Development of Aerobic Reactors for the Remediation of Textile Effluents by Marine *Streptomyces*

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ABSTRACT---- ***In this study, Streptomyces sviceus KN3 isolated from marine sediments was used to asses its potential for decolorization and detoxification of congo red ,Navy blue and textile azo dye effluent. The decolorization of was initiated on the day 1 and the rate of decolorization increased with increase in time. The maximum percentage of decolorization 90% and 60% was noticed with Congo red-21, Navyblue-28 dye respectively after 96hrs of incubation with Streptomyces sviceus KN3.The Aerobic submerged fixed bed reactors were packed with gravel and P-rings as substrates and the gravel based bioreactors exhibited higher efficiency with 99% decolourisation of effluent .The results indicates the suitability of the Aerobic submerged fixed bed reactor for fermentation of marine Streptomyces sviceus KN3 and ascertained its potential to provide a practical and cost-effective bioremediation process for the treatment of azo dye effluents.***

Keywords--- Marine Actinobacteria, Congo red, Textile effluents,Decolourisation

1. INTRODUCTION

The textile industry is one of the oldest and self -reliant among all other industries in India. The fundamental strength of this industry is based on its substantial value addition at each stage of processing which contributes to economy of India. The big challenge for textile industry is to implement more water-friendly technologies to dye either cotton or polyester, the most mass marketed textiles. Dye houses in India and China are notorious for not only exhausting local water supplies, but for dumping untreated wastewater into local streams and rivers. With escalating demand for textile products, textile mills and their wastewater have been increasing proportionally, causing a major problem of pollution in the world. Many chemicals used in the textile industry cause environmental and health problems. Among the many chemicals in textile waste water, dyes are considered important pollutants.The environmental problems associated with textile activities are caused mainly by the extensive use of dyes [12].

Reactive azo dyes are great alarm in the textile water effluents as small amount of azo dyes in waste water is highly visible [16] and the amount of dye released into the textile effluent has been estimated to be in the range of 200mg/L to 500mg/L [6].Strong and bright colors of the azodyes in waste water is the serious problem of the textile waste effluent and the release of these effluents into water bodies cause damage to the environment [15]. The colour of the azodyes may significantly interrupt the photosynthetic activity of phytoplankton by reducing light penetration and further it may be toxic to aquatic life [3] The azodyes are characterized by the presence of azo (N=N) linkages and dyes are released into the waste water due to the cleavage of azo linkage. Under natural ecosystem, the azodyes are highly resistant and recalcitrant to degradation [13]. The release of azodyes into the natural environment results in the conversion of azo group to toxic aromatic amines which are highly carcinogenic and mutagenic to humans [8]. Further, the release of textile dye effluent into water stream changes the p^H scale and increases the BOD, COD and total organic carbon of the water bodies [9]. Several physical and chemical methods like coagulation, flocculation, activated carbon adsorption and reverse osmosis are adopted for the removal of azo dyes from textile effluents. But the application of these methods produce large amount of sludge and the wide usage of chemicals demand high cost and leads to secondary pollution [4].All these conventional methods are confined to transfer dye stuff from one phase to another without resolving the dye pollution. Alternatively, the genetic diversity and enzymatic profile of microbial system provides a biological method to remediate the pollution load caused by the azo dyes. Thus, the researchers focused on the exploitation of microorganisms for decolorization and degradation of toxic azo dyes through bioremediation [17]. Keeping in view of the importance of

bioremediation, the present research is focussed on the exploitation of marine actinobacteria in the decolourisation of textile effluents by using fixed bed reactors.

2. MATERIALS AND METHODS

The reactive azo dyes Congo red - 21and Navy blue dye - 28 were collected from the retail vendors at Madanapalli and Puttur , Chittoor district, A.P. India. Starch casein agar, Maltose yeast extract agar were used for the isolation of marine actinomycetes and degradation of azo dyes respectively. All other chemicals and solvents used were of analytical grade procured from Sd-fine chemicals.

2.1 Sample collection

The marine samples were collected from the sediments of sea shore of Bay of Bengal, A.P, India. Samples were stored in sterile containers and transported to the laboratory and preserved in refrigerator at 4^0c.

2.2 Preparation of dye stock solution

The stock solution of the reactive Congo red dye was prepared by dissolving 500mg dye in 100ml distilled water.

2.3 Primary screening for dye resistant marine actinobacteria

The marine sediment samples were used as source to isolate *actinobacteria*. The sediment soil sample were dried in laminar chamber for 2 to 3 hours and heated at 70^0 c for 25min. The heated sediment samples were mixed with 100 ml of sterile natural sea water and kept in shaker at 150rpm for 30min .The samples were subjected to serial dilution and 10^{-3} and 10^{-4} dilutions were inoculated on to the culture media and incubated at 28 ± 2^0c for 7days.Starch casein agar supplemented with 100mg/l of Congo red-21 and Navy blue-28 dyes was used for primary screening. After 7 days of incubation, a loop full of medium was streaked onto sterile Maltose yeast extract agar and incubated at 28±20 c for 48hrs. The dye resistant colonies were isolated based on the colony morphology.

2.4 Secondary screening for dye decolorizing Actinobacteria

All the actinobacteria isolated during primary screening were evaluated for their dye degradation efficiency by inoculating in Maltose yeast extract amended independently with 400mg/l Congo red-21 dye and 400mg/l Navy blue-28 dye and incubated at 28±20c for different time periods. The isolate that demonstrated maximum decolorization was selected for further study.

2.5 Decolorization Assay

Decolorization experiments were carried out in 250ml conical flasks with 100ml of sterile nutrient Maltose yeast extract agar amended with different concentrations of reactive Congo red-21 and Navy blue-28 dyes. The flasks were inoculated with the isolated actinobacteria and incubated at 28 ±20C for 7 days under static conditions.Control was maintained without the inoculum. The decolorized samples were collected at regular intervals, centrifuged at 10,000rpm for 20min. After centrifugation, the supernatant of the respective dyes was collected and rate of decolorization of the Congo red-21 and Navy blue-28 dyes was monitored by measuring the absorbance of culture supernatant at 540nm and 570nm respectively using UV-spectrophotometer (Schimadzu uv 1601).The percentage decolorization of textile reactive Congo red -21dye and Navy blue -28 dyes was determined [1].

2.6 Analyzing the decolorization of effluent dyes by using Aerobic fixed bed reactors:

Two lab- Scale Aerobic fixed bed reactors designated as AFBR1 and AFBR2 were fabricated and packed with gravel and P Rings respectively . The AFBR model contains a perplex glass tube with inlet and outlet. The AFBRs were housed at a controlled room temperature at 30^0C under submerged conditions. The reactors were filled with 750ml Maltose yeast extract broth and 250ml textile effluent dye abundant with red and blue dyes and inoculated with *Streptomyces sviceus* KN3 in order to promote the formation of bio-mass. After 3 days of incubation, nutrients were added to the reactor daily in a fed- batch mode. The process was continued till the development of a good bio-film. The thin bio-film layer was observed after 7 days of preparatory period. A clear slime adhesion was noticed on the surface of the medium.

After preparatory period of 7 days, textile effluent at dye concentration 100mg/l was fed into both the reactors for acclimatization through peristaltic pump to maintain continuous regime. Steady state was observed after 3 days of acclimatization period. For experimental work dye concentration was varied gradually from 100 to 400mg/L and is operated at 24HRT. The experimental setup, with Gravel and P-ring media of AFBR model was monitored for a period of more than 10 days for evaluation of decolorization efficiency. Parameters of influent and effluent were analyzed daily. The steady-state conditions were maintained for a period to enable collection of data for performance evaluation.

3. 3. RESULTS

In the present study, marine samples collected from Bay of Bengal were screened for dye degrading actinomycetes by using Congo red -21 and Navy blue -28 dyes as model azo dyes.

3.1. Primary screening for dye resistant isolates from marine sediment samples

From the sediment samples,15 morphologically different actinobacteria were isolated and designated as KNto KN15. Based on the preliminary screening, the isolates KN3, KN5, KN8, KN11, KN13 were found to be effective dye degrading actinobacteria (Figure.1). Among these isolates, KN3 showed maximum decolorization of Congo red-21 dye (90%) and Navy blue-28dye (60%) (Figure.2).Due to higher efficiency of degradation, the isolate KN3was selected for further analysis.

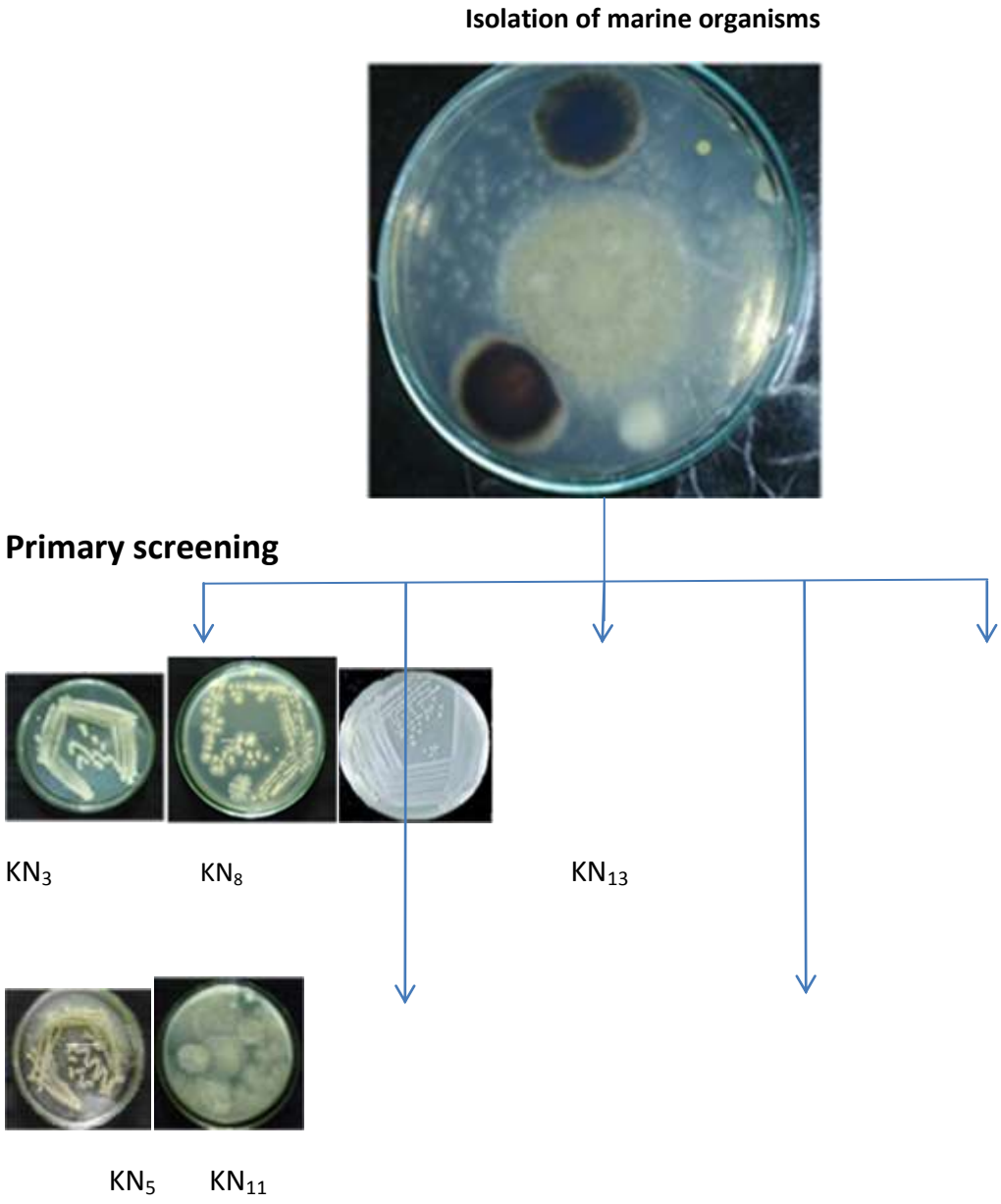

Figure 1: Morphological characteristics of dye degrading marine actinobacteria

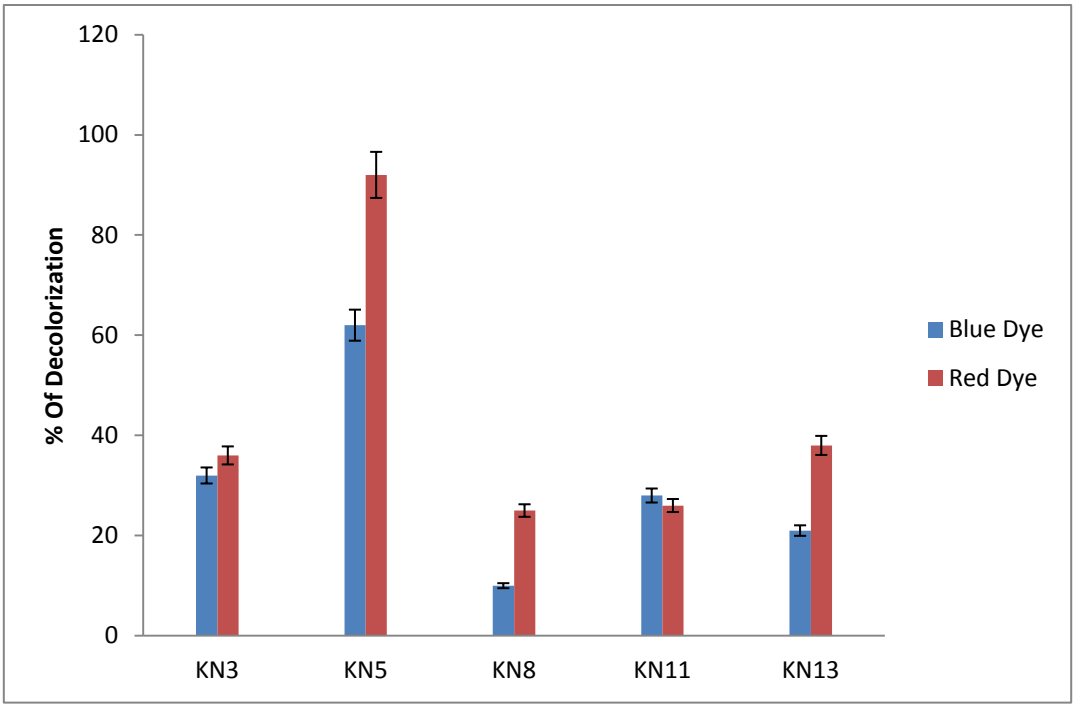

Figure2: Decolorization of Reactive azo dyes by Marine Actinobacteria.

Values are expressed as mean ± SEM of three independent experiments. Statistical significance 1% level ($p \leq 0.01$).

3.2. Decolorization of Azo dyes by *Streptomyces sviceus*

To evaluate the decolorization efficiency ,the KN3 strain was inoculated in to the Maltose yeast extract agar medium amended with Congo Red-21 dye and Navy blue-28 dye independently and incubated at 300c for different time intervals. As shown in figure.3, the decolorization of congored-21 was initiated on the day 1 and the rate of decolorization increased with increase in time. On the other hand, the decolorization was less with Navy blue-28 dye (Figure. 4).The maximum percentage of decolorization 90% and 60% was noticed with Congo red-21,Navyblue-28 dye respectively after 96hrs of incubation with KN3 isolate(Figure.5).

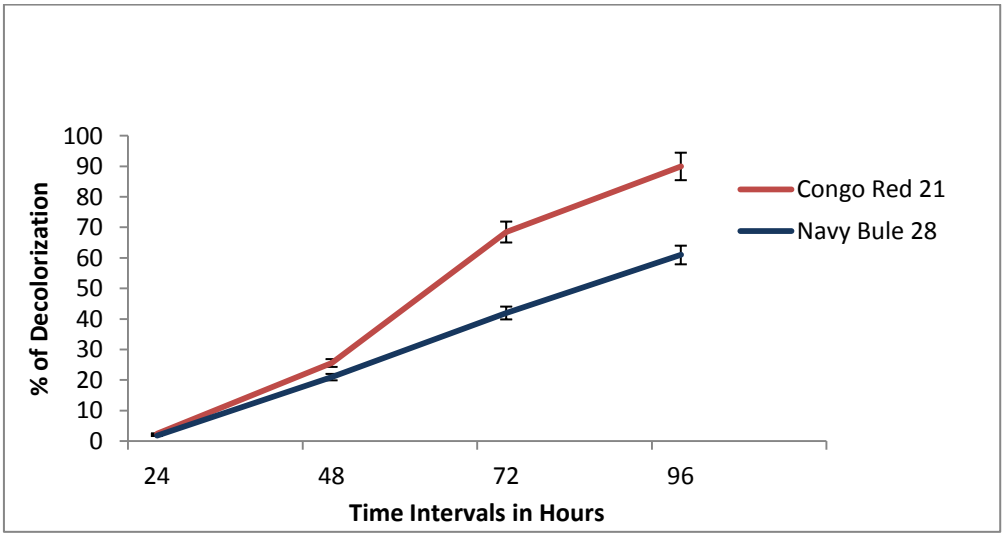

Figure3: Effect of time on rate of decolorization by KN3 isolate .

values are expressed as mean ± SEM of three independent experiments .Statistical significance 1% level ($p \leq 0.01$).

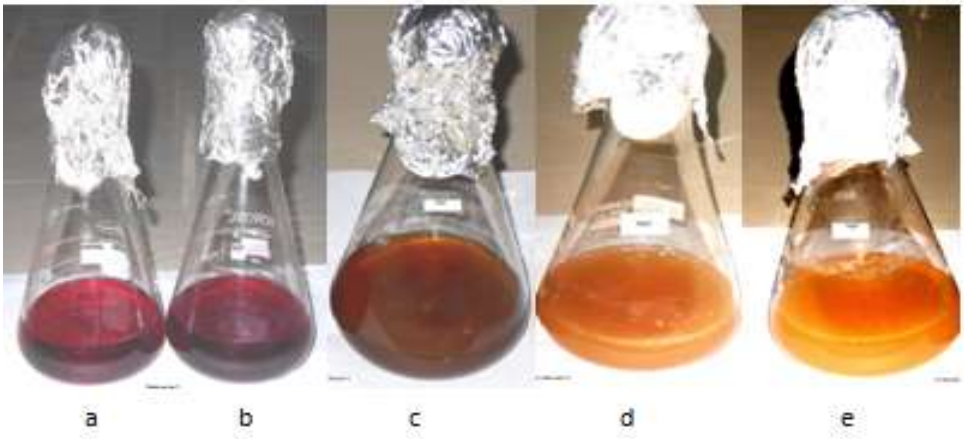

Figure4: Decolorization of Congo red-21 by *Streptomyces sviceus*

(a)Congored-21 control dye (b) Decolorization after 24h (C) 48h (d) 72h (e) 96h

Figure5: Decolorization of Navy blue-28 by isolate KN3
(a) Navy blue -28 control dye (b) Decolorization after 24h (C) 48h (d) 72h (e) 96h

3.3. Decolorization of effluent dye by Aerobic Fixed bed reactors

The Aerobic submerged fixed bed reactors were operated in continuous regime throughout the study and the impact of bed material on the decolorization process, by *Streptomyces sviceus* KN_3 at varying dye concentration, was tabulated in table.1. In AFBR1 packed with gravel, at 24hrt, the bio film efficiently removed the dyes from the effluent at all concentrations. The decolorization was found to be maximum (96%) at 100mg/l and decolorization rate was reduced at higher concentration. In the other reactor, $AFBR_2$ loaded with p-rings ,the rate of decolorisation was noticed as 94%,76%,54%,at 100,200,400mg/l respectively.

Table1: Decolorization of Textile effluents using Aerobic Fixed bed reactors

Days	% of Decolorization					
	Gravel Based Reactor			P-ring Based Reactor		
	Effluent Concentration mg/L			Effluent Concentration mg/L		
	100	200	400	100	200	400
1	30	31	29	38	25	30
2	34	39	40	40	41	36
3	48	45	41	55	34	38.3
4	60	54	47.5	57	53	43
5	74	56	50	81	60	45
6	78	60	52.5	87	65	50
7	86	74	54.6	90	71	53
8	92	74	54.6	92	71	53
9	96	74	54.6	92	71	53
10	96	78	58	94	76	54

4. DISCUSSION

In the present study, both the primary and secondary screening methods were used to screen dye resistant *actinomycetes* from marine sediment samples The isolates were differentiated based on their morphological characteristics on starch casein agar, maltose yeast extract agar and Nutrient glucose agar. Based on decolorization efficiency, the marine isolate KN_3 was employed for the degradation of two reactive azo dyes such as Congo red-21 and Navy blue-28 dyes. The reactive azo dyes demonstrated different extent of degradation. As per the literature, variation in aromatic structure significantly alters the process of decolorization and biodegradtion [11]. The isolate was identified as *Streptomyces sviceus* KN3 based on the biochemical and molecular characteristics.

Color removal, especially from textile wastewaters, has been a huge challenge over the last decades, and up to now there is no single and economically attractive treatment that can effectively remove colors. Also, the reuse of water from effluents in the production process or treatment plant leads to a reduction in costs for the textile industry[14]. The presence of dye and high concentrations of salt complicates the treatment of textile wastewaters. Many microbial species are able to decolorize some azo dyes anaerobically within a certain limit of salt, but most of them are unable to decolorize azo dyes in high salt conditions [2]. Various reactor systems such as stirred tank bioreactor, aerobic fluidized bed reactor, upflow coloumn reactor,fluidized bed r, continuous packed bed , pulsed packed bed r and air lift reactor were employed for decolorisation and degradation of textile dyes using bacteria, fungi and algae [5]. Among these bioreactors, rotating drum bioreactor (RDBR) and Upflow anaerobic sludge blank reactors immobilised with fungi are effective in the treatment of various textile dyes [10].

In the present study laboratory scale aerobic submerged reactors were constructed using gravel and p-rings as support media. The biofilm of streptomyce sviceus KN3 was allowed to develop for 7 days at 28°C. Formation of biofilm was visually monitored during this period. The results revealed the efficiency of both the substrates in the decolurisation but the Gravel media shows better performance when compared to P-ring at all concentration of textile effluents. This may be attributed to less surface area on the p-rings for the adsorption of biomass when compared to the large rough surface area of Gravel media in the reactor $AFBR_1$. This demonstrates usefulness of locally available Gravel as a media for aerobic fixed bed reactors for effective bioremediation of textile azo dye effluents.our results are in tune with the treatment of sulphur black dye by the fungal strain Aspergillus terreus using Stirred tank reactor system [7] and decolorization of Drimarene blue K2RL (anthraquinone) dye by a fungal isolate Aspergillus flavus SA2 immobilized in fluidized bed bioreactor. K.Balaji and S.Poongothai [5] reported the decolourisation of Drimarene Red X 6BN, Drimarene Blue X 3LR CDG and Drimarene Yellow X4RN by using Aerobic Fluidized bed Reactor acclimatized with the fungal biomass consortium of Pleurotus ostreatus ,Pleurotus sajor-caju ,Tremetus versicolour and Tremetus hirsute.

5. CONCLUSION

The outcome of this work indicates that an aerobic submerged fixed bed bioreactor constructed with either gravel or p-rings is an ideal model for bioremediation of textile effluents using marine streptomyce sviceus KN3.

6. REFERENCES

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