Research Article

Optimization of Nutrient and Physical Parameters for the Enhanced Dibenzothiophene Desulfurization Activity by *Streptomyces* sp. VUR PPR 102

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ABSTRACT

Aim: To determine the response of Dibenzothiophene (pollutant present in fossil fuels and responsible for sulfur dioxide pollution) desulfurization (selective release of sulfur) activity by *Streptomyces* sp. VUR PPR 102 to various types of nutrients (carbon and nitrogen ingredients) and amino acid sources and physical conditions (pH and temperature) and to find out the optimum nutrients and physical conditions for enhanced desulfurization activity. **Materials and Methods:** For all experimental studies, basal salt medium supplemented with DBT as sulfur source with individual nutrient components of various concentrations to be tested was used to inoculate the *Streptomyces* sp. VUR PPR 102 and incubated at different pH and temperature conditions. **Results:** The best nutrient sources (carbon and nitrogen) for highest dibenzothiophene desulfurization activity were reported to be glucose (3%) and potassium nitrate (1%), respectively. The amino acid source, glutamine (0.3 mg/mL) enhanced DBT desulfurization activity. The optimum physical parameters were reported to be 30°C and pH 7.0 for maximum biodesulfurization. **Conclusion:** The optimized medium and physical conditions for enhanced desulfurization activity by the test organism were determined. Such organisms can be genetically improved further and in future can be employed in refining of fossil fuels for the selective elimination of sulfur.

Keywords: Dibenzothiophene, Sulfur dioxide pollution, Biodesulfurization, Carbon, Nitrogen, Amino acids, Physical conditions, Optimized medium.

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INTRODUCTION

Sulfur is the third most abundantly available chemical element in fossil fuels (coal and crude petroleum). Various sulfur containing organic and inorganic compounds occur in fossil fuels. During their utilization, fossil fuels undergo combustion and emit most hazardous sulfur oxides due to the oxidation of sulfur compounds into the environment.[1,2] The organosulfur compounds (sulfur containing organic compounds) occurring in petroleum products constitute the primary source of sulfur oxides. The sulfur oxides released into the atmosphere are responsible for air pollution and result in acid rains.[3] The sulfur oxides cause various respiratory problems among humans. The acid rains disturb the soil and aquatic ecosystems. As a part of oil refining, the refineries employ a traditional Hydrodesulfurization [HDS] which is not effective in elimination of sulfur from fuels, in particular in removal of recalcitrant organosulfur compounds such as Dibenzothiophene.[4,5] The researchers suggested Biodesulfurization (BDS) as an alternative to HDS. The biodesulfurization process

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use microorganisms for the selective elimination of sulfur from sulfur conataining organic compounds occurring in fossil fuels. The BDS is considered as an environmental friendly process. On the other hand, HDS is not eco-friendly as it involves high temperatures and pressure.[5] The Dibenzothiophene (DBT), a highly recalcitrant organosulfur compound occurring in fossil fuels is treated as a model substrate (compound) for BDS experiments. The microorganisms act upon DBT via three major metabolic pathways *viz.,* Van Afferden, Kodama and 4S pathways.[6] Among these DBT metabolic pathways, the 4S pathway is regarded as genuine and effective one. The 4S metabolic pathway involves selective elimination of sulfur atom from the recalcitrant compound, DBT without affecting its skeleton structure and hence, the mileage of fuels will not be altered. Hence, microorganisms exhibiting 4S pathway have commercial significance.[2,6] The 4S metabolic pathway occur involving four sequential reaction steps in which DBT is converted to Dibenzothiophene sulfoxide (DBTO), Dibenzothiophene sulfone ($DBTO_2$), Hydroxyphenyl Benzene Sulfonate (HPBS) and 2-Hydoxy Biphenyl (2-HBP). The first two reaction steps are catalyzed by DBT monooxygenase, the $DBTO_2$ monooxygenase catalyzes third reaction and fourth reaction step is catalyzed by HPBS desulfinase.[7,8] The microorganisms which exhibit 4S metabolic pathway eliminate sulfur from DBT as sulphite and release 2-Hydoxy Biphenyl (2-HBP) as end product.

In the present research work, the DBT desulfurization activity by *Streptomyces* sp. VUR PPR 102 in response to different nutrient components (carbon, nitrogen and amino acid sources) and physical conditions was determined and nutrient sources and physical parameters for desulfurization activity were optimized.

MATERIALS AND METHODS

Medium

In the present study, for the conduct of all DBT desulfurization experiments, Basal Salt Medium (BSM) supplemented with 5 mM DBT (serving as sole sulfur source)[9] was used.

Preparation of inoculum

On casein starch agar medium the organism (*Streptomyces* sp. VUR PPR 102) was grown for 6 days at 30ºC. Then a homogenous spore suspension in 0.05% Tween 20 (0.2 optical density)[10] was made and used as inoculum (5 mL for 50 mL medium).

Nutrient sources

Carbon sources

The carbon sources employed in the study were Glucose, Sucrose, Starch, Mannitol, Glycerol, Fructose, Cellulose and Maltose.[11] To the BSM supplemented with DBT each carbon source (at concentrations, 1%, 2%, 3%, 4% and 5%) added separately and the organism (5 mL inoculum of spore suspension) was transferred to each medium (of different carbon sources at different concentrations) and grown at 30ºC and pH 7.0.

Nitrogen sources

To the 50 mL of BSM medium (supplemented with DBT as sulfur source) the different nitrogen sources (Urea, Peptone, Ammonium nitrate, Yeast extract, Ammonium chloride, Sodium nitrate, Casein and Potassium nitrate) were added separately each at concentrations, 1%, 2%, 3%, 4% and 5% to study their individual effect[12] on the DBT desulfurization activity. The inoculum of *Streptomyces* sp. VUR PPR 102 (5 mL of spore suspension) was added to each medium (of different nitrogen sources of varying concentrations) and incubated at 30ºC and pH 7.0. Amino acid sources

The various amino acids used for the study include L-Lysine, L-Proline, L-Arginine, L-Cysteine, L-Phenylalanine, L-Methionine, L-Tyrosine, L-Alanine and L-Glutamine.[13] Each amino acid at different concentrations (0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL and 0.5 mg/mL) was added separately to 50 mL of BSM with DBT (which was supplemented with optimum carbon and nitrogen sources). Then the inoculum was added to each medium with one type of amino acid of varying concentrations and cultivated at 30ºC and pH 7.0.

Physical parameters

Temperature

To the 50 mL of the improved medium (with optimum nitrogen, carbon and amino acid sources) the specified volume of inoculum (5 mL spore suspension of the organism) was added and cultivated at 20ºC, 25ºC, 30ºC, 35ºC, 40ºC and 45ºC (at pH 7.0).[14]

pH

The optimized media of different pH environments *viz.,* pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10, 11, and 12[15] were prepared separately. To each optimized medium with a specific pH value (ranging between 3.0 and 12) inoculum (5 mL spore suspension) was added and incubated at 30ºC Incubation period.

In all the DBT desulfurization optimization experiments, after inoculation of the medium with the organism, results were recorded after 6, 10 and 14 days of incubation periods.

Determination and estimation of dibenzothiophene desulfurization activity

The intensity of DBT desulfurization activity of *Streptomyces* sp. VUR PPR 102 by 4S metabolic reaction pathway is directly proportional to the amount of end product (2-HBP). Hence, the rate of DBT desulfurization process exhibited by the organism was denoted in terms of concentration of 2-HBP produced in each experimental set up.

Construction of 2-HBP standard graph to determine the concentration of 2-HBP

The different 2-HBP concentrations (ranging between 1 mg/L and 10 mg/L) were prepared in different test tubes. To each tube specified amount of sodium bicarbonate and Gibb's reagent were added to develop blue colour in all the tubes (with different colour intensities). The Optical Density (OD) for blue colour developed in each tube was determined in a spectrophotometer at 595 nm and the OD values were recorded. A standard graph was prepared using OD values corresponding to different concentrations (of 2-HBP).[16] The amount of 2-HBP released by *Streptomyces* sp. VUR PPR 102 in different optimization experimental step up was measured using standard graph.

RESULTS

Effect of carbon sources (Tables 1-5)

Increased DBT desulfurization activity was observed with increase of glucose amount from 1% to 3% after the incubation periods (after 6, 10 and 14 days). The biodesulfurization activity decreased with further increase of glucose above 3% (i.e., at 4% and 5%). At 3% glucose, maximum DBT desulfurization activity was shown by *Streptomyces* sp. VUR PPR 102 and least activity at 1% of glucose.

The increase of cellulose and fructose concentration from 1% to3% in the medium, enhanced the desulfurization activity in the organism and then there was decrease in biodesulfurization activity above 3% i.e., from 4-5% after 6, 10 and 14 days of incubation. Maximum desulfurization activity was observed at 3% of fructose and cellulose.

The organism showed highest desulfurization activity with the increase of glycerol, sucrose, mannitol, maltose and starch amount from 1% to 2% after the incubation periods (6, 10 and 14 days). Then there was a decrease in DBT desulfurization activity with increase of concentration above 2% of these carbon sources.

The desulfurization activity (2-HBP production) of the organism in the presence of all carbon sources at different concentrations increased with the increase of number of days of incubation period i.e., after 6, 10 and 14 days.

Table 1: Influence of 1% carbon ingredients on 2-HBP production.

Carbon Source (1%)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Glucose	1.63±0.09	1.97±0.05	2.33±0.05
Fructose	0.73±0.12	1.00±0.08	1.26±0.17
Sucrose	0.63±0.05	0.80±0.14	0.97±0.05
Maltose	1.20±0.16	1.50±0.08	1.83±0.12
Starch	1.10±0.08	1.37±0.09	1.60±0.08
Cellulose	0.33±0.05	0.53±0.05	0.73±0.05
Mannitol	0.83±0.12	1.07±0.12	1.27±0.19
Glycerol	0.93±0.05	1.23±0.05	1.40±0.08

Table 2: Influence of 2% carbon ingredients on 2-HBP production.

Carbon Source (2%)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Glucose	1.93±0.05	2.27±0.05	2.63±0.05
Fructose	0.87±0.05	1.10±0.08	1.40±0.08
Sucrose	0.80±0.08	1.03±0.05	1.27±0.12
Maltose	1.43±0.09	1.73±0.12	2.07±0.12
Starch	1.33±0.05	1.57±0.05	1.90±0.08
Cellulose	0.53±0.05	0.67±0.09	0.90±0.14
Mannitol	1.00±0.08	1.27±0.05	1.50±0.08
Glycerol	1.13±0.05	1.40±0.08	1.63±0.17

Table 3: Influence of 3% carbon ingredients on 2-HBP production.

Carbon Source (3%)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Glucose	2.30±0.08	2.73±0.05	2.93±0.09
Fructose	1.10±0.08	1.43±0.12	1.63±0.12
Sucrose	0.83±0.05	1.17±0.05	1.37±0.05
Maltose	1.03±0.12	1.37±0.19	1.57±0.09
Starch	0.93±0.05	1.23±0.05	1.43±0.05
Cellulose	0.70±0.08	0.85±0.05	1.17±0.05
Mannitol	1.27±0.05	1.47±0.09	1.70±0.14
Glycerol	1.37±0.12	1.57±0.12	1.93±0.05

Table 4: Influence of 4% carbon ingredients on 2-HBP production.

Carbon Source (4%)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Glucose	2.03±0.05	2.27±0.12	2.67±0.09
Fructose	0.93±0.12	1.27±0.09	1.43±0.05
Sucrose	0.73±0.05	1.04±0.12	1.10±0.14
Maltose	0.83±0.09	1.07±0.05	1.40±0.12
Starch	0.63±0.12	0.83 ± 0.05	1.03±0.09
Cellulose	0.63±0.12	0.79±0.17	0.97±0.19
Mannitol	1.13±0.05	1.43±0.05	1.63±0.12
Glycerol	1.27±0.09	1.47±0.08	1.77±0.05

Table 5: Influence of 5% carbon ingredients on 2-HBP production.

Carbon Source (5%)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Glucose	1.83±0.12	2.20±0.08	2.53±0.09
Fructose	0.83±0.05	1.22±0.05	1.36±0.09
Sucrose	0.61±0.05	0.96±0.08	1.24±0.08
Maltose	0.73±0.09	1.01±0.09	1.34± 0.08
Starch	0.57±0.05	0.79 ± 0.08	0.99±0.09
Cellulose	0.53±0.12	0.74± 0.09	0.93±0.08
Mannitol	1.03±0.05	1.33±0.05	1.53±0.08
Glycerol	0.92±0.08	1.42±0.08	1.67±0.09

Optimization of nitrogen sources (Tables 6-10)

In all the 1% nitrogen sources, the test organism had shown the highest biodesulfurization activity and then with the increase of concentration of nitrogen sources (1-5%) there was a gradual decrease of desulfurization activity after the specified incubation periods. The lowest 2-HBP production was observed with all nitrogen ingredients of 5%. There was an increase in 2-HBP production with all nitrogen sources (at all concentrations) with the increase of incubation periods (from 6-14 days).

Table 6: Effect of 1% Nitrogen sources on 2-HBP production.

Nitrogen source (1%)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Peptone	1.70±0.08	1.90±0.08	2.10±0.08
Yeast extract	2.17±0.17	2.33 ±0.05	2.53±0.12
Casein	1.80±0.08	1.97 ± 0.12	2.17±0.09
Urea	1.60±0.08	1.83±0.09	2.00±0.08
Ammonium chloride	2.03±0.12	2.23±0.09	2.40±0.14
Ammonium nitrate	2.43±0.17	2.67±0.12	2.97±0.09
Sodium nitrate	2.30±0.08	2.53±0.09	2.70±0.08
Potassium nitrate	3.53±0.12	4.07±0.17	4.80±0.08

Table 7: Effect of 2% Nitrogen sources on 2-HBP production.

Nitrogen source (2%)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Peptone	1.53±0.09	1.73±0.05	1.90±0.08
Yeast extract	1.90±0.08	2.13±0.17	2.33±0.09
Casein	1.63 ±0.05	1.77±0.12	1.97±0.09
Urea	1.27±0.12	1.50±0.08	1.73±0.12
Ammonium chloride	1.77±0.05	1.97±0.09	2.23±0.05
Ammonium nitrate	2.17±0.09	2.37±0.05	2.60±0.08
Sodium nitrate	1.97±0.12	2.20±0.14	2.43±0.09
Potassium nitrate	2.33±0.12	2.47±0.12	2.67±0.09

Table 8: Effect of 3% Nitrogen sources on 2-HBP production.

Nitrogen source (3%)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Peptone	1.43±0.17	1.63±0.12	1.80±0.09
Yeast extract	1.83±0.05	1.97±0.05	2.15± 0.05
Casein	1.53±0.12	1.67±0.05	1.90±0.05
Urea	1.20±0.16	1.40±0.14	1.58±0.12
Ammonium chloride	1.70±0.08	1.90±0.14	2.10±0.08
Ammonium nitrate	2.07±0.05	2.30±0.08	2.54± 0.05
Sodium nitrate	1.90±0.08	2.07±0.17	2.33±0.12
Potassium nitrate	2.23±0.05	2.40±0.08	2.23±0.09

Table 9: Effect of 4% Nitrogen sources on 2-HBP production.

Nitrogen source (4%)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Peptone	1.30±0.08	1.47±0.09	1.67±0.05
Yeast extract	1.73±0.05	1.87±0.05	2.07±0.12
Casein	1.43 ± 0.17	1.63±0.09	1.77±0.05
Urea	1.13±0.05	1.30±0.08	1.50±0.08
Ammonium chloride	1.63±0.12	1.80±0.08	1.97±0.05
Ammonium nitrate	2.00±0.08	2.17±0.12	2.43±0.05
Sodium nitrate	1.83±0.12	1.97±0.05	2.23±0.09
Potassium nitrate	2.10±0.08	2.30±0.14	2.47±0.12

Table 10: Effect of 5% Nitrogen sources on 2-HBP production.

Nitrogen source (5%)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Peptone	1.23±0.17	1.43±0.05	1.57±0.12
Yeast extract	1.53±0.12	1.67± 0.12	1.90±0.08
Casein	1.27±0.09	1.47±0.05	1.70±0.08
Urea	1.03 ± 0.17	1.20±0.08	1.43±0.05
Ammonium chloride	1.37±0.12	1.57±0.12	1.77±0.09
Ammonium nitrate	1.70±0.08	1.90±0.08	2.07±0.09
Sodium nitrate	1.57±0.05	1.80±0.08	1.97±0.12
Potassium nitrate	1.83±0.05	1.97±0.05	2.20±0.14

Optimization of amino acids (Tables 11-15)

The *Streptomyces* had exhibited an increased 2-HBP production with increase of amount of lysine, arginine and methionine from 0.1 mg/mL to 0.2 mg/L after specified incubation periods and above 0.2 mg/mL concentration of these amino acids resulted in a gradual decrease in DBT desulfurization activity.

Better 2-HBP production was observed at 0.1 mg/mL concentration of phenylalanine, alanine and tyrosine and further, there was a gradual decrease in desulfurization activity with the increase of concentration of these amino acids (0.2 mg/mL to 0.5 mg/L) at all incubation periods.

An enhanced 2-HBP production was observed with increase of the amount of glutamine and proline from 0.1 to 0.3 mg/mL after the specified incubation periods. Further increase of proline and glutamine concentration decreased DBT biodesulfurization activity. The highest 2-HBP production was recorded with glutamine and proline at a concentration of 0.3 mg/L and least at 0.1 mg/mL.

The organism had not produced 2-HBP (no desulfurization activity) in the presence of cysteine at all concentrations.

Table 11: Effect of 0.1 mg/mL amino acid sources on 2-HBP production.

Amino acid (0.1 mg/mL)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Methionine	3.40±0.16	4.03±0.12	4.53±0.12
Phenylalanine	4.03±0.12	4.50±0.08	4.90±0.14
Tyrosine	4.43±0.12	4.90±0.08	5.37±0.09
Alanine	4.63±0.24	5.13±0.19	5.57±0.12
Arginine	4.23±0.17	4.70±0.14	5.10±0.14
Lysine	3.83±0.12	4.33±0.17	4.73±0.17
Proline	4.80±0.14	5.33±0.12	5.83±0.12
Glutamine	5.03 ± 0.12	5.57±0.12	6.10±0.14
Cysteine	-	-	-

Table 12: Effect of 0.2 mg/mL amino acid sources on 2-HBP production.

Amino acid (0.2 mg/mL)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Methionine	3.63±0.05	4.23±0.12	4.56±0.16
Phenylalanine	3.70±0.14	4.10±0.22	4.50±0.08
Tyrosine	4.00±0.08	4.53±0.09	4.93±0.12
Alanine	4.93±0.09	5.37±0.17	5.87±0.09
Arginine	4.47±0.17	4.93±0.09	5.47±0.09
Lysine	4.07±0.09	4.57±0.09	5.07±0.19
Proline	5.13±0.09	5.60±0.08	6.13±0.09
Glutamine	5.37±0.12	5.90 ± 0.08	6.57±0.17
Cysteine	-	-	-

Table 13: Effect of 0.3 mg/mL amino acid sources on 2-HBP production.

Amino acid (0.3 mg/mL)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Methionine	3.83±0.05	4.33±0.19	4.63±0.19
Phenylalanine	3.47±0.09	3.87±0.12	4.23±0.21
Tyrosine	3.63±0.05	4.03±0.05	4.47±0.21
Alanine	4.70±0.08	5.23±0.17	5.77±0.09
Arginine	4.17±0.12	4.73±0.19	5.17±0.17
Lysine	4.00±0.08	4.53±0.09	4.93±0.09
Proline	5.37±0.12	5.87±0.12	6.37±0.17
Glutamine	5.67±0.12	6.20±0.22	6.77±0.12
Cysteine	-	-	-

Table 14: Effect of 0.4 mg/mL amino acid sources on 2-HBP production.

Amino acid (0.4 mg/mL)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Methionine	3.37±0.12	3.87 ± 0.09	4.50±0.08
Phenylalanine	2.87±0.05	3.30±0.14	3.77±0.21
Tyrosine	3.10±0.14	3.57±0.05	4.13±0.19
Alanine	4.33±0.09	4.90±0.16	5.47±0.19
Arginine	3.90±0.08	4.50±0.08	5.07±0.09
Lysine	3.67±0.09	4.23±0.05	4.80±0.16
Proline	4.97±0.17	5.43±0.12	5.90±0.22
Glutamine	5.20±0.16	5.70±0.14	6.20±0.14
Cysteine	-	-	-

Table 15: Effect of 0.5 mg/mL amino acid sources on 2-HBP production.

Amino acid (0.5 mg/mL)	2-HBP concentration (mg/L)		
	Incubation periods		
	6 days	10 days	14 days
Methionine	3.13±0.09	3.60±0.08	4.13±0.09
Phenylalanine	2.63±0.05	3.10±0.08	3.63±0.12
Tyrosine	2.90±0.08	3.33±0.09	3.87±0.12
Alanine	4.03±0.05	4.43±0.05	4.93±0.05
Arginine	3.60±0.16	4.07±0.09	4.63±0.05
Lysine	3.33±0.19	3.80±0.08	4.30±0.08
Proline	4.67±0.09	5.13±0.09	5.70±0.08
Glutamine	4.93±0.05	5.50± 0.12	6.07±0.09
Cysteine	-	-	-

Effect of Physical parameters (temperature and pH)

Temperature

The organism had shown the highest desulfurization activity at 30ºC and lowest at 45ºC after the specified incubation periods (6, 10 and 14 days) (Figure 1). There was a gradual increase of activity from 20-30ºC and a decrease from 30-45ºC.

pH

Maximum 2-HBP production was reported at pH 7.0 after the incubation periods (6, 10 and 14 days) (Figure 2). The *Streptomyces* sp. VUR PPR102 exhibited a gradual increase of 2-HBP production from pH 3.0 to 7.0 and decrease from pH 7.0 to 12.

DISCUSSION

The influence of various nutrient ingredients (different types of carbon and nitrogen components and various amino acids) and physical parameters on dibenzothiophene desulfurization activity of *Streptomyces* sp. VUR PPR 102 was studied in the present work. The BSM medium (with DBT as sulfur source) was employed to make all the experimental studies. In the medium the DBT serves as the only source of sulfur. The desulfurization activity was measured in terms of the amount of 2-HBP produced by the organism.

The glucose at all concentrations served as best carbon source for enhanced desulfurization activity over other carbon sources. Of all the concentrations, the glucose of 3% was determined as best for enhanced production of 2-HBP by *Streptomyces* sp. VUR PPR 102. The experimental studies of Gokhan *et al.*[17] revealed that the desulfurizing bacterium, *Sulfobolus solataricus* P2 exhibited increased growth and physiological activities in the presence of glucose when compared to other carbon sources. The glucose acts as starting substrate for glycolysis (the primary event of energy yielding process

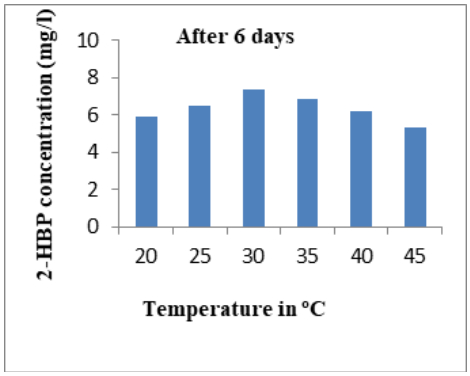

Figure 1A

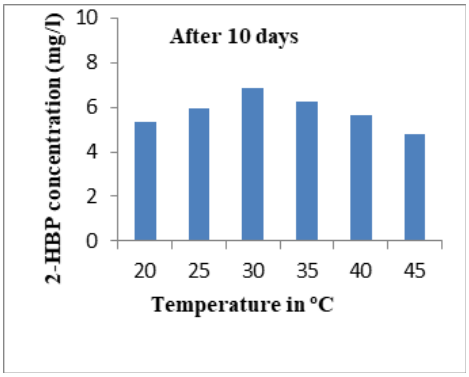

Figure 1B

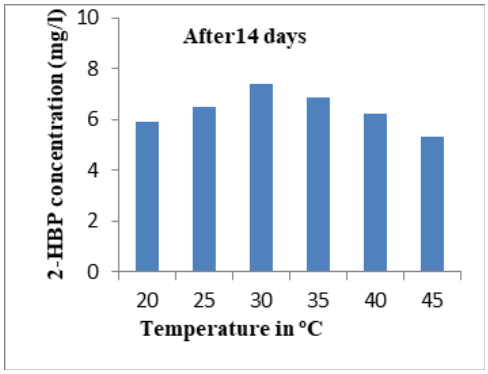

Figure 1C

Figure 1: Effect of temperature on 2-HBP production by *Streptomyces* sp. VUR PPR 102 after 6 days (B) 10 days and (C) 14

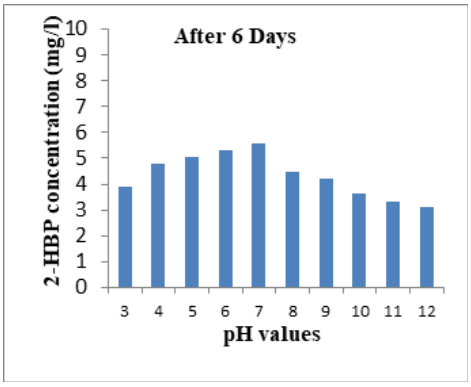

Figure 2A

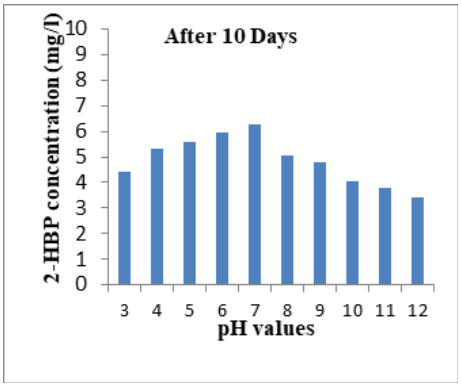

Figure 2B

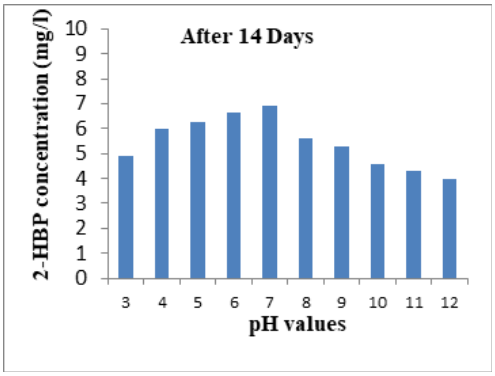

Figure 2C

Figure 2: Effect of pH on 2-HBP production by *Streptomyces* sp. VUR PPR 102 after 6 days (B) 10 days and (C) 14 days.

of respiration) hence, serves as a better carbon source. Similarly, Gunam *et al*.[18] reported in their research work that, *Agrobacterium tumefaciens* LSU20 exhibited enhanced desulfurization when the medium was supplemented with glucose.

Enhanced 2-HBP production by *Streptomyces* sp. VUR PPR 102 was observed at 1% potassium nitrate. In other earlier studies, *Streptomyces* species and potassium nitrate in other microbes were reported to be the best nitrogen source. Nitrate was found to be widely used as nitrogen source for most of the *Streptomyces* species.[19] *Streptomyces anandii var. taifiensis* had shown maximum growth in potassium nitrate-containing medium.[20] In the presence of potassium nitrate the *Streptomyces aureofaciens*[21] and *Streptomyces mesioensis*[22] exhibited maximum antibiotic production.

The *Streptomyces* sp. VUR PPR 102 had shown maximum 2-HBP production with 0.3 mg/L glutamine and no 2-HBP production (i.e., desulfurization activity was not observed) when grown in optimized medium with cysteine which is a sulfur containing amino acid. The research studies of Agarwal *et al*.[23] revealed that when *Rhodococcus erythropolis* and *Gordonia alkanivorans* were grown in the medium containing DBT and cysteine, both the organisms did not exhibit any desulfurization activity. These two organisms used cystiene as sole source of sulfur as they can use cysteine with less energy when compared to energy required for the utilization of DBT. In the presence of all types of amino acids in optimized medium, the *Streptomyces* sp. VUR PPR 102 had exhibited the enhanced 2-HBP production except with cysteine and methionine. In the presence of cysteine, there was no utilization of DBT by the organism, thus no desulfurization activity and with methionine decreased desulfurization activity was observed. When the medium is supplemented with DBT and cysteine, the cysteine was preferentially used by *Streptomyces* as it serves as the best nutrient source (sulfur source) when compared to DBT.[24] In the presence of methionine (a sulfur containing amino acid), some (decreased) desulfurization activity (production of 2-HBP) was observed. This may due to though methionine contains sulfur, it may not serve as a complete source for the synthesis of all sulfur containing precursors in the organism. Hence, the *Streptomyces* sp. VUR PPR 102 had utilized both methionine and DBT and so there was some desulfurization activity in the presence of methionine.[25]

The *Streptomyces* sp. VUR PPR 102 exhibited highest 2-HBP production at pH 7.0 and 30^{0}C. Mohammad *et al*.[26]

reported that when the *Stenotrophomonas maltophilia* strain KHO1 was cultivated in the DBT containing medium at pH 7.0 and 30ºC, it had utilized DBT and produced maximum 2-HBP (indication of enhanced desulfurization activity). Many research studies revealed that *Streptomyces* species usually grow best at pH 7.0 and 30ºC and produce maximum amount of metabolites. The *Streptomyces galbus* had exhibited best growth and enhanced antifungal production at pH 6.8 and 30ºC.[27]

CONCLUSION

In the present study, the nutrient components, amino acid sources and physical parameters pertaining to the desulfurization activity of *Streptomyces* sp. VUR PPR 102 were optimized. The best carbon and nitrogen sources for enhanced 2-HBP production were found to be glucose (3%) and potassium nitrate (1%), respectively. The optimum amino acid source for the desulfurization activity was reported to be glutamine (0.3 g/mL). An optimized medium was prepared using these best nutrient ingredients and the organism was grown at different pH and temperature conditions to determine the optimum pH and temperature for optimum desulfurization activity. The best pH and temperature for the enhanced 2-HBP production were found to be pH 7.0 and 30ºC, respectively. Further the genes of 4S DBT desulfurization pathway in *Streptomyces* sp. VUR PPR 102 can be improved by genetic engineering tools for enhanced biodesulfurization activity. And such genetically engineered strain can be eploited on large scale for fuel refining.

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CONFILCT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DBT: Dibenzothiophene; **HDS:** Hydrodesulfurization; **BDS:** Biodesulfurization; **2-HBP:** 2-hydroxy biphenyl; **BSM:** Basal salt medium.

SUMMARY

Industries and Automobiles utilize petroleum products and emit various deleterious oxides into the environment. The sulfur dioxide is one of those deleterious oxides and adversely affect humans, animals and plants. The sulfur dioxide is released due to the oxidation of sulfur-containing organic compounds occurring in fuels. The petroleum refineries use a conventional hydrodesulfurization approach to separate and remove sulfur content from crude petroleum products. The hydrodesulfurization is not an eco-friendly process as it uses high temperature and pressure. The researchers and scientists suggested a biodesulfurization process, which is eco-friendly for selective elimination of sulfur from fuels. The biodesulfurization method involves the application of microorganisms for selective elimination of sulfur content from sulfur-containing organic compounds. Among organosulfur compounds present in fossil fuels, the dibenzothiophene is regarded as model compound for biodesulfurization experiments due to its high non-biodegradable nature. The microorganisms which release sulfur element specifically from dibenzothiophene by 4S metabolic reaction pathway are commercially important strains. At the end of 4S reaction pathway, 2-HBP and sulphite are formed as end products. In fact, the sulfur present in the dibenzothiophene is released in the form of sulfite. During 4S pathway the dibenzothiophene ring structure is not degraded and the 2-HBP which is formed as end product is an intact ring structured compound and hence, mileage of the petroleum product is not changed. In the present work, the nutrients and physical parameters *viz.,* temperature and pH were optimized for enhanced dibenzothiophene biodesulfurization activity by *Streptomyces* sp. VUR PPR 102 exhibits a desulfurization pathway via 4S metabolic pathway. The optimum nutrient sources (carbon and nitrogen) for the highest dibenzothiophene desulfurization activity were reported to be glucose (3%) and potassium nitrate (1%). The glutamine (0.3 mg/mL), which enhanced DBT desulfurization activity, was reported as the best amino acid. The optimum physical parameters were reported to be 30ºC and pH 7.0 for maximum biodesulfurization.

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