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Full Length Article

Optimization of Hexavalent Chromium (Cr(VI)) Reducing Strains for Accelerated Degradation of Biphenyl and 2-Cholorbiphenyl in Tannery Wastewater

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Abstract

Bioremediation of multi-contaminated sites can be accelerated by optimization of bacterial strains having the ability to metabolize organic and inorganic compounds, individually. In this study previously identified bacterial strains, capable of hexavalent chromium (Cr(VI)) reduction, were optimized for accelerated degradation of biphenyl and 2-chlorobiphneyl. The MSM medium was amended with yeast biphenyl (10:90) and only biphenyl (100%) in separate experiments. Selected strains showed <1.0 OD_{600} in control (MSM) which decreased to 0.5–0.6 OD_{600} with yeast biphenyl MSM and further decrease to 0.06–0.09 OD_{600} in MSM with biphenyl as sole carbon source. Cr(VI) reducing capability of strain, however, was not affected by medium amendment from MSM to yeast biphenyl MSM. In various pH and temperature treatments (6–9 pH and 25–40ºC), highest percentage (84% and 93% respectively) of 2-CB and Cr(VI) transformation was achieved at pH 7 and 30ºC temperature by *Pseudomonas aeruginosa* SB. *Stenotrophomonas maltophilia* K8, on the other hand, showed 78% degradation of 2-CB and 77% Cr(VI) reduction under similar conditions. Strain SB showed significantly higher biotransformation of Cr(VI) and 2-CB (80% and 85% respectively) in yeast biphenyl MSM in comparison to glucose and starch biphenyl whereas K7 and K8 also performed better in similar medium. Glucose and starch were not found to be suitable as carbon sources for the isolates. In electron shuttles experiment, 85% degradation of biphenyl at 1 mmol L^{-1} concentration of sodium benzoate was observed with strain SB, whereas 82 and 72% degradation were observed with same concentration of hydroquinone and mannitol respectively. Result of the study suggested that maximum detoxification of Cr(VI), biphenyl and 2-CB was achieved at 7 pH, 30ºC in yeast biphenyl MSM. Whereas non-significant difference in degradation ability of strains was observed for sodium benzoate, hydroquinone, and mannitol when applied as electron shuttles in lower concentrations (1–3 mmol L^{-1}). The result of this study can be helpful in simultaneous treatment of multi-contaminated sites.

Keywords: Hexavalent chromium; 2-chlorobiphenyl; Carbon source; Environmental factors; Electron shuttles; Biodegradation

Introduction

The degradation of recalcitrant and harmful compounds is a global challenge worldwide. Industrial growth and oxidative properties of metals and compounds have resulted in the sorption of these pollutants to soils/sediments and cell membranes, which have significant mutagenic and carcinogenic effects on biotic components of ecosystem. Hexavalent chromium as well as polychlorinated biphenyls (PCBs) are few of the most common pollutants of water bodies due to their versatile use in the past (Tchounwou *et al.*, 2012; Hens and Hens, 2018). In developing countries, water pollution is further aggravated due the lack of resources and awareness to adopt proper treatment system among the local population. Availability of these chemicals in the old stockpiles and untreated industrial effluents are also among major sources of these pollutants (Eqani *et al.*, 2012).

The PCBs and Cr(VI) can bioaccumulate in the food chain due to high Kow (octanal/water partition coefficient), strong adsorption capability and permeability in cell membranes (D'Angelo and Nunez, 2010; Emadzadeha *et al.*, 2016). In US, fish consumption advisories have been regulated in over 75% of states, because of higher PCB levels in fish tissue (>2 mg kg^{-1}) (LePrevost *et al.*, 2013). Consumption of Cr(VI) and PCBs contaminated food have harmful effects on the liver, blood, endocrine system, nervous system, and reproductive system. The co-occurrence of Cr(VI) and

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organic contaminants such as PCBs in industrial wastewater has been well reported (Kumar *et al.*, 2005; Mwinyihija, 2010). Various remediation approaches have been adopted for industrial effluent treatment, but all the chemical or mechanical treatment system have their own pros and cons.

Different types of bacteria can degrade PCBs under anaerobic and aerobic conditions. Similarly, bacterial species with Cr(VI) reduction capacity through biosorption, bioaccumulation and enzymatic transformation have been well described (Jobby *et al.*, 2018). As the initial step in the detoxification of PCBs and Cr(VI) is a reduction process, so a simultaneous approach for bioremediation of both contaminants in a single system is a viable option. Several bacterial species have been reported to degrade organic compounds and metals in separate studies and only few have been used in simultaneous treatment. *Pseudomonas aeruginosa* (Schroeter, 1872; Migula, 1900) was able to simultaneously reduce Cr(VI) and phenol to a concentration of 40 mg L^{-1} of Cr(VI) (Song *et al.*, 2009), while completely degrading the aroclor of PCBs, individually, after 96 h (Mathews and Sithebe, 2018). Similar characteristics were exhibited by strain of *Stenotrophomonas maltophilia* (Palleroni and Bradbury, 1993), which reduced Cr(VI) to a trivalent from of chromium (Baldiris *et al.*, 2018) and degraded chlorobiphenyl under aerobic conditions in separate studies (Somaraja *et al.*, 2013). Simultaneous treatment of Cr(VI) and PCBs using a single strain could be therefore an option for the bioremediation of multi-pollutant sites.

Role of environmental factors on biotransformation of Cr(VI) and PCBs is crucial in determining the optimum conditions for the maximum detoxification of both contaminants. Cr(VI) reduction by *P. aeruginosa* isolated from tannery effluents showed maximum absorption (30 mg L^{-1}) at pH 8 (Chatterjee *et al.*, 2011). It has been observed that *Bacillus sphaericus* (today *Lysinibacillus sphaericus*) was resistant to 800 mg L^{-1} Cr(VI) and reduced more than 80% Cr(VI) during growth under pH and temperature of 6.0 and 25°C, respectively (Pal and Paul, 2004). Similarly, *Bacillus amyloliquefaciens* (Priest *et al.*, 1987) showed higher tolerance and fast reduction rate of Cr(VI) under optimized conditions of 100 mg L^{-1} Cr(VI), pH 7 and temperature 35°C within 45 h (Das *et al.*, 2014). Maximum potential of bacterial strains for Cr(VI) reduction has been reported between 6–7 pH range and temperature 30–35ºC. Presence of organic compounds have been observed to accelerate the electron transfer during metal reduction through bacterial strains (Mahmood *et al.*, 2015). The molecular mechanisms of PCBs degradation have been well understood but evidence about the effects of environmental factors on degradation of PCBs is limited. This study would be helpful in optimizing environmental conditions for *in situ* bioremediation of multi-contaminated sites by microorganisms reportedly having biodegradation ability of single contaminant.

Materials and Methods

Bacterial Strains and Culture Conditions

The study was designed for optimizing the environmental factors that affects bioremediation and accelerating the detoxification of multi-contaminant site in a single system. Selected strains, capable of Cr(VI) reduction were optimized for pH, temperature, carbon substrate and electron shuttles to degrade 2-chlorobiphenyl efficiently. Previously isolates bacterial strains, capable of Cr(VI) reduction, were optimized for the degradation of 2-chlorobiphenyl by MSM medium amendment and optical density measurement. Five strains were used in this study, namely *P. aeruginosa* SB, *P. pseudoalcaligenes* K7, *S. maltophilia* K8, *Providencia stuartii* SA and *B. cerus* K5 (NCBI accession numbers MG576130-MG576134). Selected strains were further optimized for different environmental conditions to achieve maximum detoxification of both contaminants by same strains in separate set of experiments. Minimal salt medium amended with yeast: biphenyl was used containing (g L^{-1}) NaCl (1.0), Na_2HPO_4 (1.0), KH_2PO_4 (1.0), $CaCl_2.2H_2O$ (0.1), $MgSO_4.7H_2O$ (0.5), yeast extract (0.5) and biphenyl (0.2). Potassium dichromate was used as source of Cr(VI) whereas 1,5-diphenyl carbazide, purchased from Merck-Millipore (Darmstadt, Germany), was used as a color complexing agent for evaluation of Cr(VI) transformation potential using a spectrophotometer (Desai *et al.*, 2008). 2-chlorobiphenyl stock solutions were prepared in acetone for enrichment in MSM medium whereas hexane was used to prepare GC-MS stocks and samples.

Effect of Media Amendment on Bacterial Growth and Cr(VI) Reduction Ability

Growth of the selected strains were assessed on biphenyl and yeast + biphenyl (10%: 90% ratio) spiked MSM medium. The optical density was measured after every 24 h at x600 nm wavelength using spectrophotometer (Peak Instruments E-1000 UV). Experiment was carried out to distinguish if a change in the media recipe could affect the Cr(VI) reduction capability of strains at the concentration of 2 mg L^{-1}. This helped in selection of a medium for further experiments and optimization of strains for biphenyl and 2-chlorobiphenyl degradation. All the strains were inoculated separately in 20 mL MSM, yeast biphenyl MSM and only biphenyl MSM and incubated under control conditions. One mL of sample was taken after every 24 h till 120 h. Each treatment was repeated three times. For the correlation among strains to assess their Cr(VI) reduction ability in yeast and yeast biphenyl MSM, selected strains were grown in 2 mg L^{-1} enriched media separately and incubated for 48 h. Twenty mL yeast and yeast biphenyl MSM was taken in serum bottles and inoculated with selected strains (OD = 0.5) in separate set of experiments. After the incubation

period, 1 mL sample was taken from each serum bottled and analyzed for Cr(VI) reduction using 1,5-DPC as color complexing agent on spectrophotometer. Experiment was laid out in triplicates and results are presented in percentage.

Effect of pH, Temperature and Varying Carbon Sources

Effect of different carbon sources was studied using two carbon sources *i.e*., starch and glucose, whereas yeast-biphenyl MSM was used as control. In the following two experiments, biphenyl was replaced by starch and glucose with the same concentration. Amended MSM was spiked with 2 mg L^{-1} Cr(VI) and 4 mg L^{-1} of 2-CB in separated experiments and pH was adjusted using NaOH or 0.01 M HCl solution. Selected strains were assayed for their biotransformation ability of both Cr(VI) and 2-CB laid out in separate experiments under static conditions in the incubator. Effect of pH ranging from 6–9 and different temperatures (25–40ºC) in amended MSM spiked with 2 mg L^{-1} Cr(VI) and 4 mg L^{-1} 2-CB with selected five strains was studied under similar conditions. Twenty mL MSM was added to autoclaved serum bottles and inoculated with 200 µl of inoculum (OD = 0.7 ± 0.02 at 600 nm) of selected strains. After 48 h of incubation, aliquots were centrifuged at 10,000 rpm for 5 min to remove the bacterial cells. Cr(VI) concentration in the supernatant was determined by the same spectrophotometer than before at 540 nm using diphenyl carbazide reagent as color complexing agent.

Role of Electron Complexes in Cr(VI) and 2-CB Biotransformation

Three different electron complexes *i.e*., sodium benzoate, hydroquinone and mannitol were used to assess their role in degradation of biphenyl, 2-CB and Cr(VI) at 200 mg, 15 and 8 mg L^{-1} reduction with three most efficient selected strains respectively. Efficacy of strains was assessed by their biotransformation ability of both contaminants (Cr(VI) and 2-CB) at pH7 and 30ºC temperature. Electron complexes were applied at the rate of 1, 3 and 5 mmol L^{-1} separately for Cr(VI), biphenyl and 2-CB. Concentration of Cr(VI) and 2-CB were the same as in previous experiments whereas concentration of biphenyl, used as co-substrate, was 200 mg L^{-1}. Experiment was performed three times separately for all the contaminants.

Cr(VI) and 2-CB Biotransformation Assay

Ethyl acetate extraction was performed for biphenyl and 2-CB analysis (Ohtsubo *et al.*, 2003). Two mL ethyl acetate was added into 5 mL sample and vortexed for 3 min. The organic solvent layer was transferred to acid rinsed clean vial and the procedure was repeated three times to transfer the entire mass of biphenyl and 2-CB from the samples. The combined solvent sample volume was evaporated by a gentle nitrogen blow down using Organomation 12-position N-EVAP nitrogen evaporator (MA, US) and 20 mg L^{-1} of internal standard was added to the sample before analysis. Extracts were diluted to 1 mL with hexane for GC-MS analysis. The Cr(VI) reduction assay was performed using 1,5-DPC as color complexing agent through spectrophotometer at 540 nm. Results of the experiment were reported in percentage for Cr(VI), biphenyl and 2-CB detoxification by the following equations:

$$\text{Reduction Percentage} = \frac{\text{Initial Conc.} - \text{Final Conc.}}{\text{Final Conc.}} \times 100 \ldots\ldots\ldots \text{(Eq.1)}$$

$$\text{Percentage biodegradation (\%)} = \frac{PCB_{control} - PCB_{sample}}{PCB_{control}} \times 100 \ldots \text{(Tu } \textit{et al.}\text{, 2011)}$$

Statistical Analysis

All the experiments were conducted in CRD design with three replicates. One sample *t-test* was performed for the bacterial growth analysis whereas one-way ANOVA was used to analyze the significance difference (P<0.05) among means for pH, temperature and carbon source experiments. Two-ANOVA and correlation among means was used for the analysis of electron shuttles role on bacterial degradation efficiency. Linear regression was used to compute the relationship between dependent and independent variable and all the results presented in percentage. All the statistical tests were performed on GraphPad Prism 8.

Results

Effect of MSM Amendment on Bacterial Growth and Cr(VI) Reduction Ability

Bacterial growth was affected by change in carbon source from yeast to yeast biphenyl and only biphenyl for the selected strains. Results suggested that maximum bacterial growth was observed until 72 h in control MSM (Fig. 1a) whereas growth of bacterial cells continues to increase until 48 h and reach steady phase afterwards (Fig. 1b) for yeast-biphenyl. In case of biphenyl as sole carbon source, bacterial cells continue to grow slowly until 120 h (Fig. 1c). The maximum OD was observed, after 72 h, by strain K7 in control MSM (1.416) (Fig. 1a) which was 0.568 OD in yeast-biphenyl MSM (Fig. 1b) and only 0.09 OD in biphenyl as sole carbon source (Fig. 1c). Similar trend was observed for rest of the strains *i.e*., increase in growth until 72 h in control and cells continue to grow in biphenyl amended MSM treatments. All the strains showed <1.0 OD in control between 24–48 h that decrease to 0.5–0.6 OD with yeast biphenyl MSM and further decrease to 0.06–0.09 OD in MSM with only biphenyl within the same time period (Fig. 1). Bacterial cells were collected during the exponential phase of growth (120 h) as measured by maximum OD in yeast + biphenyl medium for all the experiments. The decreased optical density with biphenyl spiking clearly indicated its toxicity to bacterial strains, which would ultimately affect the degradation efficiency.

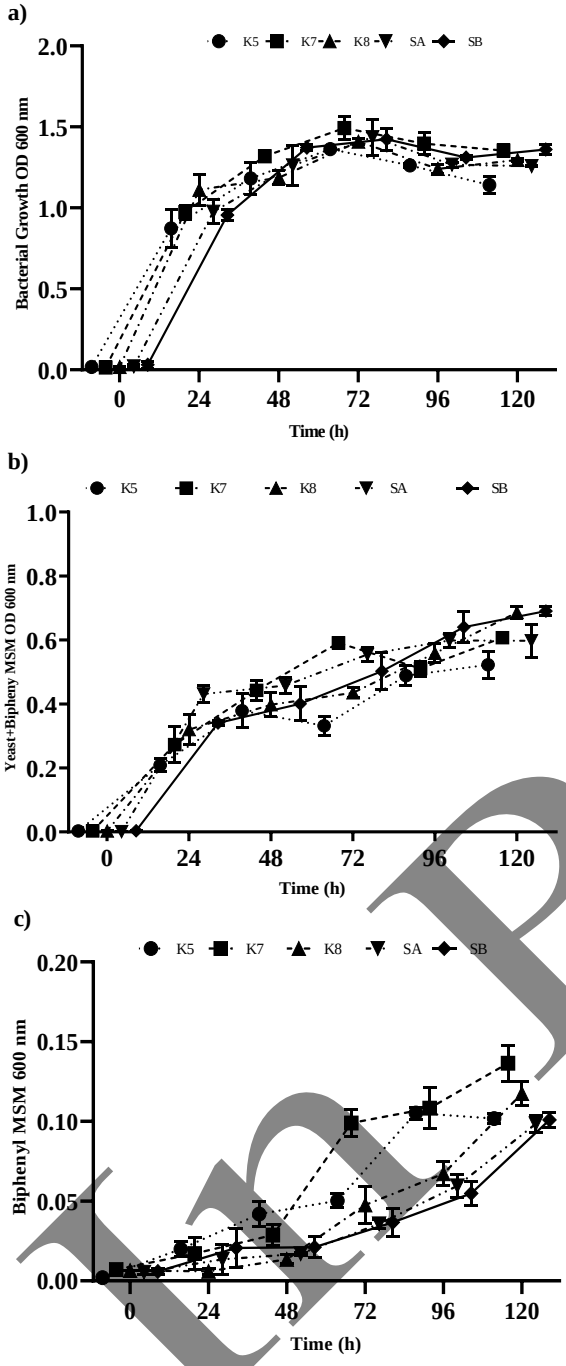

Fig. 1: Bacterial growth of selected strains (K5, K7, K8, SA, SB) in a) Yeast MSM, b) Yeast + biphenyl MSM and c) Biphenyl MSM, measuring the optical density (OD 600 nm) with a spectrophotometer

The *t-test* analysis showed significant difference among bacterial strains between the time intervals but the difference between mean was non-significant at each time point for all the strains as shown by regression analysis. Significantly higher growth pattern was shown by strains K7 and SB in all the three mediums as compared to strains K5 and SA whereas strain K8 also showed resilience to

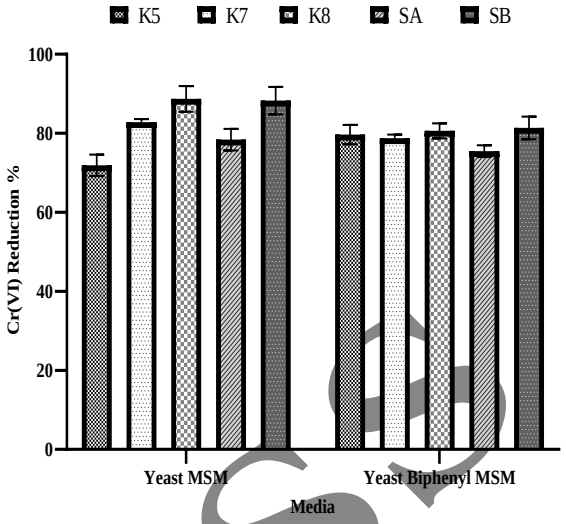

Fig. 2: Hexavalent chromium reduction by selected strains (K5, K7, K8, SA, SB) in Yeast MSM and Yeast + biphenyl MSM

adopt in changing conditions. Results of the study, however, suggested non-significant differences in the ability of strains for Cr(VI) to Cr(III) transformation in both yeast and yeast-biphenyl MSM (Fig. 2). Maximum Cr(VI) reduction was achieved in yeast MSM by strains K7 and SB (89% respectively) but it was not significantly different than yeast biphenyl MSM (81%) ($P > 0.05$). Strain K5 and SA showed non-significantly higher Cr(VI) reduction in yeast biphenyl MSM as compare to yeast MSM but it was not significantly different in both mediums. Strain K8, on the other hand, showed better growth as well as significantly higher Cr(VI) reduction.

Effect of pH and Temperature on Cr(VI) and 2-CB Biotransformation

Bacterial growth was assessed by MSM amendment to select the most suited growth medium in which both the contaminants (Cr(VI) and 2-CB) can be bio transformed simultaneously. The selected five strains were evaluated for pH, temperature as well as replacing biphenyl with two different carbon sources; glucose and starch. Results suggested maximum biotransformation of Cr(VI) and 2-CB degradation at pH7 by strain SB 85 and 81% and strain K8, 78 and 77% respectively (Fig. 3). Strains K7 showed 77% Cr(VI) reduction and 74% 2-CB degradation at pH7. Significant difference was observed among treatment from 6–9 pH levels in the Cr(VI) and 2-CB degradation ability of selected strains ($P < 0.001$). Maximum transformation of both Cr(VI) and 2-CB was observed at pH 7.

Effect of temperature was measured at 25, 30, 35 and 40°C for all the five strains. Strain SB showed highest degradation of 2-CB (93%) at 4 ppm concentration at 30°C temperature whereas 84% reduction of Cr(VI) was observed at 2 ppm concentration under same conditions.

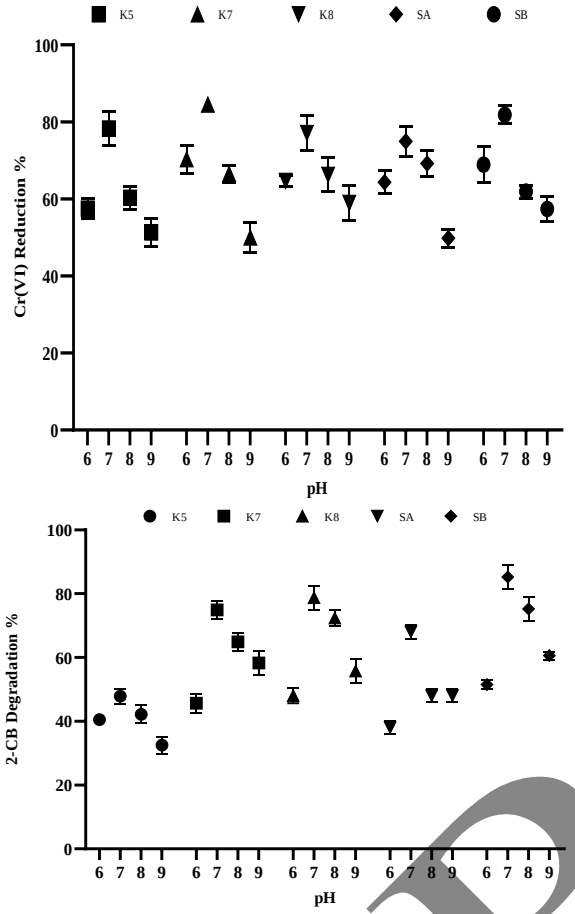

Fig. 3: Effect of pH (ranging from 6 to 9) on biotransformation of Cr(VI) and 2-chlorobiphneyl by selected bacterial strains (K5, K7, K8, SA, SB)

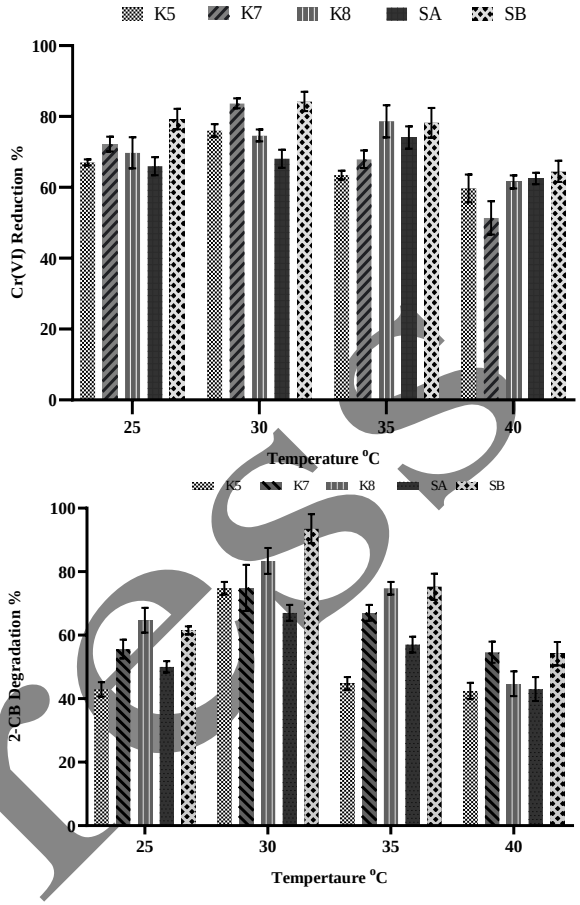

Fig. 4: Effect of temperature (25, 30, 35 and 40ºC) on biotransformation of Cr(VI) (above) and 2-CB (below) by selected strains (K5, K7, K8, SA, SB)

Strains K8 and K7 showed 83 and 74% Cr(VI) reduction with 83 and 74% 2-CB degradation at 30ºC temperature. Strains SA and K5 also showed significant degradation of both contaminants but their performance was not sustainable with variation in temperature. Non-significant difference was observed in the biotransformation ability of strains for both contaminants at temperature 25, 35 and 40ºC ($P > 0.05$) but relatively higher detoxification of both Cr(VI) and 2-CB was observed at 30ºC (Fig. 4).

Effect of Carbon Sources on Cr(VI) and 2-CB Degradation

Degradation of both Cr(VI) and 2-CB was also determined using different carbon sources in MSM medium. Amended MSM (10% yeast + 90% biphenyl) was further examined by replacing biphenyl with glucose and starch at same concentration of 200 mg L^{-1}. Results suggested higher degradation rate of the selected stains with yeast biphenyl amended MSM medium. Strain SB showed highest transformation of 80 and 85% of Cr(VI) and 2-CB at 2 and 4 ppm concentration respectively with yeast biphenyl medium which was significantly lower in case of glucose and starch amended medium. Similar results were observed with strain K7 and K8 (79% Cr(VI) and 80% 2-CB) (Fig. 5). Significantly higher biotransformation ability of strains was observed in yeast biphenyl MSM whereas significant difference among treatments was observed in biotransformation of both contaminants (2-CB, P=0.0003; Cr(VI), P= 0.0057). Cr(VI) reduction by strain SA and K5 were non-significant with change in carbon source. Whereas 2-CB degradation was significantly lower for both strains with use of starch and glucose.

Effect of Electron Shuttles on Cr(VI) and 2-CB Degradation

Selected strains were observed for Cr(VI) reduction ability using three different electron complexes *i.e.*, sodium benzoate, hydroquinone, and mannitol, at 1, 3 and 5 mmol L^{-1} concentration in yeast biphenyl medium. Result suggested non-significant difference in reduction ability of strains at 1 mmol L^{-1} for all three electron complexes. Significant decrease in reduction ability of strains was observed with sodium benzoate from 1 to 5 mmol L^{-1}.

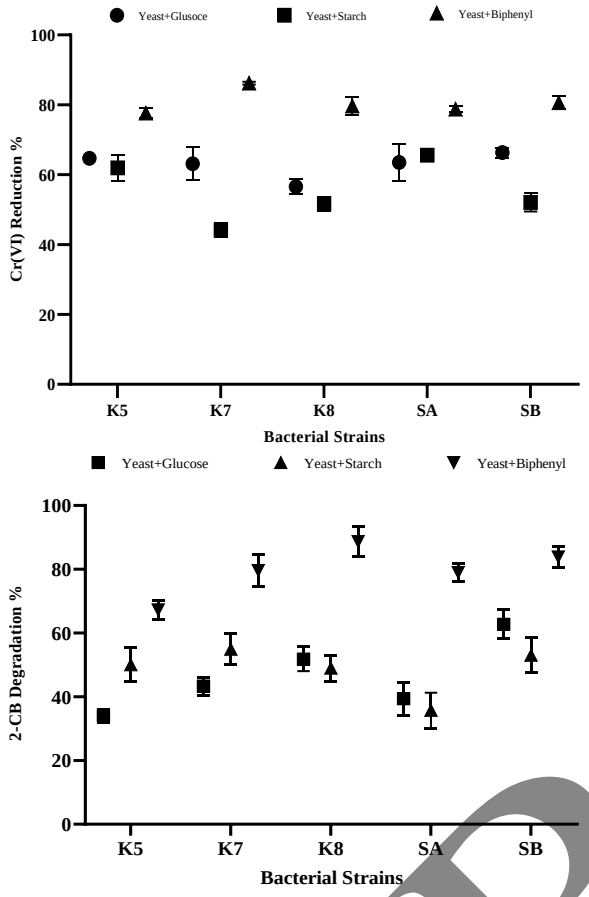

Fig. 5: Effect of different carbon sources on Cr(VI) (above) and 2-CB (below) biotransformation ability of selected strains (K5, K7, K8, SA, SB) under optimized conditions (pH 7 and 30ºC)

Strains SB showed significant difference in reduction at 1 mmol L^{-1} (85%) which was decreased to 53% at 5 mmol L^{-1} concentration of sodium benzoate. Similarly, strain K7 and K8 showed a 40% decrease in Cr(VI) reduction ability with increasing concentration of sodium benzoate from 1 to 5 mmol L^{-1}. Cr(VI) reduction ability of strains also decreased in presence of hydroquinone and mannitol as electron shuttles. Strain SB reduced 86% of Cr(VI) at 4 L^{-1} and 1 mmol L^{-1} concentration of hydroquinone whereas 85% reduction was observed with similar concentration of mannitol. Increasing the concentration of hydroquinone from 3 to 5 mmol L^{-1} showed 12 and 22% decrease in reduction ability of strains SB respectively. Strain K7 showed reduction of 81% at 1 mmol L^{-1} hydroquinone and 77% at 3 mmol L^{-1} whereas as 69% reduction in overall concentration of Cr(VI) was observed at 5 mmol L^{-1}. Strain K8 showed more resistant at 1 and 3 mmol L^{-1} hydroquinone concentration with 5% difference in Cr(VI) reduction ability whereas 75% Cr(VI) reduction was achieved at 5 mmol L^{-1} concentration. In case of mannitol, Cr(VI) reduction ability of strain K7 and K8 was non-significant at 1 and 3 mmol L^{-1} concentration which varied from 88% to 86% reduction in total concentration of Cr(VI).

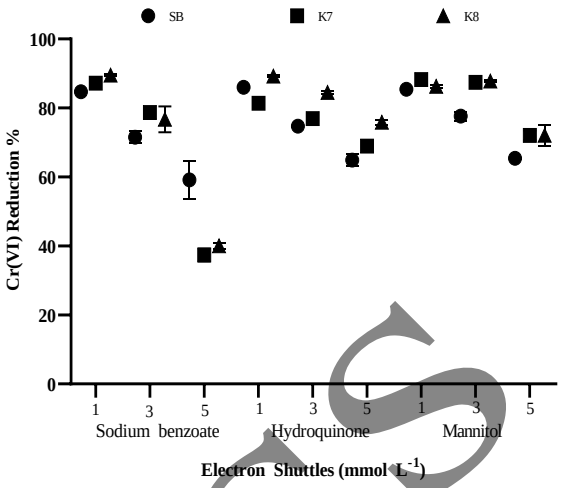

Fig. 6: Effect of electron shuttles on Cr(VI) reduction (4 mg L^{-1}) at 1, 3 and 5 mmol l^{-1} concentrations of sodium benzoate, hydroquinone and mannitol in yeast biphenyl MSM at pH7 and 30ºC under aerobic conditions

At 5 mmol L^{-1}, 76 and 72% Cr(VI) reduction was observed by both K7 and K8, respectively (Fig. 6). Strains showed more susceptibility to increasing concentration of sodium benzoate as electron shuttle and significantly higher resistance to hydroquinone and mannitol. Two-way analysis of the variables showed significant difference among treatments for Cr(VI) reduction ability of strains (P<0.05). Correlation among strains for the reduction of Cr(VI) also showed significant difference for all the three applied electron shuttles.

Role of electron shuttles on the biphenyl degradation ability of selected strains was observed using sodium benzoate, hydroquinone and mannitol at 1, 3 and 5 mmol L^{-1} concentration. Strain SB highest (82%) degradation of biphenyl applied as carbon source for co-metabolism at 200 and 1 mmol L^{-1} concentration of sodium benzoate whereas 85 and 72% degradation was observed with similar concentration of hydroquinone and mannitol, respectively. Strain K7 showed 87% degradation of biphenyl at 1 mmol L^{-1} of sodium benzoate which decreased to 74% at 3 mmol L^{-1} but remained at 71% at 5 mmol L^{-1} concentration showing non-significant effect with increasing concentration of sodium benzoate. For hydroquinone applied at 3 mmol L^{-1} concentration, K7 showed 71% biphenyl degradation which was non-significant to 70% at 5 mmol L^{-1}. Decrease in biphenyl degradation ability of K7 was observed at 1 mmol L^{-1} mannitol (76%) which further decrease to 69% at 3 mmol L^{-1} but less difference in degradation was observed at 5 mmol L^{-1} mannitol with 63% decrease in biphenyl concentration in medium. Strain K8 showed similar trends in biphenyl degradation with all the three electron shuttles applied at 1, 3 and 5 mmol L^{-1} concentration. Maximum degradation was observed at 1 mmol L^{-1} hydroquinone in yeast biphenyl MSM (88%) whereas 85 and 78% biphenyl degradation were observed at similar concentration of

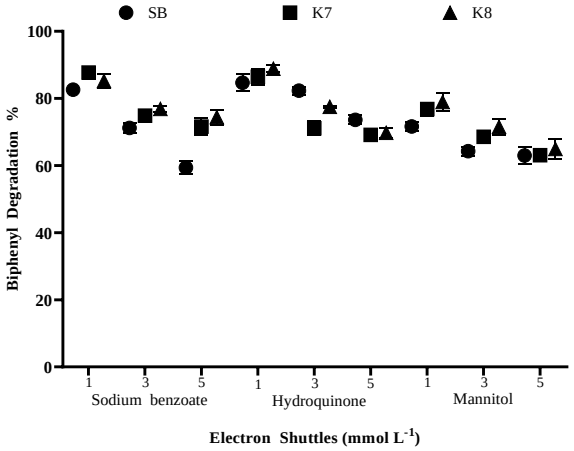

Fig. 7: Effect of electron shuttles on biphenyl degradation (200 mg L^{-1}) at 1, 3 and 5 mmol l^{-1} concentration of sodium benzoate, hydroquinone and mannitol in yeast biphenyl MSM at pH7 and 30°C under aerobic conditions

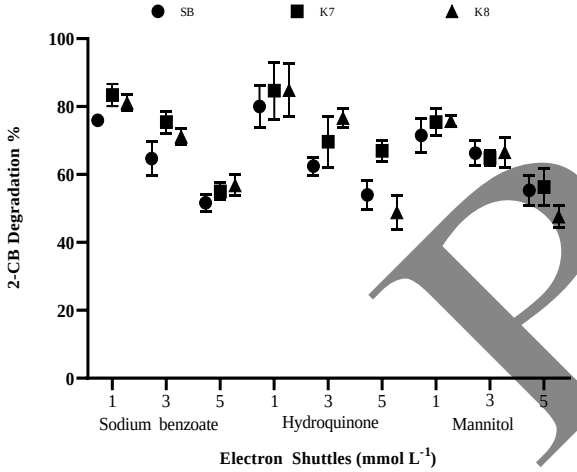

Fig. 8: Effect of electron shuttles on 2-chlorobiphenyl degradation (4 mg L^{-1}) at 1, 3 and 5 mmol L^{-1} concentration of sodium benzoate, hydroquinone and mannitol in yeast biphenyl MSM at pH7 and 30°C under aerobic conditions

sodium benzoate and mannitol respectively. At higher concentration of 3 mmol L^{-1}, SB showed decrease in biphenyl degradation ability with 76, 78 and 72% degradation observed for sodium benzoate, hydroquinone and mannitol. Similar trend was followed at 5 mmol L^{-1} concentration of all three electron shuttles with biphenyl degradation varying from 75 to 65% by strain K8 (Fig. 7). Result suggested that increase in concentration of electron shuttles significantly decreased the degradation ability of selected strains. Strain SB showed 75% degradation of 2-CB at 1 mmol L^{-1} sodium benzoate concentration whereas strains K7 and K8 showed 84 and 81% decrease under similar conditions respectively. With increase in concentration to 3 mmol L^{-1}, degradation of 2-CB decreased to 65% for strain SB, 76% for K7 and 71% for K8. Similarly, at 5 mmol L^{-1} sodium benzoate degradation significantly decrease and varied from 52 to 56% for all the three strains. Two-way analysis of the variables showed significant difference among treatments for biphenyl degradation ability of strains (P<0.05).

At 1 mmol L^{-1} of hydroquinone, SB showed 80% degradation of 2-CB at 4 mg L^{-1} concentration whereas K7 and K8 showed 84% degradation under similar conditions. Increasing the concentration from 3 to 5 mmol L^{-1} significantly decreased the degradation ability of all strains. 2-CB degradation as low as 54% by SB, 67% by K7 and 49% by K8 was observed at 5 mmol L^{-1} concentration of hydroquinone. Significant difference was observed in degradation ability of strains was observed with higher concentrations suggesting the inhibitory effect of hydroquinone on selected strains. Similar trends were observed in case of mannitol as electron shuttles at 1, 3 and 5 mmol L^{-1} concentration. Mannitol even at 1 mmol L^{-1} concentration decreased the degradation ability of all the three strains as compared to sodium benzoate and hydroquinone. The decrease in degradation percentage of 2-CB continued for 3and 5 mmol L^{-1} of mannitol with SB showing 66% and 55% degradation, K7 with 64 and 56% detoxification and K8 with 67 and 48% decrease in total applied concentration of 2-CB in yeast biphenyl MSM respectively (Fig. 8). Results suggested that mannitol as electron shuttle has more inhibitory effect on degradation ability of strains for 2-CB at 4 mg L^{-1} concentration with hydroquinone as second most ineffective electron shuttle for chlorinated biphenyls. Two-way analysis of the variables showed significant difference among treatments for 2-chlorobiphneyl degradation ability of strains (P<0.05). Correlation among strains for biphenyl and 2-CB degradation also showed significant difference for all the three applied electron shuttles.

Discussion

The study was focused on the optimization of previously isolated strains, capable of Cr(VI) reduction, for accelerated degradation of 2-chlorobiphenyl in simulated water. MSM medium was amended to asses bacterial growth under yeast biphenyl MSM and biphenyl as sole carbon source. Results of the study suggested that bacteria strains utilized readily available carbon source (yeast) and then continue to grow even in the amended yeast biphenyl MSM. Cr(VI) reduction ability of strains was not affected by the bacterial growth in yeast and yeast biphenyl MSM but non-significant growth was observed in only biphenyl medium. Also, bacterial growth can be stimulated by the amount of chromate present at the metabolic site but overall the genes involved in the metabolism of chromate are important (Zhou *et al.*, 2012). Toxic effects of chromate inhibit the reducing abilities of bacterial strains as well as damage the bacterial cell membranes which can be coped with the increased number of cells present at the site of action (Viti *et al.*, 2014).

Natural habitats have large amounts of toxic and non-toxic metals which can affect the bacterial cell numbers due to toxicity. So, study related to the effect of metallic ions on the bacterial growth is necessary before adopting any bioremediation strategy (Verma *et al.*, 2009).

The pH is a significant factor that influence the degradation activity of microorganisms and neutral pH conditions were most favorable for biodegradation of chlorinated aromatic compounds and petroleum hydrocarbons by bacterial species (Bidlan and Manonmani, 2002; Al-Hawash *et al.*, 2018). Temperature also has substantial effect on microbial growth and enzymatic activity for breakdown of aromatic compounds (Simcik *et al.*, 1999). The importance of conducting studies at varying temperatures can never be neglected since temperature influences the microbial growth, enzymatic activities as well as bioavailability of PCBs (Wiegel and Wu, 2000). Fluctuation in day and night temperature can affect different microbes under natural conditions, then those studied under controlled conditions. Both cold and high temperatures affect the bioremediation of PCBs under different environments (Robinson and Lenn, 1994; Weiland-Bräuer *et al.*, 2017). A pH range of 6.0–8.5 was optimal for maximum reduction of Cr(VI) by *Enterobacter cloacae* and *Escherichia coli* were in contrast, *B. coagulans* worked well at variable pH range of 3.0–8.0. However, at pH 7.0, the maximum initial rate of Cr(VI) reduction was shown by all bacterial cultures with 30 to 36ºC optimal temperature (Marsh *et al.*, 2000). Similar findings were observed in this study as maximum degradation rate of both Cr(VI) and 2-CB was observed at 30ºC and pH 7 by all the selected strains. Temperature affects the bacterial population available for bioremediation that has direct impact on the metabolic processes in the system.

Carbon substrates can affect the microorganisms directly or indirectly which in turn improves the living condition for other microbes. This improvement among other microbial communities may supply the dechlorinating bacteria with more suitable electron donors and nutrients (Maphosa *et al.*, 2012). Co-metabolism is the major condition for PCB degradation mostly, as soil microbes cannot use it as growth substrate. Under anaerobic conditions however, higher chlorinated PCBs act as electron acceptors as they are highly oxidized and undergo reductive dechlorination (Vasilyeva and Strijakova, 2007). Acetate, propionate, butyrate and hexanoic acid have been shown to be available in nutrient limited organic soils, whereas glucose, acetate, methanol etc. are mostly available in organic rich soils (Wiegel and Wu, 2000). Studies have shown that biphenyl in addition to serving as an enrichment substrate can also be a co-metabolite that can enhance the rate of dechlorination (Vergani *et al.*, 2017). Results of the study suggested that Cr(VI) reducing strains grow efficiently when MSM media was amended with biphenyl @ 200 mg L^{-1} concentration in combination with 500 mg L^{-1} yeast.

Selected strains *i.e.*, K7, K8 and SB, were able to efficiently degrade both biphenyl and 2-chlorobiphny as well in amended MSM which strengthen the idea that under aerobic conditions, lower chlorinated compounds are detoxified along with breakdown of biphenyl structure (Garrido-Sanz *et al.*, 2018). Degradation of biphenyl is one characteristic shared by majority of aerobic PCB degraders. Therefore, addition of biphenyl to a mixed microbial consortium or natural sample could help enrich for PCB degraders (Abraham, 2002). There are many studies on microbial reduction of Cr(VI) but very few studies have been conducted on the effect of carbon sources on the microbial community influencing Cr(VI) reduction (Desai *et al.*, 2008). The activity of microorganisms for degradation of pollutants depended upon the amount in which they are present at contaminated site. A sufficient amount of PCB is essential to activate the bacteria for metabolic and enzymatic breakdown of the contaminant (Vasilyeva and Strijakova, 2007). Presence of toxic substance enhance the production of extracellular polymerase substance among microbial communities. This helps them in defending against the toxic effects and tolerate higher concentrations of Cr(VI) (Liu *et al.*, 2017). Glucose, acetate and lactate are the common electron donors during dehalogenation process (Lee *et al.*, 2007).

Role of metal ions in microbial activity inhibition including dehalogenation and reductive dechlorination is predominant factor in adopting to a bioremediation strategy. However, the role of metal ions on microbial degradation of organic contaminants has not clearly been studied. The only studies present till date are related to organic contaminants degradation in presence of metal ions (Sandrin and Maier, 2003). A PCB degrading and metal tolerant specie, *P. pseudoalcaligenes* KF707 can effectively detoxify both under optimized conditions even the toxicity level is high (Tremaroli *et al.*, 2010). For effective dehalogenation, different studies have discussed the critical role of dehalorespiration and dissimilatory iron reduction (Li *et al.*, 2008). In dehalorespiration, halogen-free compounds and halogenated congeners are accumulated as halogenated compounds and play the role of electron acceptors (Hiraishi, 2008). Results of the study also suggested that similar type microbes were able to degrade both metallic and organic compounds under same environmental conditions which is helpful in adopting to an effective bioremediation strategy for treatment of multi-contaminated sites. Electron shuttles play a vital role in Cr(VI) reducing activities of microbes. Cr(VI) act as electron acceptor under anaerobic conditions for large number of electron donors which includes fats, hydrogen, carbohydrates, and proteins (Joutey *et al.*, 2015). Presence of metal ions in tannery effluents, due to extensive manufacturing processes and involvement of different chemicals, can affect the treatment processes negatively (Tariq *et al.*, 2006; Shah, 2014). The interference of trace metals, with the proteins or enzymes involved in the redox reaction, from strong complex with the protein molecules

and helps in reduction or completes detoxification of pollutant by deactivating the enzyme activity (Jadhav *et al.*, 2012). Trace metals, under anaerobic conditions, act as electron acceptor, but they are not soluble at neutral pH thus affecting the transfer of electron needed for bacterial growth. Organic compounds on the other hand can act as electron shuttling compounds and fast-track the transfer of electrons from a primary donor to the acceptor. Application of electron shuttles *i.e.*, sodium benzoate, hydroquinone and mannitol, at three different concentration suggested that the microbial degradation process was affected by the addition of electron shuttling agent at higher concentration of but non-significant difference in the biodegradation ability of the selected strains was observed.

Conclusion

Higher amounts of mixed pollutants containing metallic and organic compounds is one of the major hurdles for development of bioremediation strategies. So, this study was designed to optimize Cr(VI) reducing strains for accelerated degradation of biphenyl and 2-chlorobiphenyl to develop a bioremediation strategy for multi pollutant sites. Selected strains, capable of Cr(VI) reduction, degraded both biphenyl and 2-chlorobiphneyl in amended MSM but their degradation ability significantly decreased when biphenyl was replaced by glucose and starch as carbon sources. Strains were also optimized for pH and temperature at different levels and results suggested the maximum degradation at 30ºC and pH 7 in single medium by selected strains. Sodium benzoate enhanced the degradation ability of strains at higher concentration whereas hydroquinone and mannitol showed non-significant difference to control. The results of the study suggested that metal reducing bacterial strains could metabolize organic compounds under aerobic conditions and degradation process can be accelerated with optimization of environmental factors.

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