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Bioremedial Potential of Moderately Halophilic Bacteria

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Abstract: Dye discharge from textile industries causes a serious threat to the water resources. Many complicated molecular structure of dyes make the wastewater from textile industries difficult to be treated by conventional methods. Therefore, innovative treatment technologies need to be investigated. The present study focuses on to the characterization of the biological potential of moderately halophilic bacterium isolated from the marine site, with mixed consortium of bacteria that were previously studied for dye degradation. The isolate was tested for their different dyes degradation with mixed consortium of bacteria and their heavy metal tolerance capacity. The isolates found to improve degradation of the azo dyes used in the studies and were also help tolerant to heavy metals such as zinc, copper, nickel.

Keywords: halophilic, dye degradation, heavy metal tolerance

Introduction

Moderately halophilic bacteria are extremophilic microorganisms adapted to live in saline environments. These halophiles grow optimally in media containing between 3% -15% NaCl [1]. While moderate halophiles present not only an advantage with respect to their growth over a wide saline concentration, but also their ability to grow in simple media [2]. Hyper-salinity coloured wastewater is a consequence product of batch process both in the dye manufacturing and dye-consuming industries and the salt concentration is up to 15%-20%. Textile wastewater are complex waste products containing dyes, sizing agents and dyeing aids that are characterized by their deep colour and high concentration of salt [3]. One of the dyes used widely in the textile industries is acid dye. They are water soluble anionic dyes containing one or more acidic groups along with one azo group. Azo dyes, one of the greatest group of synthetic dyes have one or more azo bonds[-N=N-] and because of their solubility, low expense, stability and colour variety they

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are widely used in many application. Most of Azo-dyes are toxic, carcinogenic and mutagenic [4]. Contamination of the environment by heavy metals is a consequence of technological and industrial process [5]. This has led to the increasing concern about the effects of toxic metals as environmental contaminants. The presence of these contaminants in aquatic environments is known to cause severe damage to aquatic life [6]. The use of halotolerant and halophilic organisms in the cleanup of organic contaminants in saline environments would prevent costly remediation strategies that reduce or remove salt by dilution methods, reverse osmosis, ion exchange, or electrodylsis before biological treatment begins.

In the present study, we tried to characterize and explore the potential of a moderately halophilic marine bacterium in the process of bioremediation, including isolation, purification and characterization of moderately halotolerant bacterial species and previous study mentioned mixed consortium was used for dye degradative efficiencies for three different dyes and testing for heavy metal tolerance carry out.

Materials and Methods

Sample collection:

In the present study, the soil and water samples were collected from Tithal beach and Charvada village from Dist. valsad, State Gujarat, India. Soil samples were kept in a container and refrigerated till use and water sample collected in sterile bottle used within 24 hours.

Dyes:

In these study azo dyes, Reactive Red 31 and Reactive Black 5, Reactive yellow 42 were used. These dyes were procured from Atul Ltd., Atul, Dist. Valsad, India.

Table: 1 Absorption maxima of Reactive Dyes

Sr. No	Dyes	Absorption maxima (nm)
1	Reactive Red 31	549
2	Reactive Black 5	601
3	Reactive Yellow 42	419

Media and Chemicals:

Nutrient broth/agar medium

Modified halophilic agar medium

Other reagent and different heavy metal salts; Copper sulphate ($CuSO_4$), Zinc sulphate ($ZnSO_4$) and Nickel Sulphate ($NiSO_4$) were purchased from HiMedia company.

Preparation of the dye solutions:

Dye solutions of 1% were prepared in distilled water, stored as stock solution until for further study.

Enrichment:

1 gm of soil sample was inoculated in 10 ml sterile nutrient broth test tube. Similarly, 1 ml water sample inoculated in sterile nutrient broth test tubes. All the inoculated test tubes then incubated for enrichment at 37° C for 24 hours.

Isolation and purification of halotolerant bacterial colonies:

In order to select the potential microbes for our study, 1 ml of enrichment soil and water samples were serially diluted and the dilution from 10^{-1} to 10^{-6} were spread on the sterile nutrient agar plates containing 9% additional salt concentration and modified halophilic agar medium. After 24 hours of incubation isolated colonies which are capable of surviving in moderately halophilic condition will only grown and used for further study[13]. Purified colonies were obtained by sub culturing on similar previously used agar medium plates and preserved. Morphological characteristics and gram reaction of the isolates also observed [13].

Screening of dye degradative effect:

The isolated halotolerant bacteria were screened for its dye degradation efficiency. For this 10 ml of sterile nutrient broth medium along with 100 µg/ml dye was inoculated with enriched 1 ml of each isolated halotolerant bacterial strain in test tube, similarly 10 ml of sterile nutrient broth with 100 µg/ml dye was inoculated with each 1 ml of isolated halotolerant bacterial strain plus 1 ml of already studied Mixed consortium (MC) from laboratory [14]. The test tubes with isolated halotolerant were kept in incubator at 37° C and test tubes with MC were incubated at room temperature for up to 24-48 hours. All the incubated test tubes were studied for dye decolourization at almost every 6 hrs. of interval and observed results were noted. A control test tube was also maintained in the same way. After 24-48 hours, 5 ml of decolorized samples were taken for degradation analysis and centrifuged at 10,000 rpm for 15 min. The supernatant was taken and its optical density was determined spectrophotometrically at specific wavelength of all selected azo reactive dyes.

% Decolourization per day = OD control- OD sample/ OD control* 100

Percentage dye degradation was calculated after 24 hours using above describe formula [5].

Heavy metal tolerance tests **[5]:**

Inhibition zone assay was done to screen the heavy metals resistance patterns of the isolated bacterial strain for the different heavy metal salts; Copper sulphate ($CuSO_4$), Zinc sulphate ($ZnSO_4$) and Nickel Sulphate ($NiSO_4$) used. For this agar well diffusion method was used. Sterile nutrient agar plates with 9% NaCl concentration were prepared and spread with the isolated bacterial cultures. There after 4 wells of 8 mm diameter were dug with the help of sterile cup

borer. Five plates were prepared, in accordance with number of heavy metal salts used in the study. In each plate: 1, 2, 3, 4 wells were filled with 50µl of heavy metal salt solution at the concentration of 10mM, 50mM, 100mM, 200mM respectively. The plates were then placed in incubator at 37° C for 24 hours. The heavy metal sensitivity at each concentration was expressed by diameter of clear zone of inhibition (in mm) produced by heavy metal at the end of incubation period. Similarly, bacterial isolates and already studied mixed consortium (MC) together was spread on sterile nutrient agar plate and results were recorded for the heavy metal salt resistance [5].

Results & Discussion

Bacteria were isolated by using spread plate method. In the halophilic agar medium contain 9% NaCl concentration shows different colonies. A total of 20 different types of colonies were isolated. Among all these colonies, 4 different types of pigmented colonies were chosen and preserved for further studies. In this H-3 isolates colonies showed yellow pigment, this isolates colonies appear small in size and round. Isolate H-10 showed orange pigment while H-15 showed the light brown pigmentation. In this H-10 isolate colonies appear convex but H-15 isolate colonies appear flat. H-18 colonies produce pigment pink color and colonies size showed big and round. All the pigmented isolates were gram positive cocci.

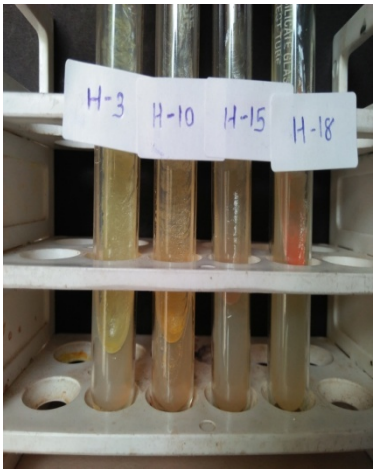


Figure: Pure isolates on modified halophilic agar medium slant

Screening of dye degradative effect

The pigmented isolates were studied for selected different three reactive azo dyes, Reactive Red 31, Reactive Black 5 and Reactive yellow 42 were used for decolourization activity. Pigmented isolates H-3, H-10, H-15 and H-18 did not showed any decolourization in the medium even after the 96 hrs of incubation at room temperature and even at 37°C.

Next we studied the same pigmented isolates with the combination of mixed consortium (MC) for decolourization of the nutrient broth media with the same dyes. From 1% dye stock solution the concentration of 100 µg/ml each during screening of degradative efficiencies indicate that the bacterial isolate was themselves not able to decolorize but improved the decolourization activity of Mixed consortium (MC) to utilize the Azo dyes as sole source of nutrient by decreasing the decolourization time very effectively. The percentage decolourization for four pigmented isolate bacteria with combination of mixed consortium (MC) in nutrient medium broth containing 9% NaCl concentration incorporated with 100 µg/ml from the 1% dye solution of three different dyes, Reactive Red 31, Reactive Black 5 and Reactive yellow 42.

In this decolourization of dye with pigmented isolated bacteria not showing dye decolourization. But with the mixed consortium (MC) decolourization of the nutrient broth media containing along with dyes at the concentration 100 µg/ml during screening of degradative efficiencies indicate that the bacterial isolate was able to improve utilize the Azo dyes. Thus it was able to show very promising decolourization activities against all selected different three reactive azo dyes, Reactive Red 31, Reactive Black 5 and Reactive yellow 42.

Previously presented study for mixed consortium (MC) showed that they are able to decolorized these selected dyes within 24 - 48 hours of incubation where in our study showed all the selected pigmented isolates improved the ability of mixed consortium (MC) for dye decolourization in less than 12 hours. While mixed consortium (MC) alone dye decolourization also observed after 24 - 48 hours. Thus our study results indicate that pigmented isolates help to improve the ability of mixed consortium (MC) for dye decolourization.

This synergistic effect can be explained by the action of one organism is responsible for causing the biotransformation of the dye, which then renders it more accessible to another organisms present in the consortium; which otherwise would not have occurred if there was only a single organisms [12].

Table 1: Azo dye decolourization potential analysis of bacteria isolates

Bacteria isolates	Reactive Red 31 decolourization	Reactive Black 5 decolourization	Reactive yellow 42 decolourization
H-3+-MC	71	71	75
H-10+MC	71	86	60
H-15+MC	86	71	65
H-18+MC	68	86	75
MC	64	68	69

Heavy metal tolerance tests:

In order to check the heavy metal resistance of the bacteria isolates, and also checked previously studied mixed consortium (MC), agar well diffusion method was used. The entire test was conducted and the zone of inhibition obtained recorded.

The isolated bacteria were tolerant to heavy metal salts such as copper sulphate, zinc sulphate, nickel sulphate at the concentration 10mM. We also checked the heavy metal tolerance of mixed consortium (MC) culture with pigmented isolates bacteria. The results obtained in reducing in zone size therefore that indicate that pigmented isolates help in the improving heavy metal resistance of Mixed consortium (MC).

We get result of all pigmented isolates and was tolerate different heavy metal salts Copper sulphate, Zinc sulphate, Nickel sulphate up to 10 mM salt concentration, which proved very good results when compared to the study done by [5],They found almost all zone of inhibition under 10mM of each selected heavy metals. In their studies moderately halotolerant strain of Klebseilla was tolerant to heavy metal salts such as Arsenic, Nickel, copper and Zinc to an extent of 5mM, 3mM, 1.5mM and 1.25mM respectively.

In the present study we focused on the same issue and tried to isolate and characterize the bacteria that have bacterial pigment and that help in improved the dyes decolourization with previously studied mixed consortium culture and also check their heavy metal resistance for different salts.

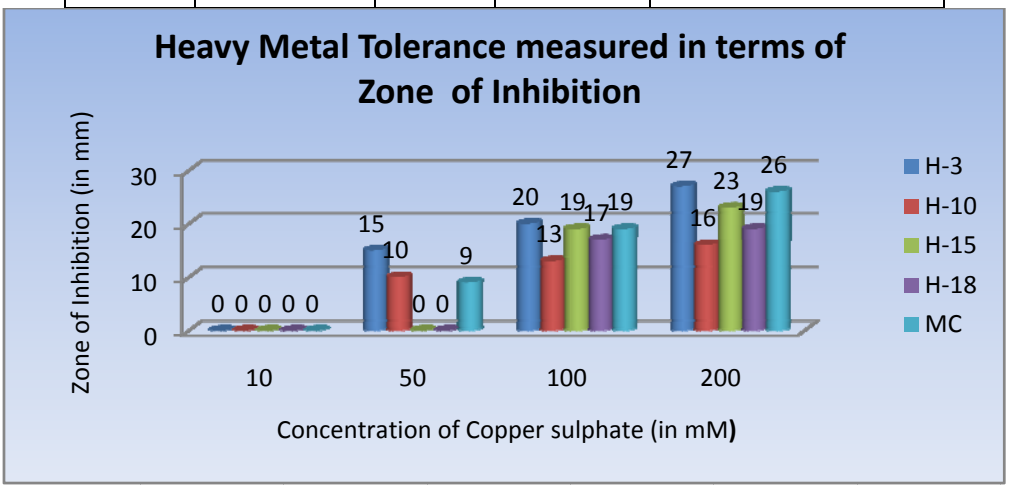
Decolourization of synthetic dyes is due to the cleavage of the chromatic group which generates colourless metabolic intermediate. These intermediate metabolites of the dye substrates are aromatic amines [8, 9]. The cleavage of the chromophoric group of dyes is a reduction process which requires redox equivalents that transfer electrons to the chromophoric group of dyes.

Some heavy metals are essential trace elements; most can be, at high concentration toxic to all branches of life, including microbes, by forming complex compounds within the cells. Because heavy metals increasingly found in microbial habitats due to natural and industrial processes. Bacteria that are resistance to grow on metals also play an important role in the biogeochemical cycling of metal ions. This is an important implication of microbial heavy metal tolerance because the oxidation state of heavy metal relates to the solubility and toxicity of the metal itself [10].

By comparing the dye degrading capability and metal resistance in future, the techniques of molecular biology and biochemistry coupled with the latest advances genomics and proteomics might offer a wide range of possibilities for enhancing the performance of these bacterial treatments of azo dye containing water [11]. On the other hand, a super bug may be constructed by the combination of heavy metal resistant gene and textile dye degrading gene. Mixed culture of textile dye degrading and heavy metal resistant bacteria can be used to improve the treatment process.

Table 2: Heavy Metal Tolerance Test by well diffusion Method of Copper sulphate ($CuSO_4$)

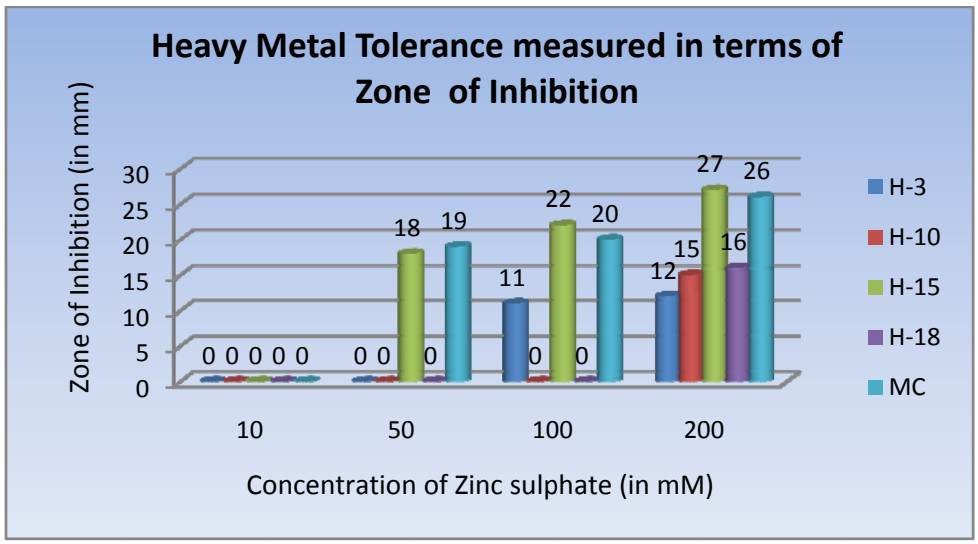
Bacteria Isolates	Copper sulphate concentration (in mM) Zone of inhibition (in mm)			
	10mM	50mM	100mM	200mM
H-3	-	15	20	27
H-10	-	10	13	16
H-15	-	-	19	23
H-18	-	-	17	19
MC	-	9	19	26



Graphical representation of heavy metal tolerance measured in terms of zone of inhibition for Copper sulphate ($CuSO_4$)

Table 3: Heavy Metal Tolerance Test by well diffusion Method of Zinc sulphate ($ZnSO_4$)

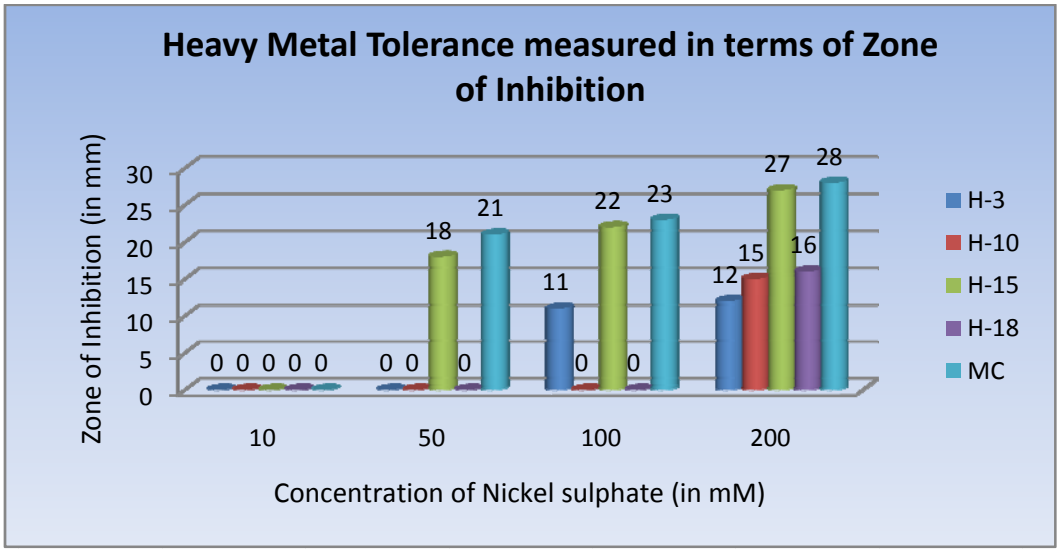
Bacteria Isolates	zinc sulphate concentration (in mM) Zone of inhibition (in mm)			
	10mM	50mM	100mM	200mM
H-3	-	-	11	12
H-10	-	-	-	15
H-15	-	18	22	27
H-18	-	-	-	16
MC	-	19	20	26



Graphical representation of heavy metal tolerance measured in terms of zone of inhibition for Zinc sulphate ($ZnSO_4$)

Table 4: Heavy Metal Tolerance Test by well diffusion Method of Nickel sulphate ($NiSO_4$)

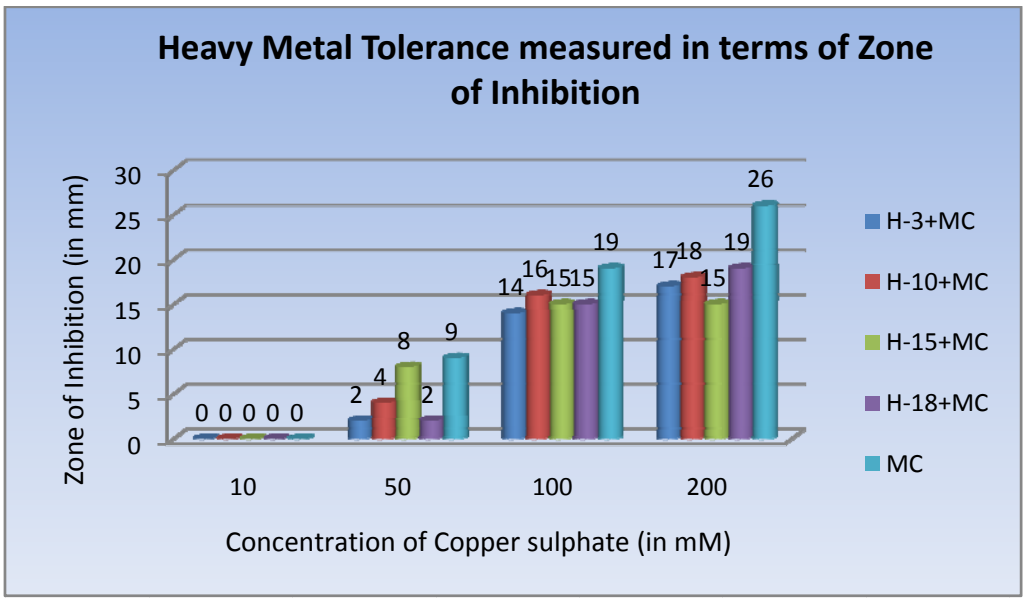
Bacteria isolates	Nickel sulphate concentration (in mM) Zone of inhibition (in mm)			
	10mM	50mM	100mM	200mM
H-3	-	-	11	12
H-10	-	-	-	15
H-15	-	18	22	27
H-18	-	-	-	16
MC	-	21	23	28



Graphical representation of heavy metal tolerance measured in terms of zone of inhibition for Nickel sulphate ($NiSO_4$)

Table 5: Heavy Metal Tolerance Test by well diffusion Method of Copper sulphate ($CuSO_4$)

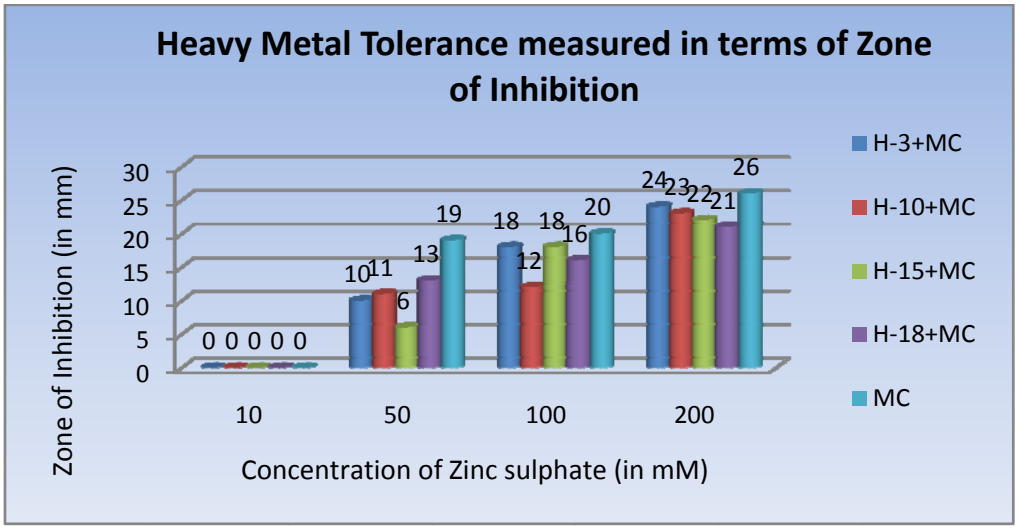
Bacteria isolates	copper sulphate concentration (in mM) Zone of inhibition (in mm)			
	10mM	50mM	100mM	200mM
H-3+MC	-	2	14	17
H-10+MC	-	4	16	18
H-15+MC	-	8	15	15
H-18+MC	-	2	15	19
MC	-	9	19	26



Graphical representation of heavy metal tolerance measured in terms of zone of inhibition for Copper sulphate ($CuSO_4$)

Table 6: Heavy Metal Tolerance Test by well diffusion Method of Zinc sulphate ($ZnSO_4$)

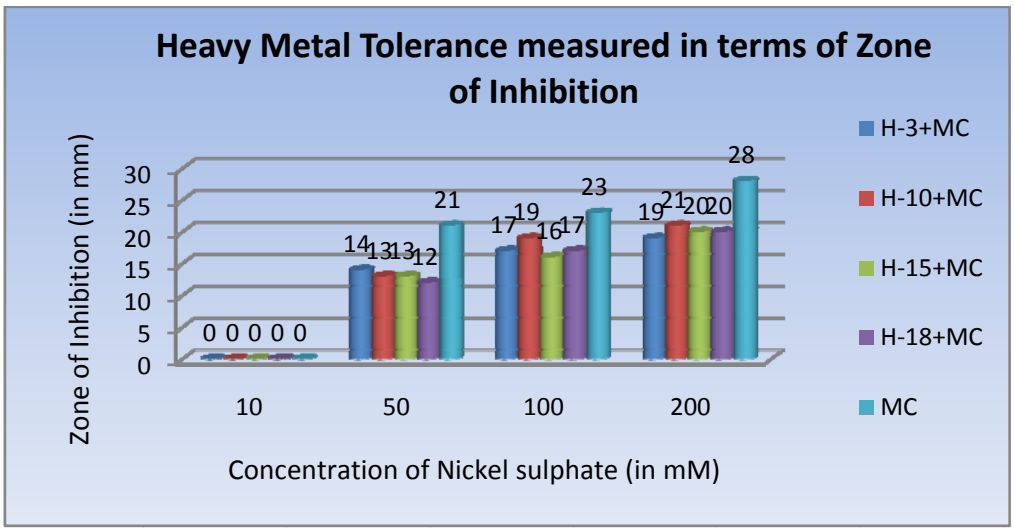
Bacteria isolates	Zinc sulphate concentration (in mM) Zone of inhibition (in mm)			
	10mM	50mM	100mM	200mM
H-3+MC	-	10	18	24
H-10+MC	-	11	12	23
H-15+MC	-	6	18	22
H-18+MC	-	13	16	21
MC	-	19	20	26



Graphical representation of heavy metal tolerance measured in terms of zone of inhibition for Zinc sulphate ($ZnSO_4$)

Table 7: Heavy Metal Tolerance Test by well diffusion Method of Nickel sulphate ($NiSO_4$)

Bacteria isolates	Nickel sulphate concentration (in mM) Zone of inhibition (in mm)			
	10mM	50mM	100mM	200mM
H-3+MC	-	14	17	19
H-10+MC	-	13	19	21
H-15+MC	-	13	16	20
H-18+MC	-	12	17	20
MC	-	21	23	28



Graphical representation of heavy metal tolerance measured in terms of zone of inhibition for Nickel sulphate ($NiSO_4$)

Conclusion:

Screening of the microorganisms resulted in isolation of four pigmented halotolerant bacteria with previously studied Mixed consortium (MC) capable of promisingly improved the decolourization capabilities of selected different three reactive azo dyes, Reactive Red 31, Reactive Black 5 and Reactive yellow 42 decolourization activity within 9 - 12 hours with dye concentration of 100 µg/ml. This halotolerant pigmented isolates also found to be improved the heavy metal salts tolerance and alkali salt tolerance of Mixed consortium (MC). From the above result we concluded that bacterial pigmented isolates with Mixed consortium help to improve the dye degradation process and also help in tolerate the heavy metal salt concentrations. Bacterial combination culture effectively used in future for textile effluent bioremediation process.

Conflict of Interest

The authors declare no conflict of interest

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