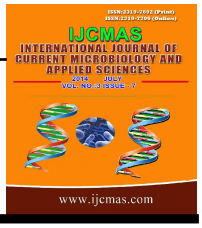
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 7 (2014) pp. 411-419**
http://www.ijcmas.com



Original Research Article

Effect of some metals on growth of *Pseudomonas aeruginosa* ARSKS20 and its decolorization ability of Reactive red 35

R.K. Soni[1], P.B.Acharya[1], and H.A.Modi[2]*

[1]P.G. Department of Microbiology, Gujarat Vidyapeeth, Sadra-382 320, Gujarat, India
[2]Department of Life Sciences, School of Sciences, Gujarat University,
Ahmadabad-380 009, Gujarat, India
**Corresponding author*

Keywords

Pseudomonas aeruginosa ARSKS20, Reactive red 35, metals, decolorization

A B S T R A C T

One of the major problem associated with textile processing effluents are the presence of heavy metals ions, which make it difficult for biological treatment. In present study effect of chromium, nickel, cobalt and copper were assessed on growth of *Pseudomonas aeruginosa* ARSKS20 and its decolorization ability of Reactive red 35 was studied in nutrient broth medium under static condition at 35°C. Growth of *Pseudomonas aeruginosa* ARSKS20 and decolorization of Reactive red 35 were adversely affected at 1.9 mM of Cr(VI), 7.5 mM of Ni(II), 6.5 mM of Co(II) and 0.5 mM of Cu(II). Thus, toxic effect was observed in Cu(II) > Cr(VI) > Co(II) > Ni(II) order.

Introduction

Modernization, industrialization, population growth and its demand increased the consumption of raw materials and production of finished goods, resulting the dumping of waste to environment. These activities result in the release of the recalcitrant and toxic compound into the environment.

Heavy metals are major toxicants found in industrial wastewater and may adversely affect the biological treatment of wastewater. Unlike other pollutants heavy metals due to their recalcitrant in nature, tend to accumulate in environmental niche and enter the food chain posing a serious threat to for all type of life (Laxman and Rao, 2001).

Metals affect the growth, morphology and metabolism of microorganisms present in soil and biological waste water treatment. Increasing metal concentration inhibit growth completely or decreased growth rate and increased lag time or may affect metabolism and thus the growth of microorganisms is reduced ultimately affect biodegradation processes (Sterritt and Lester, 1980; Kathiravan et al., 2010). The biodegradation of organic compound can be inhibited by metal toxicity in aerobic and anaerobic system. Influence of metals like Hg, Cd, Cu, Cr, Ni, Pb or Zn shows most adverse effects on biodecomposition activities (Hattori, 1992; Sandrin and Maier, 2003).

One of the major problem associated with textile processing effluents are the presence of heavy metals ions, which may arise from material used in the dying process or from metal containing dyes. In textile effluent different metals like Cu(II), Cd(II), Zn(II), Fe(II), Cr(III), Cr(VI), Mn(II), Ni(II), Pb(II) are found to be present at different concentration varies from 0.28 - 6.36 mg l^{-1} (Ali et al., 2009).

Biodegradation of dyes is mostly performed by enzymes present in microbial cells. Metals ions exert their toxicity in number of ways, including inhibition of enzyme functioning by displacement of essential metals from their normal binding site on biological molecules, altering the active site confirmation or denature protein which directly leads to the inhibition of enzymes, metals may destruct the integrity of bacterial cell membrane (Kurade et al., 2011; Anyanwu et al., 2011, Zhao et al., 2014). In presence of Mg(II), Ca(II), Zn(II), Cd(II) activities of lignin peroxidase, laccase, tyrosinase were inhibited resulting decolorization of Reactive blue HERD by *Comamonas* sp. UVS was completely inhibited (Jadhav et al., 2011). Nagai et al (2002) reported that Zn(II) and Ca(II) have significant negative effect on laccase activity in dye degrading bacteria.

Most of the heavy metal salts are soluble in water and form aqueous solutions and consequently cannot be separated by ordinary physical means of separation (Hussein et al., 2004). The most important methods for minimizing heavy metal toxicity are precipitation, sorption and chelation by organic and inorganic ligands before it exposed to biological treatment (Oleszkiewicz and Sharma, 1990).

The aim of this research was to determine the toxic effects of metals like Cr (VI), Ni (II), Cu (II) and CO (II) on growth and decolorizing ability of *P. aeruginosa* ARSKS20. The effects of metals on growth were monitored by assessing optical density and decolorizing ability was evaluated by measuring the efficiency of bacteria to decolorize Reactive red 35, a mono azo vinyl sulfon textile dye.

Materials and Methods

Dyes and chemicals

The azo dye used in this study, Reactive red 35 was obtained from local dye manufacturing market of Vatva, Ahmedabad (India). The chemical structure of this dye is shown in fig. 1. All the other chemicals and media were of highest purity and of an analytical grade from Sisco Research Laboratory, Himedia and Merck, India.

Bacteria and culture conditions

P. aeruginosa ARSKS20 (Accession no. JN817386) isolated from soil contaminated with effluent discharge of textile processing and dye manufacturing industries was used as test organism. *P. aeruginosa* ARSKS20 was grown at 35°C under static condition and maintained on nutrient agar at 4°C.

Effect of metals on growth and decolorization of Reactive red 35

Effect of metals on growth was studied by addition of 5% (v/v) active culture (O.D. 1.0 at 620 nm) of *P. aeruginosa* ARSKS20 in 250 ml conical flask containing 100 ml nutrient broth supplemented with Ni(II), CO(II) at concentration of 0.5 - 7.5 mM; Cr(VI) at 0.1 - 1.9 mM concentration and Cu(II) at 0.1 - 0.5 mM concentration at 35°C under static condition.

Growth of *P. aeruginosa* ARSKS20 was assessed by withdrawing 3.0 ml of culture broth at regular time interval and optical

density was measured at 620 nm with UV-VIS Spectrophotometer (ELICO UV-VIS Spectrophotometer 169, India).

Dye decolorization experiments were also conducted at same metal concentration with 100 mg l^{-1} of Reactive red 35 in nutrient broth at 35°C under static condition.
Controls were also kept with/without dye and metal in nutrient broth. All experiments were performed in triplicate at 35°C under static condition.

Decolorization assay

Decolorization of Reactive red 35 was checked by withdrawing 3.0 ml of broth at regular time interval and centrifuged (REMI R-24) at 6000 rpm for 15 min. Absorbance of the obtained supernatant was measured at 512 nm. Percentage decolorization has been calculated as mentioned by Modi et al (2010).

$$\text{Decolorization } (\%) = \frac{I - F}{I} \times 100$$

Where,
I = Initial absorbance
F = Absorbance of decolorized sample.

Results and Discussion

Effect of chromium on growth and dye decolorization

Effect of varying concentration of chromium on growth of *P. aeruginosa* is shown in figure 2(a). It is evident from figure that the growth of *P. aeruginosa* was decreased as Cr(VI) concentration was increased in the medium.

After 12 h of incubation the optical density of *P. aeruginosa* was recorded as 0.440, 0.425, 0.423, 0.413, 0.412, 0.407, 0.322, 0.303, 0.268 and 0.261 at 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, 1.7, 1.9 mM concentration of chromium, respectively. Whereas after 24 h of incubation the optical density were recorded as 1.110, 1.009, 0.987, 0.941, 0.937, 0.933, 0.904, 0.887, 0.874 and 0.827 at same level of chromium.

Anyanwu and Ezaka (2011) observed the inhibition of growth of *Pseudomonas* sp. at chromium concentration of 200 µg ml^{-1} and higher.

After 12 h, 74.51, 72.20, 5.12% dye decolorization in compare to control was found at 0.1, 0.3 and 0.5 mM of chromium, respectively (fig. 2b). No remarkable decolorization was observed at 0.7 mM and higher concentration of chromium after 12h of incubation. 96.13, 95.10, 94.03,91.60, 91.97, 74.68, 61.66, 17.34, 16.90, 6.29% of dye decolorization was recorded after 24 h of incubation at 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, 1.7, 1.9 mM of chromium in compare to control, respectively.

From the results it was observed that at lower concentration (0.1mM) of chromium, the growth of *Pseudomonas aeruginosa* ARSKS20 was not affected. This result point out the fact that *Pseudomonas* sp. possess an enzyme known as chromium reductase which reduced Cr(VI) to Cr(III) as reported by Kathiravan et al (2010).

Pourbabaee et al (2011) observed that the growth of *Halomonas* sp. (strain IP8) was inhibited at 0.5 mM of chromium and bacteria were failed to decolorize Cibacron black W-55. Decreased decolorization of methyl red by *Bacillus* sp. (strain UN2) was also observed by Zhao et al (2014).

Effect of nickel on growth and dye decolorization

Fig. 3(a) shows the effect of various concentration of nickel on growth of *P.*

aeruginosa. After 12h, 0.500, 0.400, 0.310, 0.250, 0.180, 0.147, 0.147, 0.068 O.D. of *P. aeruginosa* were observed at 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 mM, nickel respectively. Whereas 1.63, 1.33, 1.000, 0.720, 0.570, 0.480, 0.239, 0.150, 0.071 O.D. was observed at 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 mM nickel respectively, after 24 h. No growth was observed at 7.5 mM of nickel. Thus more than 50% growth was inhibited at 3.5mM and higher concentration of nickel.

Anyanwu et al (2011) reported the total heterotrophic count was decreased with increasing concentration of Nickel from 50 to 500ppm Nickel.

Decolorization of reactive red 35 was 89.57, 82.91, 82.44, 79.95, 59.82, 21.89, 23.04% observed at 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 mM, nickel respectively, after 12 h (fig. 3b). After 24 h 90.22, 88.39, 87.98, 83.03, 82.94, 45.84, 25.77% decolorization was observed at 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 mM nickel respectively. Decolorization of reactive red 35 was completely inhibited at 7.5 mM of nickel.

Seesuriyachan et al (2007) reported that Ni(II) at 1 mM concentration had significant negative influence on degradation rate of methyl red as it affect the cell viability and inhibit the degradation of azo dye by inhibiting the enzyme activity by denaturing enzyme.

Effect of cobalt on growth and dye decolorization

Media components and physiological conditions of both the original and the new growth medium are the important factors influence the variation in duration of lag phase. Effect of varying concentration of cobalt on growth of *P. aeruginosa* is shown in fig. 4(a). It is evident from results that due to the presence of CO(II) in growth medium above 3.5 mM concentration the lag phase of *P. aeruginosa* was extended resulting the increase in time required for dye decolorization.

After 12 h, 0.242, 0.258, 0.167, 0.097, 0.095, 0.085 and 0.078 O.D. of *P. aeruginosa* was found at 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 mM cobalt, respectively. After 24 h, 1.36, 1.051, 0.638, 0.309, 0.236, 0.117, 0.09 O.D. of *P. aeruginosa* was found at same metal concentration. Higher toxic effect of cobalt was observed from 4.5 mM of cobalt and growth was nearly inhibited at 6.5 mM. Dye decolorization was markedly declined at 4.5 mM cobalt. Only 19% and 32% dye decolorization was found after 12 and 24 h at 4.5 mM of cobalt (fig. 4b). Whereas at 7% dye decolorization was observed at 5.5 mM cobalt after 24 h and no decolorization was observed at higher concentration of cobalt.

Decolorization of Amaranthand Remazol orange by *Pseudomonas aeruginosa* BCH was strongly affected at 5 mM cobalt (Jadhav et al., 2013). Only 20% decolorization of Reactive orange 16 by *Comamonas acidovorans* was reported at 0.05 g l^{-1} of cobalt chloride (Rudukiya and Pawar, 2013).

Effect of copper on growth and dye decolorization

In present study major toxicity was found with copper. 0.415, 0.336, 0.237 O.D. was observed at 0.1, 0.3, and 0.5 mM copper after 12 h of incubation (fig.5a). While after 24 h of incubation only 0.55, 0.462, 0.28 O.D. of *P. aeruginosa* were recorded at same concentration of copper. Toxicity of copper was also influenced on decolorization of Reactive red 35 by *P.*

aeruginosa. Only 77.30, 15.26, 1.27% and 80.58, 16.93, 7.84% dye decolorization were recorded (fig. 5b) after 12 and 24 h of incubation at 0.1, 0.3, and 0.5 mM of copper, respectively.

Hattori (1992) also reported that CFU was markedly reduced at 1µM Cu(II) g^{-1} soil and so the soil microbial activity was also declined. Microbial activity was strongly affected by presence of copper during activated sludge processes (Coello oviedo et al., 2002). Growth of *Pseudomonas* sp. ACT1 strain was depicted severely at even lower concentration of Cu(II). The lag phase reached infinite and specific growth rate surged into zero at a Cu(II) ion concentration of 0.2 g l^{-1} (Gopinath et al., 2011).

In present study Cu(II) ions was found to be most toxic to the *Pseudomonas aeruginosa* ARSKS20. Cu(II) ions affect the transcription mechanism in cell which in turn affect the cell division and ultimately affect on its activities. Many researchers were concluded copper as more toxic than other metals. Gopinath et al (2011) observed that in presence of 0.15 g l^{-1} of Cu(II) the time required for decolorization of Congo red by *Pseudomonas* sp. ACT1 was increased from 27 h to 87 h. Specific decolorization rate of Reactive orange 16 by *Bacillus* sp. ADR was decreased in presence of $CuSO_4$ (Telke et al., 2009). Decolorization of Cibacron black W 55 by *Halomonas* sp. strain IP8 was only 17.525% at 0.1 mM $CuSO_4$ while completely inhibited at 0.5 mM (Pourbabaee et al., 2011). Kurade et al (2011) reported the presence of $CuSO_4$ strongly diminished the decolorization efficiency of mixture of dyes by *B. laterosporus*.

Fig.1 Chemical structure of Reactive red 35

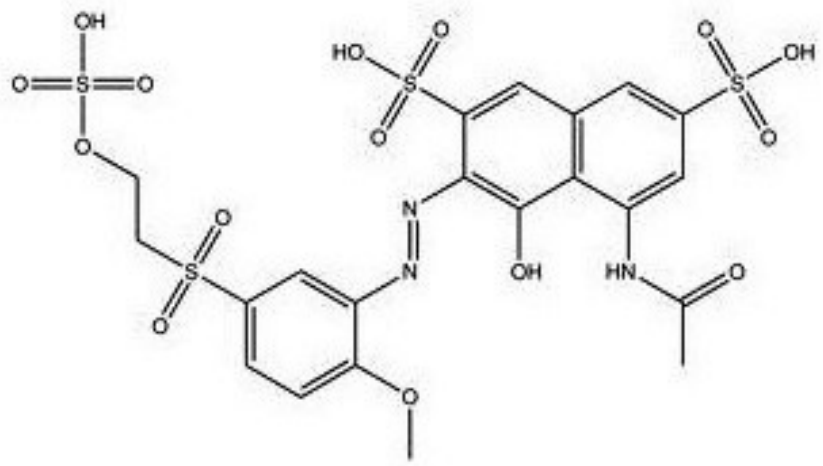
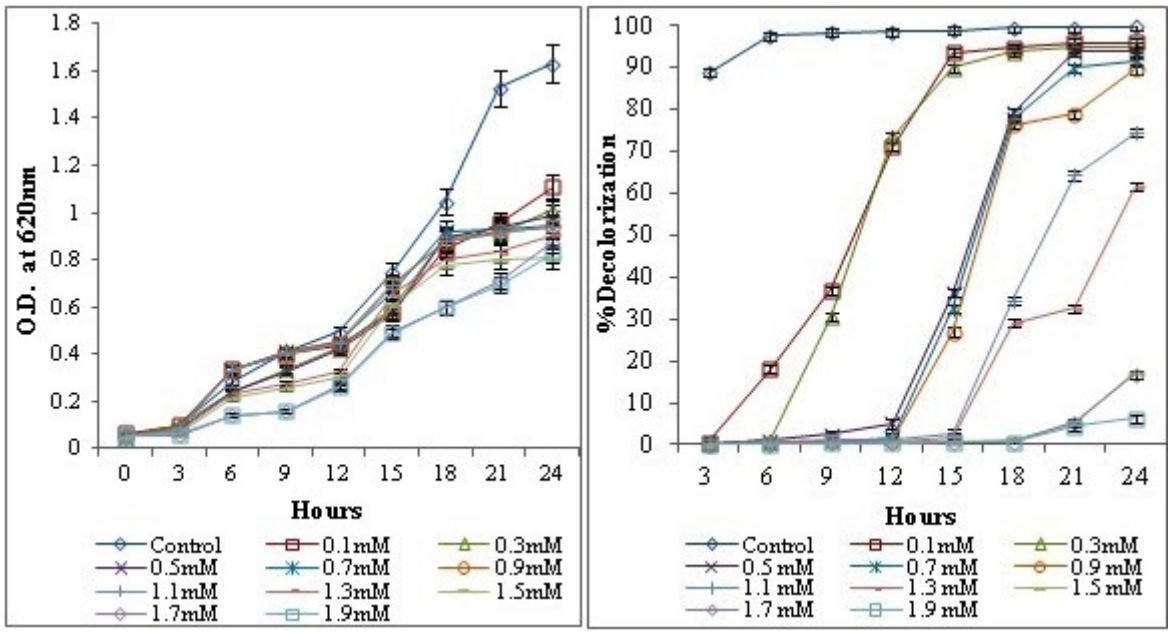
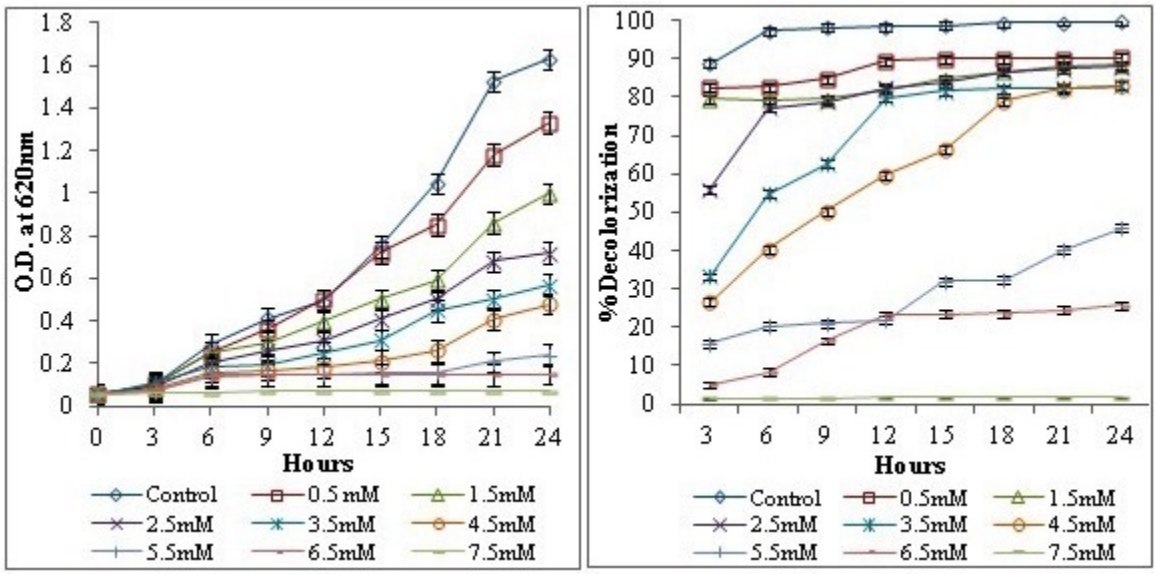


Fig. 2 Effect of chromium on growth of *P. aeruginosa* (a) and % decolorization of Reactive red 35 (b)



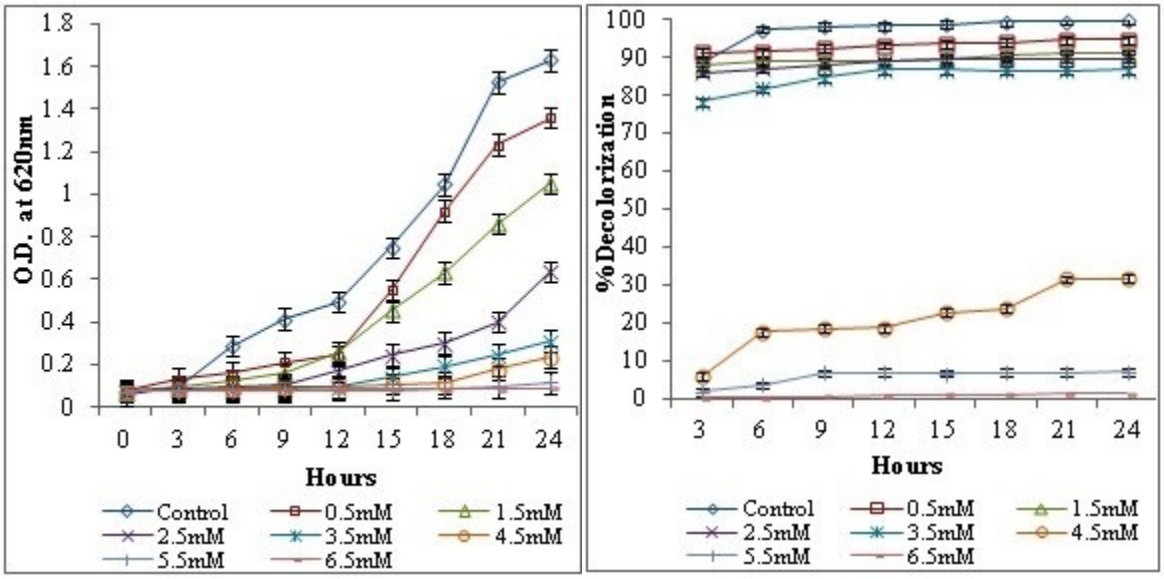
(a) (b)

Fig. 3 Effect of nickel on growth of *P. aeruginosa* (a) and % decolorization of Reactive red 35 (b)



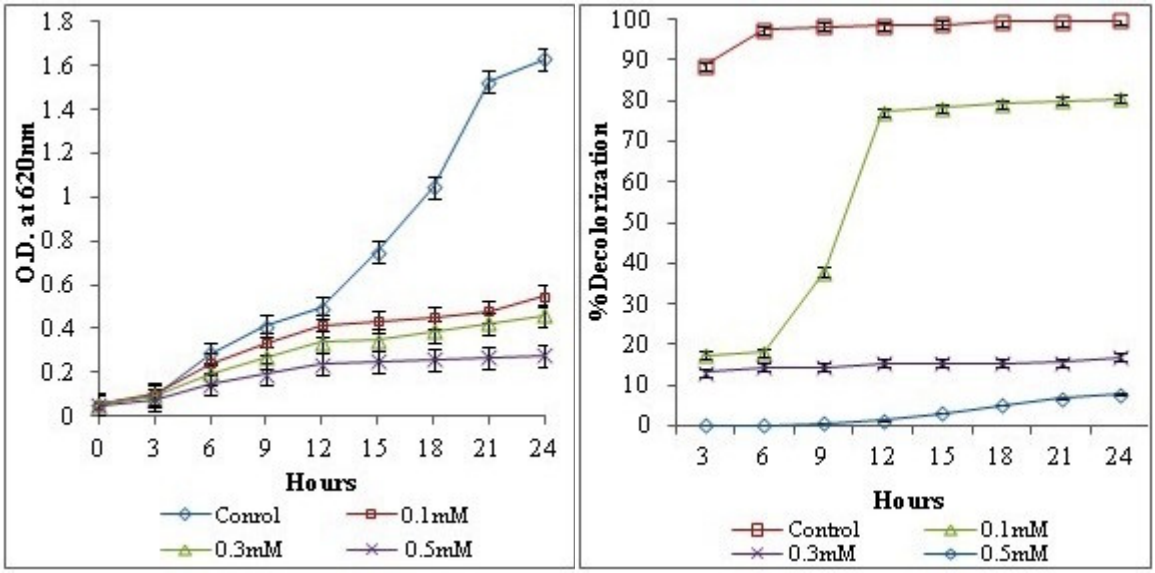
(a) (b)

Fig. 4 Effect of cobalt on growth of *P. aeruginosa* (a) and % decolorization of Reactive red 35 (b)



(a) (b)

Fig. 5 Effect of copper on growth of *P. aeruginosa* (a) and % decolorization of Reactive red 35 (b)



(a) (b)

In present study effect of metals on decolorization of Reactive red 35 was assessed along with growth of *Pseudomonas aeruginosa* ARSKS20. Experimental results revealed that presence of metals at certain level retard the growth of test organism and reduced their ability to decolorize Reactive red 35. Growth and dye decolorization ability of *Pseudomonas aeruginosa* ARSKS20 were adversely affected at 1.9 mM of Cr(VI), 7.5 mM of Ni(II), 6.5 mM of Co(II), 0.5 mM of Cu(II).

References

Ali, N., Hammed, A., and Ahmed, S. 2009. Physicochemical characterization and Bioremediation perspective of textile effluent, dyes and metals by indigenous bacteria. Journal of Hazardous Materials. 164: 322-328.

Anyanwu, C. U., Nwankwo, S. C., and Moneke, A. N. 2011. Soil bacterial response to introduced metal stress. International Journal of Basic & Applied Science. 11(1): 73-76.

Anyanwu, C. U., and Ezaka, E. 2011. Growth response of chromium (VI) tolerant bacteria to different concentrations of chromium. International Journal of Basic & Applied Science. 11(5): 60-66.

Coello oviedo, M. D., Sales Marquez, D., and Quirora Alonso, J. M. 2002. Toxic effects of metals on microbial activity in the activated sludge process. Chemical Biochemical Engineering Q. 16(3): 139-144.

Gopinath, K. P., Kathiravan, M. N., Srinivasan, R., and Sankaranarayanan, S. 2011. Evaluation and elimination of inhibitory effects of salts and heavy metal ions on biodegradation of Congo red by *Pseudomonas* sp. Mutant. Bioresource Technology. 102: 3687-3693.

Hattori, H., 1992. Influence of Heavy metals on Soil Microbial activities. Soil Science Plant Nutrition. 38(1): 93-100.

Hussein, H., Ibrahim, S. F., Kandeel, K., and Moawad, H. 2004. Biosorption of heavy metals from waste water using *Pseudomonas* sp. Electronic Journal of Biotechnology. 7(1): 38-46. http://www.scielo.cl/pdf/ejb/v7n1/a06.pdf

Jadhav, U. U., Dawkar, V. V., Kagalkar, A. N., and Govinwar, S. P. 2011. Effect of Metals on Decolorization of Reactive Blue HERD by *Comamonas* sp. UVS. Water Air Soil Pollution. 216: 621-631.

Jadhav, S. B., Patil, N. S., Watharkar, A. D., Apine, O. A., and Jadhav, J.P. 2013. Batch and continuous biodegradation of Amaranth in plain distilled water by *P. aeruginosa* BCH and toxicological scrutiny using oxidative stress studies. Environmental Science Pollution Research. 20: 2854-2866.

Kathiravan, M. N., Karthiga Rani, R., Karthick, R., and Muthukumar, K. 2010. Mass transfer studies on the reduction of Cr(VI) using calcium alginate immobilized *Bacillus* sp. In packed bed reactor. Bioresource Technology. 101(3): 853-858.

Kurade, M. B., Waghmode, T. B., and Govindwar, S.P. 2011. Preferential biodegradation of structurally dissimilar dyes from a mixture by *Brevibacillus laterosprus*. Journal of Hazardous Materials. 192: 1746-1755.

Laxman, L. S., and Rao, B. S. 2001. Biosorption/ Bioremediation of heavy metals. In: Role of Microbes in the management of environmental pollution. APH Publishng

Corporation, New Delhi.

Modi H. A., Rajput G., and Ambasana C. 2010. Decolorization of water soluble azo dyes by cultures, isolated from dye house effluent. Bioresource Technology. 101: 6580-6583.

Nagai, M., Salto, T., Watanabe, H., Saito, K., Kawata, M., and Enei, H. 2002. Purification and characterization of an extracellular laccase from the edible mushroom *Lentinula edodes*, and decolorization of chemically different dyes. Applied Microbiology and Biotechnology. 60: 327-335.

Oleszkiewicz, J. A., and Sharma, V. K. 1990. Stimulation and inhibition of anaerobic processes by heavy metals – A review. Biological Waste. 31(1): 45-67.

Pourbabaee, A. A., Bostani, S., and Amozzegar, M. A. 2011. Decolorization of Cibacron Black w-55 under Alkaline conditions by new strain of *Halomonas* sp. Isolated from textile effluent. Iranian Journal of Chemistry and Chemical Engineering. 30(4): 63-70.

Rudukiya, D. M., and Pawar, K. S. 2013. Optimization of culture condition for enhanced decolorization of Reactive orange 16 by *Comamonas acidovoran* MTCC 3364. International Journal Current Microbiology Applied Sciences. 2(10): 467-476.

Sandrin, T. R., and Maier, R. M. 2003. Impact of metals on the biodegradation of organic pollutants. Environmental Health Perspectives. 111(8): 1093-1101.

Seesuriyachan, P., Takenaka, S., Kuntiya, A., Klayraung, S., Murakami, S., and Aoki, K. 2007. Metabolism of azo dyes by *Lactobacillus casei* TISTR 1500 and effects of various factors on decolorization. Water Research. 41: 985-992.

Sterritt, R. M., and Lester, J. N. 1980. Interaction of Heavy metals with bacteria. Science Total Environment. 14(1): 5-17.

Telke, A. A., Kalyani, D. C., Dawkar, V. V., and Govindwar, S. P. 2009. Influence of organic and inorganic compounds on oxidoreductive decolorization of sulphonated azo dye C.I. Reactive Orange 16. Journal of Hazardous Materials. 172: 298-309.

Zhao, M., Sun, P. F., Wang, G., Jia, X.M., and Zhao, Y. H. (2014). Biodegradation of methyl red by *Bacillus* sp. Strain UN2: decolorization capacity, metabolites characterization, and enzyme analysis. Environmental Science Pollution Research. 21(9): 6136-45.