

Physico-Chemical Characterisation of Brewery Effluent and Its Degradation using Native Fungus - *Aspergillus Niger*, Aquatic Plant - Water Hyacinth- *Eichhornia SP* and Green Mussel – *Pernaviridis*

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Abstract

Brewery industries produce a substantial effluent stream, rich in organic matter originating from the brewing process. When this Brewery effluent discharged into municipal sewage system it leads to reduction of chemical and Biological oxygen demand. pH, EC, TSS, TDS, BOD, COD, Total Hardness (TH), Chloride, Copper and Zinc of untreated brewery effluent were estimated. The results of the study revealed that parameters were found to be above the permissible limits for disposal of effluent. Laboratory scale degradation of different concentrations of untreated brewery effluent were carried out using native fungus - *Aspergillus niger*, aquatic plant, Water hyacinth *Eichhornia sp* and green mussel - *Perna viridis* individually as they are efficient in degrading the effluent to meet the permissible limits for agricultural and aquacultural purposes.

Keywords: Brewery effluent, degradation, *Aspergillus niger*, *Eichhornia sp*, *Perna viridis*

1. Introduction

Brewery, the alcohol producing industry, is one of the major polluting industries. It involves the making of fermented alcoholic beverages, such as beer and ale from cereal grains. There are two major steps involved in the process of malting and brewing. Brewery wastes are composed mainly of liquor pressed from the wet grain and wash water from the various departments. After the distillation of the alcohol process, the residue remains is referred to as "distillery slops", or "still bottoms". The brewing industry consumes much water about 10 gallons of processed water / gallon of product. The Biochemical Oxygen Demand levels are quite high, as are the total solids, typically about half the BOD and over 90% of the suspended solids are generated in the brewing operation. There are also solid wastes spent grains hops and sludges, that are formed in this and the malting steps that must be disposed off. Disposal of such effluent without any prior treatment into water courses causes serious pollution problems (Ninnekar,1992). Such wastes when discharged into open drain undergo aerobic decompositions and create obnoxious odourous conditions. The indiscriminate disposal of untreated waste water into water courses or into land invariably pollutes the ecosystem (Mala & Saravana Babu, 2006). It also poses adverse effects to the aquatic fauna and flora and also to the ground water. Hence treatment of brewery effluent is a very important consideration before its disposal.

Though there are many physical and chemical treatment methods available, scientists have found that in managing certain wastes, the best option is the biological treatment which is more efficient and consumes no energy (Environment News, 2002). Since the complete degradation of organic chemicals in the natural ecosystem is primarily carried out by biological methods, bio technological application use biological method or their enzymes for waste treatment (Ninnekar,1992). Degradation of industrial effluents using microbes, *Eichhornia sp* and *Perna viridis* were carried out by many researchers (Sridevi, 2000; Bhavani,2000; Adekunle & Oluyode,2005; Aftab, Noorjahan, 2006). In this study an attempt has been made to analyse the parameters of untreated brewery effluent and degrade the untreated brewery effluent which is also highly polluted using native fungus - *Aspergillus niger*, aquatic plant - *Eichhornia sp* and green mussel - *Perna viridis*.

2. Materials and methods

2.1 Sample – Brewery effluent

The untreated brewery effluent of about 5 litres was collected from a brewery industry located in Poonamallee, Thiruvalluvar District, Chennai, Tamil Nadu, India, and it was stored at 25°C. Characterization of the effluent such as colour, odour, hydrogen Ion concentration (pH), electrical conductivity (EC), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD),

Total Hardness (TH), Chloride (Cl), Copper (Cu) and Zinc (Zn) were analysed as per Standard methods (APHA, 1998). Samples of about 30 litres were brought to the laboratory and the degradation of effluent using *Aspergillus niger*, *Eichhornia* sp and *Perna viridis* were carried out.

2.2 Fungus- *Aspergillus niger*

Aspergillus niger was isolated from the untreated brewery effluent using 1 ml of untreated brewery effluent which was diluted to 10⁻¹ with sterile distilled water and was cultured on malt extract agar medium (MEA) following pour plate method. Fungal species developed on the medium were observed periodically. The fungi were stained with lactophenol cotton blue and identified using the manual (Onions et al., 1981). *Aspergillus niger* was then subcultured on Potato Dextrose Agar (PDA) for 3 days separately.

2.3 Water hyacinth - *Eichhornia* sp – Experimental plant

Water hyacinth (*Eichhornia* sp) of same size were collected from Chetpet Pond, Chennai. The plants were collected carefully without causing any damage to the roots. The plants collected were washed thoroughly in tap water. They were introduced into large tubs containing tap water and maintained for 10 days for acclimatization to the laboratory conditions (Jamuna & Noorjahan, 2009).

2.4 Green mussel- *Perna viridis* Experimental animal

Green mussel - (*Perna viridis*) was collected from Ennore estuary and acclimatized to the laboratory conditions for two weeks in aerated sea water.

2.5 Degradation Study

Aspergillus niger was washed with sterile distilled water and approximately 2.5, 5 and 10 gms mycelia of fungus were transferred to Brew 1, Brew 2 and Brew 3 of untreated brewery effluent taken in different conical flasks. They were incubated at 30 ± 0.5°C for 96 hrs, on rotary shaker at 2000 rpm and centrifuged at Brew 200 rpm for 20 minutes. The degradation process was carried out by following the procedure of Noorjahan, (2002).

After acclimatization of Water hyacinth (*Eichhornia* sp) to the laboratory conditions, experiments were conducted in plastic tubs (36cm×11cm×9cm). Accordingly, the effluent was diluted to the desired percentage concentrations of Brew 1, Brew 2 and Brew 3 using aerated tap water. 5 fresh plants of same size were introduced in each concentration of the tub. It was examined for a period of 4 days (96 hrs) for degradation process and degradation was carried out by following the procedure of Sridevi, (2000).

After acclimatization of *Perna viridis* to the laboratory conditions experiments were conducted in plastic tubs. Accordingly, the effluent was diluted to the desired percentage concentrations of Brew 1, Brew 2 and Brew 3 using aerated sea water and 10 animals were introduced in each concentration and the study was carried for a period of 96 hrs. The degradation was carried out by following the procedure of Bhavani, (2000). The samples were analysed for the physico-chemical parameters before (control) and after degradation using *Aspergillus niger*, *Eichhornia* sp and *Perna verdis*. The data obtained from the experiments were statistically analysed and expressed as mean, standard deviation and percentage change.

3. Results and Discussion

The results of physico-chemical characteristics of untreated brewery effluent are depicted in Table 1. The effluent was brownish black in colour and has offensive odour. This colour and odour, attributed could be due to decomposition of organic or inorganic matter stated by Singh et al., (1998). pH was alkaline in nature. The EC (3585 µmhos/cm), TSS (10Brew 2 mg/l), TDS (2712 mg/l), BOD (260 mg/l), COD (854 mg/l), TH (480 mg/l), Chloride (804 mg/l) levels were higher than the permissible limits of CPCB, (1995) which may be due to the presence of large amount of organic and inorganic matter present in the effluent which is supported by the work of Aftab and Noorjahan, (2006). Copper (0.00581 mg/l) and Zinc (0.07566 mg/l) levels were within the permissible limit of CPCB, (1995). The presence of heavy metals in the effluent itself produce several effects on living organisms such as Skin disorders, Respiratory abnormalities, abdominal and Intestinal problems, dental disorders, impairments of central Nervous systems, skeletal muscular systems, blood disorders, malaria, chicken pox, septic wounds and congenital abnormalities, Cardiovascular disorders and lung cancer etc reported by Chukwu, (2006).

The results of degradation of different concentrations (Brew 1, Brew 2 and Brew 3) of untreated brewery effluent using *Aspergillus niger*, Water hyacinth and *Perna viridis* are presented in Table 2 & fig 1, Table 3 & Fig 2 and Table 4 & Fig 3. The colour was brownish black and had offensive odour before degradation. This colour and odour could be due to the decomposition of organic or inorganic matter. This may also be due to the presence of various aromatic and volatile organic compounds present in the different concentrations in the effluent, but after degradation for 96 hrs there is change in the colour to almost colourless and also odourless

condition in all concentrations of the effluent using *Aspergillus niger*, *Eichhornia* sp and *Perna viridis*. pH of different concentrations (Brew 1, Brew 2 and Brew 3) before degradation is alkaline in nature but after degradation for 96 hrs. pH was alkaline in nature which had changed to the neutral state. The different concentrations are coded as Brew 1 (Brew 1), Brew 2 (Brew 2) and Brew 3 (Brew 3).

EC in Brew 1 concentration of untreated brewery effluent before treatment is $900 \mu\text{mhos/cm} \pm 5.67$ which is beyond the permissible limit $400 \mu\text{mhos/cm}$ (CPCB, 1995). Untreated brewery effluent has higher level of Electrical conductivity which could reflect the presence of organic and inorganic substances and salts that would have increased the conductivity (Robinson and Stokes, 1959), Marwaha et al., (1998) but after degradation, in Brew 1 *Eichhornia* sp degraded EC to $400 \mu\text{mhos/cm} \pm 10.8$ and the percentage change is 55.55% but *Perna viridis* degraded EC to $200 \mu\text{mhos/cm} \pm 10.8$ and percentage change is 44.44% followed by *Aspergillus niger* which degraded EC to $560 \mu\text{mhos/cm} \pm 4.08$ and percentage change is 37.77%. Therefore maximum reduction of EC in Brew 1 of untreated brewery effluent was recorded using *Eichhornia* sp followed by *Perna viridis* and *Aspergillus niger*. In Brew 2 concentration EC in control was $1792 \mu\text{mhos/cm} \pm 1.63$ but after degradation for 96 hrs, *Eichhornia* sp degraded EC to $802 \mu\text{mhos/cm} \pm 0.81$ (55.24%), *Perna viridis* reduced EC to $980 \mu\text{mhos/cm} \pm 3.74$ (43.31%) followed by *Aspergillus niger* $1000 \mu\text{mhos/cm} \pm 3.74$ (44.19%). In Brew 3 concentration similar trend was recorded in degradation of EC where *Eichhornia* sp degraded EC to $3062 \mu\text{mhos/cm} \pm 2.16$ and percentage change was 12.13% when compared to control value of EC (i.e) $3585 \mu\text{mhos/cm} \pm 2.94$. Whereas *Perna viridis* reduced EC values to $3100 \mu\text{mhos/cm} \pm 2.94$ and percentage change was 12.13% followed by *Aspergillus niger* which degraded EC to $3300 \mu\text{mhos/cm} \pm 5.67$ and percentage change was 7.94%. A similar trend was recorded in Brew 2 and Brew 3 concentrations as in Brew 1 concentration of untreated brewery effluent with respect to EC. Thus in all the three different concentrations (Brew 1, 2 & 3) of untreated brewery effluent, maximum reduction of EC was recorded using *Eichhornia* sp followed by *Perna viridis* and *Aspergillus niger*. Maximum reduction (55%) of EC was recorded in Brew 1 and Brew 2 followed by Brew 3. This maximum degradation of EC by *Eichhornia* sp compared to *Aspergillus niger* and *Perna viridis* indicates the efficiency of *Eichhornia* sp in degrading untreated effluent compared to *Aspergillus niger* and *Perna viridis*.

TSS in Brew 1 concentration of untreated brewery effluent before treatment (i.e) control in Brew 1 is $300 \text{mg/l} \pm 5.67$ when compared to the permissible limit (100mg/l) prescribed in (CPCB,1995) for effluent discharge. High amount of TSS elevated the density and turbidity of water thereby affects osmoregulation and interfered with the photosynthesis by preventing sunlight (Kalita et al.,2003). But after degradation for 96 hrs, *Eichhornia* sp degraded TSS to $17 \text{mg/l} \pm 3.26$ and percentage change was 94.33% followed by *Perna viridis* which degraded TSS by 94.33% ($17 \text{mg/l} \pm 3.26$) and *Aspergillus niger* reduced TSS to $20 \text{mg/l} \pm 1.63$ (93.33%). In Brew 2 similar result was observed where *Eichhornia* sp reduced TSS to $20 \text{mg/l} \pm 1.63$ (96.19%) followed by *Perna viridis* which reduced TSS to $20 \text{mg/l} \pm 1.63$ (96.19%) whereas *Aspergillus niger* reduced TSS to $30 \text{mg/l} \pm 0.81$ (94.28%). In Brew 3 maximum reduction of TSS was recorded using *Eichhornia* sp which degraded TSS to $28 \text{mg/l} \pm 2.94$ (97.33%) followed by *Perna viridis* which reduced TSS to $28 \text{mg/l} \pm 2.94$ (97.33%) whereas *Aspergillus niger* reduced TSS to $48 \text{mg/l} \pm 2.94$ (95.42%). Thus overall maximum reduction of TSS in all three different concentrations of untreated brewery effluent was recorded using *Eichhornia* sp and *Perna viridis* followed by *Aspergillus niger*. Maximum reduction (97.33%) of TSS was recorded in Brew 1 followed by Brew 3 and Brew 2.

TDS in Brew 1 concentration of untreated brewery effluent before treatment (i.e) control in Brew 1 is $678 \text{mg/l} \pm 16.5$ which may be due to high inorganic salt contents present in the untreated effluent and also rendered it unsuitable for irrigation hence further treatment or dilution would be required before disposal as reported. If the TDS levels of water exceeded 200mg/l , it became unsuitable for bathing and drinking purposes for animals as it caused distress in cattle and livestock. But after degradation for 96 hrs. *Aspergillus niger* degraded TDS to $230 \text{mg/l} \pm 4.89$ and percentage change was 66.07% followed by *Eichhornia* sp which degraded TDS by 63.12% ($248 \text{mg/l} \pm 4.89$) and *Perna viridis* reduced TSS to $400 \text{mg/l} \pm 10.8$ (41%). In Brew 2 similar result was observed as in Brew 1 concentration where *Aspergillus niger* reduced TDS to $460 \text{mg/l} \pm 4.54$ (66.81%) followed by *Eichhornia* sp which reduced TDS to $726 \text{mg/l} \pm 4.08$ (47.61%) whereas *Perna viridis* reduced TDS to $856 \text{mg/l} \pm 2.94$ (38.23%). In Brew 3 maximum reduction of TDS was recorded using *Aspergillus niger* which degraded TDS to $908 \text{mg/l} \pm 1.63$ (66.51%) followed by *Eichhornia* sp which reduced TDS to $2622 \text{mg/l} \pm 2.16$ (3.31%) whereas *Perna viridis* reduced TDS to $2700 \text{mg/l} \pm 10.8$ (0.44%). Thus overall maximum reduction of TDS in all three concentrations of untreated brewery effluent were recorded using *Aspergillus niger* followed by *Eichhornia* sp and *Perna viridis*. Maximum reduction (66.81%) of TDS was recorded in Brew 1 followed by Brew 2 and Brew 3. This maximum reduction of TDS by *Aspergillus niger* compared to *Eichhornia* sp and *Perna viridis* may be due to consumption of inorganic and organic matter by microbes for their food which was supported by the work of Elizabeth et al., (2006).

BOD in Brew 1 concentration of untreated brewery effluent before treatment (i.e) control in Brew 1 is $65 \text{ mg/l} \pm 2.44$ which is beyond the permissible limit 30 mg/l (CPCB, 1995). The present study revealed high levels of BOD in the untreated brewery effluent which may be due to the presence of considerable amount of organic matter. High BOD levels have also been reported in tannery effluent. But after degradation for 96 hrs *Aspergillus niger* degraded BOD to $3.5 \text{ mg/l} \pm 0.16$ and percentage change was 94.61% followed by *Eichhornia* sp which degraded BOD by 69.23% ($20 \text{ mg/l} \pm 1.63$) and *Perna viridis* which reduced BOD to $40 \text{ mg/l} \pm 4.32$ (38.46%). In Brew 2 similar trend was observed where control is 140 ± 5.35 but after degradation for 96 hrs. *Aspergillus niger* reduced BOD to $10 \text{ mg/l} \pm 2.16$ (92.35%) followed by *Eichhornia* sp which reduced BOD to $30 \text{ mg/l} \pm 0.81$ (78.57%) whereas *Perna viridis* reduced BOD to Brew 2 $\text{mg/l} \pm 2.94$ (64.28%). In Brew 3 also similar trend was observed where BOD in control is $260 \text{ mg/l} \pm 2.16$ but after degradation for 96 hrs. *Aspergillus niger* degraded BOD to $14 \text{ mg/l} \pm 2.44$ (94.61%) followed by *Eichhornia* sp which reduced BOD to $190 \text{ mg/l} \pm 2.94$ (26.92%) whereas *Perna viridis* reduced BOD to $225 \text{ mg/l} \pm 1.63$ (13.46%). Thus overall maximum reduction of BOD in all three concentrations of untreated brewery effluent is recorded using *Aspergillus niger* followed by *Eichhornia* sp, *Perna viridis* and maximum reduction (94.61%) of BOD was recorded in Brew 1, Brew 3 followed by Brew 2. This maximum reduction of BOD by *Aspergillus niger* compared to *Eichhornia* sp and *Perna viridis* showed the efficient degrading capability of *Aspergillus niger* by degrading contaminants as they use it for their growth and reproduction (Hossain and Das 2001).

COD in Brew 1 concentration of untreated brewery effluent before treatment (i.e) control in Brew 1 is $213 \text{ mg/l} \pm 2.94$ for effluent discharge into inland surface waters thereby indicating large quantity of organic and inorganic matter present in the effluents which is supported by the work of Kansal et al., (2005). But after degradation for 96 hrs *Aspergillus niger* degraded COD by 85.91% ($30 \text{ mg/l} \pm 0.81$) followed by *Eichhornia* sp which degraded COD by 60.56% ($84 \text{ mg/l} \pm 3.74$) and *Perna viridis* reduced COD to $131 \text{ mg/l} \pm 2.16$ (38.49%). In Brew 2 similar trend was observed where COD of control is $430 \text{ mg/l} \pm 2.94$ but after degradation for 96 hrs, *Aspergillus niger* reduced COD to Brew 2 $\text{mg/l} \pm 2.94$ (88.37%) followed by *Eichhornia* sp which reduced COD to $209 \text{ mg/l} \pm 2.94$ (51.39%) whereas *Perna viridis* reduced COD to $300 \text{ mg/l} \pm 5.67$ (30.23%). In Brew 3 also similar result was obtained where COD of control is $854 \text{ mg/l} \pm 2.16$ but after degradation for 96 hrs. *Aspergillus niger* degraded COD to $96 \text{ mg/l} \pm 2.44$ (88.75%) followed by *Eichhornia* sp which reduced COD to $613 \text{ mg/l} \pm 2.94$ (28.22%) whereas *Perna viridis* reduced COD to Brew 2 $\text{mg/l} \pm 2.74$ (12.17%). Thus overall maximum reduction of COD in all three concentrations of untreated brewery effluent was recorded using *Aspergillus niger* followed by *Eichhornia* sp and *Perna viridis*. Maximum reduction (88.75%) of COD was recorded in Brew 2 and Brew 3 followed by Brew 1. This maximum reduction of COD by *Aspergillus niger* compared to *Eichhornia* sp and *Perna viridis* is supported by the work of Poonkothai and Parvatham (2005).

TH in Brew 1 concentration of untreated brewery effluent before degradation (i.e) control in Brew 1, $130 \text{ mg/l} \pm 5.09$. which was mainly due to the presence of carbonates and bicarbonates of calcium and magnesium ions (Rajput et al., 2004). But after degradation for 96 hrs. *Aspergillus niger* degraded TH by 76.92% ($30 \text{ mg/l} \pm 0.81$) followed by *Perna viridis* which degraded TH by 23.07% ($100 \text{ mg/l} \pm 7.25$) and *Eichhornia* sp reduced TH to $115 \text{ mg/l} \pm 5.09$ (11.5%). In Brew 2 concentration TH in control was $290 \text{ mg/l} \pm 3.74$ but after degradation for 96 hrs. *Aspergillus niger* degraded TH to Brew 2 $\text{mg/l} \pm 2.16$ (48.27%), *Perna viridis* reduced TH to $160 \text{ mg/l} \pm 2.16$ (44.82%) followed by *Eichhornia* sp which reduced TH to $166 \text{ mg/l} \pm 3.26$ (42.75%). A similar trend was recorded in Brew 2 concentration as in Brew 1 concentration of untreated brewery effluent with respect to TH. In Brew 3 concentration TH in control is $480 \text{ mg/l} \pm 2.16$ and similar trend was recorded in degradation of TH where *Aspergillus niger* degraded TH to Brew 2 $\text{mg/l} \pm 7.48$ (33.33%) followed by *Perna viridis* which reduced TH to $376 \text{ mg/l} \pm 2.44$ (21.66%) whereas *Eichhornia* sp reduced TH to $430 \text{ mg/l} \pm 2.94$ (10.41%). Thus overall maximum reduction of TH in all three concentrations of untreated brewery effluent was recorded using *Aspergillus niger* followed by *Perna viridis* and *Eichhornia* sp. Maximum reduction (76.92%) of TH was recorded in Brew 1 followed by Brew 2 and Brew 3.

Chloride in Brew 1 concentration of untreated brewery effluent before treatment is $221 \text{ mg/l} \pm 2.94$. The effluent with higher level of chloride when discharged into water body will prevent the self purification of water and may harm the fishes and other aquatic life but after degradation, in Brew 1 *Eichhornia* sp degraded chloride to $100 \text{ mg/l} \pm 7.25$ (54.75%), while *Perna viridis* degraded chloride to $170 \text{ mg/l} \pm 7.48$ (23.07%) followed by *Aspergillus niger* which degraded chloride to $180 \text{ mg/l} \pm 2.44$ (18.55%). Therefore maximum reduction of chloride in Brew 1 of untreated brewery effluent was recorded using *Eichhornia* sp followed by *Perna viridis* and *Aspergillus niger*. In Brew 2 concentration chloride in control was $413 \text{ mg/l} \pm 1.63$ but after degradation for 96 hrs. *Eichhornia* sp reduced chloride to $251 \text{ mg/l} \pm 2.16$ (21.30%), *Perna viridis* reduced chloride to $300 \text{ mg/l} \pm 5.67$ (27.36%) followed by *Aspergillus niger* which degraded chloride to $325 \text{ mg/l} \pm 1.63$ (21.30%). A similar trend was recorded in Brew 2 concentration as in Brew 1 concentration of untreated brewery effluent with respect to chloride. In Brew 3 concentration similar trend was recorded in degradation of chloride where

Eichhornia sp degraded chloride to $407 \text{ mg/l} \pm 1.63$ (49.37%) when compared to control values of chloride (i.e) $804 \text{ mg/l} \pm 2.16$ whereas *Perna viridis* reduced chloride to $700 \text{ mg/l} \pm 10.8$ (12.93%) followed by *Aspergillus niger* which degraded chloride level to $7 \text{ mg/l} \pm 4.32$ (6.71%). Thus in all the three different concentrations (Brew 1, Brew 2 and Brew 3) of untreated brewery effluent, maximum reduction of chloride was observed using *Eichhornia sp* followed by *Perna viridis* and *Aspergillus niger*. Maximum reduction (54.75%) of chloride was recorded in Brew 1 followed by Brew 3 and Brew 2. This maximum reduction of chloride by *Eichhornia sp* compared to *Perna viridis* and *Aspergillus niger* indicate the degrading efficiency of *Eichhornia sp* in reducing chloride level of the effluent which is in accordance with the reports of Zaranyika et al., (1994).

Copper present in untreated brewery effluent before treatment (i.e) control in Brew 1 was $0.0041525 \text{ mg/l} \pm 0.00000020$. The presence of heavy metals in the effluent produce several effects on living organisms as reported (Chukwu, 2006). But after degradation for 96 hrs. *Eichhornia sp* degraded copper to $0.00021 \text{ mg/l} \pm 0.00000016$ (0.02%) followed by *Aspergillus niger* which degraded copper by 73.83% ($0.00038 \text{ mg/l} \pm 0.0000033$) and *Perna viridis* reduced copper to $0.0013 \text{ mg/l} \pm 0.00020$ (10.49%). In Brew 2 concentration in control copper was $0.002905 \text{ mg/l} \pm 0.0000047$ but after degradation for 96 hrs. *Eichhornia sp* degraded copper to $0.0005 \text{ mg/l} \pm 0.00021$ (82.78%), *Aspergillus niger* reduced copper level to $0.00079 \text{ mg/l} \pm 0.000021$ (72.80%) followed by *Perna viridis* which reduced copper to $0.00095 \text{ mg/l} \pm 0.000029$ (67.29%). A similar trend was recorded in Brew 2 concentration as in Brew 1 concentration of untreated brewery effluent with respect to copper. In Brew 3 concentration similar trend was recorded in degradation of copper where *Eichhornia sp* degraded copper to $0.006 \text{ mg/l} \pm 0.0021$ (89.67%) when compared to control values of copper (i.e) $0.00581 \text{ mg/l} \pm 0.000021$ whereas *Aspergillus niger* reduced copper to $0.0015 \text{ mg/l} \pm 0.00029$ (74.18%) followed by *Perna viridis* which degraded copper to $0.0021 \text{ mg/l} \pm 0.00021$ (63.85%). Thus in all the three different concentrations (Brew 1, Brew 2 and Brew 3) of untreated brewery effluent maximum reduction of copper is carried out by *Eichhornia sp* followed by *Aspergillus niger* and *Perna viridis*. Maximum reduction (89.67%) of copper was recorded in Brew 1 followed by Brew 3 and Brew 2.

Zinc present in untreated brewery effluent before treatment (i.e) control in Brew 1 was $0.018915 \text{ mg/l} \pm 0.0000016$. The presence of heavy metals in the effluent itself produce several effects on living organisms as reported14. But after degradation for 96 hrs, *Eichhornia sp* degraded zinc to $0.005 \text{ mg/l} \pm 0.00020$ (97.35%) followed by *Perna viridis* which degraded zinc $0.005 \text{ mg/l} \pm 0.00020$ (97.35%) *Eichhornia sp*, *Aspergillus niger* which reduced zinc to $0.006 \text{ mg/l} \pm 0.0024$ (68.27%). In Brew 2 concentration zinc level in control was $0.03783 \text{ mg/l} \pm 0.000024$ but after degradation for 96 hrs. *Eichhornia sp* degraded zinc to $0.01 \text{ mg/l} \pm 0.0085$ (73.56%), *Perna viridis* reduced zinc to $0.01 \text{ mg/l} \pm 0.0085$ (73.56%) as followed by *Aspergillus niger* which reduced zinc to $0.03 \text{ mg/l} \pm 0.016$ (20.69%). A similar trend was recorded in Brew 2 concentration as in Brew 1 concentration of untreated brewery effluent with respect to zinc. In Brew 3 concentration similar trend was recorded in degradation of zinc where *Eichhornia sp* degraded zinc to $0.003 \text{ mg/l} \pm 0.0001$ (96.03%) when compared to control values of zinc (i.e) $0.07566 \text{ mg/l} \pm 0.000037$ whereas *Perna viridis* degraded zinc to $0.003 \text{ mg/l} \pm 0.0001$ (96.03%) followed by *Aspergillus niger* which degraded to $0.035 \text{ mg/l} \pm 0.0029$ (53.74%). Thus in all three different concentrations (Brew 1, Brew 2 and Brew 3) of untreated brewery effluent maximum reduction of zinc were carried out by *Eichhornia sp* and *Perna viridis* followed by *Aspergillus niger* and maximum reduction (97.35%) of zinc was recorded in Brew 1 followed by Brew 3 and Brew 2. This maximum reduction of zinc by *Eichhornia sp* followed by *Perna viridis* compared to *Aspergillus niger* may be due to the complete exhaustion of the binding sites in the plants. These results were in agreement with observations of APHA, (1998).

CONCLUSION

Thus from the foregoing discussion it is very clear that parameters such as TSS, TDS, BOD, COD, TH were found to be higher than the permissible limits of (CPCB,1995) for effluent discharge. Waste treatment, the most vital aspect in any wastewater management programme. Hence the untreated brewery effluent was degraded using *Eichhornia sp*, *Aspergillus niger* and *Perna viridis*. The results of the above study show that *Eichhornia sp*, *Aspergillus niger* and *Perna viridis* play an important role in the degradation of organic and inorganic matter present in the untreated brewery effluent. Industrial wastes / effluents were complex comprising of innumerable chemical compounds, that even the most sophisticated chemical analysis were just inadequate to identify all biological active compounds present in the waste. Biodegradation seems to be the most promising technique for untreated brewery effluent as confirmed 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. Hence after degradation the treated effluent could be used for crop cultivation or irrigation purposes.

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Table 1. Analysis of parameters of untreated brewery effluent

Sl.No.	Parameters	CPCB (1995)	Untreated brewery effluent
Physical Parameters			
1.	Colour	Colourless	Brownish black
2.	Odour	Odourless	Offensive
3.	pH	5.5 - 9.0	7.2
Chemical Parameters			
4.	Electrical conductivity ($\mu\text{mhos/cm}$)	400	3585
5.	Total Suspended Solids (mg/l)	100	1050
6.	Total Dissolved Solids (mg/l)	2100	2712
7.	Biological Oxygen Demand (mg/l)	30	260
8.	Chemical Oxygen Demand (mg/l)	250	854
9.	Total Hardness (mg/l)	300	480
10.	Chloride (mg/l)	600	804
11.	Copper (mg/l)	3	0.00581
12.	Zinc (mg/l)	1.0	0.07566
CPCB- Central Pollution Control Board			

Table 2. Analysis of parameters of untreated brewery effluent Brew 1 (25%) Concentration before (control) and after degradation using A.niger, Eichhornia sp and P.viridis (96 hrs.)							
Sl.No.	Parameters	CPCB (1995) (Values)		Control	Fungi - <i>A.niger</i>	Aquatic plant - <i>Eichhornia sp</i>	Green mussel - <i>Pernaviridis</i>
Physical Parameters							
1.	pH	5.5 - 9.0	Mean ± S.D % Change	7.8 ± 0.16	7.1 ± 0.16 8.97%	7.0 ± 0.16 10.25%	7.2 ± 0.21 7.69%
Chemical Parameters							
2.	Electrical Conductivity (µmhos/cm)	7.0	Mean ± S.D % Change	900 ± 5.67	560 ± 4.08 37.77%	400 ± 10.8 55.55%	500 ± 10.8 44.44%
3.	Total Suspended Solids (mg/l)	100	Mean ± S.D % Change	300 ± 5.67	20 ± 1.63 93.33%	17 ± 3.26 94.33%	17 ± 3.26 94.33%
4.	Total dissolved solids (mg/l)	2100	Mean ± S.D % Change	678 ± 5.67	230 ± 4.89 66.07%	250 ± 4.89 63.12%	400 ± 10.8 41.00%
5.	Biological Oxygen Demand (mg/l)	30	Mean ± S.D % Change	65 ± 2.44	3.5 ± 0.16 94.61%	20 ± 1.63 69.23%	40 ± 4.32 38.46%
6.	Chemical Oxygen Demand (mg/l)	250	Mean ± S.D % Change	213 ± 2.94	30 ± 0.81 85.91%	84 ± 3.74 60.56%	131 ± 2.16 38.49%
7.	Total Hardness (mg/l)	300	Mean ± S.D % Change	130 ± 5.09	30 ± 0.81 76.92%	115 ± 5.09 11.5%	100 ± 7.25 23.07%
8.	Chloride (mg/l)	600	Mean ± S.D % Change	221 ± 2.94	180 ± 2.44 18.55%	100 ± 7.25 54.75%	170 ± 7.48 23.07%
9.	Copper (mg/l)	3	Mean ± S.D % Change	0.0041525 ± 0.00000020	0.000380 ± 0.00000033 73.83%	0.00021 ± 0.00000016 85.54%	0.0013 ± 0.00020 10.49%
10.	Zinc mg/l	1.0	Mean ± S.D % Change	0.018915 ± 0.0000016	0.006 ± 0.0024 68.27%	0.0005 ± 0.00020 97.35%	0.0005 ± 0.00020 97.35%
CPCB- Central Pollution Control Board							

Sl.No.	Parameters	CPCB (1995) (Values)		Control	Fungi - <i>A.niger</i>	Aquatic plant - <i>Eichhornia sp</i>	Green mussel - <i>Pernaviridis</i>
Physical Parameters							
1.	pH	5.5 - 9.0	Mean ± S.D	3.5 ± 0.21	7.3 ± 0.21	7.5 ± 0.16	7.1 ± 0.16
			% Change		10.85%	11.42%	8.97%
Chemical Parameters							
2.	Electrical Conductivity (µmhos/cm)	400	Mean ± S.D	1792 ± 1.63	1000 ± 3.74	802 ± 0.81	980 ± 3.74
			% Change		44.19%	55.24%	45.31%
3.	Total Suspended Solids (mg/l)	100	Mean ± S.D	525 ± 2.16	30 ± 0.81	20 ± 1.63	20 ± 1.63
			% Change		94.28%	96.195	96.19%
4.	Total dissolved solids (mg/l)	2100	Mean ± S.D	1386 ± 2.44	460 ± 4.54	726 ± 4.08	856 ± 2.94
			% Change		66.81%	47.61%	38.23%
5.	Biological Oxygen Demand (mg/l)	30	Mean ± S.D	140 ± 5.35	10 ± 2.16	30 ± 0.81	50 ± 2.94
			% Change		92.85%	78.57%	64.28%
6.	Chemical Oxygen Demand (mg/l)	250	Mean ± S.D	430 ± 2.94	50 ± 2.94	209 ± 2.94	300 ± 5.67
			% Change		88.37%	51.39%	30.23%
7.	Total Hardness (mg/l)	300	Mean ± S.D	290 ± 3.74	150 ± 2.16	166 ± 3.26	160 ± 2.16
			% Change		48.27%	42.75%	44.82%
8.	Chloride (mg/l)	600	Mean ± S.D	413 ± 1.63	325 ± 1.63	251 ± 2.16	300 ± 5.67
			% Change		21.30%	39.22%	27.36%
9.	Copper (mg/l)	3	Mean ± S.D	0.002905 ± 0.0000047	0.00079 ± 0.000021	0.0005 ± 0.000216	0.00095 ± 0.000029
			% Change		72.80%	82.78%	67.29%
10.	Zinc mg/l	1.0	Mean ± S.D	0.03783 ± 0.000024	0.03 ± 0.016	0.01 ± 0.0085	0.01 ± 0.0085
			% Change		20.69%	73.56%	73.56%

CPCB- Central Pollution Control Board

Table 4. Analysis of parameters of untreated brewery effluent Brew 3 (100%) Concentration before (control) and after degradation using A.niger, Eichhornia sp and P.viridis (96 hrs.)						
Sl.No.	Parameters	CPCB (1995) (Values)	Control	Fungi - A.niger	Aquatic plant - Eichhornia sp	Green mussel - Perviridis
Physical Parameters						
1.	pH	5.5 - 9.0	Mean ± S.D 7.2 ± 0.21 % Change	7.0 ± 0.16 2.77%	6.9 ± 0.29 4.16%	7.1 ± 0.16 1.38%
Chemical Parameters						
2.	Electrical Conductivity (µmhos/cm)	400	Mean ± S.D 3585 ± 2.94 % Change	3300 ± 5.67 7.94%	3062 ± 2.16 14.58%	3150 ± 2.94 12.13%
3.	Total Suspended Solids (mg/l)	100	Mean ± S.D 1050 ± 2.94 % Change	48 ± 2.94 95.42%	28 ± 2.94 97.33%	28 ± 2.94 97.33%
4.	Total dissolved solids (mg/l)	2100	Mean ± S.D 2712 ± 2.16 % Change	908 ± 1.63 66.51%	2622 ± 2.16 3.31%	2700 ± 10.8 0.44%
5.	Biological Oxygen Demand (mg/l)	30	Mean ± S.D 260 ± 2.16 % Change	14 ± 2.44 94.61%	190 ± 2.94 26.92%	225 ± 1.63 13.46%
6.	Chemical Oxygen Demand (mg/l)	250	Mean ± S.D 854 ± 21.6 % Change	96 ± 2.44 88.75%	613 ± 2.94 28.22%	750 ± 2.74 12.17%
7.	Total Hardness (mg/l)	300	Mean ± S.D 480 ± 2.16 % Change	320 ± 7.48 33.33%	430 ± 2.94 10.41%	376 ± 2.44 21.66%
8.	Chloride (mg/l)	600	Mean ± S.D 804 ± 2.16 % Change	750 ± 4.32 6.71%	407 ± 1.63 49.37%	700 ± 10.8 12.93%
9.	Copper (mg/l)	3	Mean ± S.D 0.00581 ± 0.000021 % Change	0.0015 ± 0.00029 74.18%	0.0006 ± 0.00021 89.67%	0.0021 ± 0.00021 63.85%
10.	Zinc mg/l	1.0	Mean ± S.D 0.07566 ± 0.000037 % Change	0.035 ± 0.0029 53.74%	0.003 ± 0.0001 96.03%	0.003 ± 0.0001 96.03%
CPCB- Central Pollution Control Board						