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TOXICITY EVALUATION OF THROUGH FISH BIOASSAY RAW BULK DRUG INDUSTRY WASTEWATER AFTER ELECTROCHEMICAL TREATMENT

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ABSTRACT
Considering the high pollution potential that the synthetic Bulk Drug industry Wastewater (BDW) possesses due to the presence of variety of refractory organics, toxicity evaluation is of prime importance in assessing the efficiency of the applied wastewater treatment system and in establishing the discharge standards. Therefore, in this study the toxic effects of high strength bulk drug industry wastewater before and after electrochemical treatment on common fish Lebistes reticulatus-(peter) were studied under laboratory conditions. Results indicated that wastewater being very strong in terms of color, COD and BOD is found to be very toxic to the studied fish. The LC_{50} values for raw wastewater and after electrochemical treatment with carbon and aluminium electrodes for 24, 48, 72 and 96 hours ranged between, 2.5-3.6%, 6.8–8.0%, 5.0–5.8% respectively. Carbon electrode showed marginally better removals for toxicity than aluminium electrode. It was evident from the studies that electrochemical treatment reduces toxicity in proportion to the removal efficiency shown by both the electrodes. The reduction in toxicity after treatment indicates the intermediates generated are not toxic than the parent compounds. Furthermore, as the electrochemical treatment did not result in achieving disposal standards it could be used only as a pre-treatment and the wastewater needs further secondary treatment before final disposal.

Key Words*:* Acute toxicity; Bulk drug wastewater; Electrochemical treatment; Lebistes reticulatus-(Peter)

INTRODUCTION
Bulk drug and pharmaceutical units usually involve complex manufacturing processes that consume large quantity of organic and inorganic materials and generate wastewater, which is never consistent in characteristics. These wastewaters normally consist of solids in dissolved form and substances that are toxic and refractory natured (Venkatamohan *et al*., 2001). These refractory organics either due to their complex chemical structure or due to their toxic nature are resistant to biodegradation. Organic, inorganic and toxic substances present in the bulk drug effluents have direct impact on the aquatic life and hence have high ecological relevance. It is very difficult to relate observed effects to specific pollutants present in the industrial effluents, which remains a confused field due to the presence of unknown complex and often-fluctuating characteristics of the effluent. BDW has high Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Suspended Solids (SS) and Total Dissolved Solids (TDS) and cannot be discharged without proper treatment. BDW effluents apart from being hazardous to public health have been also found to cause serious damage to the fauna of the region.

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The fishes have been considered as useful index for the purity of water and no river should be considered in a satisfactory condition unless fishes thrive well in it (Klein 1969). Bioassay studies for effluents from wastewater treatment plants provides a complete response of test organisms to compounds present in wastewater and to understand the discharge capacity of the raw and treated wastewaters (Shrinivas *et al*., 1984; Movahedian *et al*., 2005). Many industrial effluents have been used for toxicity evaluation on different fresh water fishes (Klein 1969; Kumar *et al*., 1995; Vanerkar *et al*., 2004). In recent years more attention is being given to acute toxicity evaluation for industrial effluents due to the imposition of stringent laws on discharge standards (Maleki *et al*., 2005). Chemical industries/chemical based pharmaceutical industries are the ones which generate highly toxic wastes and create acute environmental problems (Shahtalebi and Sarrafzadeh 2011). A detailed literature survey regarding the fate and mobility of several pharmaceutical compounds on different aquatic species is reported (Enick and Moore 2007). Toxicity evaluation of spent broth from an antibiotic unit on *Lebiscus recticulatus* has been cited in the literature. Report says that spent broth is too toxic to fish with 0.28% LC_{50} for 96 hours. For spent broth waste after anaerobic treatment only marginal reduction in toxicity was observed (Satyanarayan *et al*., 2005). While, for herbal pharmaceutical wastewater this was found toxic to *lebistus recticulatus* even after anaerobic treatment (Vanerkar *et al*., 2004).

Several studies on electrochemical treatment of industrial wastes have been reported towards destruction/detoxification of organics (Bazrafshan *et al*., 2007; Chen 2004), while none of the studies towards toxicity evaluation on electrochemically treated wastewaters has been reported so far. During electrochemical treatment there is always possibility of formation of more toxic intermediates either due to structural modification of original compounds or the risk of formation of organo- chlorinated by-products under presence of high chlorides. So it was decided to study in detail the acute toxicity of BDW and to assess the potential of electrochemical treatment in removing toxicity. For the purpose of electrochemical treatment widely available and cost effective electrode materials such as aluminium and carbon have been used. Bioassay studies were carried by choosing the locally available fish *lebistus reticulatus (Peter)* which is widely found in natural water bodies in India. This article discusses in detail the acute toxicity studies of both raw and electrochemically treated BDW to fish *lebistes reticulates*.

MATERIALS AND METHODS

Bulk drug industry wastewater

The wastewater used for the present investigations was collected from a bulk drug manufacturing unit engaged in manufacture of drug Ranitidine and located in Pune in Maharashtra state of India. Wastewater was collected on hourly basis for 24 hours and composited as per flow. Raw and electrochemically treated effluents were characterized for important parameters as per standard methods (Franson *et al.* 1998) and presented in Table 1. The heavy metals concentration was determined by Inductivity Coupled Plasma - Atomic Emission Spectrophotometer (ICP-AES): Jobin Yuon Model JY-24, France equipped with a computer, while the multielemental standards for the metals were procured from E-Merk Germany.

Electrochemical treatment studies

Electrochemical experiments were conducted in batch reactor with 150 ml of wastewater. The electrodes were placed vertically parallel to each other spaced at 1 cm distance with effective area of 25 cm^2 exposed to the solution. For supplying the current, the electrodes were connected with a DC regulated supply [APLAB 7101, 0-15V, 0-2A] and for both the electrodes the applied current density was 80 A/m^2, while electrolysis time for aluminium electrode was 25 min and with carbon electrode experiment lasted for 90 min which was the electrolysis time required for the optimal removal. Mechanical stirrer, having a plastic rod with a paddle at one end dipped in solution carried the mixing of electrolyte. Thus the treated wastewater was allowed to settle for one hour. Supernatant liquid was separated and subjected to routine analysis and used for bioassay studies.

Fish bioassay studies

Static bioassay tests were conducted at room temperature using fish Lebistes reticulatus (peter) as test organism. This fish was used for the experiments based on their easy availability and being sturdier in nature. Healthy specimens were chosen for the experiments with length and weight of the fish used ranged between 1.5-2.0 inches and 15–25 grams respectively. Fish required for bioassay studies were procured form local pond and toxicity evaluation on fish was followed as cited in the literature (Doudoroff *et al.* 1951; Sprague 1969). The dilution water for bioassay studies was prepared from the tap water after dechlorination by passing through a column of GAC (granular activated carbon) and aerated to keep the dissolved oxygen at saturated level. Bioassay studies were carried out in 10 litre glass aquaria using 20 fishes in each dilution. Fish were stored for 48 hours prior to start of the experiments. The test fish were exposed to fresh test solution of similar concentration once in every 24 hours by replacing the test solution. The total number of dead fishes was recorded every 24, 48, 72 and 96 hours and the fish was considered to be dead when it did not respond to external stimuli.

RESULTS

The physicochemical characterization of the raw and electrochemically treated wastewater is presented in Table 1 and for the dilution water used for fish bioassay is shown in Table 2. The toxicity

Table 1: Physicochemical characteristics of raw and after electrochemical treatment of bulk drug industry wastewater

Parameters*	Raw Wastewater (Average values)	After Electrochemical Treatment	
		Using Aluminium electrode	Using Carbon electrode
pH	6.8	7.4	7.8
Color (Visual)	Dark brown	Yellow	Brownish yellow
Pt-Co units	790	240	525
Conductivity, ms cm^{-1}	23.5	19.6	13.9
Total solids	29150	21150	18600
Total suspended solids	6250	75	1400
Total Kjeldhal Nitrogen	370	85	40
Oil and Grease	110	15	45
Chlorides	7150	5900	4330
Sulphides	330	170	80
BOD_5 @20^0 C	21000	14300	13200
COD	34400	26150	22700
Heavy Metals			
Zn	0	0	0
Pb	0.520	0.028	0.426
Cd	0.117	0.005	0.065
Ni	0.318	0.021	0.248
Co	0.210	0.028	0.175
Mn	0.298	0.035	0.135
Fe	4.530	0.200	0.557
Cr	0.190	0.031	0.075
Cu	0.030	0.009	0.025

* All values are expressed in mg/L except pH, Color and conductivity

Table 2: Physicochemical characteristics of the dilution water for fish toxicity

Parameter	Values*
Temperature, ^{0}C	26-28
pH	7.6-7.8
Dissolved oxygen	7.0-7.6
Total Alkalinity as $CaCO_3$	160-184
Total hardness a $CaCO_3$	146-168
Calcium hardness as $CaCO_3$	68-82
Magnesium hardness	78-86
Calcium as Ca	30-36
Magnesium as Mg	18-20
Sodium as Na	26-28
Potassium as K	2-5.4

*All the values are expressed in mg/L except pH and temperature

data for the raw bulk drug wastewater and after electrochemical treatment is presented from Table 3 to Table 5, respectively. The results obtained were subjected to statistical evaluation and to confirm the authenticity of the experiments LC_{50} values, 95% confidence interval, slope function and regression value (R^2) were calculated as is indicated in Table 6. LC_{50} values were calculated as quoted in literature (Litchfeild and Wilcoxin 1949) and further based on LC_{50} values SAR (safe application rate) and SAFE concentration were also calculated (Basak and Konar 1977).

Table 3: Toxicity data for the raw bulk drug industry wastewater to *Lebistes reticulatus* (Peter)

(A) Toxicity data for the Raw bulk drug industry wastewater														
Exposure period (h)	Concentration of raw pharmaceutical wastewater with dilution water, % by volume													
	1.8*	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4
	% animals dead after hours(% Mortality)													
24	0	0	0	10	20	20	30	40	40	50	60	70	80	90
48	0	0	10	20	30	30	50	60	70	80	80	90	100	-
72	0	10	20	20	40	50	60	70	80	90	100	-	-	-
96	10	20	30	40	50	60	70	80	90	100	-	-	-	-

*(Indicates 1.8% PW/98.2% dilution water to make total volume (100%) of the test solution)

Table 4: Toxicity data for the treated bulk drug industry wastewater after electrocoagulation by Aluminium electrodes to (Peter)

Exposure period (h)	Concentration of BDW with dilution water (treated by electrocoagulation), (%) by volume														
	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0
	animals dead after hours(% Mortality)														
24	0	0	0	0	10	20	30	40	50	60	70	80	80	90	100
48	0	0	0	10	20	40	50	50	60	70	80	90	100	-	-
72	0	0	10	20	30	50	60	60	70	80	90	100	-	-	-
96	0	10	20	30	50	60	70	80	90	100	-	-	-	-	-

Table 5: Toxicity data for the treated bulk drug industry wastewater after electrooxidation by Carbon electrodes to *Lebistes reticulatus* (Peter)

Exposure period (h)	Concentration of BDW (treated by electrooxidation) with dilution water,(%) by volume															
	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0	8.2	8.4	8.6	8.8	9.0	9.2
	% animals dead after hours(% Mortality)															
24	0	0	0	0	10	10	20	30	40	50	60	60	70	80	90	100
48	0	0	0	10	20	30	30	40	50	60	70	80	90	100	-	-
72	0	0	10	20	30	40	50	60	70	80	90	100	-	-	-	-
96	0	10	20	30	50	60	70	80	90	100	-	-	-	-	-	-

DISCUSSION

BDW that is generated during the manufacture of Ranitidine (an antacid) during manufacturing, is quite strong waste as observed from the physicochemical characterisation and indicated in Table 1. The main source of wastewater in bulk drug manufacturing industry is during the manufacture of medicines. The manufacturing processes involves numerous raw materials, organic and inorganic constituents including spent solvents, acid/alkalis, catalysts, reactants and intermediates and products as well in addition to the usual manufacturing streams such as pump-seal waters, waste scrubber wastewaters, boiler blow down and floor washings.

The synthesis of ranitidine requires variety of chemicals and the solvents such as dimethyl formaldehyde, chloroform and acetone. The synthesis process carried in three stages results in formation of intermediate compounds which include methyl iso-cynate, nitrometane, methanol, k-salt, dimethyl sulphamic oxide, furfuryl alcohol, p-formaldehyde, dimethyl amine, cystamine, furamine, ranitidine and methyl mercaptan gas which generates concentrated and highly odorous streams. The process wastewater therefore contains all these unconverted raw materials and the intermediate products in different stages and finally form a cocktail mixture of these compounds dissolved in the solvents. The wastewater also contains variety of trace metals that happens to have entered due to the reaction of wastewater with storage containers and metal pipes and fittings.

Table 6: Calculated LC50, 95% confidence interval, slope function, and regression values for raw and after electrochemical treatment

Exposure period	Parameters	Raw Wastewater	After Electrochemical treatment	
			Carbon electrode	Aluminium Electrode
24 hours	LC_{50} values were	3.6%	8.0%	5.8%
	95% confidence interval	2.72 – 4.75	7.20 – 8.80	5.1 – 6.58
	Slope function	y = 36.9640x – 77.6190	y = 35.882x – 237.54	y = 40x – 182
	Regression value	0.9751	0.9634	0.9718
48 hours	LC_{50} values were	3.0%	7.4%	5.4%
	95% confidence interval	2.32 – 3.87	6.6 – 8.14	4.81 – 6.05
	Slope function	y = 44.5050x – 85.8240	y = 40.659x – 263.52	y = 45.055x – 199.45
	Regression value	0.9856	0.9787	0.9782
72 hours	LC_{50} values were	2.7%	7.0%	5.2%
	95% confidence interval	2.07 – 3.51	6.14 – 7.98	4.48 – 6.00
	Slope function	y = 50.9090x – 93.455	y = 48.077x – 305.13	y = 48.077x -207.31
	Regression value	0.9924	0.9947	0.9848
96 hours	LC_{50} values were	2.5%	6.8%	5.0%
	95% confidence interval	1.92 – 3.25	6.07 – 7.6	4.25 – 5.85
	Slope function	y = 50x - 80	y = 57.273x – 355.64	y = 59.273x – 241.09
	Regression value	1.000	0.994	0.994
96 hrs SAFE % / SAR %		0.44% / 1.1%	0.775% / 5.27%	0.70% / 3.5%

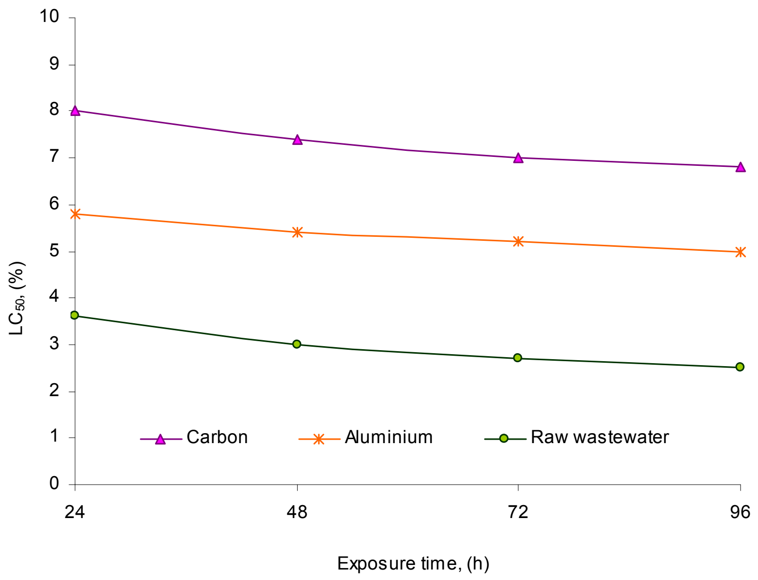

Fig. 1: Variation of LC50 as a function of exposure time for raw BDW and electrochemically treated effluents

Apart from the above substances gel, oil and grease is also generated. Soluble gel is generally used for making the cover of capsules which also gets mixed in the wastewater contributing to pollution load; thus primary treatment is necessary before secondary biological treatment. Hence, the BDW was first subjected to electrochemical method as a primary treatment which resulted in marginal reduction in COD/BOD and TDS as indicated in the Table 1. For bioassay studies all the experimental procedures using the test organisms were performed in compliance with the relevant laws and institution approved ethical guidelines. Raw BDW exerted more toxicity due to the presence of high TKN which concentration of 370 mg/l. Equally, sulphide concentration was 330 mg/l which is also very high compared to stipulated standard of 2.0 mg/l. Presence of sulphide also causes odour to the surrounding environment. BDW also contained oil/grease to the tune of 110 mg/l. This gets coated on the gills creating respiratory problems. Surface layer of floating oil reduces the dissolved oxygen content of the water. All these parameters would impart toxicity to fish; hence toxicity evaluation through fish bioassay was carried out to arrive at a dilution factor for raw wastewater prior to its discharge into surface water bodies.

The fish Lebistes reticulatus is supposed to be very sturdy in nature but during the acute toxicity tests it exhibited different degrees of susceptibility to the various wastewaters. However LC_{50} values still provide information for the gross comparison of toxicity of the pollutants to fishes. During the acute toxicity test the fish exhibited several type of reactions, for instance raw BDW, which depicted more toxicity, made the fish more restless immediately after the addition of the wastewater. They came to the surface of the water to gulp the air at initial stages. Quick opecular movements were observed initially and after few hours they started slow movements and finally the fishes became calm and settled at the bottom of test chamber. Loss of balance was observed in case of all experimental fishes whereas, in case of raw BDW, body of fish became dark and body bending was seen.

From the LC_{50} values as indicated in Table 6, it is clear that the raw BDW exerts more toxicity. Electrochemical treatment using cost effective electrodes like carbon and aluminium resulted in marginal reduction in COD/BOD and TDS. In case of aluminium electrode COD/BOD reductions were 23.98% and 35% respectively. While in case of carbon the reductions were higher than aluminium electrode. The COD/BOD reductions with respect to carbon were 34.0% and 40.0% respectively. It is quite evident that the carbon electrode is comparatively more superior in terms of treatment to aluminium electrode. Hence this treatment can be applied as a primary treatment to reduce organic load on the secondary biological systems.

Electrochemical treatment methods have been reported to be quite efficient in eliminating toxicity of the organics due to its structural modification. However, it is also likely that the intermediates after treatment are more toxic than the parent substances. Also, presence of high chlorides (> 7000 mg/L) content in raw wastewater, could lead to formation of organo-chorinated by-products after the electrochemical treatment. Therefore, it was also intended to assess if the electrochemical treatment leads to increase/decrease in toxicity of treated samples before a biological treatment. Effluents after electrochemical treatment using carbon and aluminium electrode showed reduction in toxicity values which was in proportion to the amount of COD removals shown by the respective electrodes. The reduction in toxicity values does not indicate formation of any toxic intermediates. The variation of LC_{50} values for raw and treated BDW as a function of exposure time is shown in Figure 1. The LC_{50} values for raw BDW were 3.6%, 3.0%, 2.7% and 2.5% for 24, 48, 72 and 96 hours exposure, respectively. For BDW treated by aluminium electrodes the LC_{50} values were 5.8%, 5.4%, 5.2% and 5.0% for 24, 48, 72 and 96 hours respectively. Similarly, after treatment with carbon electrodes the LC_{50} values were 8.0%, 7.4%, 7.0% and 6.8% for 24, 48, 72 and 96 hours, respectively. LC_{50} values for raw and treated BDW did not show wide variation indicating toxic nature of the wastewater and treated effluent. The regression coefficient (R^2) between 0.96 and 0.99 indicates high correlation between % concentration and % mortality.

Apart from 96 hours LC_{50}, full range of lethal concentration (LC_0 - LC_{100}) should be taken into consideration while assessing the susceptibility of any organism to any toxic effluent. Based on 96 hours LC_{50} values it is possible to calculate the safe application rate and also the safe concentration. Hence SAFE (Safe factor) and SAR (Safe Application Rate) were calculated on the basis of 96 hours acute toxicity tests. The formula quoted in literature was used as indicated below (Basak and Konar 1977):

$$\frac{LC_0 \text{ at 96 hours}}{LC_{100} \text{ at 96 hours}}$$

$$\text{SAR} = \text{96 hours } LC_{50} \times \text{SAFE}$$

The results provide baseline information in formulating strategy for controlled release of treated industrial effluents into the receiving water bodies. For application of toxicity data in regulation of wastewater discharges and prediction of environmental affects both acute and chronic toxic levels have to be determined to conserve aquatic life. Therefore, from the studies it can be inferred that raw BDW is very toxic to the fish Lebistus reticulates whereas, electrochemical treatment did reduce the toxicity to certain extent, but this treatment is not enough. However it can certainly be applied as a pre-treatment method to reduce the pollutants to the tune of 30-35% thus reducing the load on secondary treatment.

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REFERENCES

Basak, P.K., and Konar, S.K., (1977). A new method for the determination of safe concentration of insecticides to protect fishes. Indian J. of Environ. Health, **19(4)**: 283-292.

Bazrafshan, E., Mahvi, A.H., Nasseri, S., Shaieghi, M., (2007). Performance evaluation of electrocoagulation process for diazinon removal from aqueous environments by using iron electrodes, Iran J. of Environ. Health Sci. & Eng., **4(2)**: 127-132.

Chen, G., (2004). Electrochemical technologies in wastewater treatment. Sep. & Purif. Tech., **38:** 11–41.

Doudoroff, P., Anderson, B.G., Burdick, G.E., Galtsaff, P.S., Hart, W.B., Patrick, E.R., Strong, E.R., Surbes, E.W., Vanitron, W.M., (1951). Bioassay methods for the evaluation of acute toxicity of industrial wastewater to fish. Sewage & Ind. Wastes, **23**: 130-136.

Enick, O.V., and Moore, M.M., (2007). Assessing the assessments: Pharmaceuticals in the environment. Environ. Imp. Assess. Rev., **27**: 707–729.

Franson, M.A.H., Eaton, A.D., Clesceri, L.S., Greenberg, A.E., (1998). Standard methods for the examination of water and wastewater, 20th Ed.; APHA, AWWA, WPCF: Washington D.C., USA.

Kumar, S., Sahay, S.S., Sinha, M.K., (1995). Bioassay of common distillery effluent on common guppy Lebistes Recticulatus (Peter). Bull. Environ. Contamin. & Toxicol., **54**: 309-316.

Litchfeild, J.T. Jr, Wilcoxin, F.A., (1949). Simplified method of evaluating dose effect experiments, J. Pharmacol. & Exper. Thera., **96**: 99-113.

Maleki, A., Mahvi, A. H., Vaezi, F., Nabizadeh, R., (2005). Ultrasonic degradation of phenol and determination of the oxidation by-products toxicity, Iranian J. of Environ. Health Sci. & Eng., **2(**3**)**: 201-206.

Movahedian, H., Bina, B., Asghari, G.H., (2005). Toxicity evaluation of wastewater treatment plant effluents using *Daphnia Magna,* Iranian J. of Environ. Health Sci. & Engg, **2(**2**)**: 1-4.

Satyanarayan, S., Kotangale, J.P., Satyanarayan, A., Choudhary, P.R., (2005). Toxicity of spent broth from an antibiotic unit to fish-*lebistes reticulatus* (peter). Poll. Res., **24(**2**)**: 439-442.

Shahtalebi, A., and Sarrafzadeh, M. H., (2011). Application of nanofiltration memberane in the separation of amoxicillin from pharmaceutical wastewater. Iranian J. of Environ. Health Sci. & Engg., **8(**2**)**: 106-110.

Shrinivas, P., Banerji, G., Rao, P., (1984). Preliminary observations on the effect of industrial effluents to certain animals, Ind. J. of Environ. and Health, **26**(1): 57-59.

Sprague, J.B., (1969). Review paper: Measurement of pollutant toxicity to fish 1-Bioassay methods for acute toxicity, Water Res., **3**: 793-796.

Vanerkar, A.P., Satyanarayan, S., Dharmadhikari, D.M., (2004). Toxicity of herbal bulk drug industry wastewater on fish- *lebistes reticulatus* (peter), J. of Environ. Sci. and Health, **B39 (**1**)**: 115-123.

Venkatamohan, S, Prakashan, R.S., Satyavathi, B., Annapurna, J., Ramakrishna, S.V., (2001). Biotretability studies of pharmaceutical wastewater using an anaerobic suspended film contact reactor, Water Sci. and Tech., **43(**2**)**: 271-276.