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Effect of metal ions on the Growth and Dibenzothiophene biodesulfurization activity of *Streptomyces* species isolated from oil contaminated soil sites

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***Abstract*:** *When fuels are burnt, various hazardous gases are released into the atmosphere. In particular, sulfur oxides released as a result of oxidation of organosulfur compounds present in fuels cause ill effects to humans, air pollution and are sources of acid rain. Hydrodesulfurization (HDS), a traditional process, is routinely employed to remove sulfur content from fossil fuels during oil refining. But, HDS is not effective in removal of sulfur content from all organosulfur compounds especially from heterocyclic organosulfur compounds like Dibenzothiophene. Biodesulfurization (BDS), which employs microbes is an effective and alternative method to HDS to remove sulfur from fossil fuels. The present paper deals with the influence of various metal ions on the growth and biodeulfurization efficiency of two Streptomyces species, Streptomyces sp. VUR PPR 101 and Streptomyces sp. VUR PPR 102 isolated from oil contaminated sites, which can specifically remove sulfur from Dibenzothiophene (DBT), a model compound for biodesulfurization studies, via 4S pathway without breaking the DBT ring. In the present investigation, Fe^{2+} and Mn^{2+} were found to be inhibitory towards biodesulfurization activity of both the Streptomyces species. However, Zn^{2+} ions played an augmented role in enhancing the DBT biodesulfurization activity.*

***Keywords*:** *Biodesulfurization, Dibenzothiophene, Hydrodesulfurization, Metal ions, 4S pathway, Streptomyces.*

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I. Introduction

Sulfur constitutes the third most abundant element in fossil fuels (crude petroleum oils and coal). A wide range of sulfur containing organic and inorganic compounds exists in fossil fuels. On combustion, fossil fuels release hazardous sulfur dioxides into the atmosphere [1, 2]. These sulfur oxides cause air pollution and are responsible for acid rains [3]. The particulate sulfate material in oils reduces the life of motor vehicle engines by causing corrosion [2]. The traditional hydrodesulfurization (HDS) employed in oil refining process is carried out at very high temperature and pressures. Moreover, HDS is not effective in removing the sulfur content from all sulfur containing organic compounds especially from recalcitrant compounds like Dibenzothiophene (DBT) [4,5]. An alternative to HDS, for efficiently removing sulfur content from organosulfur compounds present in crude oil, biodesulfurization (BDS) had been developed and introduced. Biodesulfurization involves removal of sulfur content from fossil fuels by employing microbial cells. Unlike HDS, BDS is economical and does not involve high temperatures and pressures [5]. The DBT, a heterocyclic polyaromactic organosulfur compound, is regarded as a model organosulfur compound for biodesulfurization studies because of its recalcitrant, toxic and oncogenic to human beings and hazardous to environment. Microbes metabolize DBT via three major pathways, Kodama, Van afferden and 4S pathways [6]. Among these pathways, the 4S pathway is prominent one as microbes in this pathway do not attack the carbon skeleton of DBT and degrade the ring structure. Thus, calorific value of the fuel is not affected. Therefore, microbes which exhibit DBT biodesulfurization via 4S pathway are commercially important. Various potential DBT biodesulfurizing bacteria isolated include *Rhodococcus erythropolis* XP, *Rhodococcus erythropolis* IGTS8, *Gordonia alkanivorans* RIPI90A etc. [6, 2]. The 4S pathway involves four steps in which DBT is consecutively converted into Dibenzothiophene sulfoxide (DBTO), Dibenzothiophene sulfone ($DBTO_2$), Hydroxyphenyl benzene sulfonate (HPBS) and 2-Hydrxy biphenyl (2-HBP) [7]. The first two steps are catalyzed by DBT monooxygenase, third reaction is catalyzed by $DBTO_2$ monooxygenase and final reaction by HPBS desulfinase [8]. The dsz operon genes, dszA, and C encode flavin dependent $DBTO_2$ monooxygenase and DBT monooxygenase, respectively and dszB gene synthesize HPBS desulfinase [9].

Metal ions are important for the growth and metabolism of microbial cells as well as in the maintenance of stability of cell wall and formation of protein synthesizing apparatus [10]. Metal ions play a key role in functioning of many cellular enzymes by acting as electron donors/acceptors, lewis acids and involve in regulation of various structures [11]. The present paper deals with effect of various metal ions on the growth and DBT biodesulfurization activity of *Streptomyces* sp. VUR PPR 101and *Streptomyces* sp. VUR PPR 102 isolated from oil contaminated sites of mechanical workshops of Karimnagar town, Telangana, India.

II. Materials and Methods

The basal salt medium (KH_2PO_4 - 4.0 gm, Na_2HPO_4 - 4.0 gm, NH_4Cl - 2.0 gm, $MgCl_2$ -0.2 gm, and distilled water-1000 ml) with DBT (5 mM per liter) as sole sulfur source and glucose (5 gm/l) as sole carbon source was prepared. Metal ions viz., Fe^{3+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Mn^{2+}, MoO_4^{2-} and Zn^{2+} were selected for the study. The basal salt medium with DBT and glucose and all ions was designated as Negative control and the basal salt medium with DBT and glucose but without any ions as Positive control. To study the effect of absence of each ion, the medium without that ion but with all other ions selected for the study was setup as treatments [12]. The cultures of *Streptomyces* sp. VUR PPR 101 and *Streptomyces* sp. VUR PPR 102 were grown separately on Starch Casein agar plate for six days at 30^0C. Spores were then harvested and a homogeneous spore suspension (0.2 OD) in 0.05% Tween 20 was prepared. To all the flasks (Negative control, Positive control and treatments) containing 50 ml of the basal medium, spore suspensions (5ml) of *Streptomyces* sp. VUR PPR 101 and *Streptomyces* sp. VUR PPR 102 were inoculated separately and incubated at pH 7.0 and 30^0C temperature. After every 6, 8, 10, 12 and 14 days of incubation, 100 µl of Gibb's reagent was added to the culture broths of the test tubes and the intensity of the blue colour developed was measured at 610 nm in terms of OD values using Spectrophotometer. The desulfurization activity in terms of concentration of 2-HBP produced, directly proportional to colour intensity, was measured using standard graph. The growth (dry weight) of the bacteria was also determined at the same incubation periods.

A standard graph of 2-HBP was prepared to measure the concentration of 2-HBP produced by *Streptomyces* species by desulfurizing DBT. Five ml solutions of 2-HBP ranging from 1mg/l to 12mg/l were prepared in BSM medium supplemented with glucose and DBT and adjusted the pH to 8.0 by using sodium bicarbonate. To each test tube, 100 µl of Gibb's reagent was added and incubated at 30^0C for 30 minutes for the development of blue colour. After 30 minutes, OD values of the colour complexes in all the tubes were measured at 610 nm by spectrophotometer and a standard graph of OD values versus known 2-HBP concentrations was plotted [1].

III. Results and Discussion

The present study results (Tables 1 & 2) on the effect of absence/and or presence of certain metal ions on desulfurization activity and growth of *Streptomyces* sp. VUR PPR 101 and *Streptomyces* sp. VUR PPR 102, revealed that 2-HBP production and growth increased in the absence of Fe^{3+} and Mn^{2+} ions when compared to that in Negative control (DBT containing medium with all ions) and Positive control (DBT containing medium without any ions). Infact, the 2-HBP production as well as growth of both the *Streptomyces* species showed a gradual increase with increase in the incubation period from 6 days to 14 days. Nearly one third of total enzymes activity is known to be influenced by metal ions. In such enzymes, metal ions alter electron flow in substrate or enzyme, and regulate the enzyme catalysis [13]. On the other hand, some metal ions may inhibit growth and various activities in living cells. One of the important roles of metal ions is their involvement in the formation of protein synthetic machinery [10]. Proteins, as the building blocks of living cells, perform various functions like transport, pigmentation, catalysis of metabolic reactions (enzymes) etc., which are part of cell growth and metabolism. Thus, metal ions directly or indirectly influence the metabolic reactions (enzyme activity) and growth of living cells.

The increased production of 2-HBP by both the *Streptomyces* species in absence of Fe^{3+} and Mn^{2+} observed in this study was in good accordance with earlier reports on inhibition of malic dehydrogenase activity of *Aspergillus niger* at 0.1 mM Fe^{3+} ion concentration [14] and inhibition of nuclease, RNAase and alkaline phosphatase production in *Lysobacter enzymogenes* at Mn^{2+} ions concentration of more than 0.01 mM [15]. Absence of Ca^{2+}, Co^{2+}, Cu^{2+} and MoO_4^{2-} decreased the 2-HBP production i.e., rate of biodesulfurization which was also well supported by some earlier works on activities of different enzymes. The Ca^{2+} ions had increased the activity of cellobiose dehydrogenase significantly in *Myriococcum thermophilum* and *Humicola insolens* [16]. Spencer *et al.* [17] reported that in *Bacillus licheniformis*, Co^{2+} ions enhanced the synthesis and activation of alkaline phosphatase. In Cucumber calli, the activity of ascorbate oxidase enzyme was increased by Cu^{2+} ions [18]. Al-Issawi *et al.* [19] reported that application of MoO_4^{2-} ions had increased the synthesis and activity of antioxidant enzymes in two wheat cultivars.

Absence of Zn^{2+} had adversely affected both the 2-HBP production and growth of both the *Streptomyces* species indicating its important role in biodesulfurization process. Similar results were reported by Alves [12], who observed the increase of biodesulfurization activity and growth in the absence of Fe^{3+} and a tremendous decrease in biodesulfurization activity and growth in DBT desulfurizing *Gordonia alkanivorans* strain 1B in the absence of Zn^{2+}. Ohshiro *et al.* [20] reported that the DBT desulfurizing enzyme DBT sulfone monoxygenase activity had increased when Al^{3+}, Cd^{2+} and Zn^{2+} ions were incorporated into the medium. Overall, Zn^{2+} ion can be regarded as one of the key ions essential for DBT biodesulfurization process in *Streptomyces* species.

IV. Conclusion

Both the DBT desulfurizing *Streptomyces* species of the present work had exhibited decreased desulfurization activity and growth in the absence of majority of ions selected for the study. In the absence of only two metal ions viz., Fe^{3+} and Mn^{2+} the organisms had shown increased desulfurization activity and growth. This infers that Fe^{3+} and Mn^{2+} ions might have some inhibitory effect on 4S desulfurization pathway enzymes. Among other metal ions, especially Zn^{2+} was found be playing a key role in the enhancement of biodesulfurization activity as in its absence the *Streptomyces* species showed tremendous decrease in 2-HBP production i.e., reduced biodesulfurization activity.

References

[1]. M R Ardakani, A Aminsefat, B Rasekh, F Yazdiyan, B Zaragar, M Zarei and H Najafzadeh, Biodesulfurization of Dibenzothiophene by a newly isolated, Strenotrophomonas maltophilia Strain Kho1, World Applied Sciences Journal, 1(3), 2010, 272-278.

[2]. N K Bordloi, P Bhagwati, M Chaudhuri and A K Mukherjee, Proteomics and Metabolomics Analyses to Elucidate the Desulfurization Pathway of Chelatococcus sp., PLOS ONE. 11(4), 2016, 1-21.

[3]. F L Li, P Xu, C Q Ma, L L Luo and X S Wang, Deep desulfurization of hyrodesulfurization treated diesel oil by a facultative thermophilic bacterium Mycobacterium sp. X7B, FEMS Microbiology Letters, 223, 2003, 301-307.

[4]. L Rashidi, D J Towfighi and K Khosravi-Darani, Biodesulfurization: Biochemical and Genetic Engineering Aspects. Dynamic Biochemistry, Process Biotechnology and Molecular Biology, Global Science Books, 1(1), 2007, 24-31.

[5]. P Derikvand, Z Etemadifar and H Saber, Sulfur removal from Dibenzothiophene by newly isolated Paenibacillus validus Strain PD2 and process optimization in aqueous and Biphasic (Model-Oil) Systems, Polish Journal of Microbiology, 641(1), 2015, 47-54.

[6]. H W C de Araujo, M C de Freitas Silva, C I M Lins, A E do Nasciment, C A A da Silva and G M Campos-Takaki, Oxidation of dibenzothiophene (DBT) by Serratia marcescens UCP 1549 formed biphenyl as final product, Biotechnology for fuels. 5(33), 2012, 1-9.

[7]. W Wang, T Ma, K Lian, Y Zhang, H Tian, K Ji and G Li, Genetic Analysis of Benzothiophene Biodesulfurizatin Pathway of Gordnia terrae Strain C-6. PLOS ONE, 8 (12), 2013, 1-8.

[8]. B R Folsom, D R Schieche, P M Digrazia, J Werner and S Palmer, Microbial Desulfurization of Alkylated Dibenzothiophenes from a Hydrodesulfurized Middle Distillate by Rhodcoccus erythropolis 1-19. Applied and Environmental Microbiology, 65(11), 1999, 4967-4972.

[9]. G F Duarte, A S Rosado, L Seldin, W de Araujo and J D V Elsa, Analysis of Bacterial Community Structure in Sulfurous-Oil Containing Soils and Detection of Species Carrying Dibenzothiophene Desulfurization (dsz) Genes. Applied and Environmental Microbiology, 67(3), 2001, 1052-1062.

[10]. P A Joshi, S Singh and D B Shekhawat, Effect of metal ions on growth and bisurfactant production by Halophilic bacteria. Advances in Applied Science Research, 6(4), 2015, 152-156.

[11]. J F Riordan, The Role of Metals in Enzyme Activity. Annals of Clinical and Laboratory Science, 7(2), 1977, 119-129.

[12]. L M G Alves, Dibenzothiophene desulfurization by Gordonia alkanivorans strain 1B. University of Libson, Libson, Portugal. 2007.

[13]. J P Glusker, A K Katz and C W Block, Metal ions in Biological Systems. The Rigaku Journal. 16(2), 1999, 8-16.

[14]. K Jernejc and M Legisa, The influence of metal ions on malic enzyme activity and lipid synthesis in Aspergillus niger, FEMS Microbiology Letter. 217(2), 2002, 185-190.

[15]. R G V Tigerstrom, The effect of magnesium and manganese ion concentrations and medium composition on the production of extracellular enzymes by Lysobacter enzymogenes. Microbiology, 129, 1983, 2293-2299.

[16]. C Schulz, R Ludwig, P O Micheelsen, M Silow, M D Toscano and L Gorton, Enhancement of enzymatic activity and catalytic current of cellobiose dehydrogenase by calcium ions. Electrochemistry Communications. 17, 2012, 71-74.

[17]. D B Spencer, C P Chen and F M Hulett, Effect of cobalt on synthesis and activation of Bacillus licheniformis alkaline phosphatase. Journal of Bacteriology, 145(2), 1981, 926-933.

[18]. J Sekiya, K Mizuno, O Kimura and N Shimose, Ascorbate oxidase in Cucumber Calli and enhancement of enzyme activity by copper sulfate. Soil Science and Plant Nutrition, 36(1), 1990, 43-51.

[19]. M Al-Issawi, H Z Rihan, H Al-Shmgani and M P Fuller, Molybdenum application enhances antioxidant enzyme activity and COR15a protein expression under cold stress in wheat. Journal of Plant Interactions, 11(1), 2016, 5-10.

[20]. T Ohshiro, T Kojima, K Torii, H Kawasoe and Y Izumi, Purification and characterization of dibenzothiophene (DBT) sulfone monooxygenase, an enzyme involved in DBT desulfurization, from Rhodococcus erythropolis D-1. Journal of Bioscience and Bioengineering, 88(6), 1999, 610-616.

Table-1: Effect of Metal ions on Desulfurization activity (2-HBP production) and Growth (Dry weight) of *Streptomyces* sp. VUR PPR 101

Presence/Absence of different metal ions	After 6 days		After 8 days		After 10 days		After 12 days		After 14 days	
	HBP mg/l	Dry wt mg/ml	HBP mg/l	Dry wt mg/ml	HBP mg/l	Dry wt mg/ml	HBP mg/l	Dry wt mg/ml	HBP mg/l	Dry wt mg/ml
Negative control (contains all ions)	3.00 ± 0.08	3.33 ± 0.12	3.37 ± 0.17	3.47 ± 0.09	3.53 ± 0.17	3.70 ± 0.08	3.67 ± 0.05	3.87 ± 0.05	3.83 ± 0.05	4.07 ± 0.05
Positive control (contains no ions)	1.67± 0.05	1.93 ± 0.05	1.83 ± 0.05	2.17 ± 0.05	2.03 ± 0.05	2.33 ± 0.05	2.23 ± 0.17	2.57 ± 0.05	2.47 ± 0.09	2.67 ± 0.05
Fe^{3+} absence	3.70 ± 0.08	2.60 ± 0.08	3.77 ± 0.05	2.70 ± 0.08	3.97 ± 0.17	2.87 ± 0.12	4.07 ± 0.05	3.07 ± 0.12	4.23 ± 0.05	3.20 ± 0.14
Co^{2+} absence	2.87 ± 0.05	2.93 ± 0.05	3.07 ± 0.12	3.03 ± 0.05	3.27 ± 0.05	3.27 ± 0.05	3.47 ± 0.09	3.60 ± 0.08	3.67 ± 0.12	3.77 ± 0.12
Cu^{2+} absence	2.37 ± 0.17	3.27 ± 0.05	2.63 ± 0.05	3.37 ± 0.05	2.83 ± 0.12	3.60 ± 0.08	3.03 ± 0.05	3.80 ± 0.16	3.13 ± 0.05	3.93 ± 0.05
Mn^{2+} absence	3.53 ± 0.09	2.80 ± 0.08	3.67 ± 0.19	2.93 ± 0.05	3.77 ± 0.05	3.13 ± 0.05	3.93 ± 0.12	3.37 ± 0.05	4.13 ± 0.17	3.47 ± 0.12
MoO_4^{2-} absence	2.77 ± 0.05	3.57 ± 0.05	2.93 ± 0.05	3.67 ± 0.09	3.10 ± 0.08	3.87 ± 0.17	3.27 ± 0.19	4.03 ± 0.17	3.47 ± 0.09	4.27 ± 0.05
Zn^{2+} absence	2.23 ± 0.05	2.03 ± 0.12	2.53 ± 0.09	2.27 ± 0.05	2.70 ± 0.08	2.53 ± 0.05	2.87 ± 0.05	2.67 ± 0.05	3.07 ± 0.05	2.83 ± 0.12
Ca^{2+} absence	2.60 ± 0.14	3.03 ± 0.05	2.77 ± 0.05	3.23 ± 0.05	2.97 ± 0.05	3.53 ± 0.12	3.17 ± 0.12	3.67 ± 0.12	3.27 ± 0.19	3.80 ± 0.08

Table-2: Effect of Metal ions on Desulfurization activity (2-HBP production) and Growth (Dry weight) of *Streptomyces* sp. VUR PPR 102

Presence/Absence of different metal ions	After 6 days		After 8 days		After 10 days		After 12 days		After 14 days	
	HBP mg/l	Dry wt mg/ml	HBP mg/l	Dry wt mg/ml	HBP mg/l	Dry wt mg/ml	HBP mg/l	Dry wt mg/ml	HBP mg/l	Dry wt mg/ml
Negative control (contains all ions)	2.87 ± 0.12	3.17 ± 0.12	3.00 ± 0.08	3.30 ± 0.08	3.10 ± 0.08	3.47 ± 0.05	3.23 ± 0.05	3.57 ± 0.09	3.37 ± 0.12	3.67 ± 0.12
Positive control (contains no ions)	1.40 ± 0.08	1.83 ± 0.09	1.53 ± 0.05	1.97 ± 0.05	1.60 ± 0.08	2.07 ± 0.09	1.70 ± 0.08	2.17 ± 0.12	1.83 ± 0.05	2.27 ± 0.05
Fe^{3+} absence	3.43 ± 0.12	2.43 ± 0.09	3.60 ± 0.16	2.53 ± 0.05	3.77 ± 0.12	2.60 ± 0.08	3.87 ± 0.09	2.70 ± 0.08	3.97 ± 0.05	2.80 ± 0.08
Co^{2+} absence	2.77 ± 0.12	2.70 ± 0.08	2.90 ± 0.08	2.83 ± 0.09	2.97 ± 0.12	2.93 ± 0.05	3.17 ± 0.12	3.03 ± 0.09	3.30 ± 0.08	3.17 ± 0.09
Cu^{2+} absence	2.27 ± 0.09	2.97 ± 0.05	2.37 ± 0.12	3.10 ± 0.08	2.47 ± 0.05	3.23 ± 0.12	2.57 ± 0.05	3.37 ± 0.05	2.67 ± 0.09	3.47 ± 0.09
Mn^{2+} absence	3.07 ± 0.17	2.53 ± 0.12	3.23 ± 0.09	2.63 ± 0.09	3.37 ± 0.09	2.77 ± 0.09	3.50 ± 0.08	2.93 ± 0.05	3.63 ± 0.09	3.03 ± 0.12
MoO_4^{2-} absence	2.63 ± 0.12	3.30 ± 0.08	2.73 ± 0.12	3.47 ± 0.09	2.83 ± 0.17	3.60 ± 0.08	2.97 ± 0.05	3.73 ± 0.12	3.10 ± 0.08	3.87 ± 0.05
Zn^{2+} absence	2.07 ± 0.05	1.93 ± 0.12	2.20 ± 0.14	2.07 ± 0.05	2.30 ± 0.14	2.23 ± 0.12	2.47 ± 0.09	2.37 ± 0.12	2.60 ± 0.08	2.47 ± 0.05
Ca^{2+} absence	2.37 ± 0.12	2.83 ± 0.12	2.47 ± 0.05	3.00 ± 0.08	2.57 ± 0.12	3.10 ± 0.08	2.70 ± 0.08	3.20 ± 0.08	2.83 ± 0.12	3.33 ± 0.12

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