HALO BACTERIA *BREVIBACILLUS* SP. ON BIODEGRADATION OF TEXTILE EFFLUENT CONTAINS DIAZO DYE – CONGO RED

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Abstract : Azo dyes are composed of pollutants such as suspended solids and toxic materials. These dyes are discharged into waterbodies without any treatment. Thisalters the quality and physico – chemical parameters of water.Therefore, the water is unfit for consumption and the flora and fauna inhabiting suchfresh water bodies are severely affected. In order to reduce toxicants in textile azo dye effluent suitable microbes which can tolerate wide environmental conditions areused.Thehalophilic bacterial strains isolated from solar salt pans have the capacity to degrade the selected diazo dye Congo red. The present study was conducted to investigate the degradation of Congo red dye effluent by using halo bacteria *Brevibacillus* sp. This halo bacterium was inoculated into the flask containing Congo red dye effluent and incubated for 5 days.The*Brevibacillus* sp. treated diazo Congo red dye effluent showed reduction of impurities such as pH 13.33 ± 0.03%, temperature 9.09± 00 %, BOD 61.29± 12.65 %, COD 49.91 ± 22.94 %, TDS 57.43 ± 48.11 %, chloride 42.61 ± 39.73 % and total hardness 48.73 ± 40.64 % when compared to raw dye effluent.

***IndexTerms* – Xylanase, *Brevibacillus* sp., Diazo dye- Congo red**

1. Introduction

The textile industries require enormous quantity of water for dyeing process which generates substantial amount of effluent containing organic and inorganic matters with high concentration (Soares *et al*., 2002).Dyes are formed of synthetic aromatic amine compounds which are used as colouring agent in food, drug and textile industries(Aksu*et al*., 2005). These are classified based ontheir origin, physical, chemical properties and characteristics related to the application process (Mishra and Tripathy, 1993). Azodyes are extensively applied in various industrial purposes which having one or more azo bond (-N=N-) that cannot be easily broken down and more over some of them making hazard to animals (Fu and Viraraghavan, 2001). Congo red is a benzidine based anionic diazo dye toxic to many organisms and is suspected carcinogen and mutagen which is difficult to degrade due to its stability (Dafare *et al*., 2013). Nearly about 10 -15 % of unused dyes with various colours are let out into the fresh water bodies with pollutant concentration of 10-200 ppm (Andra predesscu and Avran nicolae, 2012). The biological techniques used for dye degradation that provides prominent benefits over the conventional techniques of waste water management and is more ecologically friendly, economically valuable and produces harmless sludge (Kapdan*et al*., 2003). The bacterial strains and their extra cellular enzymes which can able to tolerate wide environmental fluctuations can be used to degrade the azo dye compounds. Biodegradation is a pollution control process that uses microorganisms to catalyze the degradation of various hazardous chemicals into less harmful substances and this process of degradation reduces the contaminants in the natural environment is an important alternative to conventional degradation methods (Vidali, 2001).

2. Materials and methods

2.1. Isolation, identification and screening of bacterial strain

The bacterial strain *Brevibacillus* sp. was isolated from sediment sample of hypersaline environment situated at Kovalam, Kanyakumari District, Tamilnadu. The collected sediment sample was serially diluted

as per standard procedure (Bergey's manual of Systematic Bacteriology, 2000). The isolated colonies were screened for xylanase activity by using birch wood xylan agar (Teather and Wood, 1982).The xylanase activity was determined by DNS method (Miller, 1959). The bacterial biomass was determined as per method followed by Bakri *et al*., 2009.

2.2. Optimization of the bacterial strain

The*Brevibacillus* sp. strain was optimized by physico – chemical parameters such as temperature, pH, NaCl concentration and incubation time for xylanase activity. The optimum activity of xylanase enzyme was determined by keep the reaction mixture with different temperature conditions (30°C - 50°C), pH range (6 - 10), NaCl concentration (1 - 5%) and different incubation periods (12, 24, 48, 72 and 96 hours).

2.3. Physico-chemical parameters

Each 5 ml (18 $\times 10^2$CFU/ml)of the optimized bacterial strain *Brevibacillus* sp. was inoculated in three replicates of flask containing 500 ml of Congo red dye effluent and kept in an incubator for 5 days at 35˚C temperature. Then, the parameters like pH, temperature, BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), TDS (Total Dissolved Solids),chlorides and hardness were determined in raw Congo red dyeeffluent and bacterial strain *Brevibacillus* sp. treated effluent after 5 days of incubation. The samples were analyzed as per the standard methods prescribed in APHA, 2005.

2.4. Dye degradation

The degradation in the dye treated with bacterial strains expressed as a percentage was determined by using the formula given below.

$$\text{Dye degradation (\%)} = \frac{\text{Initial reading–Final reading}}{\text{Initial reading}} \times 100$$

Initial reading – Physico chemical parameters of raw effluent

Final reading – Physico chemical parameters of bacterial strain treated effluent

3. RESULTS AND DISCUSSION

The *Brevibacillus* sp.was optimized by various physico - chemical parameters. The bacterial strain *Brevibacillus* sp. showed minimum xylanase activity 67 U/ml and biomass production29.15mg/ml respectively were observedat 50˚C temperature and maximum 85 U/ml and 39.43 mg/ml respectively were observed at 35˚C temperature. Similarly, at pH 10 lowest xylanaseactivity 64 U/ml and biomass 27.54 mg/ml were observed and highest production 88 U/ml and 41.08 mg/ml respectively observed at pH 8 (Table 1). The findings of Irfan*et al*., 2016 in*B.subtilis*andGhasemi*et al*., 2014 in*Sphingobacterium*sp. SaH-05 showed that maximum xylanase was produced at35˚C and an alkaline pH 8 were resembled with our study.The*Brevibacillus* sp. strain showed minimum xylanase activity 7U / ml and biomass production 1.5 mg / mlat 5 % NaCl concentration and maximum 34.0 U / ml and 13.0 mg / ml respectively at 1 % NaCl concentration. Similarly, the lowest production of xylanase enzyme 60 U / ml and biomass 23.55 mg / ml respectively at 12 hours of incubation and highest production 93 U /ml and 42.63 mg /ml at 48 hours of incubation (Table 2). The result of Padmavathi Tallapragada and Kavya (2011) in fungal strain *Aspergillus niger* and Sharma *et al*.,2011 in*Bacillus* sp. showed that maximum xylanase produced at1% NaCl and 48 hours of incubation were coincide with our investigation in the bacterial strain *Brevibacillus* sp.

Table 1: Effect of temperature and pH on the xylanase activity in*Brevibacillus* sp.

Temperature				pH			
S.NO	(°C)	EA (U/ml)	BM(mg/ml)	S.NO	pH	EA (U/ml)	BM(mg/ml)
1	30	81	36.08	1	6	68	30.17
2	35	85	39.43	2	7	75	34.24
3	40	79	35.65	3	8	88	41.08
4	45	74	33.00	4	9	72	31.91
5	50	67	29.15	5	10	64	27.54

*EA- Enzyme Activity *BM - Biomass

Table 2: Effect of NaCl concentration and incubation time on the xylanase activityin*Brevibacillus* sp.

NaCl concentration				Incubation time			
S.NO	(%)	EA (U/ml)	BM(mg/ml)	S.NO	(Hours)	EA (U/ml)	BM (mg/ml)
1	1	34	13.0	1	12	60	23.55
2	2	19	7.5	2	24	87	40.59
3	3	11	3.4	3	48	93	42.63
4	4	8	1.9	4	72	84	36.85
5	5	7	1.5	5	96	68	26.31

*EA- Enzyme Activity *BM – Biomass

The bacterial strain *Brevibacillus* sp.showed an excellent effect ondegradation ofdiazo dyeCongo red after5 daysof incubation (Table 3). The bacterial strain *Brevibacillus* sp. treated raw Congo red dye effluent showed reduction of impurities when compared untreated dye. The optimized strain *Brevibacillus* sp. treated Congo red dye effluent recorded a reduction of pH 13.33 ± 0.03 %, temperature 9.09 ± 00%, BOD 61.29 ± 12.65 %, COD 49.91 ± 22.94%, TDS 57.43 ± 48.11%, chlorides 42.61 ± 39.73% and total hardness 48.73 ± 40.64% when compared to raw dye effluent. The present results were in accordance with the findings of Amar*et al*., 2010. According to them, the reduction of parameters such as BOD, COD and total hardness were observed in the bacterial strain *Pseudomonas* sp. SU-EBTtreated azo dye - Congo red effluent. Similarly, the bacterial strain *Micrococcus* sp. treated Congo red and Acid Orangeeffluent showed the reductions of pH, Temperature and TDS were resembled with our present study(Islam *et al*., 2017).

Table3: Biodegradation of azo dye Congo red with the optimized bacterial strain *Brevibacillus* sp.

S. No	Parameters	Congo red		
		Raw	Treated	Reduction (%)
1	pH	9 ± 0.01	7.8± 0.05	13.33± 0.03
2	Temperature (°C)	33± 00	30±00	9.09±00
3	BOD (mg/l)	447± 11.26	173± 13.92	61.29± 12.65
4	COD (mg/l)	613 ± 23.14	307± 21.86	49.91± 22.94
5	TDS (mg/l)	1109± 46.58	472± 48.94	57.43± 48.11
6	Chlorides (mg/l)	542± 40. 36	311± 39.42	42.61± 39. 73
7	Total hardness (mg/l)	790± 39. 59	405± 43. 50	48.73 ± 40.64

4. CONCLUSION

The conventional method of textile waste water treatment is expensive and becomes uneconomical and causes severe environmental pollution. Therefore, ecofriendly and economically viable techniques using halo bacteria can be applied for reducing toxic ingredients in textilewaste water. The biodegradation procedures are cheaper and effective alternative for removal of pollutants in textile azo dye effluent. Thus, the halo bacteria *Brevibacillus* sp. acts as a good biodegradation agent for textile waste water treatment.

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