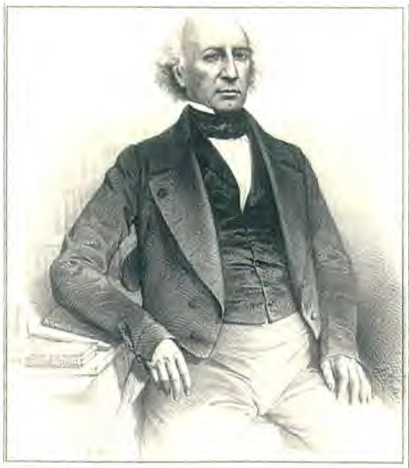

Mathieu Joseph Bonaventure Orfila (1787–1853), often called the "Father of Toxicology," was the first great 19th-century exponent of forensic medicine. Orfila worked to make chemical analysis a routine part of forensic medicine, and made studies of asphyxiation, the decomposition of bodies, and exhumation. He helped to develop tests for the presence of blood in a forensic context and is credited as one of the first people to use a microscope to assess blood and semen stains. He also worked to improve public health systems and medical training.

Chapter IV

TOXICITY ASSESSMENT OF TEXTILE EFFLUENT BEFORE AND AFTER BIOLOGICAL TREATMENT WITH *P.OLEOVORANS PAMD_1* BACTERIA AND AZOREDUCTASE

ABSTRACT

Toxic effluents containing azo dyes are discharged from various industries which adversely affect water resources, soil fertility, aquatic organisms and ecosystem integrity. The objective of this study was to compare the toxicity of untreated, treated textile effluent with *P. oleovorans PAMD_1* and purified azoreductase using Nile Tilapia (*Oreochromis niloticus*) as test organism. The acute (up to 96 hrs) static bioassay experiments were conducted to determine the median lethal dose (LD_{50}) and hematological changes on *Oreochromis niloticus* for the toxicity analysis. Results showed statistical differences in hematological parameters which were significant when compared to the control groups. The reduction in erythrocyte counts to the 50 % level was similar in sensitivity with fish mortality of 100 % for the maximum dose in control. The textile effluent was analyzed for physicochemical parameters including BOD, COD, TDS, and color intensity prior to and after biological treatment with *P. oleovorans PAMD_1* and enzymatic treatment with azoreductase. Hematological profile of *Oreochromis niloticus* was also analyzed with the same manner to assess the toxicity. The results indicates remarkable overall BOD reduction from 371 to 89 mgl^{-1} (77.5 %), COD from 895 to 158 mgl^{-1} (82 %) and TDS from 4400 to 3250 mgl^{-1} and all the hematological parameters levels too greatly elevated when compared to untreated and bacterial treated effluent.

4.1 INTRODUCTION

Increasing industrialization and urbanization leads to environmental pollution now-a-days. The discharges of wastes from various industries adversely affect water resources, soil fertility, aquatic organisms and ecosystem integrity. Waste water from textile industries is the major source of azo dyes. There are more than 10,000 dyes used in textile industry and 280,000 t of textile dyes are discharged every year worldwide (Mass *et al.,* 2005). Azo dyes are the most versatile class of dyes and account for more than 50 % of the dyes produced annually (Puvaneswari *et al.,* 2006). Azo dyes are characterized by nitrogen to nitrogen double bond (-N=N-). The color of dyes is due to azo bond and associated chromophores (Muruganandham *et al.,* 2004), so the disposal of azo dyes into surface water not only affects the aesthetic but also cause biotoxicity.

Dyeing factory effluents that alter the color and quality of the water bodies has been proved to be hazardous to aquatic organisms (Khan *et al.,* 1995). In most of the ecotoxicological studies, fish has been considered as an ideal test organism to examine both acute and chronic toxicity of pollutants. Changes in the water quality cause several physiological and biochemical disturbances in fish. The biological changes in fish that are related to the exposure or to the effects of contaminants are called biomarkers. Toxic compounds from the textile effluent strongly affect the rate of feeding, absorption, and metabolism in fishes. As a result the dye effluent brings, growth reduction, protein content of muscles, liver, and gills (Amutha *et al.,* 2002).

Azo dyes are aromatic hydrocarbons, derivatives of toluene, benzene, naphthalene, phenol and aniline. A wide variety of dyes like anthraquinone, polycylic and tripehnylmethane groups are being extensively used in textile, paper and printing industries. The reductive cleavage of azo bond under anaerobic conditions produces aromatic amines, which are more toxic than intact dye molecules (Chung *et al.,* 1978) and hence need further treatment.

Main aim of the azo dye containing wastewater treatment is for the reduction of associated toxicity. Many reports cite textile wastewater as a significant contributor to toxic load on aquatic ecosystems (Rajaguru *et al.,* 2002; Umbuzeiro *et al.,* 2005; Puvaneshwari *et al.,* 2006). There are several methods like chemical precipitation, coagulation, flocculation and absorption are currently employed for the removal of colors from the textile waste water, each with its own demerits. Chemical decolorization often requires biogenic reductants. These biogenic compounds are sometimes not effective and often require further treatments (Vandevivere *et al.,* 1998; Hao *et al.,* 2000). Microbial decolorization and detoxification, is a cheaper and eco-friendly alternative compared to chemical and physical methods (Verma *et al.,* 2003). Generally, color removal by bacteria has been linked to oxidoreductase enzymes such as laccase and azoreductase (Telke *et al.,* 2009; Kalyani, 2009).

Azoreductase catalyzes the reductive cleavage of azo groups under mild conditions. Several bacterial strains possess unspecific cytoplasmic enzymes, which acts as 'azoreductases' and transfers electrons via soluble flavins/NAD(P)H to azo dyes. A FMN dependent NADH-azoreductase of *E.coli* has been purified and n-terminal sequence of azoreductase was confirmed (Puvaneswari *et al.,* 2006).

The importance of azoreductase gene has been identified in other bacteria, such as *Bacillus subtilis, Pseudomonas aeruginosa, B. stearothermophilus, Mycoplasma pneumonia,* and *Azospirillum brasilens* (Yasuhiko *et al.*, 2001).

This case study summarizes the research undertaken to determine the contribution of textile effluent associated with toxicity. The significant contributions of this study are (i) the illustration of the toxic characteristics of textile effluent on water- bodies (ii) establishment of the potential application of azoreductase in bioremediation.

4.2 MATERIALS AND METHODS

4.2.1 Source of sample

The dye house effluent was collected from Textile Industry, Tirupur, Tamil Nadu, India. Effluent sample was collected in a clean plastic container and transported immediately to the laboratory and stored at 4 °C prior to analysis. Physicochemical characteristics of wastewaters were analyzed within 24 hrs of collection using standard methods (Aneja, 1993; APHA, 1989).

4.2.2 Fish bioassay

4.2.2.1 Test organism and maintenance

Nile Tilapia (*Oreochromis niloticus*) is a freshwater fish species in the Cichlids family, was used as the model organism in this study. It has well-documented general biology, short development time, easy culturing and year round reproduction. The average body weight of 75 gmfish^{-1} was obtained from Kelavarapalli Reservoir, Avalapalli Village (Via) Hosur, Tamil Nadu, India. This source was selected

for the supply of fish, since it has government approved aquarium and there are no industries nearby. The fishes were acclimatized for at least 15 days prior to the experiment in a glass aquarium (15 l) filled with dechlorinated water containing good plankton population to serve as food and *Ceratophyllum demersum* (a submerged hydrophyte) to oxygenate water. Thereafter, the acclimatized fishes were used as test organism.

4.2.2.2 Experimental design

A total of 60 healthy adult Nile Tilapia (*Oreochromis niloticus*) of both sexes were used. The average body length and weight of the fish at the beginning of the experiment were 12.5 cm (12- 13 cm) and 75 g (60 -90 g), respectively. There was no statistical difference between the study groups and controls regarding the size of the fish. This may be important because smaller animals are generally more active than large ones, so metabolism could also be higher in smaller animals (Canli, 1993).

The fishes were batch distributed (10 per batch) into 6 glass tanks. Batch 1 was kept without treatment to serve as control. Batch 2 to 6 were exposed to five different concentration of the textile effluents (12.5, 25, 50 75 & 100 %) for determining acute toxicity (96 hrs).

4.2.2.3 Determination of LC_{50} and mortality rate

Graphical method of Miller and Tainter

The 96 hrs LD_{50} value of textile effluent for the fish *Oreochromis niloticus* was determined by this method (Miller and Tainter, 1944). The observed percentage mortality was converted into probit referring to the probit table (Table-1)

(Abridged from table IX of Fisher and Yates: *Statistical Tables of Biological, Agricultural and Medical Research,* Oliver and Boyd, Edinburgh.). The percentage dead for 0 and 100 are corrected before the determination of probits as under (Ghosh, 1984):

Corrected % formula for 0 and 100 % mortality:

For 0 % dead : 100 (0.25/n)

For 100 % dead : 100 (n-0.25/n)

Where, n - No. of animals used.

The probit values are plotted against log-dose and then the dose corresponding to probit 5, which is 50 %.

4.2.2.4 Hematological Examination

At the end of the different hour exposure (24, 48, 72, 96) with untreated textile effluent, 2 sample fish were taken from respective group (a total of 10 fish). Variations in blood parameters were monitored by taking approximately 1 ml of blood samples from the caudal vessels using heparinized syringes with a 1.10 x 40 mm injection needle. Blood samples transferred to tubes containing ethylenediamine tetra acetic acid (EDTA) as an anticoagulant. Hematological parameters including the total number of erythrocytes was done using Dacie and Lewis, (2001). Blood was diluted to 1:200 with Hayem's fluid and then counted with a Neubauer counting chamber under a light microscope. The MCV gives an indication of the status or size of the red blood cells. The average size of the red blood cells was expressed in femtoliters (ft). MCV was calculated by dividing the hematocrit (as percent) by the RBC count in millions per microliter of blood, then multiplying by 10.

The percentage of morphological abnormalities such as shape and size of RBC was calculated from the blood smear prepared according to Lee *et al.*, (1993) by observing approximately 100 RBCs in 10 microscopic fields (10x X 100x) using an oil immersion. Morphometric measurements of RBCs were made with an occulometer, standardized with micrometer scale as parallel magnification. Similar experiments were carried out in biologically treated and azoreductase treated effluent exposed *Oreochromis niloticus* fishes of same experiment design.

The data observed in the experiments were statistically analyzed for the calculation of standard error of mean (SEM). The data shown are the average of three replicates ± SE and statistical significance was tested at $p<0.05$ level.

4.2.3 BOD/COD assay

4.2.3.1 Determination of total dissolved solids (TDS)

A clean dish was dried of suitable size at 102 – 105 °C in an oven to a constant weight. 100 - 250 ml of thoroughly mixed effluent sample was accurately pipetted into a dish, weighed and evaporated to dryness on a steam bath. The residue was dried in an oven for about 1 hr at 103 – 105 °C and re-weighed after cooling to room temperature. The total dissolved solid was calculated by (APHA, 1989; Aneja, 1993; Ademoroti, 1996) from untreated, biologically and enzymatically treated samples.

$$TDS\ (mgL^{-1}) = (B-A)/V \times 10^{-6}$$

Where, A- Initial weight of the dish (g)

B- Final weight of the dish (g)

V- Volume of the water sample taken (ml)

4.2.3.2 Determination of Biological Oxygen Demand (BOD)

Biological Oxygen Demand (BOD) is a measure of the uptake rate of dissolved oxygen used by microorganisms in a body of water. The untreated sample of the effluent was first analyzed for BOD within 24 hrs. The biologically and enzymatically treated sample were also analyzed for BOD as earlier reported (Marr and Cresser, 1983; APHA, 1989; Aneja, 1993).

BOD measures the rate of oxygen uptake by micro-organisms in a sample of water at a temperature of 20 °C and over an elapsed period of five days in the dark. The BOD test was carried out by diluting the sample with oxygen saturated de-ionized water, inoculating it with a fixed aliquot of seed, measuring the dissolved oxygen (DO) and then sealing the sample to prevent further oxygen dissolving in. The sample is kept at 20 °C in the dark to prevent photosynthesis (and thereby the addition of oxygen) for five days, and the dissolved oxygen is measured again. The difference between the final DO and initial DO is the BOD. The apparent BOD for the control was subtracted from the control result to provide the corrected value. The loss of dissolved oxygen in the sample, once corrections have been made for the degree of dilution, is called the BOD5. BOD was calculated by:

$$BOD\ (mgl^{-1}) = D_1 - D_2$$

Where, D_1 – initial DO in sample (mgl^{-1})

D_2 – DO after 5 days incubation (mgl^{-1})

4.2.3.3 Determination of Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is related to Biological Oxygen Demand, another standard test for assaying the oxygen-demanding strength of waste waters.

COD is used as a measure of oxygen requirement of a sample that is susceptible to oxidation by strong chemical oxidant. The untreated sample of the effluent was first analyzed for COD immediately after collection. The biologically treated and azoreductase treated sample was also analyzed for COD as earlier reported (Aneja, 1993; APHA, 1989; Asamudo *et al.,* 2005).

A closed water sample was incubated with a strong chemical oxidant under specific conditions of temperature and for a particular period of time. A commonly used oxidant in COD assays is potassium dichromate ($K_2Cr_2O_7$) or potassium permanganate ($KMnO_4$) which was used in combination with boiling sulfuric acid (H_2SO_4). Because this chemical oxidant is not specific to oxygen-consuming chemicals that are organic or inorganic, both of these sources of oxygen demand are measured in a COD assay. COD can be calculated by:

$$COD\ (mgl^{-1}) = 8 \times C \times (B-A)/S$$

Where, C= Concentration of titrant ($mmoll^{-1}$)
A= Volume of titrant used for blank (ml)
B = Volume of titrant used for sample (ml)
S = Volume of water sample taken (ml)

4.2.4 Biological treatment with bacteria

Isolated *Pseudomonas oleovorans PAMD_1* (Accession No. GU357740) inoculum was developed by 100 ml of nutrient broth which was inoculated with 50 ml of glycerol stock culture and incubated for 24 hrs at 37 ^{0}C under static conditions. Textile industrial effluent was inoculated with 10 % inoculum and kept under static conditions. Effluent without inoculum was kept as control. Physiochemical parameters

of the effluent and the hematological parameters of the *Oreochromis niloticus* fishes were measured after the bacterial residence time of 24 hrs.

4.2.5 Enzymatic treatment with azoreductase

Purified *Pseudomonas oleovorans PAMD_1* azoreductase was directly incubated with textile industry effluent at 35 °C under static conditions. Effluent without enzyme was kept as control. Physiochemical parameters of the effluent and the hematological parameters of the *Oreochromis niloticus* was measured as same as bacterial treatment.

4.3 RESULT AND DISCUSSION

4.3.1 Fish toxicity assay

4.3.1.1 Mortality

Fish mortality was mainly dose dependent during acute exposure, the percentage of mortality was calculated from Table 1 (probit table). Table 2a showed the increasing and corrected percentage of mortality for different exposure concentrations of textile effluent to the untreated *Oreochromis niloticus* (control). Similarly Table 2b depicts the probit analysis of biologically and enzymatically treated *Oreochromis niloticus*. From the Fig 1, the 96 hrs LD_{50} value of raw textile effluent for the *Oreochromis niloticus* was found to be around 68 %, whereas it increased significantly to 78 % and 93 % for biologically and enzymatically treated textile effluent respectively. This shows the reduction in toxicity to a greater extent by azoreductase compared to *Pseudomonas oleovorans PAMD_1* bacteria.

4.3.1.2 Hematological profile

RBC counts:

Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the hematological parameters (Van Vuren, 1986). In our study when compared to control fish, RBC counts and hemoglobin concentration were decreased in fish exposed to textile effluent. The erythrocyte of healthy control showed the mean value of 8.22 x 10^6 mm^{-3}. The fishes exposed to different concentration of textile effluent shows mean value of RBCs as 7.53, 6.28, 5.53, 4.60, and 4.10 x 10^6 mm^{-3} for 12.5, 25, 50, 75, & 100 % respectively. The results showed a drastic reduction in the total count of RBCs (table 3), similar results with significant reduction of RBC in fishes exposed to different concentration of textile effluent and toxicants have been reported previously by Pratima Soni *et al.,* (2006), Goel *et al.*, (1981) and Banu *et al.*, (2007). The damaging of toxicant on erythrocytes may be secondary, the primary action of toxicant may be on erythropoietic tissues which causes a failure in red cell production and or may be due to increase in the of erythrocyte destruction (Vosyliene and Jankaite 2006).

RBC shape and size (Poikilocytosis and Anisocytosis)

Poikilocytosis and anisocytosis assessments are the most sensitive parameter for examining toxicity of untreated and biologically treated wastewater (Pratima Soni *et al.,* 2006; Shweta Sharma *et al.,* 2006). RBCs in control fish were ovoid (Fig 2) whereas in textile effluent treatments they were in various shapes (poikilocytosis) such as beaked, tear-drop, spherical, elliptical and pentagonal (Fig 3). During acute exposure, the percentage of poikilocytosis (morphologically abnormal RBCs)

increased, with an increase in concentration of textile effluent. The cytotoxicity of dyes and textile dye wastewater to erythrocytes in terms of poikilocytosis is well documented by Murugesan *et al.*, (1989) and Sharma *et al.*, (2007).

Anisocytosis is variation in cell size (Fig 4). The discrepancy in red cell size is due to the presence of larger than normal red blood cells (macrocytic) or smaller (microcytosis) than normal red blood cells. Thus, variations in RBC size were found to be the maximum, thereby establishing it to be the most sensitive index for expressing textile effluent toxicity. The MCV was significantly decreased from 34.14±1.05 femtoliter (Ft) in control fish as showed in (Table 3) which reflects an abnormal cell division during erythropoiesis. The decrease in MCV indicates that the RBC have shrunk during exposure to textile effluent (Alwan *et al.*, 2009). MCV values are completely depends on the factors of PCV (packed cell volume), RBC count and hemoglobin concentration. In the present study, the MCV, RBC and hemoglobin concentration decreased significantly ($p<0.05$) at higher concentration of untreated textile effluent. Similar reductions have been reported by Drastichova *et al.*, (2004) and by a number of research works in different fish (Garg *et al.*, 1989). Thus, the present study has shows relevance of data on fish hematological profile for measuring acute toxicity of pollutants.

The hematological parameter results of *Oreochromis niloticus* exposed with the five days of biologically treated textile effluent and azoreductase enzymatic treatment shows toxicity reduction when compared with raw textile effluent treatment. The RBC count was greatly increased from 4.1 to 6.10x 10^6 mm^3 when compared to 5.42 x 10^6 mm^3 of biologically treated fishes and the hemoglobin concentration from

8.5 to 16.1 gdl^{-1} when compared to 14.3 gdl^{-1} of biologically treated fishes and so on (Table 4a and 4b). The results obtained after enzymatic treatment showed a remarkable reduction in toxicity of the textile industry effluent.

4.3.2 BOD/COD assay

All the industrial effluents gave biological oxygen demand (BOD) values higher than the recommended tolerance level of 50 mgl^{-1} (Akan *et al.*, 2009). These high levels of BOD and COD values observed in the industrial effluents may due to high amount of organic matter from various chemicals used during the soaking, tanning and post tanning processing chemicals used in textile industries for mercerizing. It has been reported that only about 20 % of the large number of chemicals used in the tanning and textile process is absorbed by leather and clothes, the rest is released as waste (UNIDO, 2005), thereby increasing the levels of BOD and COD in the effluent.

Table 5, represents the physiochemical peoperties obtained from the raw textile wastewater sample analysis. The most frightening values are for COD, BOD and TDS which may cause a real threat to the environment (CPP, 1999). Fig 5 (a), (b) and (c) shows the parameters COD, BOD, TDS levels respectively and the percentage reduction in the different hours of biological and enzymatic treatment. The BOD and COD level decreased from the initial concentration of 371 mgl^{-1} to 89 mgl^{-1}, 895 mgl^{-1} to 158 mgl^{-1} respectively after the enzymatic treatment as shown in Table 6a and 6b. BOD/COD ratio increased rapidly from the initial level 0.41 to 0.56 after 96 hrs of incubation with azoreductase. Thus the results obtained after enzymatic treatment showed a very good relationship with other methods which had

been reported to reduce the COD load of effluents below the upper limit of 250 mgl^{-1} (Pourbabaee *et al.*, 2006). The TDS value of the untreated effluent was initially high and in the azoreductase treated effluent the overall reduction was observed about 26.1 % when compared to 15.2 of biologically treated effluent (Fig 6). The results indicated that enzymatic treatment is more effective for reducing the toxic nature of textile effluent, which is in agreement with previous results reported by Ong *et al.*, 2005 and Pourbabaee *et al.*, 2006.

4.4 CONCLUSION

Disposal of textile industrial effluents into water bodies poses a major threat to the ecosystem. This study established the potential application of azoreductase from *Pseudomonas oleovorans PAMD_1* a newly isolated bacteria to make a satisfactory system than compared to use of whole organism and physiochemical methods to tackle the textile industry waste water pollution. This obtained results after enzymatic treatment was showed a good relationship with changes in physiochemical parameters and reduction in toxicity. Hence, it seems to be a good enzymatic tool for further research in large-scale treatment of textile industry effluent.

Table 1: Probit table (Abridged from table IX of Fisher and Yates: *Statistical Tables of Biological, Agricultural and Medical Research,* Oliver and Boyd, Edinburgh.).

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

Table 2a: Percentage corrected probit analysis LD_{50} of textile effluent treated fishes

Group	Dose	Log Dose	% dead	Corrected %	Probit*
1	0	0	0	2.5	3.04
2	12.5	1.1	0	2.5	3.04
3	25	1.4	0	2.5	3.04
4	50	1.7	20	20	4.16
5	75	1.88	60	60	5.25
6	100	2.0	100	97.5	6.96

* Average of triplicate

Table 2b: Probit analysis LD_{50} of *P. oleovorans PAMD_1* and azoreductase treated fishes

Group	Dose	Log Dose	Probit*	
			P. oleovorans PAMD_1	Azoreductase
1	0	0	3.04	3.04
2	12.5	1.1	3.04	3.04
3	25	1.4	3.04	3.04
4	50	1.7	3.75	3.04
5	75	1.88	5.00	4.16
6	100	2.0	5.74	5.44

* Average of triplicate

Table 3: Hemoglobin profile of the control and textile effluent treated *Oreochromis niloticus*

Group	% Dose (v/v)	No of RBCs (10^6 mm^{-3})	Hb Conc. (gdl^{-1})	Hematocrit (%)	MCV (ft)
1	0	8.22 ± 0.04	18.5 ± 0.03	28 ± 0.05*	34.06
2	12.5	7.53 ± 0.02*	14.96 ± 0.04*	24 ± 0.08	31.87
3	25	6.80 ± 0.02*	12.5 ± 0.03*	21 ± 0.08	30.88
4	50	5.53 ± 0.03*	11.0 ± 0.02*	13 ± 0.04*	23.50
5	75	4.60 ± 0.03	9.10 ± 0.03*	10.5 ± 0.04*	22.82
6	100	4.10 ± 0.01*	8.5 ± 0.26	9.0 ± 0.02*	21.95

* indicates significant ($p<0.05$), each value is mean ± SD of 3 observations

Table 4a: Hemoglobin profile of the biologically treated textile effluent on *Oreochromis niloticus*

Group	% Dose (v/v)	No of RBCs (10^6 mm^{-3})	Hb Conc. (gdl^{-1})	Hematocrit (%)	MCV (ft)
1	0	8.34 ± 0.04*	18.3 ± 0.03*	29 ± 0.05*	34.77
2	12.5	7.90 ± 0.05*	17.9 ± 0.06	25 ± 0.03*	31.64
3	25	7.10 ± 0.02*	17.6 ± 0.03*	22 ± 0.08	31.00
4	50	6.20 ± 0.07	17.1 ± 0.02*	16 ± 0.07	25.09
5	75	5.50 ± 0.06	15.4 ± 0.03*	13 ± 0.01*	23.63
6	100	5.42 ± 0.01*	14.3 ± 0.26*	12.5 ± 0.02*	23.06

* indicates significant ($p<0.05$), each value is mean ± SD of 3 observations

Table 4b: Hemoglobin profile of the azoreductase treated textile effluent on *Oreochromis niloticus*

Group	% Dose (v/v)	No of RBCs (10^6 mm^{-3})	Hb Conc. (gdl^{-1})	Hematocrit (%)	MCV (ft)
1	0	8.18 ± 0.04*	18.3 ± 0.02*	29 ± 0.02*	33.91
2	12.5	8.12 ± 0.03*	18.2 ± 0.04*	27.5± 0.05*	33.86
3	25	7.80 ± 0.04*	18.0 ± 0.06	26 ± 0.02*	33.33
4	50	6.40 ± 0.06	17.8 ± 0.04*	20 ± 0.08	31.25
5	75	6.15 ± 0.07	16.6 ± 0.05*	18.5 ± 0.05*	30.08
6	100	6.10 ± 0.03*	16.1 ± 0.07	18 ± 0.02*	29.50

* indicates significant ($p<0.05$), each value is mean ± SD of 3 observations

Table 5: Physiochemical parameters of raw textile effluent

Sl. No	Parameters	Raw effluent	NEQS standard*
1	pH	5.49	6 – 10
2	Color (A-Absorbance) 480 nm	>2.0	-
3	Temperature	32°C	40°C
4	Total Dissolved Solid (mgl^{-1})	4400	3500
5	BOD (20 °C) (mgl^{-1})	371	80
6	COD (mgl^{-1})	895	150

* National Environmental Quality Standards, 1999.

Table 6a: Physiochemical properties of biologically treated effluent at different time interval

Parameters	Control	Incubation time (hrs)*				
		24	48	72	96	120
BOD (mgl^{-1})	371	234	162	148	134	118
COD (mgl^{-1})	895	524	336	268	244	220
BOD/COD ratio	0.44	0.44	0.48	0.55	0.55	0.54
TDS (mgl^{-1})	4400	4300	4050	3950	3850	3730
(A-Absrobance) 480 nm	>2.0	>2.0	1.6	1.3	0.85	0.83

* Average of the triplicate

Table 6b: Physiochemical properties of enzymatic treated effluent at different time interval

Parameters	Control	Incubation time (hrs)*				
		24	48	72	96	120
BOD (mgl^{-1})	371	195	131	114	108	89
COD (mgl^{-1})	895	415	264	223	194	158
BOD/COD ratio	0.41	0.46	0.49	0.51	0.56	0.56
TDS (mgl^{-1})	4400	4260	3900	3750	3580	3250
(A-Absrobance) 480 nm	>2.0	1.2	0.52	0.34	0.30	0.28

* Average of the triplicate

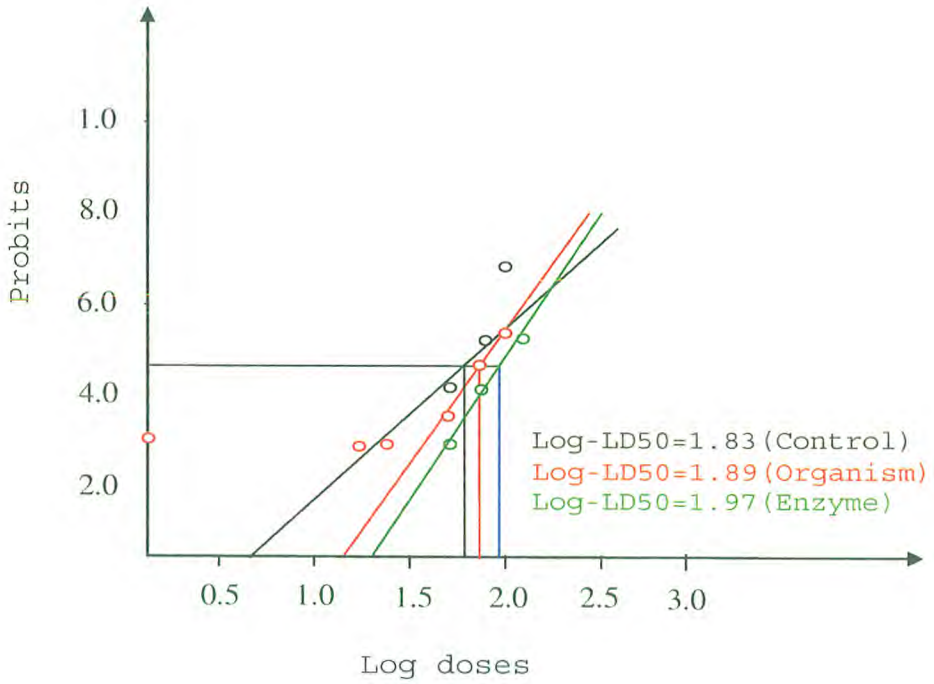

Figure 1: Plot of log-doses versus probits from Table 2 for calculation of LD_{50}

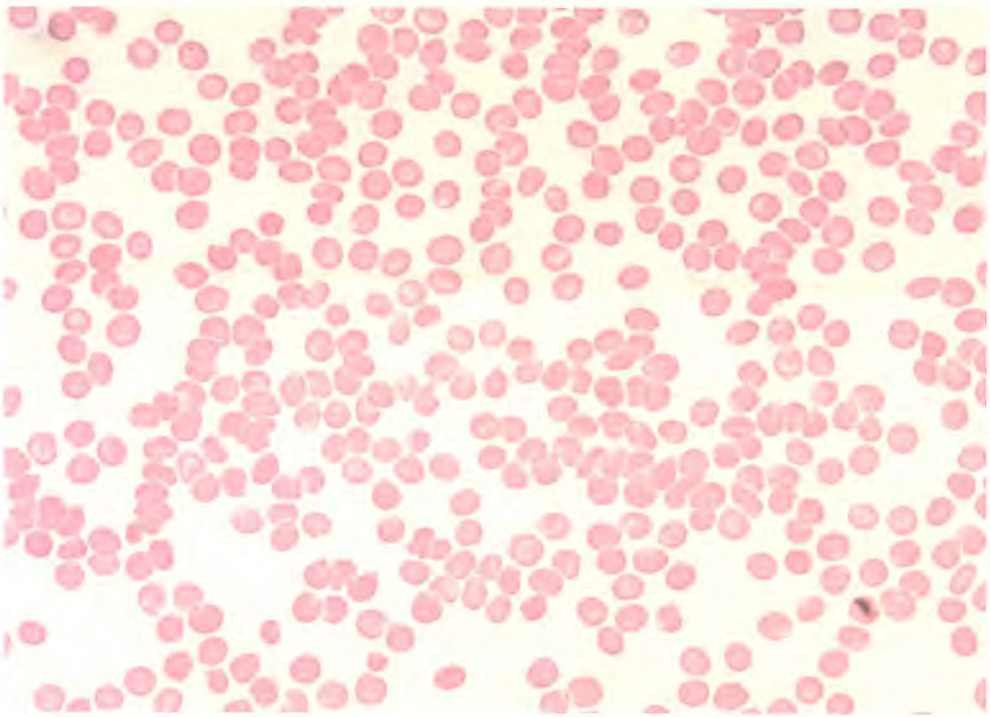

Figure 2: RBCs of normal (control) *Oreochromis niloticus.*

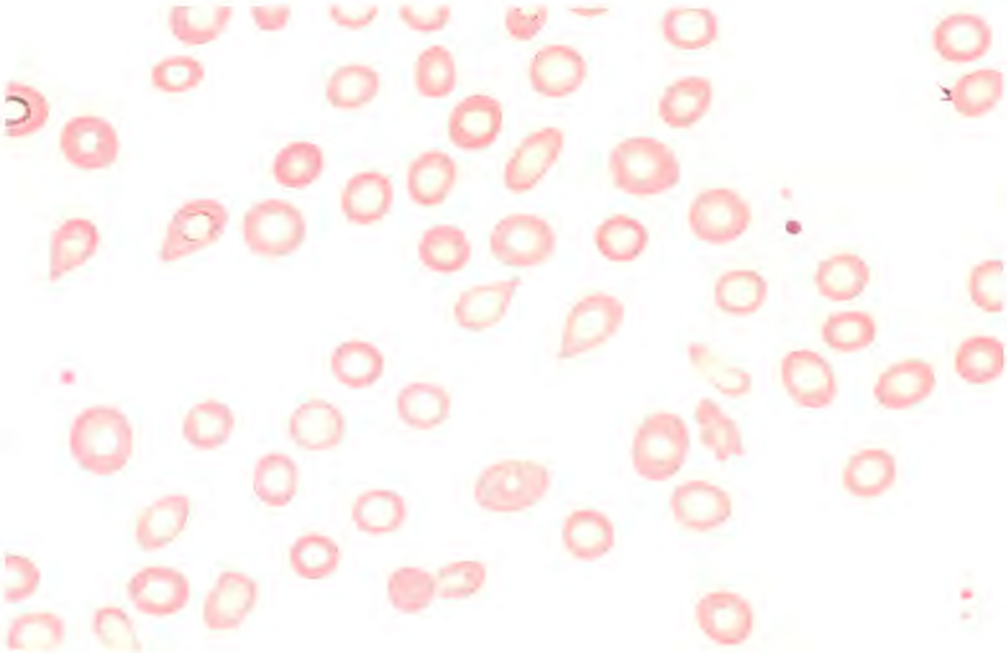

Figure 3: Poikilocytic appearance of RBCs in *Oreochromis Niloticus* exposed to textile effluent.

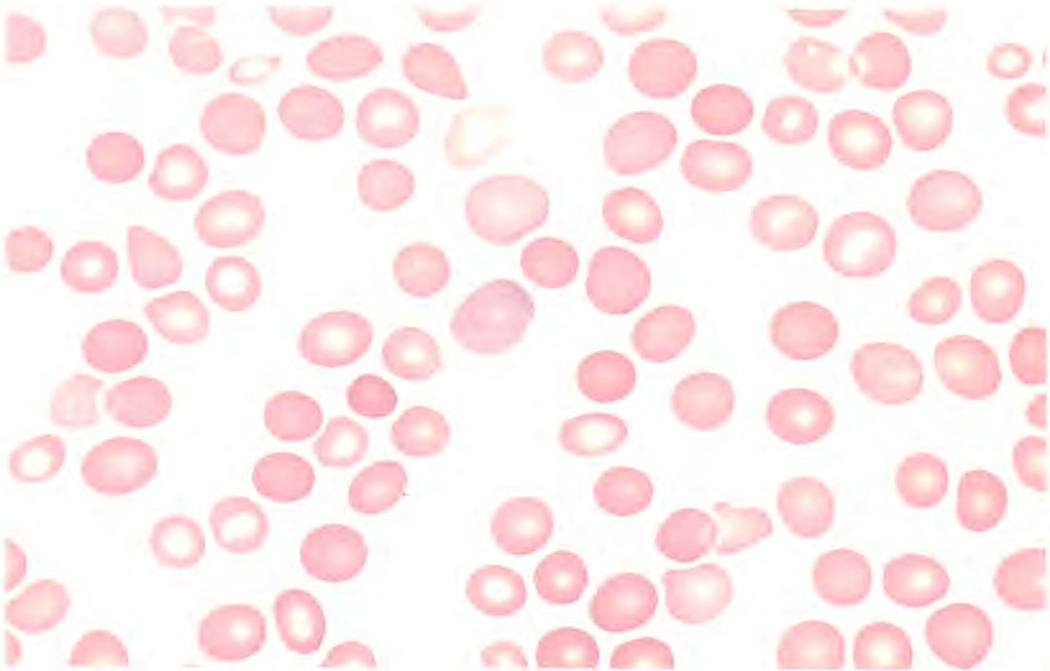

Figure 4: Anisocytic appearance of RBCs in *Oreochromis Niloticus* exposed to textile effluent.

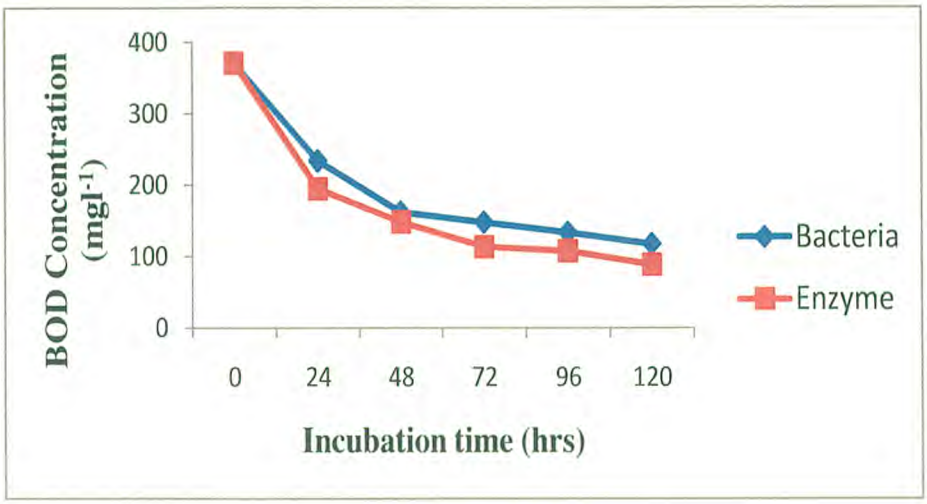

Figure 5 (a): BOD removal after biological and enzymatic treatment at different time incubation.

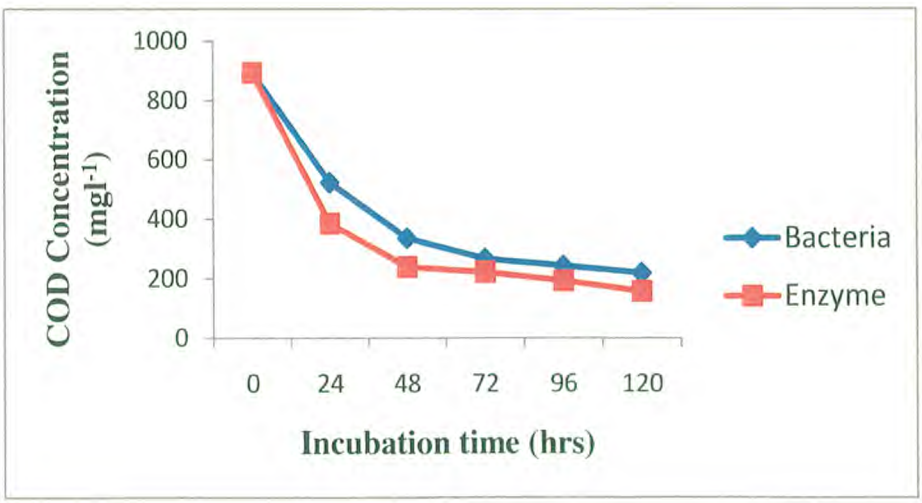

Figure 5(b): COD removal after biological and enzymatic treatment at different time incubation.

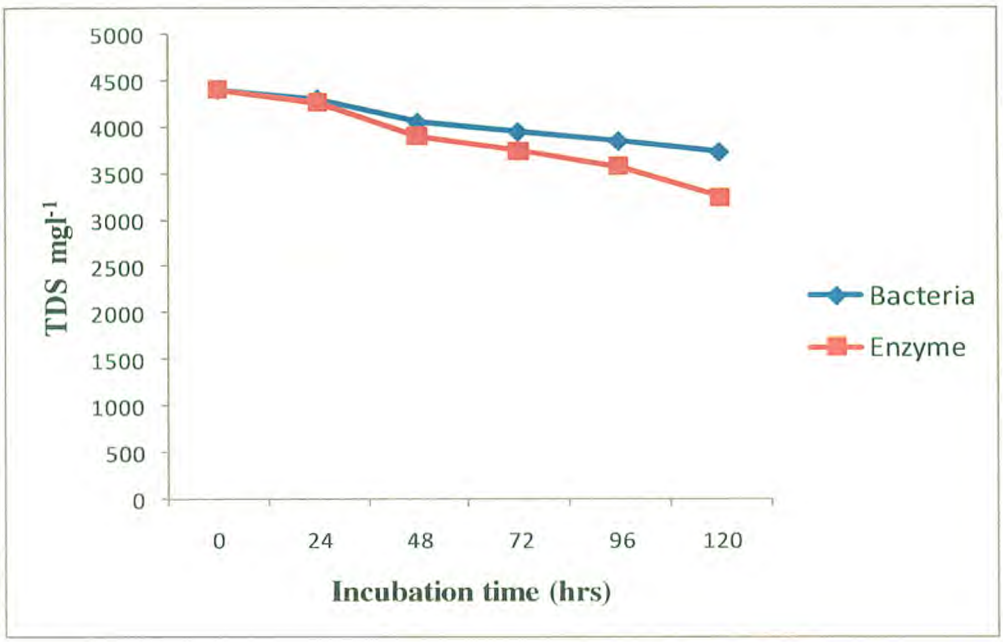

Figure 5 (c): TDS removal after biological and enzymatic treatment at different time period.

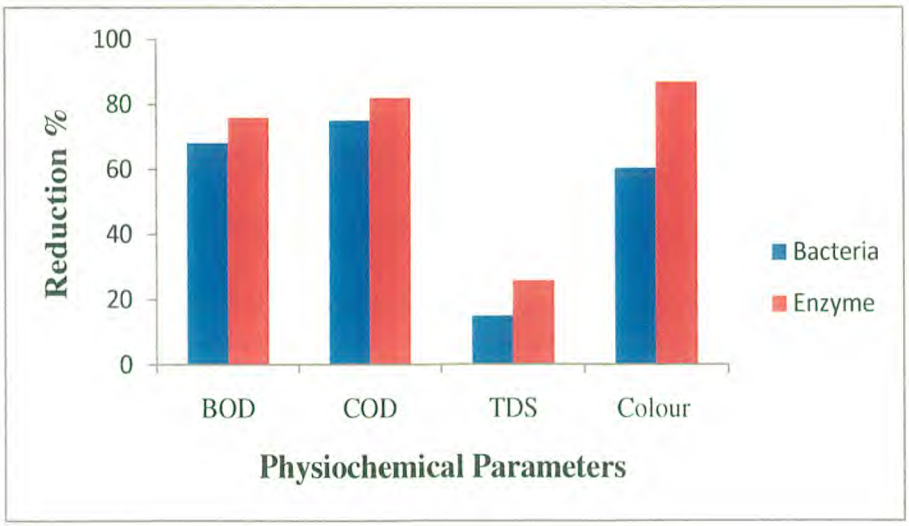

Figure 6: Comparative profile of textile effluent treated with bacteria and azoreductase