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EFFICACY OF IMMOBILISED BACILLUS CEREUS DURING THE BIODEGRADATION OF TEXTILE INDUSTRY EFFLUENTS

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ABSTRACT

Textile industry effluents contain high levels of dyes which are recalcitrant and toxic to aquatic flora and fauna. Besides, they also contain very high levels of total dissolved solids (TDS), total suspended solids (TSS), COD, BOD and hardness. As per some estimates, about 10-15% of dyes end up in the effluents during their synthesis and dyeing processes. Biological treatment by employing microbes is a better alternative, when compared to conventional physical and chemical methods of effluent treatment. In the present study, efforts were made to understand the efficacy of immobilsed microbial cells upon the degradation of textile effluent. One of the commonly used azo-dyes namely, "Reactive Brilliant Red X3b"present in the untreated textile industry effluent was used as a representative dye in the present study and Bacillus cereus was the bacterium used for degradation. The effluent sample was collected from Tirupur, Tamil Nadu, India bears latitude-longitude coordinates as 11.1085° N, 77.3411° E. Very high levels of COD of 821 mg/l and very high levels of BOD of 211 mg/l were recorded for the effluent sample. In the present study, highest biodegradation was achieved at 96 hours of incubation at 20 grams concentration of the bio-beads containing entrapped Bacillus cereus.

KEYWORDS: Bacillus cereus, biodegradation, immobilization, optical density, reactive brilliant red x3b

INTRODUCTION

The textile industry generates huge volumes of wastewater, and the textile industry effluents contain dyes which are very recalcitrant and toxic to the flora and fauna present in the environment (Pearce et al., 2003; Liu et al., 2004). Azo dyes are used widely in many industries due to their bright color, excellent colorfastness and ease of application (Mahony et al., 2002). Azo dyes, account for 60-70% of all textile dyestuffs used (Carlyle et al., 1995). As per some estimates, about 10-15% of the total production of colorants is lost during their synthesis and dyeing processes (Easton, 1995; Maguire, 1992). Almost 50% of the initial dye load is found in the dye bath effluents in the case of reactive dyes. Several physico-chemical methods have been used to treat the colored effluents in wastewater. But they are generally expensive, produce large amounts of sludge. More often, these conventional modes of treatment lead to the formation of some harmful side products. (Azmi et al., 1998; Moreria et al., 2000; Rajeshkannan et al., 2010; Rajeshkannan et al., 2011).

Therefore, the biological mode of treatment of textile industry effluents using microbial biodegradation of dyes is a better alternative (An et al., 2002). The biological treatment of effluents is more economical and leads to less accumulation of relatively harmless sludge. Moreover, biological treatment of dye bath effluents is ecofriendly, and it causes mineralization of dyes to simpler inorganic compounds, which are not lethal to life forms. Some microorganisms, including bacteria, fungi and algae can degrade or absorb a wide range of dyes (Robinson et al., 2001).

Tirupur, located in Tamil Nadu state comprises of around 5,000 textile units, which are involved in one or the other activities of Textile value chain and contribute to about 54.57% of the total quantity of Indian textile exports (Rajkumar and Nagan, 2011). The Noyyal river gets polluted when it passes through Tirupur, due to discharges of 96.1 million liters per day (MLD) of colored effluent with high total dissolved solids (TDS) in the range of 6000 to 7000 mg/L, by the textile bleaching and dyeing units in Tiruppur (Rajkumar and Nagan, 2011). Hence, in the present study, Tirupur was selected as the place for the collection of the textile effluent sample.

Immobilization of living microorganisms has been described as useful in biological wastewater treatment (Zeroual et al., 2001). They offer many advantages of reusability, control of reactions and the non-contamination of products (Engasser, 1988). Immobilized cultures tend to have a higher level of activity and are more tolerant to environmental troubles such as pH, or exposure to toxic chemical concentrations than suspension cultures (Perullini et al., 2010; Rodriguez, 2009). The microbial cells immobilized in gel beads can be stored in sterile containers under refrigeration conditions for use, as and when required (with the prior revival of the beads in the NB medium 2 h before use). They can also be used repeatedly (at least three times) without changes in their efficiency, making this study an important contribution to safe wastewater management in industrial applications (Devi et al., 2017). The present investigation was carried out with the following objectives-

- The determination of the efficacy of immobilization of Bacillus cereus upon the degradation of the representative also dyes present in the textile industry effluent with respect to different concentrations of cells immobilized in the gel bead.
- The determination of the efficacy of immobilization of Bacillus cereus upon the degradation of the representative also dyes present in the textile industry effluent with respect to the number of gel beads containing the entrapped Bacillus cereus.

MATERIALS AND METHODS

Sampling Station and Sample Collection

The untreated textile effluent sample was collected from the effluent discharge point around Tirupur town, Tamil Nadu, India with latitude-longitude co-ordinates, 11.1085° N and 77.3411° E. The sample was collected in 500 ml sterile, reagent glass bottles, sealed with adhesive tape and immediately stored on ice in an insulated box. The sample was brought to the laboratory as early as possible and stored in a refrigerator till further use.

Selection of the Representative Azo-Dye Commonly Present in Textile Industry Effluents

One of the most commonly used Azo-dyes in the textile industry is “Reactive Brilliant Red X-3b”, and hence it was selected as a representative dye in the effluent sample. The molecular formula of the dye is $C_{19}H_{10}Cl_2N_6Na_2O_7S_2$ and

the molecular weight of the dye is 615.324 g/Mol. Its IUPAC name is "Disodium; 4-[(4, 6-dichloro-1, 3, 5-triazin-2-yl) amino]-5-oxido-6-phenyldiazenyl-7-sulfonaphthalene-2-sulfonate". Its molecular structure is given in Figure 1.

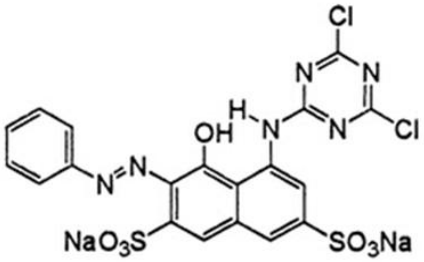

Figure 1: The molecular structure of the azo-dye, Reactive Brilliant Red X-3b

In our previous study, the absorbance maximum of the dye was determined to be 540 nm. Hence, throughout the present investigation, the levels of reactive brilliant red x3b, were used as an index of degree of biodegradation.

Analysis of Physico-Chemical Properties of the Effluent

Various physico-chemical parameters viz., Temperature, pH, Electrical Conductivity (EC), Colour, Odour, Total dissolved solid (TDS), Total suspended solids (TSS), Chemical oxygen demand (COD), Biological oxygen demand (BOD), Dissolved Oxygen (DO), Total Hardness, Chloride, Ca Hardness and Mg Hardness of the effluent samples were analyzed by using standard protocol (APHA, 1999).

Selection of Bacterial Isolate, Exhibiting Highest Degradability of the Selected Azo-Dye

The bacterial isolate exhibiting highest degradative ability upon the representative azo-dye reactive brilliant red x3b was identified to be Bacillus cereus in our previous study, and was employed in the present investigation for degrading the dye present in the untreated textile effluent sample.

Biodegradation Assays Using Immobilization of Cells

Biodegradation assays were carried out to find out the efficacy of immobilized Bacillus cereus to degrade the representative dye present in the untreated textile industry effluent sample, both at different cell densities and the weight of the gel beads with entrapped cells. The assays were carried out in 250 ml Erlenmeyer flasks. The flasks were sterilized with 50 ml nutrient broth. To this, 100 ml raw effluent was filter sterilized and added into the flask. Just before the incubation, a sample of 5 ml was drawn and the absorbance of supernatant was measured at 540 nm using a UV-Visible double beam Spectrophotometer, Systronics 2201.

Immobilization of Cells

Sodium alginate and polyvinyl alcohol were used for immobilization. In the present study, 0.5g of sodium alginate and 0.5 g of Poly Vinyl Alcohol (PVA) were added to 35 ml of boiling water and autoclaved at 121°C for 15 min and then allowed to cool at room temperature. The immobilization of cells was done at the inoculum volume of 10 ml. 10 ml of liquid culture of Bacillus cereus at mid logarithmic growth phase was mixed thoroughly in it and extruded drop by drop into a cold, sterile 5% calcium chloride solution with the help of a sterile 5 ml syringe. Gel beads of approximately 2 mm

diameter were thus obtained. The beads were hardened by resuspending it into a fresh calcium chloride solution for 24 hrs at 4°C with gentle agitation. Finally, these beads were washed with distilled water to remove excess calcium ions and un-entrapped cells. Degradation studies were conducted by using the immobilized cells of Bacillus cereus at four different concentrations of 5 g, 10 g, 15 g and 20 g of bio-beads. The samples were collected at every 12 hours and the absorbance at 540nm was measured.

RESULTS

Analysis of Physico-Chemical Properties of the Effluent

The physico-chemical analysis of the untreated effluent revealed very high levels of TDS (2212 mg/l), TSS (157 mg /l), COD (821 mg /l), BOD (211 mg/l), total hardness (314 mg/l) and chloride of (1180 mg/l) (Table 1).

Table 1: Physico-chemical properties of the untreated textile industry effluent samples

Parameter	Textile Industry Untreated Effluent Sample
Color	Black
Odour	Pungent
Temperature	35^0C
pH	8.4
Total dissolved solids (TDS) (mg/l)	2212
Total suspended solids (TSS)(mg/l)	157
Chemical Oxygen Demand (COD)(mg/l)	821
Biochemical Oxygen Demand (BOD)(mg/l)	211
Total Hardness (mg/l)	314
Chloride (mg/l)	1180
Ca-Hardness (mg/l)	215
Mg-Hardness (mg/l)	66.13

Biodegradation Assays

The results of the biodegradation assay employing the cells of Bacillus cereus, immobilized in gel beads, at four different masses are given in Table 2. The maximum degradation (0.317) was achieved at the end of 96 hours of incubation and at 20 gram concentration of the bio-beads (Table 2).

Table 2: Absorbance values reflecting biodegradation of the representative dye, Reactive Brilliant X3b at different concentrations of bio-beads

Duration of Incubation (Hrs)	Weight of Bio-Beads (In Grams)			
	5	10	15	20
0	2.312	2.316	2.315	2.317
12	2.013	1.915	1.824	1.516
24	1.864	1.742	1.716	1.225
36	1.611	1.512	1.478	0.997
48	1.346	1.313	1.237	0.715
60	1.135	1.126	0.997	0.640
72	0.991	0.985	0.884	0.523
84	0.925	0.913	0.807	0.416
96	0.861	0.853	0.720	0.317

DISCUSSIONS

Analysis of Physico-Chemical Properties of the Effluent

It is observed in the present study that, Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) were observed to be very high in all the three untreated effluent samples (Table 1). The textile industry waste water contains acids/alkalis, common salt (NaCl), heavy metals, sulphides, chlorine and mineral oils. As a result, the dye wastewaters are extremely toxic to both aquatic fauna and flora, crop plants, including human beings (Sharma et al., 1999). Observations of high concentration of TDS and TSS in the textile industry effluent elsewhere have also been reported (Kaur et al., 2010). The presence of high levels of BOD and COD affect the aquatic flora and fauna and destroys the ecosystem (Shah and Patel, 2014).

Biodegradation Assays

In recent times, the application of immobilized cell has been receiving increased attention in the field of wastewater decolorization, since this method not only simplifies separation and recovery of immobilized bacteria and the binding agent, but also makes the application reusable, which reduces the overall cost. On the whole, immobilized cells are more tolerant to local perturbations like changes in temperature, pH and presence of inhibitor compounds (Tallur et al., 2009). It has been stated that sodium alginate is a suitable matrix material, because it is non-toxic and the method used for its gelation is mild towards the microorganisms (Sriamornsak, 1998).

In the present study, highest biodegradation was achieved as the concentration of the bio-beads increased (Table 2). The highest biodegradation (0.317) was achieved at 96 hours of incubation at 20 grams concentration of the bio-beads, when compared to other incubation durations and bio-bead concentrations. In the present study, as the number of days of incubation increased to 96 hours, the highest degradation was achieved (Table 2). On the contrary, the immobilized consortium, PMB11 showed complete degradation of dye solution in flask within 15 h (Patil, 2016). Suganya and Revathi (2016) observed in their study that, sodium alginate immobilized cells of B. licheniformis showed maximum degradation against RY 17 and RB 36. However, Liu et al. (2012) observed in their study that, to achieve highest degradation of the dye, after 7 days of incubation with the bio-beads. They also observed faster degradation of the dye with immobilized cells rather than freely suspended cells. The fast decolourization in immobilized system may be due to the protection of bacterial cells from the direct toxic effect of the dye (Moawad, 2013). In the present study, immobilized Bacillus cereus was employed for the degradation the dye and achieved higher degradation (Table 2). The results of the present study are in agreement with those of Shrungare (2014), who employed immobilized Bacillus spp. for the degradation of the dye in the textile effluent and achieved higher degradation of the dye.

CONCLUSIONS

It is evident from the results of the present study that immobilized Bacillus cereus can be employed to achieve biodegradation of the representative dye, reactive brilliant red x3b. The immobilization of the microbial cells, helps in easy handling, reduces toxicity of dye upon microbial cells and achieves higher degradation.

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