International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Optimization of Growth Parameters of Dibenzothiophene Desulfurizing *Streptomyces* Species Isolated from Oil Contaminated Sites

Praveen Reddy P, Umamaheshwara Rao V*

Department of Botany & Microbiology, Acharya Nagarjuna University, Nagarjunanagar-522510, Guntur Dt., Andhra Pradesh, India

Abstract: ***Fossil fuels on combustion release hazardous gases into the environment and sulfur dioxideis one among them. Sufur dioxide is released as result of oxidation of organosulfur compounds occurring in fossil fuels. Presently hydrodesulfurization process is employed to remove sulfur content from fuels which is not an effective method. An alternative process that employs microbes is biodesulfurization, an efficient method for the desulfurization of sulfur even from recalcitrant organosulfur compounds like dibenzothiophene which considered as model compound for biodesulfurization studies. In the present study, optimum growth parameters required for two dibenzothiophene desulfurizing Streptomyces species namely Streptomyces sp. VUR PPR 101 and Streptomyces sp. VUR PPR 102 isolated from oil contaminated sites were determined. Glucose was found to be the optimum carbon source for both the species.. The optimum nitrogen sources for Streptomyces sp. VUR PPR 101 and Streptomyces sp. VUR PPR 102 were yeast extract and potassium nitrate, respectively. Both the species had shown highest growth with methionine. The optimum temperature and pH for both the species were 30°C and 7.0, respectively. Finally, with optimum nutrient sources and optimum physical parameters, both the Streptomyces*** **species showed enhanced growth at 1% salt concentration.**

Keywords: Fossil fuels, dibenzothiophene, desulfurization, *Streptomyces,* optimum, growth parameters

1. Introduction

In fossil fuels, which include crude oil and coal, sulfur is the third most abundant element. Numerous types of organic and inorganic sulfur containing compounds are present in fossil fuels. When fossil fuels are oxidized, sulfur dioxide which is deleterious to environment and human health is released (Mohammad *et al.,* 2010; Naba *et al.,* 2016). Sulfur dioxide is a major potent air pollutant and it is one of the major sources of acid rains (Fu *et al.,* 2003). Frequent exposure to sulfur dioxide causes respiratory infections and pulmonary impairment in humans (Badenhorst, 2007; Prashant, 2010). Sulfur dioxide reduces the photosynthetic rate in plants. It enhances the increase of stomata which leads to loss of water in plants (Varshney *et al.,* 1979). To remove sulfur from fuels, oil refineries usually employ a conventional hydrodesulfurization process (HDS) which operates under high temperatures and pressure (Sung-Keun *et al.,* 1998). The HDS is not effective removal of sulfur from all organosulfur compounds especially from recalcitrant compounds like dibenzothiophene (DBT) (Peyman *et al.,* 2015; Rashidi *et al.,* 2007). There are some microorganisms in nature which selectively degrade the organosulfur compounds without breaking their ring structure and remove sulfur via 4S pathway. Such microorganisms are called biodesulfurizing microbes and the process by which they remove sulfur from organosulfur compounds is known as biodesulfurizaiton (BDS). Unlike HDS, BDS work out under normal conditions and does not involve high temperature and pressure. Microbes which have the ability of desulfurizing ability are commercially important (Peyman *et al.,* 2015). The DBT is considered as a model compound for BDS studies as it is highly recalcitrant and not affected by HDS process (Mervat *et al.,* 2014). The present study was aimed to isolate the bacteria capable of desulfurizing DBT via 4S pathway and to study the optimization of growth parameters.

2. Materials and Methods

Inoculum preparation

In the present study, two bacteria viz., *Streptomyces* sp. VUR PPR 101 and *Streptomyces* sp. VUR PPR 102, capable of desulfurizing DBT via 4S pathway, isolated from oil contaminated sites of mechanical workshops of Autonagar region in Karimnagar, Andhra Pradesh, India and identified by 16S rRNA sequencing were used. To prepare the inoculum, cultures were grown on starch casein agar plates for six days at 30°C. Spores were then harvested and a homogeneous spore suspension (0.2 OD) in 0.05% Tween 20 was prepared. Five ml of this suspension was used as inoculum for the optimization experiments.

The basal medium (50 ml) was distributed into each of 250 ml Erlenmeyer flask and sterilized. Glucose, Fructose, Sucrose, Maltose, Starch, Cellulose, Mannitol and Glycerol were added separately as carbon sources into the basal medium at 1% concentration. Various nitrogen sources such as Peptone, Yeast extract, Casein, Urea, Ammonium chloride, Ammonium nitrate, Sodium nitrate and potassium nitrate were provided separately into basal medium at 1% concentration. Five ml of spore suspension of each *Streptomyces* species was inoculated into carbon and nitrogen supplemented basal medium separately and incubated at 30°C for 7 days. A variety of amino acids such as Methionine, Pheylalanine, Tyrosine, Alanine, Arginine, Lysine, Proline, Glutamine and Cysteine were amended at a concentration of 0.1 mg/ml in the improved medium containing optimized carbon and nitrogen sources. Five ml of the spore suspension of each of the cultures was inoculated into the medium and incubated at 30°C for 7 days (Kumar and Kannabiran, 2010).

The effect of incubation temperatures (20°C, 25°C, 30°C, 35°C, 40°C and 45°C) and pH (3.0, 4.0, 5.0, 6.0, 7.0, 8.0,

9.0, 10, 11 and 12) were studied separately by inoculating 5 ml of the spore suspension of each culture into the above improved medium (Kumar and. Kannabiran, 2010; Rakesh *et a*l., 2014). The effect of salt concentration (NaCl) on growth of the cultures was carried out by growing the cultures in various NaCl concentrations of 0.5%, 1.0%, 1.5% and 2.0% in the basal medium while keeping other parameters at optimum level (Ripa *et al.,* 2009).

Measurement of growth (biomass)

The biomass from the culture filtrate separated by means of centrifugation was transferred to pre-weighed dry filter paper using a clean spatula and then placed in an oven at 55°C overnight to reach a fixed weight. Growth in terms of biomass accumulation was expressed as mg/ml of the culture medium (Kumar and Kannabiran, 2010).

3. Results

Table 1: Effect of different carbon sources on the growth of *Streptomyces* species

S. No	*Carbon Source*	*Dry wt. in mg/ml (Growth)*	
		Streptomyces sp. VUR PPR 101	*Streptomyces sp. VUR PPR 102*
1	Glucose	3.77 ± 0.17	3.36 ± 0.05
2	Fructose	2.13 ± 0.05	1.80 ± 0.08
3	Sucrose	1.43 ± 0.09	1.13 ± 0.05
4	Maltose	3.16 ± 0.13	2.56 ± 0.13
5	Starch	3.06 ± 0.09	2.30 ± 0.16
6	Cellulose	1.26 ± 0.05	0.90 ± 0.08
7	Mannitol	2.26 ± 0.13	2.00 ± 0.08
8	Glycerol	2.73 ± 0.09	2.06 ± 0.09

Table 1 depicts the response of DBT desulfurizing bacteria towards different carbon sources. Both the species had shown maximum growth in the presence of glucose and least with cellulose. The dry wt. of both the species was found to be in that decreasing order of Glucose > Maltose > Starch > Glycerol > Mannitol > Fructose > Sucrose > Cellulose. *Streptomyces* sp. VUR PPR101 exhibted relatively more growth than *Streptomyces* sp. VUR PPR102 in presence of all the carbon sources.

Table 2: Effect of different nitrogen sources on the growth of *Streptomyces* species

S. No	Nitrogen source	Dry wt. in mg/ml (Growth)	
		Streptomyces sp. VUR PPR 101	*Streptomyces* sp. VUR PPR 102
1	Peptone	3.00 ± 0.08	2.26 ± 0.13
2	Yeast extract	4.43 ± 0.13	2.80 ± 0.08
3	Casein	2.00 ± 0.08	2.36 ± 0.17
4	Urea	1.76 ± 0.09	1.53 ± 0.05
5	Ammonium chloride	3.43 ± 0.17	2.77 ± 0.21
6	Ammonium nitrate	3.76 ± 0.13	3.36 ± 0.13
7	Sodium nitrate	3.93 ± 0.09	2.93 ± 0.05
8	Potassium nitrate	4.03 ± 0.20	3.53 ± 0.09

The optimal nitrogen sources for *Streptomyces* sp. VUR PPR 101 and *Streptomyces* sp. VUR PPR 102 were found as yeast extract and potassium nitrate, respectively. Least growth was shown by both bacteria with urea (Table 2). Except the casein, all the tested nitrogen sources favoured more growth of *Streptomyces* sp. VUR PPR 101 than that of *Streptomyces* sp. VUR PPR 102.

Table 3: Effect of different amino acid sources on the growth of Streptomyces species

S.No	Amino acid	Dry wt. in mg/ml (Growth)	
		Streptomyces sp. VUR PPR 101	*Streptomyces* sp. VUR PPR 102
1.	Methionine	5.90 ± 0.08	5.23 ± 0.13
2.	Phenylalanine	4.43 ± 0.13	3.93 ± 0.05
3.	Tyrosine	4.56 ± 0.13	4.13 ± 0.13
4.	Alanine	4.60 ± 0.14	4.20 ± 0.16
5.	Arginine	4.53 ± 0.05	4.10 ± 0.14
6.	Lysine	4.30 ± 0.16	3.83 ± 0.13
7.	Proline	4.76 ± 0.17	4.33 ± 0.09
8.	Glutamine	4.90 ± 0.08	4.36 ± 0.13
9.	Cysteine	5.33 ± 0.09	4.80 ± 0.08

Both the *Streptomyces* species exhibited highest growth in methionine containing medium and lowest growth in lysine supplemented medium (Table 3). The increasing order of growth exhibited by both the species in presence of different aminoacids was found to be as Phenylalanine < Arginine < Tyrosine < Alanine < Proline < Glutamine < Cysteine < Methionine. The growth of *Streptomyces* sp. VUR PPR 101 was relatiely high than *Streptomyces* sp. VUR PPR102 with all the aminoacids used.

Table 4: Effect of temperature on the growth of *Streptomyces* species

S. No.	Temperature in ^{0}C	Dry wt. in mg/ml (Growth)	
		Streptomyces sp. VUR PPR 101	*Streptomyces* sp. VUR PPR 102
1	20°C	5.43±0.13	4.60±0.16
2	25°C	6.23±0.17	5.27±0.13
3	30°C	6.67±0.09	5.80±0.08
4	35°C	6.50±0.14	5.53±0.13
5	40°C	5.67±0.13	4.77±0.09
6	45°C	4.73±0.05	3.90±0.08

At 30°C temperature, both the *Streptomyces* species had shown maximum growth and thereafter the growth decreased gradually and attained least growth at 45^{0}C within the tested range of temperatures (Table 4).

Table 5: Effect of pH on the growth of *Streptomyces* species

S.No	pH	Dry wt. in mg/ml (Growth)	
		Streptomyces sp. VUR PPR 101	*Streptomyces* sp. VUR PPR 102
1	3	4.60±0.14	3.97±0.17
2	4	5.20±0.08	4.47±0.13
3	5	5.47±0.13	5.00±0.08
4	6	5.93±0.17	5.47±0.17
5	7	6.60±0.08	5.80±0.08
6	8	5.10±0.08	4.43±0.13
7	9	4.77±0.13	4.10±0.14
8	10	4.30±0.14	3.87±0.13
9	11	3.83±0.13	3.53±0.17
10	12	3.53±0.17	3.20±0.08

Both the *Streptomyces* species exhibited an increased growth from pH 3.0 to pH 7.0 and thereafter shown a gradual decrease with least growth at pH 12 (Table 5).

Table 6: Effect of salt concentration on *Streptomyces* species

S.No	Salt concentration	Dry wt. in mg/ml (Growth)	
		Streptomyces sp.VURPPR 101	*Streptomyces* sp. VUR PPR 102
1	0.5%	4.97 ± 0.09	4.33 ± 0.09
2	1.0%	6.63 ± 0.13	5.73 ± 0.13
3	1.5%	4.50 ± 0.14	3.87 ± 0.05
4	2.0%	4.00 ± 0.16	3.47 ± 0.17

The salt concentration of 1% was found more favourable for the growth of both the *Streptomyces* species. The increase in salt concentration over 1% resulted in decreased growth of both species (Table 6).

4. Discussion

Different carbon sources were supplemented into the basal medium to study their effect on the growth of the two desulfurizing *Streptomyces* species isolated from oil contaminated soils. Both the organisms formed highest biomass when glucose was added in the medium. Other carbon sources are also favoured the growth but to relatively less extent when compared with glucose. This observation draws support from the earlier study of Gokhan *et al.,* (2015) who reported the glucose as a better carbon source for the growth of *Sulfobolus solfataricus* P2, a DBT desulfurizing bacterium, in comparison with the other carbon sources. When growth of the two *Streptomyces* species was determined with various nitrogen sources, *Streptomyces* sp. VUR PPR 101 and *Streptomyces* sp. VUR PPR 102 produced maximum biomass when yeast extract and potassium nitrate were used as nitrogen sources, respectively. This gains support from the findings of maximum growth by *Brevibacillus invocatus* C19 and *Rhodococcus erythropolis* IGST8, two DBT desulfurizing bacteria, in DBT containing medium with yeast extract as nitrogen source (Mervat *et al.,* 2014) and *Streptomyces anandii var. taifiensis* in potassium nitrate containing medium (Zeinat and Salha, 1986).

To check the optimal amino acid suitable for the *Streptomyces* species, they were grown in medium containing their optimal carbon and nitrogen sources with various amino acids. The methionine was found to be the best amino acid source for both the *Streptomyces* species. To determine the effect of salt concentration the *Streptomyces* species were grown in various salt concentrations ranging between 0.5% and 2%. To determine the effect of physical parameters (temperature and pH), *Streptomyces* species were grown at different temperatures and in different pH conditions. For both the *Streptomyces* species, temperature of 30^{o}C and pH 7.0 were found to be optimum for better growth. Kumar and Kannabiran (2010) also reported that temperature of 30^{o}C and pH 7.0 as optimal conditions for the maximum growth of *Streptomyces* VITSVK9, isolated from Bay of Bengal, India, when grown in basal medium supplemented the glucose as the carbon source. This report is in great concurrence with our observation. The optimum salt concentration for the better growth of *Streptomyces* species was observed as 1.0%.

5. Conclusion

In the present study, optimum parameters required for the growth of two DBT desulfurizing bacteria viz., *Stretptomyces* sp. VUR PPR 101 and *Stretptomyces* sp. VUR PPR 102 were determined. Both the species exhibited better growth when glucose is used as carbon source. Yeast extract and potassium nitrate were the optimum nitrogen sources for *Stretptomyces* sp. VUR PPR 101 and *Stretptomyces* sp. VUR PPR 102, respectively. Temperature of 30^{o}C, pH 7.0 and 1.0% salt concentration were the optimum physical parameters for both the DBT desulfurizing *Streptomyces* species. *Streptomyces* sp. VUR PPR 101 showed highest growth in all nutrient sources under different physical parameters when compared to *Streptomyces* sp. VUR PPR 102. The future work concentrates on the enhancement of DBT desulfurizing activity of these two *Streptomyces* species for the useful application in biodesulfurization of various fuels.

References

[1] Badenhorst C.J, 2007. Occupational health and safety risks associated with sulfur dioxide. The Journal of the Southern African Institute of Mining and Metallurgy. 17: 299-303.
[2] Gokhan GUN., Yuda YURUM., Gizem DINLER DOGANAY, 2015. Revisiting the biodesulfurization capability of hyperthermophilic archeon *Sulfobolus solfataricus* P2 revealed DBT consumption by the organism in an oil/water two-phase liquid system at high temperatures. Turkish Journal of Chemistry. 39: 255-266.
[3] Kumar Saurav and K. Kannabiran, 2010. Diversity of Optimization of process parameters for the growth of *Streptomyces* VITSVK9 spp. Isolated from Bay of Bengal, India. Journal of Natural & Environmental Sciences 1(2): 55-65.
[4] Mervat A. Abo-State., Nour Sh. EI-Gendy., Seham A.EI-Tentamy., Hesham M. Mahdy., Hussein N. Nassar, 2014. Modification of Basal Salts Medium for Enhancing Dibenzothiophene Biodesulfurization by *Brevibacillus invocatus* C19 and *Rhodococcus erythropolis* IGTS8. World Applied Sciences Journal. 30 (2): 133-140.
[5] Mohammad Roayaei Adrakani., Ayeh Aminsefat., Behnam Rasekh., Fatemeh Yazdiyan., Behrouz Zargar., Mohammad Zarei., Hosein Najafzadeh, 2010. Biodesulfurization of Dibenzothiophene by a newly isolated, *Stenotrphomonas maltophilia* Strain Kho1. World Applied Sciences Journal. 1(3): 272-278.
[6] Naba K. Bordloi., Pabitra Bhagwati., Mihir Chaudhuri., Ashis K. Mukherjee, 2016. Proteomics and Metabolomics Analyses to Elucidate the Desulfurization Pathway of *Chelatococcus*sp. PLOS ONE. 11(4): 1-21
[7] Peyman Derikvand., Zahra Etemadifar., Hossein Saber, 2015. 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): 47-54.
[8] Prashant Mehta, 2010. Science behind Acid Rain: Analysis of Its Impacts and Advantages on Life and

Heritage Structures. South Asian Journal of Tourism and Heritage. 3(2): 123-132.

[9] Rakesh K.N., Dileep N., Prashit Kekuds, 2014. Optimization of culture conditions for production of antibacterial metabolite by bioactive *Streptomyces* species SRDPTK-07, An International Journal of Advances in Pharmaceutical Sciences 5(1): 1809-1816.

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

[11] Ripa F.A.., Nikkon F.., Zaman S., Khondkar P, 2009. Optimal conditions for antimicrobial metabolites production from a new *Streptomycecess*sp. RUPA-08PR from Bangladeshi soil, Mycobiology, Korean society of Mycology 37(3): 211-214.

[12] Sung-Keun Rhee., Je Hwan Chang., Yong Keun Chang., Ho Nam Chang, 1998. Desulfurization of Dibenzothiophene and Diesel Oils by a newly isolated *Gordona* Strain, CYKS1. Applied and Environmental Microbiology. 64(6): 2327-2331.

[13] Varshney C.K., Garg J.K., Lauenroth W.K., Heitschmidt R.K, 1979. Plant responses to sulfur dioxide pollution. C R C Critical Reviews in Environmental Control. 9(1): 27-49.

[14] Zeinat Kamel and Salha H Al-Zahrani, 1986. Optimizaiton of a Growth Medium for Antibiotic Production by *Streptomyces anandii var. Taifiensis.* Perspectives in Biotechnology and Applied Microbiology. 143-152.