk media.

Optimization of Bacteria for Extracellular Enzyme Activity

The bacterial strains were optimized with physico chemical and nutritional source for maximum production of extracellular enzyme.

Effect of pH

The effect of different pH ranging from 5 to 11 was examined for the growth efficiency of the bacterial isolates. Nutrient agar medium added with NaCl were used as suitable for culturing bacterial strains. The incubation temperature was maintained at 37°C. The pH was adjusted by using 0.01 M Hcl and 0.01M NaOH.

Effect of Temperature

The isolated bacteria were studied at different temperature such as 30°C, 35°C, 40°C, 45°C, 50°C and55°C. Each bacterial strain was optimized with different temperature to produce maximum extracellular enzyme.

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Effect of Incubation Time

The incubation time varying from 24-120 hours were examined for the detection of optimum incubation time required for the growth of the bacteria. The incubation temperature was maintained at 37°C.

Effect of Salt Concentration

The effect of salt concentration on optimization of isolates was determined by increasing of NaCl concentration from 1 to 8 % concentration of NaCl was incubated at optimized conditions of all the above parameters understatic condition.

Effect of Carbon Source

The effect of several sources of carbon such as paddy glucose, fructose, sucrose, xylose and starch were used to study the growth of the bacterial strains and their respective extracellular enzyme activity. In 100ml of nutrient agar medium, 1% of respective carbon source was added individually. The incubation temperature was maintained at 37°C.

Effect of Nitrogen Source

The effect of various organic nitrogen sources such as beef extract, yeast extract, peptone, gelatin, casein and urea were used to study growth of the bacterial strain and their respective extracellular enzyme activity. In 100ml of nutrient agar medium 1% of respective nitrogen sources were added individually. The incubation temperature was maintained at 37°C.

Effect of Metal Ions

To evaluate the effect of various metal ions on the growth of bacterial strains and extracellular enzyme activity the metal ions such as sodium nitrate, ammonium sulphate, ammonium chloride, ferrous sulphate, calcium chloride and magnesium chloride were used. Each of the metal ions was separately added at 1 mM concentration in the 100 ml nutrient agar medium to study the growth of the bacterial strains and extracellular enzyme activity.

Biomass Production by the Bacteria

Pre-dried and pre-weight measure filter paper were placed on a petriplate .Samples were added filter paper placed in petriplates. Then dried at 105°C overnight in a hot air oven. After drying the final weight was determined by using an analytical weighing balance.

Tannery Effluent Collection

In the present study, tannery wastewater was collected from common effluent treatment plant, Pallavaram, Chennai. Samples were collected in sterilized glass bottles aseptically and transported to the laboratory in an ice pack condition. The collected samples were preserved at 4°C in refrigerator for further analysis.

Physico-chemical Characterization of Samples

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In the present study, tannery wastewater was collected from common effluent treatment plant, Pallavaram, Chennai. The collected tannery effluent sample was used to study parameters like pH, temperature, Biological Oxygen Demand, Chemical Oxygen Demand,Total Dissolved Solids,chloride and hardness in order to determine the quality of raw textile effluent, bacterial strain treated effluent and extracellular enzyme effluent.

pH

pH meter is the equipment used to find the pH. The effluent sample was added to a conical flask (100 ml) and the pH was measured. The pH electrode was rinsed with distilled water and calibrated using standard solution before use.

Temperature

Temperature is a measure of heat in terms of a standardized unit. It is an important ecological factor and an environmental variable, not just seasonal- as it also fluctuates on daily or even hourly bases.

Biochemical Oxygen Demand (BOD)

DO determination of one group of flasks has been done, at the 5 days incubationtime (20°C) of second group of flask.Then the incubated sample of DO was determined after 5 days. To the contents of the BOD bottle 2 ml of manganoussulphate solution and 2ml solution of alkali-iodide azide was added. Thoroughly,the bottle was stopped and gently mixed. There is a formation of precipitate which is brown in color called basic manganic oxide, settle down the precipitate for the formation of clear supernatant.For completing dissolution process few ml of conc.H_2SO_4 were added to the sides of bottle, and then stopped, gently mixed. The titrationof this contents against sodium thiosulphate (0.025N) and utilize indicator starch. The titration was continued until it becomes colorless.

$$\text{BODmg/L} = \frac{(DO_0 - DO_5 - BC) \times \text{Volume of diluted sample}}{\text{Volume of the sample taken}}$$

For titration process, the volume of sodium thiosulphate (0.025N) used = DO in mg/l

DO_0 = Initial DO

DO_5 = DO after 20 ºC incubation for 5 days

BC = Blank correction (Difference in DO of blank on the initial day and after 5 days incubation

Chemical Oxygen Demand (COD)

A 250mlof refluxing flask was fitted with double surface condenser having 300mm into which glass cap was fitted. Into this flask 50ml of the sample was transferred. 1gm of mercuric sulphate, few glass beads and 5ml of sulphuric acid-silver sulphate reagent was added, mixed well and allowed to cool. 25ml of 0.125N potassium dichromate was pipetted into the flask. The flask was connected to the condenser and cooling water was turned on. 70ml of sulphuric acid

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reagent was added through open end of condenser with swirling and mixing. Water circulation was started and refluxed for 2hrs. For dilution process, the contents were added to 350ml of distilled water. It titrated against solution of FAS (0.125N) after adding few drops of indicator ferroin. The color turned into reddish brown from blue green. A blank with distilled water instead of sample along with reagents was used. The same procedure was followed as for the sample.

$$\text{COD in mg/L} = \frac{(\text{Titre value of blank} - \text{Titre value of sample}) \times 0.125 \times 1000 \times 8}{\text{Volume of the sample taken}}$$

Total Dissolved Solids (TDS)

250ml of the effluent sample was filtered using a glass microfiber filter paper. Then the filtrate were boiled in a tared porcelain dish , preheated at 105°C and then at 550°C for in a 1 hrs in muffle furnace and cooled. The dish was kept at 180°C for one hour, cooled and weighed.

$$\text{Total dissolved solids (TDS) mg/L} = \frac{(A-B) \times 1000}{\text{Volume of sample taken}}$$

A = weight of dried residue + dish, mg
B = weight of dish, mg

Estimation of Chloride

20 ml of sample was taken and the pH was adjusted to 7-9.5. Titration against solution of standard silver nitrate with continuous stirring after adding potassium chromate indicator of 1ml, till red color formed in precipitate. The titration was repeated with 100ml blank containing distilled water (chloride free) instead of sample.

$$\text{Chloride mg/L} = \frac{(A-B) \times N \times 35.45 \times 1000}{\text{Volume of sample taken}}$$

A = Volume of silver nitrate for sample
B = Volume of silver nitrate for blank
N = Normality of silver nitrate

Total Hardness

20 ml of tannery effluent placed in a beaker that requires 20 ml EDTA titrant (0.02 M) and after adding buffer, within 5 minutes titration have to be complete. Using distilled water 25ml have to be diluted to 50ml. For getting 10.0 to 10.1 pH, 1 to 2 ml buffer solution have to add. And also provide few drops of indicator solution of Erichrome Black T or approximate amount powder. Until, the disappearance of red tinge, slowly add titrant of EDTA, with constant mixing. At 3 to 5 seconds of interval, last 2 to 3 drops have to be added. The solution is blue at end point.

$$\text{Hardness mg/L} = \frac{A \times B \times N \times 1000}{\text{Volume of sample taken}}$$

A: Volume of EDTA
N: Normality of EDTA
B: Equivalent weight of $CaCO_3$

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Chromium Removal

Chromium content of tannery wastewater was determined before and after the experiment. The candidate bacterial species was inoculated (1%, v/v) and incubated in tannery wastewater at room temperature (30 ± 2 °C) for seven days. The wastewater was first digested using HNO_3 followed by colorimetric determination using a UV- visible spectrophotometer at 357.9 nm (Clescer*iet al*., 1999).

Influence of Bacterial Strain on Biodegradation of Tannery Effluent

The rate of degradation expressed in percentage of effluent treated with bacterial strains. Absorbance reduction at absorption maxima was observed for the determination of percentage. Effluent sample contained in un inoculated nutrient medium was taken as a reference. Reaction mixture samples of 2 ml were centrifuged at 10,000 rpm. For separating biomass 10 minutes is required. At 660 nm, the determination of dye concentration has to be done. Spectrophotometer is used for measuring absorbance. Depends on the ratio of percentage the efficiency of removing color was stated, following the equation.

$$\text{Dye Degradation (\%)} = \frac{\text{Initial OD} - \text{Final OD} \times 100}{\text{Initial OD}}$$

Influence of Extracellular Enzymes on Biodegradation of Tannery Effluent

The rate of degradation expressed in percentage of effluent treated with extracellular enzymes. Absorbance reduction at absorption maxima was observed for the determination of percentage. Effluent sample contained in un inoculated nutrient medium was taken as a reference. Reaction mixture samples of 2 ml were centrifuged at 10,000 rpm. For separating biomass 10 minutes is required. At 660 nm, the determination of dye concentration has to be done. Spectrophotometer is used for measuring absorbance. Depends on the ratio of percentage the efficiency of removing color was stated, following the equation [16].

$$\text{Dye Degradation(\%)} = \frac{\text{Initial OD} - \text{Final OD} \times 100}{\text{InitialOD}}$$

GC-MS

GC-MS of metabolites was carried out by using PerkinElmer (UK) equipped with a PE auto system XL gas chromatograph and a PE-5MS capillary column (20 m × 0.18 mm internal diameter, 0.18 mm film thickness). Helium was used as a carrier gas (flow rate: 1 mL min-1) using split less injector (injector temperature was 280 °C). The column temperature was programmed as 50 °C (5 min); 50-300°C (10 °C min-1, hold time: 5 min). The MS transfer line and ion source temperatures were kept at 200 and 250 °C, respectively. A solvent delay of 3.0 min was selected. In full-scan mode, the electron ionization (EI) mass spectra were recorded in range of 30-550 (m/z units) at 70 eV. Retention time and fragmentation patterns were analysed.

Data Analysis

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The data were analyzed the present study, following the statistical analysis described by Zar (1974).

Standard Déviation (SD)

$$SD = \sqrt{\frac{(\Sigma d^2)}{N}}$$

Where,
d- Refers to the deviation of each scope from mean
N- The total number of samples.

Analysis of Variance (ANOVA)

One way and two way ANOVA were found with the help of the software PASW statistics data editor and Ky plot respectively. Means were compared at 0.05 % for Two Way ANOVA and 0.001 % level.

Results and Discussion

Isolation of Bacterial Strains

Keratinase Enzyme Producing Bacteria

The halophilic bacterial strain *P. aeruginosa* sthc002 streaked at the centre of the sterile skimmed milk agar plate showed a dumb bell shaped zone around the bacterial colonies at 37 °C and 24 hours of incubation, it was shown in fig: 1.

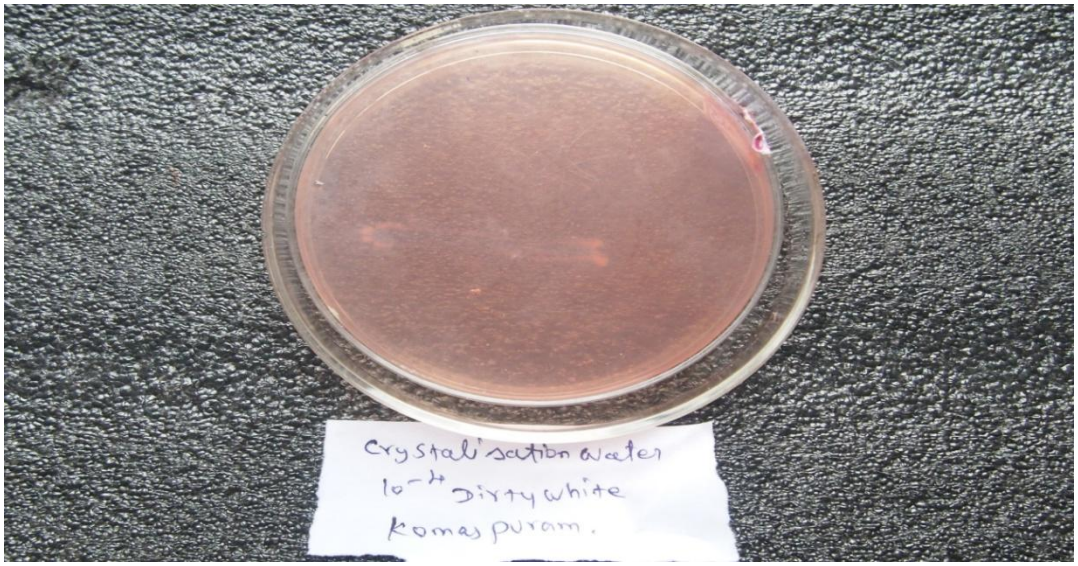

Fig 1: Screening of *P. aeruginosa* sthc002 (Accession number: MW332065) for Keratinase activity

Optimization of Bacteria for Extracellular Enzyme Activity

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Bacterial strains were optimized with nutritional source for maximum production of extracellular enzyme.

Influence of ph and Temperature

Ph 8, temperature 40 °C

Influence of NaCl Concentration on *P. aeruginosa sthc002* AJ for Keratinase Production

Max produ 4%, Enzyme production 68.3 ± 0.05 U / ml. Biomass 22.5 ± 0.05 mg / ml

Min produ 2%, Enzyme production 14.0 ± 0.03 U / ml. Biomass 4.5 ± 0.03 mg / ml

Influence of Incubation Time on *P. aeruginosa sthc002* AJ for Keratinase Production

Max produ 72hr, Enzyme production 69.4 ± 0.02 U / ml. Biomass 24.4 ± 0.04 mg / ml

Min produ 2%, Enzyme production 19.0 ± 0.04 U / ml. Biomass 5.2 ± 0.03 mg / ml

Influence of Carbon Sources on *P. aeruginosa sthc002*AJ for Keratinase and Biomass Production

Glucose, maltose, fructose, xylose and starch

Max produ maltose, Enzyme production 65.2 ± 0.03 U / ml. Biomass 22.2 ± 0.06 mg / ml

Min produ glucose Enzyme production 51.5 ± 0.01 U / ml. Biomass 15.5 ± 0.02 mg / ml

Influence of Nitrogen Sources on *P. aeruginosa sthc002* AJ for Keratinase and Biomass Production

Beef extract, yeast extract, casein, peptone, gelatin

Max produ –Yeast extract, Enzyme production 83.3 ± 0.03 U / ml. Biomass 17.8 ± 0.03 mg / ml

Min produ- beef extract Enzyme production 57.3 ± 0.03 U / ml. Biomass 8.3 ± 0.06 mg / ml

Effect of Metal Ions on *P. aeruginosa sthc002* AJ for Keratinase and Biomass Production

Sodium nitrate, ammonium sulphate, ammonium chloride, ferrous sulphate, calcium chloride, manganese chloride

Max produ –calcium chloride, Enzyme production 74.5 ± 0.06 U / ml. Biomass 29.5 ± 0.01 mg / ml

Min produ- sodium nitrate, Enzyme production 38.4 ± 0.02 U / ml. Biomass 8.3 ± 0.02 mg / ml

Physico-chemical Characterization of Samples

The collected tannery effluent sample was used to study parameters like pH and temperature. Here the figure 2 shown the difference of pH and temperature in pretreated, *P. aeruginosa sthc002*AJ treated and keratinase treated sample. Figure 3 represent the chemical properties like

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BOD, COD,TDS, chloride and hardness in order to determine the quality of raw textile effluent, bacterial strain treated effluent and extracellular enzyme effluent. The samples were analyzed according to the standard methods.

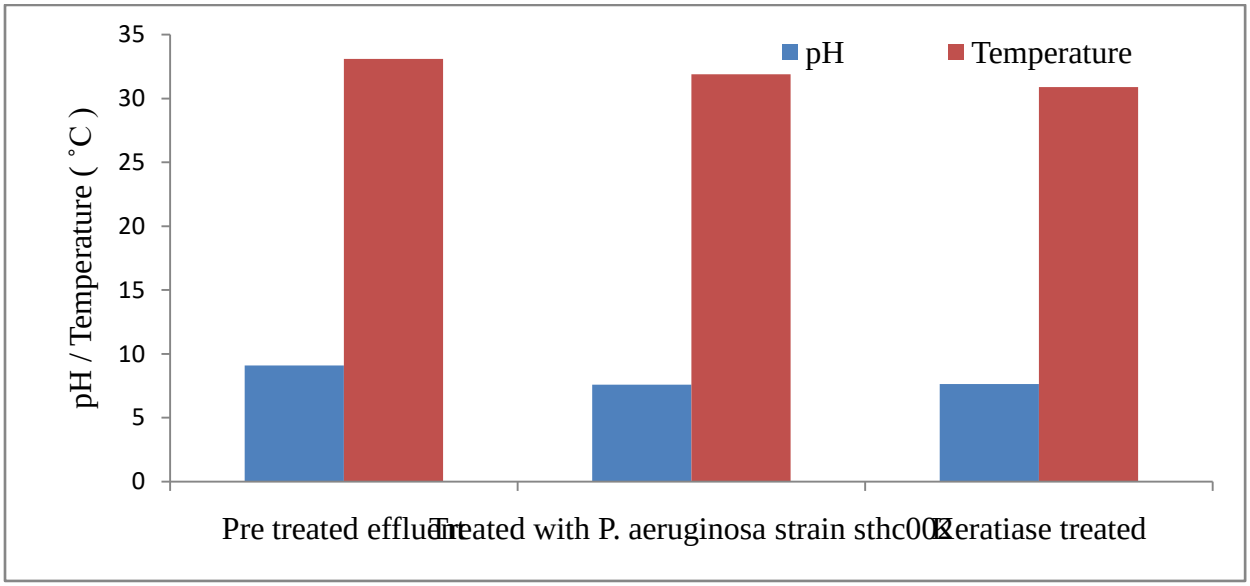

Fig 2: difference of pH and temperature in pretreated, *P. aeruginosa sthc002* AJ treated and Keratinase treated sample

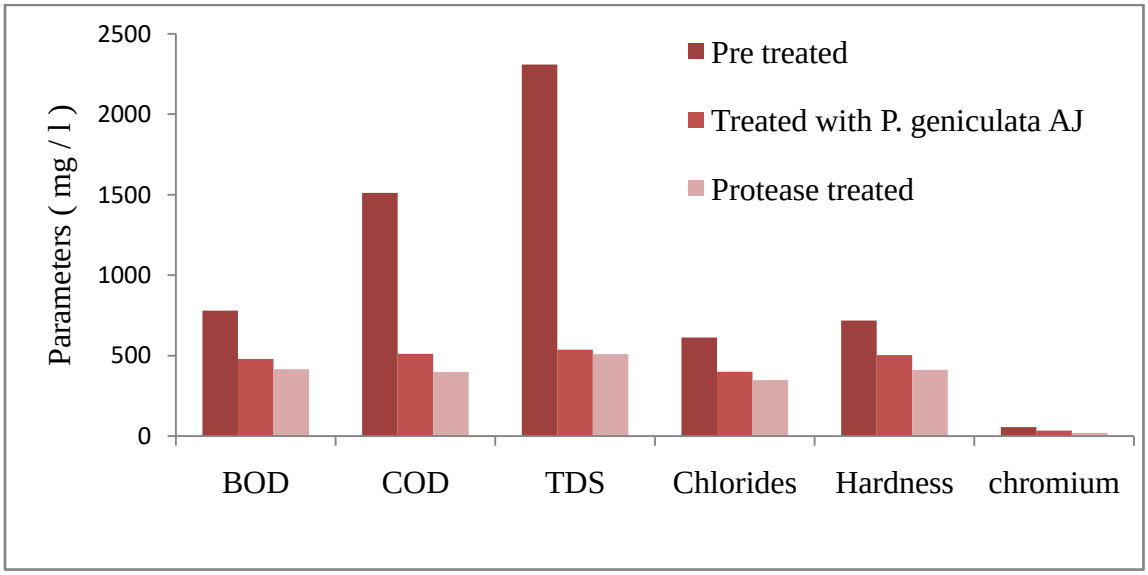

Fig 3: physico-chemical properties of raw textile effluent, bacterial strain treated effluent and extracellular enzyme effluent.

GCMS Analysis

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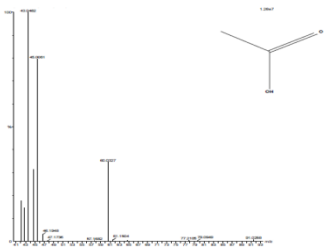
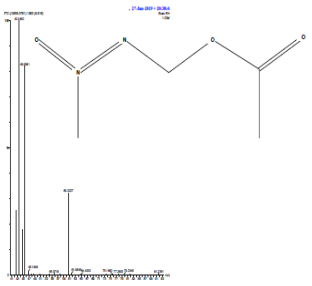
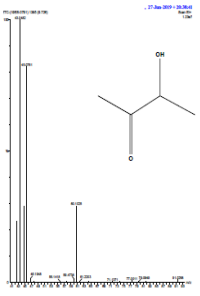
The list of identified compounds from both pretreated and treated sample details including name, retention time, molecular weight, formula and structure were given in table 1 and 2.

Table 1: GC-MS Analysis of Raw Tannery Effluent

S. No	Name of the compound	Retention time (Minites)	Area ٪	Molecular weight (g / mol)	Molecular formula	Molecular structure
1	Acetic acid	9.296	10.282	60	$C_2H_4O_2$	
2	Methanol, (methyl-one-azoxy)-, acetate (ester)	9.616	18.265	131	$C_5H_9O_3N$	
3	2-butanone, 3-hydroxy	9.726	10.716	88	$C_4H_8O_2$	

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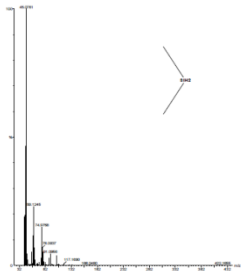
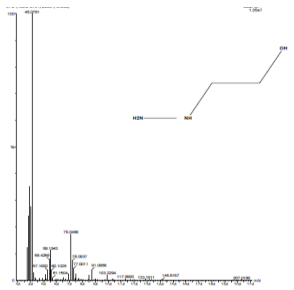
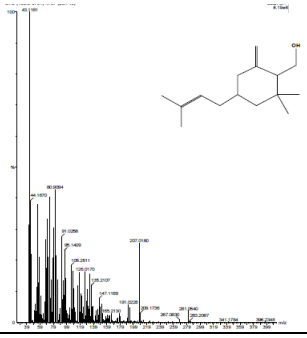
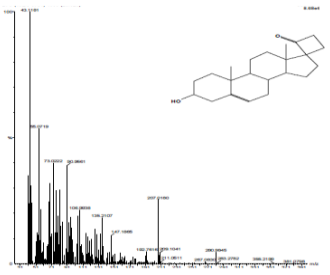
4	Silane,dimethyl	15.473	7.773	60	C_2H_8SI	
5	2-Hydroxyethylhydrazine	15.603	8.883	76	$C_2H_8ON_2$	
6	methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl	26.743	7.510	222	$C_{15}H_{26}O$	
7	spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3.beta., 17.beta.)	26.823	9.328	328	$C_{22}H_{32}O_2$	

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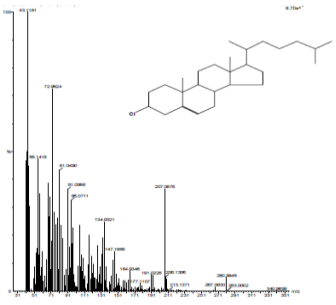
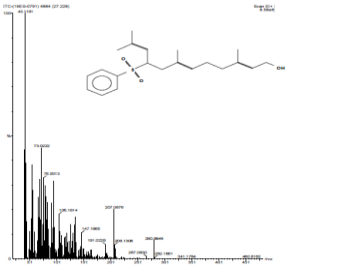
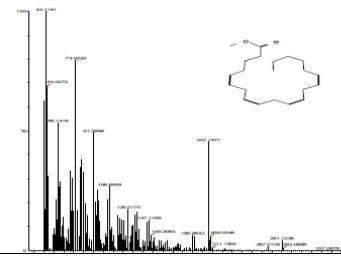
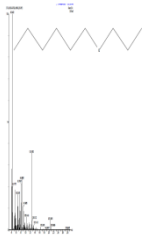
8	3-chloro-5-cholestene	26.913	14.329	404	$C_{27}H_{45}Cl$	
9	2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-9-(phenylsulfonyl)-, (e,e)	27.228	6.317	362	$C_{21}H_{30}O_3S$	
10	5,8,11,14-eicosatetraenoic acid, methyl ester, (all-z)	27.333	6.597	318	$C_{21}H_{34}O_2$	

Table 2: GC-MS Analysis of Treated Tannery Effluent

S.No	**Name of the compound**	**Retention time**	**Area ٪**	**Molecular weight**	**Molecular formula**	**Molecular structure**
1	hexane, 1-propoxy	23.057	23.777	144	$C_9H_{20}O$	
2	2,6,6-trimethyl-bicyclo[3.1.1]hept-3-ylamine	23.827	24.378	153	$C_{10}H_{19}N$	

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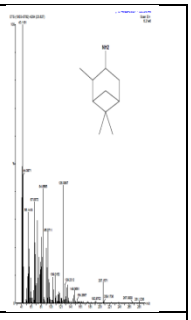
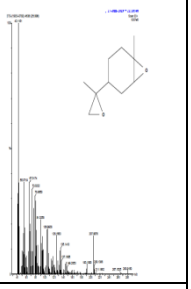
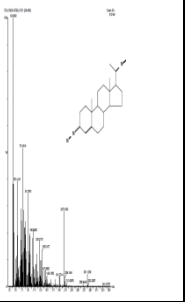
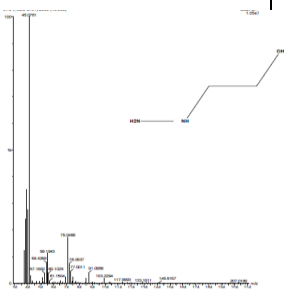
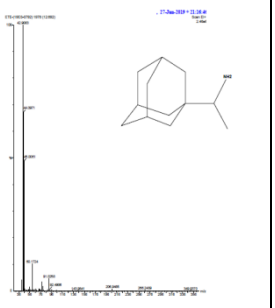
						
3	7-oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)	25.998	16.044	168	$C_{10}H_{16}O_2$	
4	Diazoprogesterone	26.463	19.613	338	$C_{21}H_{30}N_4$	
5	2-Hydroxyethylhydrazine	15.603	16.183	76	$C_2H_8ON_2$	
5	1-adamantanemethylamine, .alpha.-methyl	12.692	11.584	179	$C_{12}H_{21}N$	
6	3-Amino-2-	13.928	21.376	102	$C_3H_6O_2\ N_2$	

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	oxazolidinone					

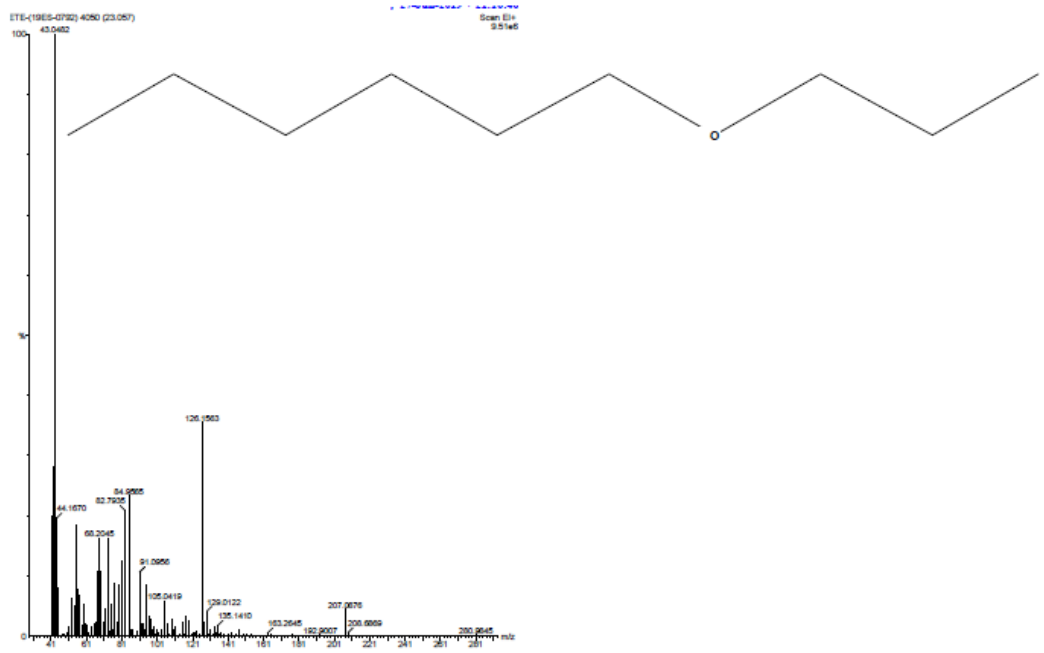

Fig 4: GC-MS Spectrum of Hexane, 1-propoxy(RT 23.757) Detected in Treated Tannery Effluent.

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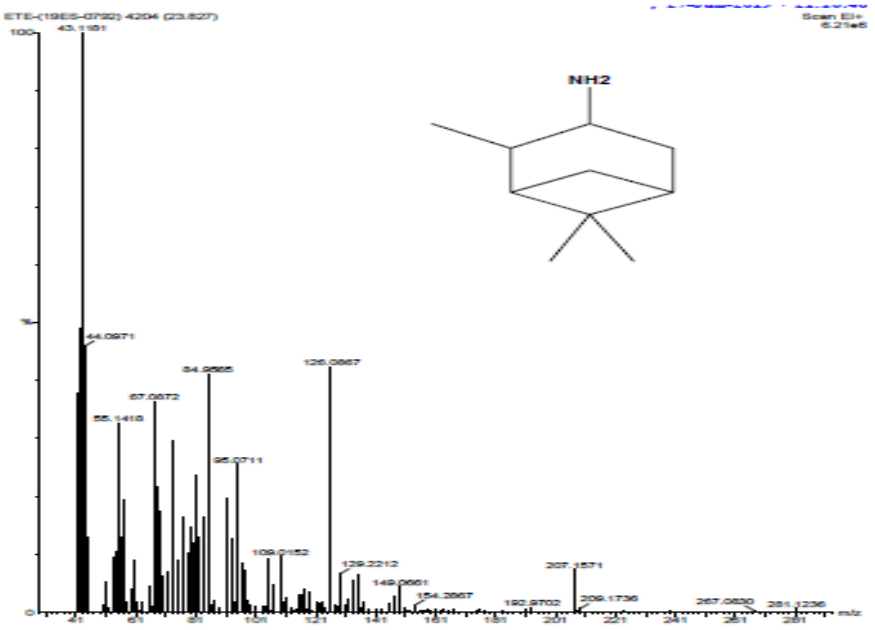

Fig 5: GC-MS Spectrum of 2,6,6-trimethyl-bicyclo[3.1.1]hept-3-ylamine (RT 23.378) Detected in Treated Tannery Effluent.

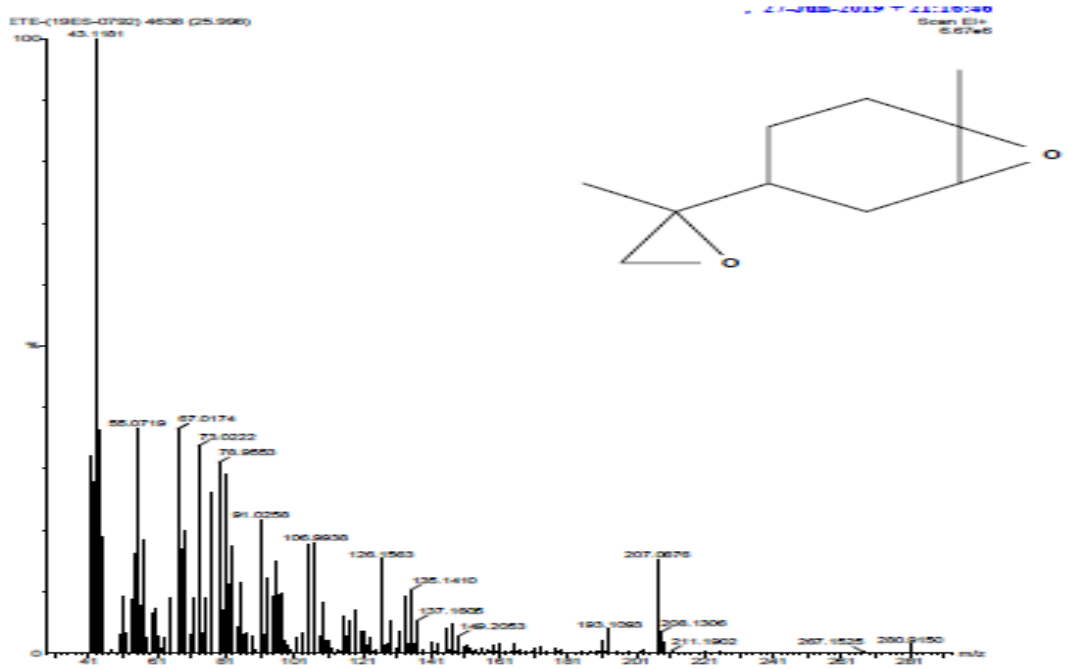

Fig 6: GC-MS Spectrum of 7-oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)- (RT 16.044) Detected in Treated Tannery Effluent.

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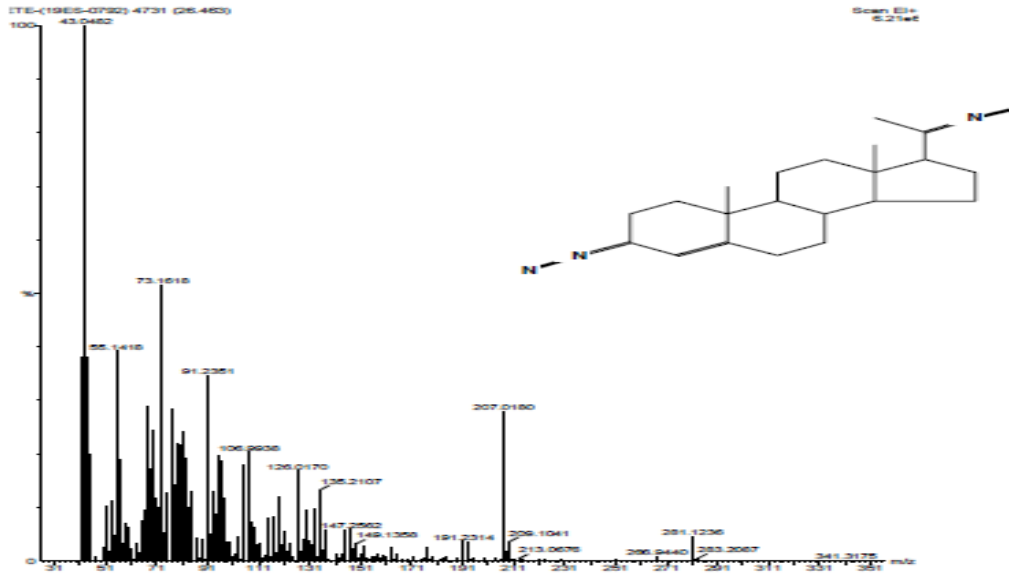

Fig 7: GC-MS Spectrum of Diazoprogesterone (RT 19.613) Detected in Treated Tannery Effluent.

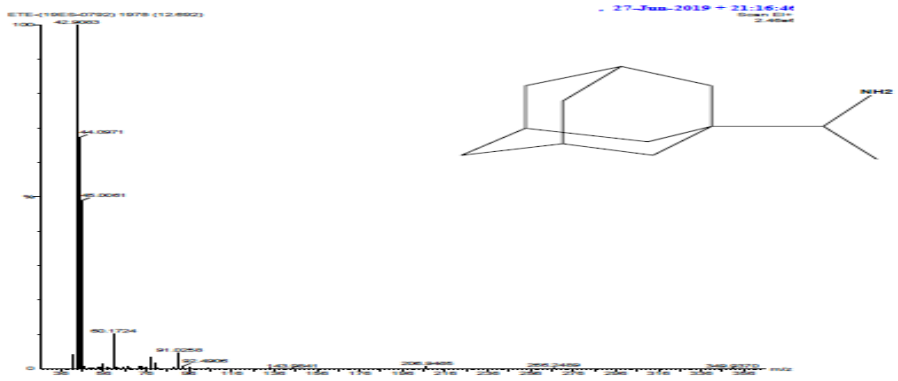

Fig 8: GC-MS Spectrum of 1-adamantanemethylamine, .alpha.-methyl (RT 12.69) Detected in Treated Tannery Effluent

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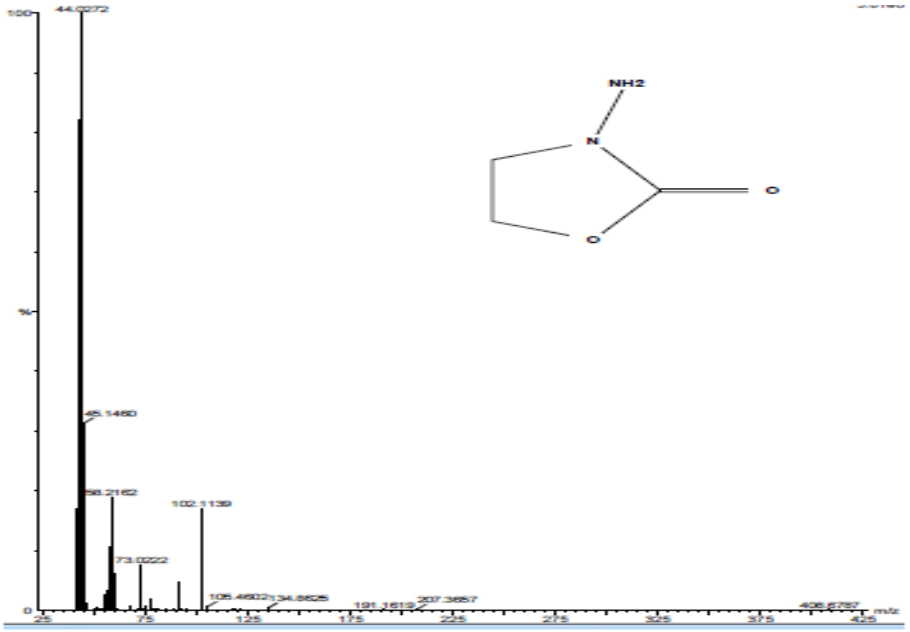

Fig 9: GC-MS Spectrum of 3-Amino-2-oxazolidinone (RT 13.928) Detected in Treated Tannery Effluent

The above project work results that tannery effluent was grey colored, turbid with a disagreeable odor, acidic in pH, with high organic and inorganic load indicating high values of TDS, total hardness, calcium, magnesium, sodium chloride, sulphate, heavy metal and chromium. From the present findings, the level of turbidity (O.D), pH and Physico-chemical parameters found to decreases from high to moderate or optimum level. The most attractive feature of *P. aeruginosa sthc002*micro organism is very effective against tannery effluent and microbe treated water can be used for irrigation purpose. The study on this topic explains that the *P. aeruginosa sthc002*would play an important role in tannery effluent treatment. We check both pre treated and treated tannery effluent sample.

Saravanan. R et al. explained in his work the tannery samples shows similar results when it treated with Bacillus subtilis (MTCC 1427).In another work Sivaprakasam et al donebacteria which are tolerant to salt were isolated from marine and tannery saline wastewater samples were discovered as *P.aeruginosa*, *B. flexus*, *E. homiense* and *S. aureus*. Growth factors of the identified strains were optimized. They were identified salt inhibition effects on chemical oxygen demand removal rate and comparative analysis was made by treating the tannery effluent with activated sludge obtained from CETP. Natural environmental microorganisms present in raw tannery saline wastewater .

Compare with other studies in our study we done the GCMS for the treated and pretreated tannery effluent sample so that we can find out the presented compounds, by using these results we can easily identify the quantity and presence of different compounds. From figure 4 to 9, it represent the GC-MS spectrum of treated tannery effluent. From the figure 3 you can understand the amount of chromium was very low in pretreated and treated (Keratinase and *P. aeruginosa*

*sthc002*AJ) samples, similarly you can see the TDS value was higher in pretreated sample. In the case of temperature and pH was almost similar in treated and pretreated samples.

Bioremediation has been used as a strategy to remove pollutants. It may be due to the concurrent process of absorption and metabolism properties of the microbial consortia, includes the heavy metal tolerance by permeability barrier, intra-cellular and extra-cellular sequestration, active transport efflux pumps, enzymatic methods and also reduction in the sensitivity of targeted cellular organelles to metal ions It is evident that Keratinase and *P. aeruginosa sthc002*AJ are very effective against tannery effluent. Based on these results the enzyme and microbes treatment method can be applied for large scale tannery effluents treatment.

References

1. AlexanderM, Biodegradation of chemicals of environmental concern. Science, 2(11), 2006, 132-138.
2. Alfonso OMD, Borquez R, Review of the treatment of sea food processing waste water sand recovery of proteins there in by membrane separation processes –prospects of the ultrafiltration of wastewaters from the fishmeal industry, Desalination, 142, 2002, 29-45
3. Adamse AD, Deinema MH, Zehnder AJB, Studies onbacterial activities in aerobic and anaerobic waste waterpurification, J Microbiol Serol, 50, 1984, 665-682
4. Ravishankar.R, Sindhu, Gnanadesigan.M, Manivannan.N,Saravannan.T, Preliminary analysis of tannery effluenttreatment using microbial consortia, Indian J Microbiol Res,2(1), 2015, 40-45.
5. Subramani.T, Haribalaji.D. Biodegradation of TanneryEffluent and Designing the Reactor for Clarifier andActivated Sludge Process, International Journal of ModernEngineering Research, 2(3), 2010, 774-781.
6. Javorekova, Stevlikova.S.T, Labuda.R, Ondrisik.P, Influenceof xenobiotics on the biological soil activity, Journal ofCentral European Agriculture, 55(2), 2001, 190-196.
7. Per larson, Lenmart Okla, and lars Tranvik, MicrobialDegradation of Xenobiotic Aromatic Pollutants in HumicWater, Appl Environ Microbiol, 54(7), 2001, 1864-1867
8. Ankita and Baljeet Singh Saharan, Studies on Pseudomonasotitidis as a novel azo dye degrading strain isolated fromgrit tank of tannery effluent treatment plant, International J.Microbial Resource Techno, 2(1), 2013
9. Sonenshein AL. CodY, a global regulator of stationary phase and virulence in Gram-positive bacteria. Curr Opin Microbi-ol. 2005;8(2):203–7
10. Jaouadi B, Aghajari N, Haser R, Bejar S. Enhancement of the thermostability and the catalytic efficiency of Bacillus pum-ilus CBS protease by site-directed mutagenesis. Biochimie. 2010;92(4):360–9.
11. Vavrová Ľ, Muchová K, Barák I. Comparison of different Bacillus subtilis expression systems. Res Microbiol. 2010; 161(9):791–7.
12. Lanyi JK. Salt-dependent properties of proteins from extremely halophilic bacteria. Bacteriol Rev. 1974;38(3):272–290.
13. Roberts MF. Organic compatible solutes of halotolerant and halophilic microorganisms. Saline Systems. 2005;1:5.
14. Aksoy SÇ, Uzel A, Hameş Kocabaş EE. Extracellular serine proteases produced by Thermoactinomyces strains from hot springs and soils of West Anatolia. Ann Microbiol. 2012; 62(2):483–92

15. Demir T, Gübe Ö, Yücel M, Hameş Kocabaş EE. Increased alka-lotolerant and thermostable ribonuclease (RNase) produc-tion from alkaliphilic Streptomyces sp. M49-1 by optimizing the growth conditions using response surface methodolo-gy. World J Microbiol Biotechnol.
16. Chen CN, Porubleva L, Shearer G, Svrakic M, Holden LG, Dover JL, Johnston M, Chitnis PR, Kohl DH (2003).Associating protein activities with their genes: rapid identification of a gene encoding a methylglyoxal reductase in the yeast Saccharomyces cerevisiae. *Yeast* 20(6):545-54.
17. Saravanan. R, Senthilkumar. K, Dhachinamoorthi. D and Haneesha. D 92017). Journal of Pharmacy Research 2017,11(2),116-119.
18. Sivaprakasam S, Mahadevan S, Sekar S, Rajakumar S. Biological treatment of tannery wastewater by using salt-tolerant bacterial strains. *Microb Cell Fact*. 2008;7:15. Published 2008 Apr 29.
19. Shawa.L.J, Beatonb.Y, Gloverc.L.A, Killhamb.K, Meharga.A, Re-inoculation of autoclaved soil as a non-sterile treatment for xenobiotic sorption and biodegradation studies, Applied Soil Ecology, 5 (11), 2000, 217-226.
20. Ankita and Baljeet Singh Saharan, Studies on Pseudomonas otitidis as a novel azo dye degrading strain isolated from grit tank of tannery effluent treatment plant, International J. Microbial Resource Techno, 2(1), 2013, 40-46.
21. Andrea, J.C.Semiao and AndreaI, xenobiotics removal by memebrane, Environmental Processes and Mitigation Strategies, 52(16), 2010, 1-14.
22. Waites MJ, Morgan NL, Rockey JS, Higton G, Industrial Microbiology an Introduction, London, Blackwell Publisher, 2008.