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Studies on effect of marine actinomycetes on amido black (azo dye) decolorization

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ABSTRACT

In the present study, an attempt was made to examine the potential of marine actinomycets for decolorization of amidoblack azo dye. Actinomycetes from marine sediment and dye waste were subjected to decolorization under aerobically at 5, 10 and 100 ppm. Effectiveness of azo dye decolorization was determined by UV–Visible spectrum and High Performance Liquid Chromatography (HPLC). Biochemical properties and carbohydrates assimilation studies revealed that the isolated organism designated as Micromonospora sp, Streptomyces sp and Micropolyspora sp. The significant decolorization (88%) of amidoblack was found at 5ppm and moderately at 10 ppm (66%). Decolorization potential increased the applicability of these marine actinomycetes for the dye removal. The present work was concluded that the marine actinomycets can be used as a useful tool to treat waste water containing reactive dyes.

Keywords: Amidoblack, Marine, pH and Static condition.

INTRODUCTION

Environmental problems such as appearance of color in discharges from various industries, combined with the increasing cost of water for industrial sector, have made the treatment and reuse of effluent increasingly attractive to the industry in India with over 1000 industries. The textile waste water is rated as the most polluting among all in the industrial sectors [1]. Azo dyes represent the largest and most versatile class of synthetic dyes. More than 3000 different varieties of azo dyes are extensively used in the textile, paper, food, cosmetics and pharmaceutical industries [2]. Azo dyes are considered as electron deficient xenobiotic compounds because they possess the azo (N=N) and sulfonic (-so3) electron with drawing groups, generating electron deficiency in the molecule and making the compound less susceptible to oxidative catabolism by bacteria. As a consequence, azo dyes tend to persist under aerobic environmental conditions [3]. These dyes are poorly biodegradable because of their structures and treatment of waste water containing dyes usually involves physical and chemical method such as adsorption, coagulation, flocculation, oxidation, filtration and electrochemical methods. Over the past decades, Biological decolorization has been investigated as a method to transform, degrades or mineralize azo dyes. Such, decolorization and degradation in an environmentally friendly and cost competitive alternative to chemical decomposition possess. Most azo dyes are recalcitrant to aerobic degradation by bacterial cells [4]. The bacterial decolorization and degradation of these dyes has been of considerable interest since it can achieve a higher degree of biodegradation and mineralization, is applicable to a wide variety of azo dyes, is inexpensive and environmentally-friendly, and produces less sludge [5]. In recent years, there has been an intensive research on marine actinomycetes decolorization of dye waste water. It is turning into a promising alternative to replace or supplement present treatment processes [6].

Actinomycetes are the group of Gram positive filamentous bacteria which are widely distributed different terrestrial and aquatic habitats. In aquatic habitats, actinomycetes play a great role in carbon cycle due to their ability to grow

at low concentrations of carbonaceous substances and to degrade recalcitrant organic matter. They make up large part of microbial population of aquatic systems. Diversity and bio prospecting studies on actinomycetes are mainly pertaining to the marine ecosystems and less importantly from fresh water systems [7]. Actinomycetes have a profound role in the marine environment apart from antibiotic production. The degradation and turnover of various materials are a continuous process mediated by the action of a variety of microorganisms especially actinomycetes [8]. The actinomycetes degradation and decolorization of amido black dye have received considerable attention from point of view of treating Industrial Waste water containing dyes. Amido black dyes are considered as xenobiotic compounds that are very recalcitrant to biodegradation processes. Economic and safe removal of polluting dye is still important issue. Currently, wide variety of microorganism's ability to degrade and absorb dyes from wastewater environments has been identified as an eco friendly, cost less alternative for disposal of waste effluent [9-10]. The present study focused that the potential of actinomycetes isolated from marine sediments and their decolorization efficiency of the azo group of amido black dye.

EXPERIMENTAL SECTION

For isolation and identification of actinomycetes from marine sediments sample was collected from Gulf of Mannar costal region, Kayalpatinam, located at Tuticorin district, Tamil nadu, India. The central portion of the marine soil sediments were aseptically transferred to the sterile bottles and brought up to laboratory with help of ice bag. The sediments sample was blackish brown color and of a sandy texture. One gram sediments were serially diluted up tp10^{-6} dilution. One ml of diluted sample was permitted in to the actinomyctes agar medium supplemented with cyclohexamide 10µg/ml. The pure strain of actinomycetes were developed after 7-15 days at 28^{o}C and maintained on 25% v/v glycerol stocks at 4°c. The slide culture technique is used to observe morphologic of molds without disturbing the arrangement of spores and conidiogenous cells. Other important application of this technique lies in studying the conidial ontogeny over a period of time in a given area of the preparation.

Screening of amido black degradation by plate assay method:
The screening process was carried out by minimal medial agar (MM) with 5 ppm of azo dye and then subjected to 25, 50 and 100 ppm level. After 24 h of incubation, the bacterial isolate which tolerated higher concentration of the azo dye was isolated by streak plate method. The azo dye decolorizing bacteria was identified from several aspects including morphology characters, biochemical tests as described in Bergey's manual of determinative bacteriology (indole, methyl red, voges-proskauer test, citrate, catalase, oxidase, starch and urea) Utilization of various sugars such as arabinose, mannose, maltose, lactose, inositol and sucrose as sole carbon source was determined by inoculating the isolate into carbohydrate broth supplemented with respective carbon source. After inoculation the tubes were incubated at 37°C for 24-48 h.

Aerobic biological decolorization of amido black on minimal medium:
Under aseptic condition, minimal medium was supplemented with 5, 10 &100 ppm of amido black. The isolated actinomycetes were inoculated and incubated under aerobic conditions at 28^{0}C for 7 days. Further aeration was carried out in a shaker at 150 rpm to promote oxidation of the degradation products. The percentage of decolorization was measured in a UV-Vis spectrophotometer at 540nm. The percentage decolorization and average decolorization rate and their pH were measured.

Percentage of decolorization = Initial absorbance- Final absorbance / Initial absorbance ×100

Compounds separation and detection by TLC
The decolorized broth was centrifuged and mixed with dichloromethane. The organism phase were collected and dried at 50°C and the residues were re dissolved in methanol. The extracted sample was placed on TLC plate were kept in to the solvent system to separate the compound until the solvent reaches ¾ and Dried. After the plates were dried and band were observed under 254nm and were separated.

HPLC and UV analysis for decolorization
High performance liquid chromatography (HPLC) was carried out as previously described [11]. Decolorized azo dye was centrifuged and the cell free aqueous was mixed with dichloromethane (1:1) and kept under 250 rpm for overnight. The solvent phase was concentrated and redissolved in methanol for HPLC detection using 1:1 acetonitrile/methanol mobile phase.

RESULTS AND DISCUSSION

Preliminary screening of azo dye degradation

Totally 6 actinomycetes were isolated from marine sediment. Study on spore morphology and Mycelium reveals that the isolated actinobacteria belongs to *Miromonospora* sp (KPMS 1 & KPMS 9) *Streptomyces* sp (KPMS2, KPMS5 & KPMS7) and *Micropolyspora* sp (KPMS4). All the isolated actinomycetes were morphologically differ on the basis of colony color, types of mycelium, spore and pigmentation. Colonies were 3-4 mm in diameter, initially had a smooth appearance but later developed a weft of aerial mycelium which is granular or powdery in nature. The aerial and substrate mycelium are fragmented and showed the production of conidiospores and arthrospore on the mycelium. Among the *Streptomyces* isolate 50% of them produced spiral chains of spore and less often spirally coiled and refractive features on substrate mycelium (Table1). *Micropolyspora* sp produced chain of spore are differentiated from *Streptomyces* sp by the fragmenting nature of the aerial mycelium [12]. The dominance of *Streptomyces* sp was reported by many workers as a rich and sustainable source in marine sediments [13].

Table 1: Study of spore and morphology dye degrading Actinomycetes

Isolate Code	Type of Mycelium	Spore morphology	Possible Genus
KPMS1	AM/SM	Rarely branched septate hyphae with Monospore	*Micromonospora sp*
KPMS 9	AM/SM	septate hyphae with Monospore	*Micromonospora sp*
KPMS 4	AM/SM	chain of spore on fragmented hyphae	*Micropolyspora sp*
KPMS2	AM/SM	Spiral chain of spore and refractile	*Streptomyces sp*
KPMS 5	AM/SM	Spiral chain of spore	*Streptomyces sp*
KPMS 7	AM/SM	Long chain of spore and refractile	*Streptomyces sp*

In initial screening to identify bacterial strains that would be most efficient at degrading amido black was performed by plate assay. It was observed among six isolate, three isolates namely *Streptomyces* sp, two isolates of *Micromonospora* sp and *Micropolyspora* sp were effectively removed the azo dye on minimal media by intracellular absorption. All the actinomycetes isolates were strictly have Gram positive cell wall, indole, methyl red positive and failed to utilize citrate and capable to utilize multiple sugar except arabinose (Table 2 and 3).

Table 2: Biochemical properties of Isolated Actinomycetes

Isolate code	Gram's stain	Indole	MR	VP	Citrate	Catalase	Oxidase	Starch	Urease
KPMS1	+	+	+	+	-	+	+	+	+
KPMS2	+	+	+	+	_	+	+	+	_
KPMS 4	+	+	+	_	_	_	_	±	_

Table 3: Utilization of various carbohydrates by isolated Actinomycetes

Isolate code	Arabinose	Mannose	Maltose	lactose	Inositol	Sucrose
KPMS1	-	++	++	++	+	++
KPMS2	-	+	++	++	++	++
KPMS 4	-	+	++	++	++	++

+ : weakly positive , ++ : Positive & - : Negative

Aerobic bio degradation of amido black on M9 broth

Basal salt medium, made from phosphate buffer (1 mol) pH 5, 6, 7 and 9 along with yeast extract (200 mg/ l) and dye (5,10 and100 ppm) was used in the studies. Experiments were performed in flask containing 100 ml of media inoculated with enriched microbial consortia and incubated at 30°C. *Micromonospora* sp (KPMS1), *Streptomyces* sp(KPMS2) and *Micropolyspora* sp (KPMS4)were found to be effectively decolorize the amido black. *Micromonospora* sp completely decolorized the tested azo dyes (amido black) in a static/agitated sequential process only in the presence of simple carbon glucose and yeast extract, which has been the most commonly, used a nutrient additive for dye bio-decolorization processes [14]. The percentage decolonization of azo dye by *Miromonospora* sp (KPMS1) showed almost 88 %, *Streptomyces* sp (KPMS2) showed 57 % and 76 % by *Micropolyspora* sp(KPMS4) at 5 ppm was observed after 2 days of incubation. Similarly, the effectiveness of decolorization from 10 ppm to 100 ppm was significantly reduced (Table 4). The percentage of decolorization was 66% 51% & 57 % at 10 ppm respectively by *Micromonospora* sp (KPMS1), *Streptomyces* sp(KPMS2) and *Micropolyspora* sp(KPMS4). The least dye degradation was observed at 100 ppm even after 15 days of incubation at 30°C. The percentage of decolorization was 56%, 42% and 42% respectively by *Micromonospora* sp (KPMS1), *Streptomyces* sp (KPMS2) and *Micropolyspora* sp (KPMS4). In the present study, actinomycetes culture exhibited decolorization activity in the range of 5-10 ppm effectively with final p^H 7.2. Actinobacterial cultures generally exhibit maximum decolorization p^H value at near 7[15].

It was found that decolorization, increased with decreasing dye concentration although the three strains showed decolorization at 5 &10 ppm. Only two strains (KPMS1& KPMS4) found to be effectively degrading the dye and less significant decolorization was recorded on samples treated at 100 ppm. It is well known that the dye concentration has a direct effect on the decolorization rate as well [16]. An enzyme azoreductase is considered to be responsible for the cleavage of azo bond (-N = N-). Since cleavage of the azo bond by an azoreductase is invariably the first step in the biotransformation of azo dyes, organisms capable of opening aromatic ring or removal of functional groups, can lead to decolorization [17].

Table 4: Percentage of Decolorization of Amido Black (5ppm) by Actinomycetes on MM9 mediumat 37° C under 100rpm

Isolate code	Percentage of Decolorization			pH
	5ppm	10 ppm	100 ppm	
KPMS1	88%	66%	56%	7.23
KPMS2	57%	51%	42%	7.13
KPMS 4	76%	57%	42%	7.12

TLC and HPLC Characterization

TLC analysis reveals the effectiveness of degradation of azo dye which shows five fractions from *Miromonospora* sp (KPMS1) and four from *Miropolyspora* sp (KPMS4). The R_f value fractions differ from control dye indicates the dye was degraded by these two strains. Amido black chromatograms showed the presence of five different fractions followed by decolorization. Figure 1 shows the result of HPLC analysis on the metabolites of amido black decolorization by the *Micromonospora* sp reveals 5 peaks with retention time of 2.0, 3.1, 3.7, 4.9, 8.01 min, respectively). Similarly Figure 2 indicates HPLC spectrum of *Micropolyspora sp* showed 5 peaks with the following retention time 2.4, 4.4, 4.9, 9.03, 10.38 min (Table 5) which is completely differ from *Micromonospora* sp. This result is reasonable to suggest that peaks at 4.9 represent the decolorization metabolites confirming the formation of additional aromatic metabolites. High performance liquid chromatography (HPLC) has been used for analysis of various dyes in waste water and metabolites from various degradation procedures [18].

Table 5: Retention time of decolorized azo dye

Fraction	KPMS1	KPMS 4
	Ret.time	Ret.time
1	2.0	2.4
2	3.1	4.4
3	3.7	4.9
4	4.9	9.0
5	8.0	10.3

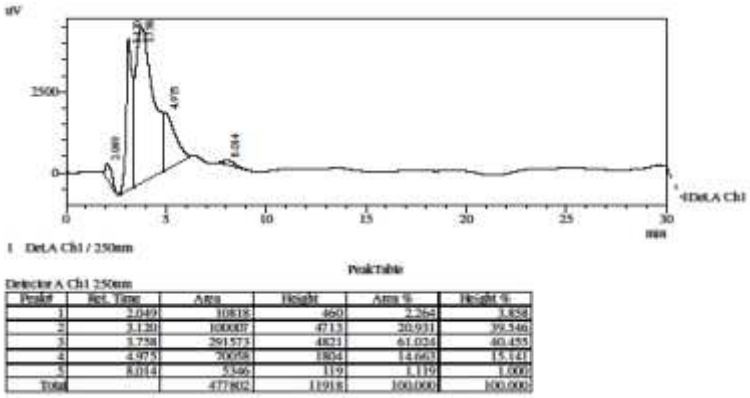
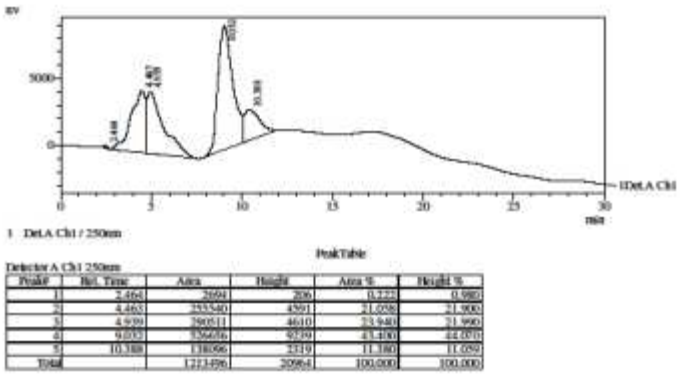


Figure 1: HPLC analysis of *Micromonospora* sp KPMS 1



PeakTable

Detector A Ch1 250nm

PeakF	Ret. Time	Area	Height	Area %	Height %
1	3.466	2660	206	0.222	0.980
2	4.465	355580	4591	27.058	21.900
3	4.939	390511	4610	73.940	21.990
4	9.053	376606	9759	43.400	44.071
5	10.388	138096	2319	11.380	11.059
Total		1223496	20964	100.000	100.000

Figure 2: HPLC analysis *Micropolyspora* sp KPMS 4

CONCLUSION

In conclusion, the immense biological diversity in the marine ecosystem, it is increasingly recognized that a large number of novel chemical entities exists in the marine sediments. As marine microorganisms, particularly actinomycetes, have evolved with the greatest metabolic diversity. Efforts should be directed towards exploring marine ecosystem as a decolorization and degradation products mediated by the action of marine actinomycetes. Decolorization is a promising process and the results were suggesting a great potential for marine actinomycetes used to remove color from dye wastewater. Interestingly, the actinomyctes species used to carry out the decolorization of azo dye amido black. This finding has established that the ability of the actinomycetes strain to be tolerate, decolorize azo dyes at high concentration gives it an advantage for treatment of both waste water and textile industry effluent.

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