EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 8
1071-1077
Year: 2017

BIODEGRADATION OF INDUSTRIAL EFFLUENT USING LIPASE ENZYME OF *BACILLUS* SP AND ITS REUSE

Dr. C. M. Noorjahan*

Assistant Professor, P.G. and Research Department of Zoology, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Chennai-600 018, Tamil Nadu, India.

***Corresponding Author: Dr. C. M. Noorjahan**
Assistant Professor, P.G. and Research Department of Zoology, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Chennai-600 018, Tamil Nadu, India.

Article Received on 20/06/2017 **Article Revised on 11/07/2017** **Article Accepted on 02/08/2017**

ABSTRACT
Water pollution is caused by the discharge of effluents from various industries into nearby water bodies that pose deleterious effects on the living organisms. Hence a Research was carried out to study physico-chemical parameters of untreated industrial effluent, to degrade the effluent by using lipase enzyme of bacteria, *Bacillus* sp and also to reuse the biotreated water for culture of zooplankton, *Mesocyclops* sp which in turn forms a live feed for the growth of ornamental fish, *Poecilia* sp. The results of analysis of physico chemical parameters of untreated industrial effluent showed that colour was black with unpleasant odour, pH was alkaline in nature and EC, TSS, TDS, BOD, COD and total chromium were found to be higher than the permissible limits of CPCB. After degradation the results showed that almost all the parameters were reduced to permissible levels by using Lipase enzyme thereby satisfying the CPCB guidelines for effluent disposal. Moreover the treated water was reused for the growth of plankton *Mesocyclops* sp and the results of the study showed that maximum growth of zooplankton was recorded in biotreated sample compared to that of untreated sample. This zooplankton was used as a live feed for the growth of ornamental fish, *Poecilia* sp. The results of the study showed that maximum growth of fish was recorded in biotreated sample than that of untreated effluent.

KEY WORDS: Industrial Effluent, Physico Chemical Parameters, Biodegradation, Lipase Enzyme, *Mesocyclops* sp and *Poecilia* sp.

INTRODUCTION
Industries release various harmful substances into the environment thereby polluting the surrounding medium and in turn it forms a threat to humanity itself. There are various industries such as tannery, paper and pulp, sago, sugar, distillery etc. which contribute to environmental pollution. The rapid industrialization has affected the ecosystem to a very large extent due to the pollutants discharged from their operations.[1] The Industrial effluents entering into the surface water are one of the most important sources of toxic contamination in the environment.

Tannery Industry and Tannery Effluent
Tannery industry is reputed globally as a major industry, which contributes to water pollution, owing to the major consumers of water and usage of mineral tanning agents.[2] Tannery wastes produce serious consequences of pollution on fresh water streams and lands for agriculture.[3] Tannery contains large amount of wastes especially tannins are toxic to plants, animals and soil as well as water microorganisms. Disposal of such wastes with high pollution load into water course or onto land, with or without prior treatment, creates a great problem to the environment in the vicinity. So, it has become essential to treat the waste to a certain degree prior to its disposal.

Methods of Treatment
The treatment of industrial wastes varies with its character, quantity and the nature of receiving media and the dilution available. The treatment methods can be broadly classified into the following types: Physical treatment method, Chemical treatment method and Biological treatment method.

Biodegradation
Though there are physical, chemical and biological means of waste water treatment are available, scientists have found that in managing certain wastes, the best option is microbiological and enzymatic treatments which are more efficient and consumes no energy. Since the complete degradation of organic chemicals in the natural ecosystem is primarily carried out by micro-organisms, bio technological application using microbes or their enzymes for waste treatment are efficient.[2] However the physical and chemical treatment does not solve the problem because of the transfer of

contaminants from one phase to another. Whereas in biological treatment, the microorganisms degrade the organic pollutants, using them as a carbon source to produce metabolic energy for survival.[4]

Role of Microbial Enzymes in Biodegradation
Enzymes have a great industrial potential and are widely found in various sources like plants, animals and microbes. Biological systems degrade target chemicals mainly due to their enzymes. Hence enzymes, both intracellular and extracellular can be explored as means for wastewater treatment as they are highly specific and extremely efficient catalysts[5] and can selectively degrade the target pollutant without affecting the other components in the effluent. Thus enzymatic treatment is suitable for effluents that contain relatively large amounts of the target pollutants. Hence an innovative application of enzymes in an industry is an upcoming process for effluent treatment.[6]

Biodegradation Using Lipase Enzyme
• [7]utilized the enzymes for the waste water management generated from the food industry.[8] reported about the use of enzyme for the degradation of wastewater generated from the pulp and paper industry.[9] stated that both *pseudomonas aeuginosa* LP602 cells and the lipase were shown to be usable for lipid-rich wastewater treatment.[10] presented a review paper on production and use of microbial enzymes for dairy processing.

Recycling/Reutilization of degraded industrial (tannery) water: A useful technique in ameliorating the environmental hazards of the effluents would be to recycle the wastewater and to reuse them for a specific purpose. Recycling of water pertaining to the use of polluted water after suitably up leading its quality by the means of treatment will also eliminate the water scarcity throughout the world. Hence degraded water is used for aquaculture and agriculture.[11]

The treated water can be used for the culture of plankton which in turn forms the live food for the fish thereby indicating that the waste of one will become food for the other.[12] Live food becomes imperative for high survival and growth of fish.[13] Fishes are important aquatic animals and are particularly sensitive to wide variety of toxicants. As fish fauna serves as a food source, it is essential to know the impact of water pollution on these organisms. There have been several reports on fish mortality which has been attributed largely to pollution affecting various organ systems.[14] and [15] reused the biotreated tannery water for culture and growth of fish, *Tilapia mossambica*.[16] has studied the recycling of tannery effluent efficiently for fish culture.

Based on all the above literature cited, an attempt has been made to analyse the physio-chemical parameters of untreated industrial (tannery) effluent, to degrade the effluent using lipase enzyme and also to reuse the biotreated water for culture of plankton, *Mesocyclops* sp. which in turn form the food for the culture of ornamental fish, *Poecilia* sp. (Molly).

AIMS AND OBJECTIVES
- To analyse the physicochemical parameters of industrial (tannery) effluent as a means of monitoring the pollution.
- To isolate and identify the enzymes from bacteria, *Bacillus* sp.
-
- To use the enzymes (lipase) of Bacillus sp for bioremediation of industrial effluent.
- To reutilize the enzymatically treated water for culture of planktons *Mesocyclops* sp. which in turn form the live feed for aquaculture purpose (fish culture – *Poecilia* sp. Molly).

MATERIALS AND METHODS
Collection of Untreated Industrial (Tannery) Effluent
Untreated industrial (tannery) effluent was used as the material in this study. The untreated sample was collected in polythene containers from the point where in the effluent from different processing units are discharged together from tannery industry situated in Chennai, Tamil Nadu, India. They were brought to the laboratory with due care and stored at 25°C for further analysis. The Physico-chemical parameters such as Colour, Odour, pH, Electrical Conductivity (EC), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and heavy metals- Chromium of untreated tannery effluent were determined by following the Standard Methods outlined by.[17]

Isolation of lipase enzyme
Lipase Enzyme (*Bacillus* sp.) for Degradation of Industrial (Tannery) Effluent.
Bacteria, *Bacillus* sp. was obtained from Microbiology laboratory, Chennai. The culture was maintained in Nutrient agar medium. Lipase enzyme was extracted from the culture of bacteria, *Bacillus* sp. and identified by following the Method of Tributyrin Assay Method of.[18]

Extraction of Lipase Enzyme from the Bacterial Culture: Extraction of lipase enzyme from the bacterial culture was carried out by following the procedure of.[19] *Bacillus* sp. was cultured in Production medium. Culture medium with *Bacillus* sp. were centrifuged at 10,000 rpm for 10 minutes at 4°C and the supernatant that contains lipase enzyme was used for biodegradation of untreated industrial effluent.

Bio degradation of untreated industrial (tannery) effluent using lipase enzyme (Bacteria, *Bacillus* sp.)
Conical flask with effluent (1000 ml) and lipase enzyme (250 ml) of bacteria, *Bacillus* sp (experimental) were incubated separately at 30±0.5˚C on rotary shaker at 2000 rpm for 96hrs.. After incubation, the samples were

centrifuged at 5000 rpm for 20 minutes. Experiment was carried out in triplicates. The procedure for degradation process was carried out by following the procedure of.[20] The supernatant were analysed for physico chemical parameters like pH, EC, Total Suspended Solids, Total Dissolved Solids, Biological Oxygen Demand, Chemical Oxygen Demand and heavy metals- Chromium. Physico chemical parameters of the effluent were analysed before bio treatment (control) and after bio treatment by following the Standard procedure of.[17]

Growth of Plankton in 100% Untreated and Biotreated (Lipase Enzyme) Industrial (Tannery) Effluent: Plankton was collected from a lake located in Chennai, India by towing the plankton net horizontally at 50 cm depth for about 10 – 15 minutes during early hours of the day. Samples were brought to the laboratory and *Mesocyclops* sp. was separated by following the method of.[21] They were maintained in glass tank containing pre-aerated and filtered tap water. Baker's yeast solution was added as the feed. Multiple generation of plankton were reared for experimental studies. After degrading the untreated industrial (tannery) effluent using lipase enzyme, the degraded water was reused for the culture of zooplankton *Mesocyclops* sp. which were assessed for a period of 60 days with a view towards utilizing the treated water for zooplankton culture.

For experimentation 10 adults (5 males and 5 females) were introduced in separate experimental beakers containing untreated and biotreated samples. Equal number of *Mesocyclops* sp. was maintained in pre stored tap water which served as the control. The experiment was carried out for a period of 60 days and the sampling was done at an interval of 10 days. The number of adults, ovigerous females, nauplii and copepodid were counted and recorded.[21]

Procurement of ornamental fish, *Poecilia* sp (Molly) for growth in 100% Untreated and Biotreated (Lipase Enzyme) Industrial (Tannery) Effluent

Poecilia sp. is a tropical fish belonging to the family Poecilidae was selected for the study. Healthy fingerlings, *Poecilia* sp. were purchased from an aquarium located in Chennai in clean containers ensuring that they were not harmed either physically or physiologically during collection and transportation. The fish were acclimatized to the laboratory condition by following the procedure of[22] for a week by keeping them in a clean glass tank of 25 litres containing dechlorinated and aerated tap water and fed with formulated fish feed.

Fish Culture: The degraded water were alsoreutilized for the culture of ornamental fish, *Poecilia* sp. Healthy fingerlings (10 nos) whose size ranging from 1.36-1.68cms in length and body weight of 0.12gms were introduced in 10 litres of control (tap water), untreated and biotreated (lipase enzyme) tannery samples separately. The fish were fed with live feed –plankton, *Mesocyclops* sp. daily and aeration was provided throughout the experimental study. The growth in terms of length, breadth and weight were estimated on 0, 10, 20, 30, 40, 50 and 60th day by following the procedure of.[14] Three trials were carried out simultaneously. The same number and same size of fish were introduced in 10 litres of aerated tap water which act as control was also run simultaneously.

Statistical Analysis: The data obtained from the experiments was analysed and expressed as mean and standard deviation.

- S.D.= $\sqrt{\frac{1}{N=1}\sum x^2 - \frac{\left(\sum x^2\right)}{N}}$

where N =Number of individual observation

$\sum x_2$ =Sum of square of individual observation

$(\sum x_2)$ = Square of the total individual observation.

- The percentage change was calculated between the control and experimental for the various experiments using the formula.

$$\% \text{ change} = \frac{\text{Control - Experimental}}{\text{Control}} \times 100$$

- Anova

RESULTS AND DISCUSSION

Table. 1: Analysis of physicochemical parameters of untreated Industrial effluent.

S. No.	Parameters	CPCB 1995	Untreated Industrial effluent
1.	Colour	Colourless	Blackish
2.	Odour	Odourless	Unpleasant
3.	pH	5.5-9.0	8.0±0.0187
4.	Electrical Conductivity (EC) (μmhos/cm)	400	8550±1.8708
5.	Total Suspended Solids (TSS)(mg/l)	100	1155±1.8756
6.	Total Dissolved Solids (TDS)(mg/l)	2100	5700±1.8794
7.	Biochemical Oxygen Demand (BOD) (mg/l)	30	610±1.8759
8.	Chemical Oxygen Demand (COD) (mg/l)	250	2355±1.8708
9.	Total Chromium (mg/l)	2	43.0±0.1870

± Standard Deviation.

Table. 2: Analysis of physicochemical parameters of untreated Industrial effluent before (control) and after biodegradation (96hrs) using lipase enzyme (*Bacillus* sp.).

S. No	Parameters	CPCB (1995)	Control (untreated) sample	Biotreated (Lipase enzyme) sample
1.	Colour	Colourless	Blackish	Colourless
2.	Odour	Odourless	Unpleasant	Odourless
3.	pH	5.5 – 9.0	7.9±0.1	7.1±0.1 (1.2%)
4.	EC(μmhos/cm)	400	8615±3.16227	4160±1.9235 (51.7%)
5.	TSS (mg/l)	100	1184±3.03315	94±1.5811 (92%)
6.	TDS (mg/l)	2100	5950±3.1622	3500±1.5811(41.1%)
7.	BOD (mg/l)	30	700±1.5811	150±1.5811 (78.5%)
8.	COD (mg/l)	250	2399±1.5811	500±1.5811 (79.1%)
9.	Chromium (mg/l)	2	0.0724±0.00015	0.055±0.0015 (99.2%)

± Standard Deviation.
% Percentage Change.

Table. 3: Culture of Zooplankton, *Mesocyclops* sp. using untreated and biotreated (Bacterial lipase enzyme) effluent for a period of 60 days.

Parameters	Samples	10th Day	20 th Day	30 th Day	40 th Day	50 th Day	60 th Day
No. of Ovigerous female	Control (Tap water)	3±1*	53±1*	100±1*	147±1*	200±1*	248±1*
	Untreated effluent	1±1*	30±1*	59±1*	80±1*	100±1*	120±1*
	Bio-treated (Lipase enzyme - *Bacillus* sp)	4±1*	58±1*	106±1*	150±1*	201±1*	246±1*
No.of Copepodid	Control (Tap water)	100±1*	150±1*	189±1*	230±1*	280±1*	340±1*
	Untreated effluent	70±1*	110±1*	125±1*	150±1*	190±1*	210±1*
	Bio-treated (Lipase enzyme - *Bacillus* sp)	105±1*	159±1*	190±1*	231±1*	280±1*	340±1*
No.of Nauplius larva	Control (Tap water)	11±1*	200±1*	300±1*	390±1*	460±1*	550±1*
	Untreated effluent	5±1*	90±1*	156.6667± 57.74369*	230±1*	330±1*	400±1*
	Bio-treated (Lipase enzyme - *Bacillus* sp)	10±1*	206±1*	310±1*	391±1*	462±1*	549±1*
No.of Zooplankton	Control (Tap water)	300±1*	430±1*	590±1*	650±1*	690±1*	730±1*
	Untreated effluent	175±1*	240±1*	390±1*	420±1*	490±1*	530±1*
	Bio-treated (Lipase enzyme - *Bacillus* sp)	313±1*	424±1*	595±1*	651±1*	692±1*	734±1*

*-Satisfactorily significant at 0.05% level.

Table. 4: Culture of ornamental fish, *Poecilia* sp. using untreated and biotreated (bacterial lipase enzyme) effluent for a period of 60 days.

Samples	Parameters	0 th Day	10 th Day	20 th Day	30 th Day	40 th Day	50 th Day	60 th Day
Control (Tap water)	Length (cms)	1.9±0.1	3.4±0.1	4.1±0.1	4.6±0.1	4.7±0.1	4.9±0.1	5.2±0.1
	Breadth (cms)	0.6±0.1	1.1±0.1	1.4±0.1	1.7±0.1	2.1±0.1	2.4±0.1	2.4±0.1
	Weight (gms)	0.13±0.1	1.9±0.1	4.4±0.1	1.1±0.1	7.8±0.1	8.5±0.1	10.6±0.1
Untreated Effluent	Length (cms)	1.9±0.1	-	-	-	-	-	-
	Breadth (cms)	0.6±0.1	-	-	-	-	-	-
	Weight (gms)	0.13±0.1	-	-	-	-	-	-
Bio-treated (Lipase enzyme-*Bacillus* sp) sample	Length(cms)	1.9±0.1*	3.2±0.1*	4.1±0.1*	4.4±0.1*	4.6±0.1*	4.9±0.1*	2.3±0.1*
	Breadth (cms)	0.6±0.1*	1.1±0.1*	1.6±0.1*	1.6±0.1*	1.8±0.1*	2.4±0.1*	2.3±0.1*
	Weight (gms)	0.13±0.1*	2±0.1*	4.1±0.1*	7.1±0.1*	8±0.1*	8.6±0.1*	10.5±0.1*

*- Values are satisfactorily significant at 0.05%.
- Mortality of fish before 10th day of exposure to untreated industrial effluent.

Environment was under great pressure due to the liquid emanating from the industrial (tannery) industry.[23] The waste water from the tannery industry is a major source of pollution among all industrial sectors considering both volume and composition of effluent. During leather processing, a number of size reduction, levelling and purification operations are carried out which results in generation of untanned and tanned proteinaceous waste materials which pose as a major environmental problems if not managed effectively. The presence of colour, oil and turbidity of tannery makes it unfit for domestic purpose. The indiscriminate discharge of the effluent into water bodies or on land is causing a serious problem of water pollution and environmental degradation. Hence the present investigation is an attempt to study the physico-chemical parameters of untreated (industrial) tannery effluent and to use bacterial enzymes to biodegrade the effluent.

Analysis of Physicochemical Parameters of Industrial (Tannery) Effluent

Results of the analysis of the physico chemical parameters of untreated industrial (tannery) effluent are depicted in Table - 1. The result of the study revealed that colour of the untreated industrial effluent was blackish in colour with unpleasant odour. This colour and odour of the untreated effluent could be due to decomposition of organic or inorganic matter.[20]

pH was 8.0± 0.0187. Alkaline nature of the tannery effluent may be attributed to the presence of carbonates and bicarbonates present in the effluent Electrical conductivity of the effluent was 8500 µmhos/cm±1.8708**.** Electrical conductivity of the sample was high due to the presence of inorganic substances and salts.[20]

TSS was 1155 mg/l ±1.8756 which was found to be beyond the permissible limits (100mg/l) of CPCB (1995) This high amount of suspended particles has adverse effects on aquatic organisms, reduced the diversity of life in aquatic system, depletes oxygen and cause silting in ponds during rainy season.[24]

Whereas TDS was 5700 mg/l ±1.8794 which surpassed the CPCB (1995) permissible limits (2100mg/l). High level of TDS in the effluent may be due to high salt content and also renders it unsuitable for irrigation.[25]

BOD was 610 mg/l ±1.8759 which was beyond the permissible limit (30mg/l) of CPCB (1995). The effluent sample exhibits a high BOD value indicating a high microbial population or may be due to the presence of considerable amount of organic matter which causes adverse effects on aquatic biota. This report is in accordance with the work of.[20]

COD was 2355 mg/l ± 1.8708 which has exceeded the permissible limit (250mg/ l) of.[26] This elevated amount of COD in the present study may be due to high amount of organic compounds which are not affected by the bacterial decomposition.[27] Heavy metals – chromium was 43.0 mg/l ± 0.1870. The tanning process is responsible for the generation of large quantities of chromium waste, which is also loaded with organic matter and salts.[20] Statistical analysis for the experiments was also carried out.

Thus the analysis of physicochemical parameters of untreated industrial effluent confirms that the effluent released from the industry was black in colour with unpleasant odour, pH was alkaline with high pollution load such as EC, TSS, TDS, BOD, COD and Chromium which surpassed the permissible limits of[26] for its disposal indicating high pollution potential of the effluent. Hence it is important to treat the effluent before its disposal.

Biodegradation of untreated industrial (Tannery) effluent using Lipase enzyme

Microbial lipases constitute an important group of biotechnological valuable enzymes, mainly because of the versatility of their applied properties and ease of mass production. Microbial lipases are widely diversified in their enzymatic properties and substrate specificity, which makes it attractive for industrial application. Thus the lipase of bacteria was extracted and used in the degradation of the industrial (tannery) effluent.

The results of degradation of untreated industrial effluent using lipase enzyme are presented in Table - 2. The colour of industrial sample was blackish before degradation which may be due to presence of large quantity of organic and inorganic pollutants[28] or may also be due to microbial activities.[27] But after degradation using lipase enzyme, colour of the effluent was changed from black to almost colourless nature of the effluent. The odour of effluent was unpleasant in nature before degradation but after degrading the effluent using lipase enzyme samples shows odourless condition. This may be due to the action of lipase enzyme (bacteria – *Bacillus* sp.) which decomposed the toxic pollutants present in the effluent and changed the colour and odour of the effluent. This is supported by the work of.[20]

pH of effluent before degradation is alkaline in nature but after degrading the sample using lipase enzyme, alkaline nature of pH has changed to the almost neutral state, which may be due to accumulation of organic acids and also indicating the efficiency of the lipase enzyme to biodegrade the effluent. This is in agreement with the reports of.[20]

EC of the effluent before treatment is 8615 (µmhos/cm) ± 3.16227 which is beyond the permissible limit (400 µmhos/cm) of[26] but after degradation, lipase enzyme degraded EC to 4160 µmhos/cm ± 1.9235 and the percentage change of EC is 51.7%. TSS of the Industrial effluent before treatment is 1184 mg/l ± 3.03315 which is beyond the permissible level (100 mg/l) of[26] for disposal, but after degradation, lipase enzyme of *Bacillus* sp degraded TSS to 94 mg/l ± 1.5811 and the percentage change is 92%. TDS of the effluent before treatment is 5950 mg/l ± 3.1622 which is beyond the permissible limit (2100 mg/l) of[26] but after degradation, lipase enzyme degraded TDS to 3500 mg/l ± 1.5811 and the percentage change is 41.1%. Since TSS and TDS are the major pollutants, the above biodegradation results are encouraging. The results of the study are in accordance with the reports of.[20]

BOD of industrial effluent before treatment is 700 mg/l ± 1.5811 which is beyond the permissible limit (30 mg/l) of[26] for disposal but after degradation, lipase enzyme degraded BOD to 150 mg/l ± 1.5811 and the percentage change is 78.5%. COD of the effluent before treatment is 2399 mg/l ± 1.5811 which is beyond the permissible limit (250 mg/l) of[26], but after degradation, lipase enzyme degraded COD to 500 mg/l ± 1.5811 and the percentage change is 79.1%.

Chromium of industrial effluent before treatment is 0.0724 mg/1 ± 0.00015 which is within the permissible limit (3 mg/l) of[26] but after degradation, lipase enzyme degraded chromium to 0.055 mg/l ± 0.0015 and the percentage change of chromium is 99.2%..

Thus from the above results it can be inferred that the maximum reduction (51- 99%) of toxic substances was recorded in biotreated sample using lipase enzyme compared to untreated effluent.

Thus from the foregoing discussion it is very clear that microbes play a important role in the biodegradation of organic and inorganic matter. During biodegradation the key element is the micro-organisms. They have enzymes that allow them to use environmental contaminants as food and hence make them ideal for biodegradation. Besides their characteristics like rapid growth, metabolism and a remarkable ability to adjust to a variety of environments make them very useful in biodegradation. How successful are the microorganisms in degrading the environmental contaminants depends on the type of microbes, contaminant and on the nature of contaminated site. From the present study, the results of the biodegradation of untreated tannery effluent using lipase enzyme of *Bacillus* sp, showed efficient degrading capabilities of the enzyme by degrading the contaminants as they use it for their growth and reproduction. Organic compounds are a source of carbon which forms one of the basic building blocks of new cell contaminants. In addition to the carbon source, they require nitrogen and phosphorus as primary nutrient and traces of inorganic salts through a series of complex enzymatically catalysed reaction, the toxic organic contaminant is converted to innocuous chemical compound, obtain energy by catalysing energy producing chemical reactions and this energy is used in the production of new cells[29] finally resulting in carbon-di-oxide and water.

Plankton Culture

The ideal solution to aquatic pollution problems is to develop methods so that wastes can be recovered and reused. Experiments were conducted to study the possibility of recycling and reusing the industrial effluent after biotreatment using lipase enzyme for the culture of zooplankton. Since zooplankton forms an important link in aquatic food chain and are highly valued as preferred live feed for fish and prawn larvae, the cyclopoid copepod, *Mesocyclops* sp. was selected for this study. It is cultured in tap water (control), untreated and biotreated (lipase enzyme) samples. Zooplankton population such as number of ovigerous female, larval production (nauplii and copepodid) and zooplankton (adult) were studied on 10th, 20th, 30th, 40th, 50th and 60 days and the results are depicted in Table – 3. The results of the study revealed that there is a remarkable increase in the number of ovigerous females, larval production in terms of nauplii and copepodid stages and zooplankton (adult) population in the biotreated samples like that of control than that of untreated samples when comparison is made between the different population individually. The maximum increase in number of plankton (Mesocyclops sp.) population is recorded in lipase enzyme of *Bacillus* sp. Thus it is clearly evident that biotreated samples using lipase enzyme are effective in stimulating zooplankton (*Mesocyclops* sp.) production. The data obtained are statistically significant at 0.05% level.

Fish Culture

Rearing of fish larvae with either artificial feed or encapsulated food did not produce better results. Larva rearing with suitable live food will produce consistent results. Zooplankton is used as live food to feed the larvae of different species of fishes. Hence the present study was further extended to culture ornamental fish, *Poecilia* sp. using *Mesocyclops* sp. as live food. The results of the growth (length, breadth and weight) of *Poecilia* sp. recorded for 60 days are presented in Table – 4. The results of the study showed that the maximum growth of fish for a period of 60 days are recorded in biotreated (lipase enzyme) samples like that of control (tap water) and 100% mortality is observed in the growth of fish, *Poecilia* sp. before 10th day of culture in 100% untreated sample. This result of the study is in accordance with the work of.[14]

Waste water treatment is the most vital aspect in any wastewater management programme. The results of this study have shown that the biological treatment almost satisfied the irrigation of water guidelines for disposal. Thus degradation by lipase enzyme (bacteria – *Bacillus* sp.) seems to be most promising technique for 100% untreated tannery effluent as evidenced in the present investigation It is well known that water of good quality and free of pollutants are primary requirements for agricultural and piscicultural practice. After degradation the treated water could be used for crop cultivation or irrigation and aquaculture purpose as proved in the above study.

ACKNOWLEDGEMENT

I am very grateful for the financial assistance provided by the University Grants Commission under XI th plan guidelines for this project.

REFERENCES

1. Subramani, TD and Haribalaji. Biodegradation of tannery effluent and designing the reactor for

clarifier and activated sludge process. IJMER, 2012; 2(3): 774-781.

2. Devi, R. Bioremediation of tannery effluent by Aspergillus flavus. Poll. Res, 2011; 6(4): 141-148.
3. Leta, S, Assefa, F, Gumaelius, L and Dalhammar, G. Biological nitrogen and organic matter removal from tannery wastewater in pilot operations in Ethiopia. Applied microbial. Biotechnology, 2004; 66: 333-339.
4. Maria Claudia Hasegawa, Aneli M, Barbosa, and Keiko Takashima. Biotreatment of industrial tannery effluent wastewater using Botryosphaeria rhodina. J. Serb. Chem. Soc., 2011; 76(3): 439-446.
5. Nelson, C and Cox, M. Principles of Biochemistry, 4^{th} (ed.), WH. Freeman, Newyork, 2004; 47-50.
6. Josephine Jenitta, V, Daphne Vivienne Gnanasalomi, J and Joel Gnanadoss. Treatment of leather effluents and waste using fungi. International Journal of Computing Algorithm, 2013; 2: 294-298.
7. Shoemaker, S. The use of enzymes for water management in food industry. In Biotechnology in Food Processing, (eds.),. Harlander, SK and Labuza. Nayes Publications, Park Ridge, NJ, 1986; 259-267.
8. Hakulinen R. The use of enzymes for wasetewater treatment in pulp and paper industry- a new possibility. Water Science and Technology, 1988; 20(1): 251-262.
9. Dharmsthiti. S, and Kuhasuntisuk. B. Lipase from Pseudomonas aeuginosa LP602: Biochemical properties and application for wastewater treatment. J Industr. Microbial. Biotechnol., 1998; 21: 75-80.
10. Neelakantan, S, Mohanty and Jai, K. Kaushik. Production and use of microbial enzyme for dairy processing. Current Science, 1999; 77(1): 143-146.
11. Maggirwar, RC. Biodegradation of Tannery effluent by using tannery effluent isolate. International Multi disciplinary Research Journal, 2012; 2(3): 43-44.
12. Rajesh, GP. Impact of Dairy effluent on the biochemical constituents in the fish, Tilapia mossambica. M.Sc. Dissertation, University of Madras, Chennai, 1998.
13. Ovie, SI, Adenji, HA and Olowe, DI. Isolation and growth characteristics of a fresh water zooplankton for feeding early larval and fry stages of fish. J. Aqua. Trop., 1993; 8: 187-196.
14. Jamuna, Sand Noorjahan, CM. Biodegradation of sewage wastewater using aquatic plant and its reuse for growth of fish. Toxicol. Int., 2009; 2(10): 110-116.
15. Najamunisha Begum. Physico-chemical characteristics, treatment of tannery effluent using aquatic plant, water hyacinth- Eichhornia sp and its reuse for culture, growth and biochemistry of fish, Tilapia mossambica. B.Sc Dissertation, University of Madras, Chennai, 2010.
16. Ramalingam, K, Usha, R, and Bharathi Rajan, UD. Effect of sewage, cypermethrin and cypermethrin cum vibrio inoculation on the tissue of Oreochromis mossambicus. J. Ecotoxicol. Environ. Monit., 2007; 17(1): 1-14.
17. APHA. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC. 17^{th} (ed)., 1998.
18. Selva mohan, T, Palavesam, A and Immanvel, G. Bacillus strains from oil mill waste, African Journal of Biotechnology, 2008; (15): 2728-2735.
19. Veronica Ravamani. Isolation and extraction of lipase enzyme from microbes. M.Sc Dissertation, University of Madras, Chennai, 2002.
20. Noorjahan, CM. Physicochemical characteristics, identification of fungi and biodegradation of industrial effluent. Journal of Environment and Earth Science, 2014; 4: 2214-2224.
21. Altaff, K, Raghunathan, NB, Suresh Kumar, R and Suriyanarayanamoorthy, M. Harvest of zooplankton live food for ornamental fishes from natural ponds in North Chennai, Tamil nadu. Indian Hydrobiology, 2002; 5(2): 115-118.
22. Behringer, MPR. Techniques and materials in Biology, Mc Graw Hill Publ, 1972; 120-122.
23. Saravana Babu, S. Treatability studies of tannery effluent by bacteria. Indian Stream Research Journal-, 2011; 1(4): 15 – 20.
24. Brightlin,C. Physico-chemical characteristics, isolation of fungi, degradation of Tannery Effluent using native and non-native fungus, reuse of biotreated water for germination, growth and biochemical estimation of Ornamental Plant, Impatiens balsamina, B.Sc Dissertation, University of Madras, Chennai, 2012.
25. Shyamili. Biodegradation of tannery effluent using fungus. B.Sc Dissertation, University of Madras, Chennai, 2013.
26. CPCB. Pollution control, acts, rules and modifications issued their under Central Pollution Control Board, New Delhi. 1995.
27. Nagarajan, K and Sasikumar, R. Effect of sago effluent on selected physiological aspects of the fresh water fish, Labeo rohita., J. Ecotoxicol. Environ. Monit, 2002; 12(3): 233-238.
28. Goel, PK.Water pollution causes, effects and control. New Age International (P) Ltd., Publ. New Delhi, 2000; 269.
29. Goudar, CT and Subramanian, P. Bioremediation for hazardous waste management. IJEP, 1996; 16(2): 124-128.