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Assessment of Mercury Detoxification Potentiality of Isolated *Streptococcus* sp. MTCC 9724 under Different Environmental Conditions

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Abstract The objectives of the present study were to isolate and identify a suitable mercury resistance bacterial strain and to explore their detoxification potentiality of mercury in different environmental conditions. A strain of *Streptococcus sp.* MTCC 9724 was isolated from the soil of waste dumping site adjacent to East Calcutta wetlands, West Bengal, India. Minimum inhibitory concentration of mercury was estimated. The bacteria were resistant to lead, arsenic, chromium and copper and antibiotics like Tetracycline, Ofloxacin, Amoxicillin, Ampicillin, Chloramphenicol and Cefadroxil. The bacteria were exposed containing 2, 5 and 10 mg/l of mercury containing media with variable pH, phenol and chloride ions as co-pollutants. It was observed that acidic pH was not suitable for bacterial growth and also for mercury detoxification. The presence of phenol in wastewater actually supported bacterial population growth and also mercury removal within 16 hours time periods, which indicated that utilization of phenol as a sole carbon source for bacterial metabolism, where as chloride ions up to 21 g/l did not inhibit the bacterial growth but significantly decreased the detoxification potentiality of *Streptococcus sp.* MTCC 9724.

Keywords Mercury, *Streptococcus Sp.*, Detoxification Potentiality

1. Introduction

Compounds of mercury such as mercuric chloride and organomercurials are toxic to both eukaryotic and prokaryotic cells. Mercury is available in different part of ecosystem and even in the food chain [1] and bind with thiol (SH) groups in proteins, thus causing damage to membranes and inactivating enzymes. Mercury is also genotoxic; inorganic Hg(II) is capable of strong reversible interactions with the nitrogens in purines and pyrimidines, and organic mercury compounds, e.g. methylmercury, also produce irreversible damage to nucleic acids [2]. The most serious ecological disaster resulting from the frequent use of this heavy metal and its compounds is the indiscriminate discharge of mercury-contaminated effluents into water bodies and adjoining soils, resulting in an unprecedented rise in pollution levels. The major sources of mercury pollution are chlor-alkaly plants, compost incinerators, mining, paper pulp, paints, fungicides, electrical equipments, instrumentation and amalgamation industries etc. Mercury droplets from thermometer and other medical laboratory equipments also are source of pollution in home, pathological laboratories and hospitals [3, 4]. Pacyna and Pacyna [5] provide estimates of global total anthropogenic mercury emissions over the period 990 to 2000 by United State. Mercury contamination has been found in marine sediments of Southern Brazil for the periods of 1998 to 2008 [6]. Some fishes collected from the same areas also contaminated with mercury ranging from 12.4 ng/g to 216 ng/g [7]. Although the problem of mercury pollution in India is yet to reach an alarming stage as compared with that observed in other developing countries, the presence of mercury in the coastal sea waters like Arabian Sea [8,9], the Gobind Sagar reservoir located in Singrauli, Madhya Pradesh, a part of Central India [10], the Yamuna River in Delhi [11] has raised serious concern regarding the safety of aquatic biota. Fishes and prawns in Mumbai, Orissa and Kolkata have reported high levels of Hg [12, 13]. Surprisingly, Directorate of General of the Commercial Intelligence, India (DGCI) had observed that import of organomercurials, including pesticides and biocides, has jumped from 0.7 tonnes to 1812 tonnes during 1996 and 2002 time period. India now consumes 50% of global production of Hg[14].

Unfortunately, very little has been done for the detoxification of mercury from polluted sites. Clean-up technologies that are capable of treating large volumes of soil, water, and sediment contaminated with mercury in a cost-effective way are urgently needed. A number of microorganisms had evolved resistance mechanisms to deal with mercury compounds. Volatilization of mercury to its elemental form by *Pseudomonas putida* strain [15] and precipitation of mercuric sulfide by the activity of sulfate reducing bacteria [16] is well known technology for mercury bioremediation. The basic principle of this process is enzymatic transformations of Hg^{2+} to Hg^0 by microorganisms [17]. Mercury tolerance *Bacillus pallidus*, *Ureibacillus thermosphaericus* [18], ten strains of *Escherichia coli* [19] from aquatic environment of India had already isolated for mercury bioremediation. Transgenic bacteria also play very crucial role in mercury removal at laboratory conditions [20, 21]

The aim of this work is to search for better mercury resistant bacteria from metal contaminated soils and an attempt has also been made to identify, characterize and observe their growth in different physicochemical conditions (i.e. pH, phenol and chloride) which are co-pollutants in mercury contaminated industrial wastewater.

2. Materials and Methods

2.1. Isolation and Identification of Microorganism

Suspensions of contaminated soil collected from waste dumping site adjacent to East Calcutta wetlands, were plated onto Mueller-Hinton (MH) agar plates [22] supplemented with $HgCl_2$ (up to 20 µg/ml) and the plates were incubated at 30^{o}C for 48 h. Colonies were isolated from the MH agar plates containing mercury and repeatedly sub-cultured on MH agar containing $HgCl_2$ (10 µg/ml) to obtain pure isolate. An efficient mercury (II) resistant bacterium was identified based on cultural, physiological, and biochemical characteristics using Bergey's Manual of Determinative Bacteriology. Further detailed identification was carried out at the Institute of Microbial Technology (IMTECH), Chandigarh, India. Mercuric chloride was added after sterilisation. Liquid cultures were grown in Erlenmeyer flasks of 250 mL containing 100 mL medium.

2.2. Determination of Optimal Growth Conditions

The optimal growth conditions with reference to pH and temperature were determined. The isolates were grown in MH liquid medium with nine different pH values i.e. 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 & 9 and incubated at different temperatures i.e. 25 °C, 30 °C, 37 °C and 42 °C. Optical density (O.D.) of the log phase of all above growing cultures was measured at 600 nm to determine the optimum growth. All experiments were replicated thrice [23].

2.3. Determination of Minimum Inhibitory Concentration (MIC) for Mercury and other Heavy Metals

24-hour old bacterial cultures maintained in MH broth were used for determination of MIC of mercury by ditch plate method. Composition of the agar media was: beef extract: 2 (g/l), casein: 16 (g/l), starch 1.5 (g/l), agar 1%. Hg concentrations in the media were maintained at the levels of 100, 200 and 300 mg/l. Zone of inhibition was measured after 24 hours. MIC was determined plotting diameter square (mm^2) versus concentration of Hg (mg/l). The same methods were followed for determination of MIC for arsenic (As^{+5}), lead (Pb^{2+}), chromium (Cr^{6+}) and copper (Cu^{2+}).

2.4. Determination of Antibiotic Resistance

24-hour old bacterial cultures maintained in MH broth were used for determination of resistance against some common antibiotics namely Tetracycline, Ofloxacin, Amoxicillin, Ampicillin, Chloramphenicol and Cefadroxil.

2.5. Determination of Mercury Removal Efficiency, Specific Growth Rate, and Mercury Detoxification Kinetics

Mercury removal efficiency was assessed under the same conditions described above. Triplicates of these experimental set up were incubated aerobically at ambient temperature and 200 rpm. Samples were taken at 2, 4, 8, 12 and 16 hour intervals for estimation of mercury in the media. Mercury removal (%) from the medium was calculated using the formula:

Mercury removal (%) = [(Initial Hg conc. in test – Residual Hg conc. in test) – (Initial Hg conc. in control – Residual Hg conc. in control)]/ Initial Hg conc. in test

2.6. Specific Growth Rate (µ)

$\mu = (X_2 - X_1) / (t_2 - t_1) X^{-1}$, where, X is the initial cell mass, $(X_2 - X_1)$ is the increase of cell mass at the time interval $(t_2 - t_1)$. Bacterial cell mass for growth curve was measured gravimetrically using weighing machine (AG135, Metler TOLEDO) Averages of 6 separate weights were taken [24].

2.7. Mercury Detoxification rate ($R_{Hg.d}$)

$R_{Hg.d} = \Delta[Hg]/ \Delta t .^{-1}X_{avg}$, Where, X_{avg} = Average cell conc. over the time interval (Δt)[25].

2.8. Determination of Mercury (II) Detoxification with Varying Phenol and Chloride Concentration

Batch culture experiments for the detoxification of mercury (II), using the isolated microbial strain, were carried out individually in Erlenmeyer flasks (250 ml) containing 100 ml of previously sterilized MH media. All experiments were carried out in triplicates. The flasks were inoculated

with 10% v/v of the bacterial culture and incubated aerobically at 30°C with agitation at 200 rpm. Culture samples were taken at 2, 4, 8, 12 and 16 hour intervals. Control experiments were set up in parallel to estimate the levels of abiotic losses. Different sets of experiments were performed by varying level of pH, phenol and chloride (as NaCl) of model wastewater (Table 1).

Table1. Experimental protocol for bioremediation study

Parameter (Variable)	Concentration variations in model wastewater					Composition of media
Mercury inflow in wastewater (μg/ml)	2	5	10			Beef extract 2g/l, Casein 16 g/l and Starch 1g/l pH 7.4
pH	4	5	6	7	8	
Phenol (g/l)	0.3	0.5	0.7	0.9	1.1	
NaCl (g/l)	7	14	21	28		
Incubation period (hr)	2	4	8	12	16	

2.9. Determination of Residual Mercury

5 ml sample was taken for residual mercury analysis. After digestion with HNO_3 and H_2SO_4, sample was used for estimation of mercury using cold vapor mercury analyzer (Model MA 5840, ECIL). Sample oxidation and subsequent reduction of Hg^{2+} to Hg^{0+} was performed by $KMnO_4$ and $SnCl_2$ respectively.

Table 2. Cultural features of *Streptococcus sp.* MTCC 9724

Colony Morphology	
Configuration	Round
Margin	Entire
Elevation	Convex
Surface	Smooth
Density	Transparent
Pigment	White
Gram' reaction	+ve
Shape	Cocci
Arrangement	Chains and pairs
Spore	-
Motility	±
Fluorescence (UV)	-
Physiological test	
Growth temperature	
4 °C – 10 °C	-
15 °C -37 °C	++
42 °C	+
45 °C – 65 °C	-
Growth pH	
pH 5 – pH 6.3	+
pH 7	++
pH 7.7 – pH 9	+
Growth on NaCl (%)	
2.5 - 5	++
5 – 6.9	+
7- 10	-

++ Good growth; + moderate growth; – no growth

3. Results

3.1. Isolation and Identification of Bacterial Strain

Thirty bacterial strains were isolated from the soil sample of East Calcutta wetlands, which all were able to grow in presence of 1 mg/l of Hg^{2+}, but only one efficient mercury resistance strain was identified as *Streptococcus sp.* MTCC 9724 (Table 2 and 3), which can tolerate mercury upto 10 mg/l in liquid media. *Streptococcus sp.* MTCC 9724 showed optimum growth at 30 °C and pH 7.0 and it could able to grow in culture media containing upto 7% of sodium chloride.

Table 3. Biochemical characteristics of *Streptococcus sp.* MTCC 9724

Biochemical test	
Growth on McConkey agar	-
Indole test	-
Methyl red reduction	+
Voges-Proskauer test	-
Citrate utilization	-
Gas production from glucose	-
Casein hydrolysis	-
Starch hydrolysis	-
Urea hydrolysis	-
Nitrate reduction	-
Hydrogen sulphide production	-
Cytochrome oxidase	-
Catalase test	+
Oxidation/Fermentation (O/F)	F
Gelatin hydrolysis	-
Arginine dihydrolase	+
Lysine decarboxylase	+
Ornithine decarboxylase	+
Acid production from carbohydrate	
Adonitol	-
Arabinose	+
Cellobiose	+
Dextrose	+
Duleitol	-
Fructose	+
Galactose	+
Inositol	-
Lactose	-
Maltose	-
Mannitol	-
Melibiose	±
Raffinose	-
Rhamnose	-
Salicin	-
Sorbitol	-
Sucrose	-
Trehalose	-
Xylose	-

+ present; – absent

Table 4. MIC of *Streptococcus sp.* MTCC 9724 for selected heavy metals

Metals	MIC (mg/l)
Pb	1.6
Cr	4.8
Cu	0.4
As	1.1
Hg	0.44

3.1. MIC of Mercury and other Heavy Metals

MIC of *Streptococcus sp.* MTCC 9724 for Hg^{2+} was 44.21

mg/l in solid medium. The bacteria could also able to grow in lead, arsenic, chromium and copper containing media (Table 4).

Table 5. MIC of *Streptococcus sp.* MTCC 9724 for (µgm/l) for selected antibiotics

Antibiotics	MIC ((µgm/l)
Tetracycline	0.91
Ofloxacine	0.68
Ampicilline	0.3
Cefadroxil	0.72
Chloramphenicol	0.81
Amoxicillin	No zone found

3.2. MIC of Antibiotics

The *Streptococcus sp.* MTCC 9724 was not resistant to Chloramphenicol and amoxicillin with the supplied dose, whereas highest MIC was observed in Tetracycline(0.91µg/l) followed by Chloramphenicol, Cefadroxil, Ofloxacine and Ampicilline (Table 5).

3.3 Determination of Mercury Removal Efficiency and Growth of Streptococcus Sp. MTCC 9724

The ability of the *Streptococcus sp.* MTCC 9724 to detoxify their ambient by removing mercury was tested in separate batch cultures (Fig 1). Upto 99.4% of mercury removal was observed after 16 hour time periods. The mercury removal efficiency was negatively correlated with mercury concentration in media (Table 6).

Table 6. Pearson correlation (r) and P values of mercury removal efficiency by *Streptococcus sp.* MTCC 9724 with several variables of batch culture study

		Hg removal by Streptococcus sp. MTCC 9724	Incubation period	Mercury level in media	pH	Phenol	Chlorides
Incubation period	r	0.448					
	P	0.01					
Hg level in media	r	-0.893	0.000				
	P	0.001	1.000				
pH	r	0.607	-0.067	-0.059			
	P	0.001	0.609	0.650			
Phenol	r	-0.024	-0.046	-0.041	*		
	P	0.836	0.694	0.727	*		
Chlorides	r	-0.757	0.000	0.000	*	*	*
	P	0.001	1.000	1.000	*	*	*

*No significant correlation found

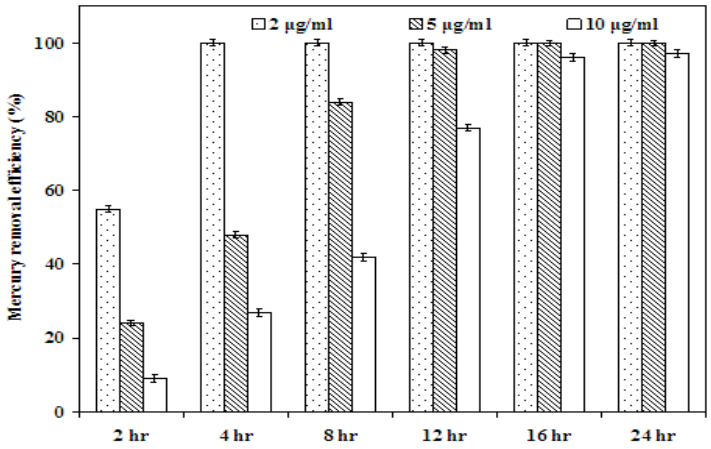

Figure 1. Batch scale mercury removal efficiency of *Streptococcus sp.* MTCC 9724 at 2 µg/ml, 5 µg/ml and 10 µg/ml mercury concentration in media

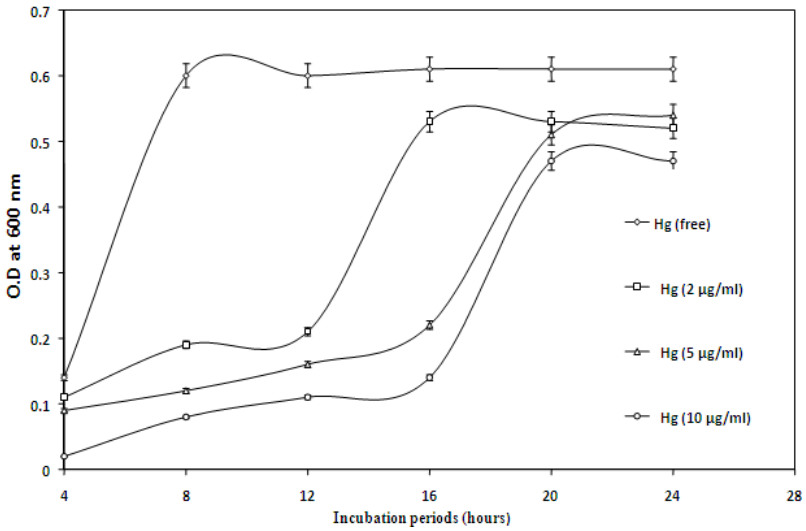

Figure 2. Growth curve of *Streptococcus sp*. MTCC 9724 at 2 µg/ml, 5 µg/ml and 10 µg/ml mercury concentration and mercury free media of batch culture study

Streptococcus sp. MTCC 9724 had shown prolong lag phase in mercury containing media in compared to mercury free media (Fig 2).

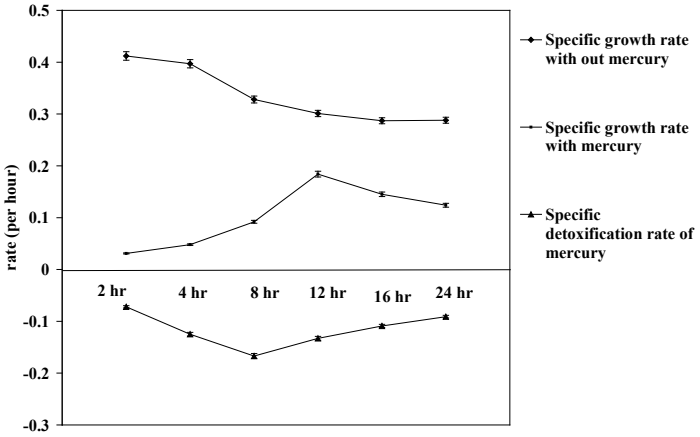

Figure 3. Status of specific growth rate and specific detoxification rate study at 10 µg/ml mercury concentration by Streptococcus sp. MTCC 9724

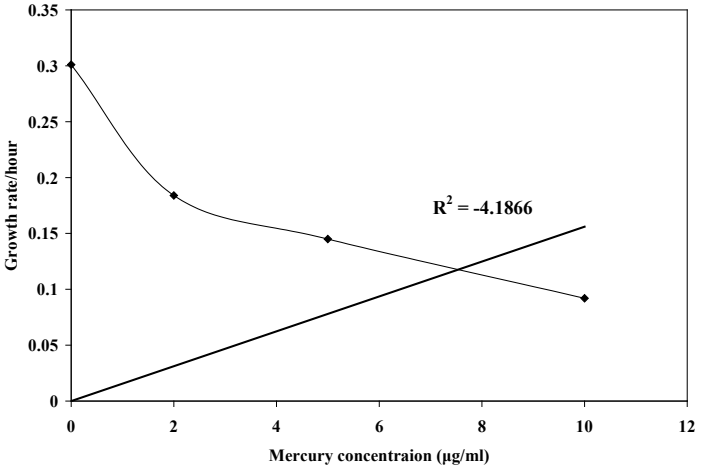

Figure 4. Effect of mercury concentration on specific growth rate of *Streptococcus sp.* MTCC 9724

3.4. Determination of specific growth rate, and mercury detoxification kinetics

Highest specific growth rate was observed after 4 hours time period without mercury present in the media but the specific growth rate was decreased when mercury was present in the media. The highest detoxification rate was observed after 8 hours, then the detoxification rate gradually decreased with time (Fig 3). The specific growth rate had a negative correlation with mercury present in media (Fig 4).

3.5. Effect of pH on Mercury Removal Efficiency of *Streptococcus sp.* MTCC 9724

In lower pH, the bacterial growth and mercury removal efficiency was limited (Fig 5). Significant bacterial growth and mercury removal efficiency was observed at pH 7 and also at 8 with varying concentration of mercury (2, 5 and 10 mg/l).

3.6. Effect of Phenol Concentration on Mercury Removal Efficiency of Streptococcus Sp. MTCC 9724

The isolated bacteria *Streptococcus sp.* MTCC 9724 successfully removed mercury in presence of phenol at different concentrations (Fig 6). The highest mercury removal was observed at 0.3 g/l of phenol concentration after 16 hours in batch culture study having 2, 5 and 10 mg/l of mercury concentration in media. The increasing phenol concentration did not actually affect so much at 2 mg/l of mercury concentration, but mercury removal was inhibited with increasing phenol concentration and at 5 and 10 mg/l of mercury concentration in media.

3.7. Effect of Chloride Concentration on Mercury Removal Efficiency of Streptococcus Sp. MTCC 9724

The highest mercury removal efficiency was observed after 16 hours in presence of both 7 and 14 g/l chloride concentration (Fig 7), whereas mercury removal was almost limited in 28 g/l chloride level. The increasing concentration of chloride had a negative effect on mercury removal efficiency of *Streptococcus sp.* MTCC 9724 (Table 6).

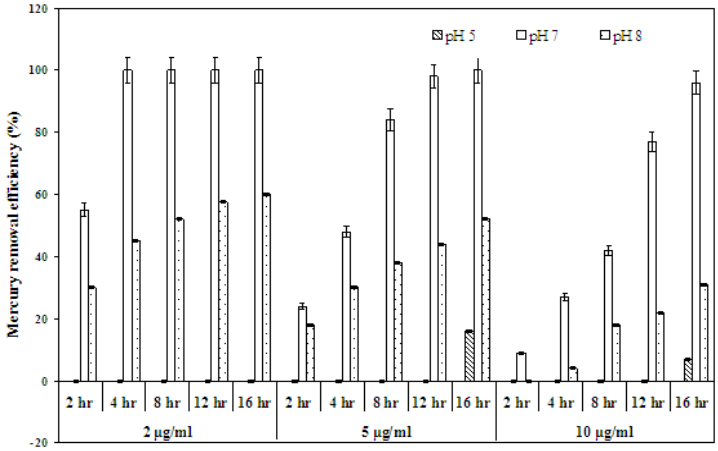

Figure 5. Effect of pH on mercury removal efficiency of *Streptococcus sp.* MTCC 9724 at 2 µg/ml, 5 µg/ml and 10 µg/ml mercury concentration in media after 2,4,8,12 and 16 hour incubation period

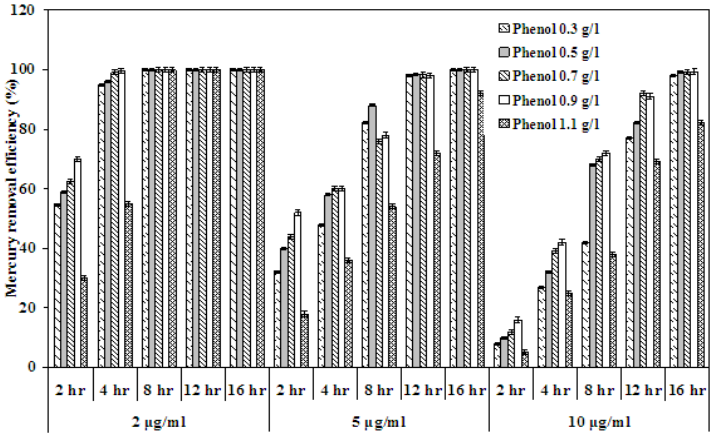

Figure 6. Effect of phenol concentration on mercury removal efficiency of *Streptococcus sp.* MTCC 9724 at 2 µg/ml, 5 µg/ml and 10 µg/ml mercury concentration in media after 2,4,8,12 and 16 hour incubation period

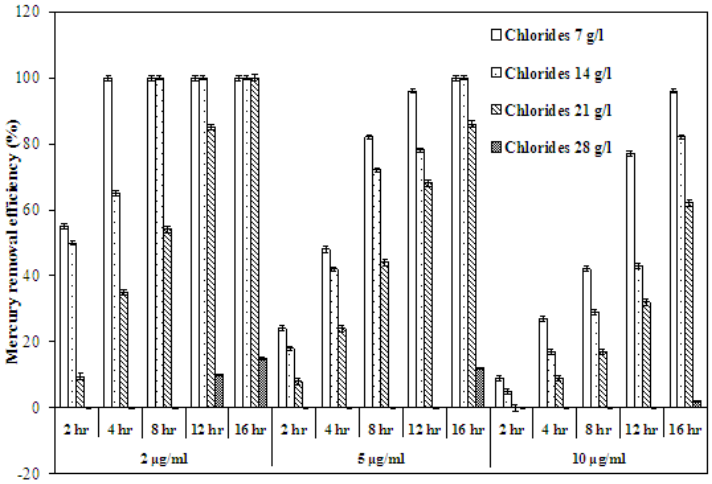

Figure 7. Effect of chloride concentration on mercury removal efficiency of *Streptococcus sp.* MTCC 9724 at 2 µg/ml, 5 µg/ml and 10 µg/ml mercury concentration in media after 2,4,8,12 and 16 hour incubation period

4. Discussions

Mercury contaminated wastewater is coming from chlor-alkali plants, pharmaceutical industries, leachet of hospital and municipal wastes dumping ground etc, where the effluents has wide variability among pH, organic loads, chloride concentration. Some industries used to neutralize their effluents applying sodium hydroxides, sulfuric acids and hydrochloric acids, which also generate salts like sodium sulphate, chlorides etc. These salts some time inhibit the bacterial growth and ultimately disrupt the bioremediation procedure. Bioremediation techniques that involve the use of microorganisms to remove environmental contaminants have gained an increasing interest in the last few years.

In the present study thirty bacterial strains were initially isolated from soils of East Calcutta Wetland area. After successive screening only one mercury resistant strain were selected for further study. East Calcutta Wetland area is an age old dumping ground and sewage irrigated vegetable field. Soils of those areas are also contaminated by considerable amount of mercury and other heavy metals [26, 27]. It was predicted that the soils of the areas might have the best habitat for mercury resistant organism. Sadhukhan et al [28] had also isolated mercury resistant *Bacillus, Escherichia, Klebsiella, Micrococcus, Pseudomonas, Salmonella, Sarcina, Shigella, Staphylococcus* and *Streptococcus* from gills and guts of fresh water fish from the same area. An another investigation by Pahan et al [29] isolated mercury resistant bacterial strains form fish gills and soil sample collected from different parts of West Bengal, India. The current isolated strain was Gram positive and identified as *Streptococcus sp* -MTCC 9724 (Table 2 and 3). Eight metal resistant bacterial strains successfully isolated from contaminated site near industrial dumping ground [30]. Previous studies established that both *Enterococcus* and *Streptococcus* species had mercury resistance extra chromosomal gene for detoxify mercury. Pike et al [31] had isolated mercury resistant *Streptococcus sp* from the teeth with mercury amalgam.

An interesting observation was found that *Streptococcus sp* -MTCC 9724 were resistant to other heavy metals and antibiotics (Table 4 and 5). Some previous investigation had also supported the present observations[25, 32] where, a direct agar diffusion assay determined the MIC of sixteen heavy metals of two marine chromogenic and non-chromogenic bacteria isolated from contaminated Arabian Sea. Another observation was also found by Mergeay [33] in soil ecosystem where soil bacteria were lived in present of toxic heavy metals. Previous investigation of Silver and Mishra [34] and Ahmed et al [35] had already established the multiple metal resistances phenomenon of the bacteria. Chattopadhyay [36] isolated bacterial species having co-tollerance ability to both mercury and antibiotics from a chemotherapy unit of a hospital. [37] They had also studied on antibiotic and heavy metal tolerance ability of an isolated *Streptococcus* species.

There had been previous attempts to estimate mercury detoxification efficiency through batch culture study [38]. The present study also revealed that *Streptococcus sp* -MTCC 9724 could successfully remove mercury within 16 hours incubation period (Fig 1). The growth of the isolated bacterial strain was also depended on the mercury concentration of the media (Fig 2). The growth with mercury (2 to 10 µg/l) exhibited a 10 to 12 hours lag phase which was absent in the mercury free media. Prolong lag phase of *Pseudomonas sp.* and *Bacillus sp*. isolated from Andaman Island, India was also exhibited long lag phase after exposing metal containing environment [39]. De et al [40] previously suggested that their isolated bacterial strain had exhibited prolong lag phase in mercury containing media. It has been assumed that, once the organism entered into the log phase, the generation time would become lower due to mercury shock. In the present study, *Streptococcus sp* -MTCC 9724 took almost eight hours more time to reach stationary phase (Fig 2). This observation could be compare with the work of Sarkar et al [41], where they could not able to estimate

growth pattern of *Microbacerium* due to its metal induced prolong log phase of more than 48 hours.

Comparative study among growth rate, specific growth rate in mercury containing and mercury free media revealed that specific growth rate of *Streptococcus sp* -MTCC 9724 was higher in mercury containing media after 12 hours (Fig 2). Almost similar findings were reported by several workers[25, 42,43] that, presence of toxic heavy metal and stress conditions had increased specific growth of metal resistance strains with compare to other. The highest mercury detoxification rate observed after 12 hour incubation (Fig 3). The specific growth rate was also varied in different concentration of mercury in media (Fig 4). Previous experiment [38], revealed that specific growth rate of Pseudomonas aeruginosa was highest at 2 µg/ml mercury concentration and as the concentration of mercury increased the specific growth rate decreased gradually.

In the present study Streptococcus sp -MTCC 9724 was exposed to pH ranging from 3 to 8. It was observed that their mercury removal efficiency was also varied (Fig 5) and highest removal was observed at pH 7. Almost similar observation was found in removal of mercury in chloroalkali effluent, in which four strain of Pseudomonas had removed mercury at pH 7 in a technical scale bioreactor [15]. In present findings, the percentage removal of mercury from wastewater were decreased simultaneously, with increasing mercury concentration (5 and 10 µg/l) but almost all mercury had removed after 16 hours incubation period (Fig 5). Bioremediation by Pseudomonas sp after neutralization of acidic effluent was common for several lab studies [44]. The isolated strain could not be able to remove mercury in acidic media, but its removal efficiency was almost 90% in pH 8 at 2 and 5 µg/l mercury containing media. Investigation revealed [45] that the mercury could be successfully removed by immobilized mercuric reductase at pH 7. It was predicted that the working strain probably detoxify mercury by means of their intracellular mercuric reductase enzyme. Another investigation [46] reported that the strain Thiobacillus ferrooxidans successfully removed mercury at pH 7 also.

The isolated bacteria Streptococcus sp -MTCC 9724 was also able to remove mercury in phenol containing media (Fig 6). Earlier workers had shown that mercury resistant organism could also able to use phenol as a carbon source [16, 40, 47, 48, 49]. Phenol biodegradation was most sensitive after addition of mercury and phenol degradation would be decreased at 0.1 and 0.7 µg/ml mercury in media [50]. A few workers [51, 52] had also established that, phenol could be assimilated by bacteria in presence of mercury as an inhibitor. In the present study mercury removal efficiency was positively correlated with phenol concentration had also in media (Table 6) as the phenol concentration increased the mercury removal efficiency was also increased. This observation ultimately helped to predict that phenol was also utilized by Streptococcus sp -MTCC 9724 as a sole carbon source for their metabolism.

Chloride inhibition was the major problem for mercury removal through the process of bioremediation [15, 53]. In present study increasing concentration of chloride had a negative effect on mercury degradation in column reactor (Table 6 and Fig 7). It appeared that, mercury removal efficiency was negatively correlated with chloride concentration in media. Almost same observation had been found in mercury removal of industrial waste of chlor-alkali plant in a technical scale bioreactor [44]. It was assumed that the bioavailability of Hg^{2+} was reduced at high chloride concentrations due to the formation of mercurochloro complexes. Using a geochemical equilibrium speciation model [17] had calculated the concentrations of different species of Hg^{2+}complexes in various assay solutions. In particular, the concentrations of $HgCl_3^-$ and $HgCl_4^-$ in the presence of different chloride concentrations were calculated. Using a *mer lux* $HgCl_4^-$ reporter strain[54, 55] were able to show that the light emitted by the reporter strain decreased as the concentration of NaCl increased from 1 to 100 mM simultaneously with significant increasing concentration of mercuro-chloro complexes. Any inhibition of mercury removal was not detected in the presence of 12 g/l of NaCl in wastewater. The effects of NaCl concentrations greater than 12 g/liter had not been studied with a defined system. In current study, *Streptococcus sp.* MTCC 9724 did not inhibit in mercury reduction activity up to 21 g/liter of chloride concentration (Fig 7). These observations could not be compared directly to those of Barkay et al. work [55], these study could rather compared with work of von Canstein et al [15], where mercury tolerance *Pseudomonas sp.* grew well in varying salt containing media.

5. Conclusions

Bioremediation of mercury (II) by batch cultures of isolated *Streptococcus sp.* MTCC 9724 in varying pH, phenol and chloride concentration had studied in ambient temperature. The experimental results indicated that mercury from waste water could effectively remove at optimum condition of pH (pH 7); phenol (1.1 g/l) and chloride (7 g/l) were present in media. When, however, the concentration of chloride were higher than 21 g/l the bacterial growth and mercury removal were limited. Increasing phenol concentration had a significant positive role for bacterial mercury removal efficiency. Though the isolated strain was not ever exposed to mercury concentration above 10 mg/l, this is an important observation that with minimum retention time *Streptococcus sp.* MTCC 9724 could able to remove mercury successfully. Volesky, [56] suggested that the existing physicochemical mercury removal techniques can also be applied at the end of the pipe treatment, but their efficiency is poor when mercury level is low (<100 mg/l). In this situation the strain could have been useful for mercury removal from industrial wastewater.

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