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SULFIDE OXIDATION IN A BATCH FLUIDIZED BED BIOREACTOR USING IMMOBILIZED CELLS OF ISOLATED THIOBACILLUS SP. (IICT-SOB-DAIRY-201) AS BIOCATALYST

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Abstract

A study is conducted to test the biological conversion of sulfides using immobilized cells of *Thiobacillus sp.* in fluidized bed bioreactor. The *Thiobacillus sp* are isolated from aerobic sludge of distillery and dairy effluent treatment plant using standard methods of isolation and enrichment. Experiments were conducted in a batch fluidized bed bioreactor using calcium alginate immobilized cells of isolated *Thiobacillus sp.* The batch fluidized bed bioreactor is operated for 168 hours with an initial sulfide concentration of 150 mg/l, at the end of 168 hours 100% sulfide oxidation is achieved by the continuous supply of sterile air as oxidant. During the operational period, biomass concentration, pH, sulfate formation is recorded.

Keywords: Sulfide, *Thiobacillus* sp., Immobilization, Fluidized bed bioreactor.

1. Introduction

Use of chemoautotrophic microorganism for the oxidation of sulfide is advantageous due to their simple nutritional requirement. A number of microbiological processes utilizing bacteria of the Thiobacillus genus for the oxidation of sulfide have been

Nomenclatures

A	Ml of iodine solution
B	Normality of the iodine solution
C	Ml of $Na_2S_2O_3$ solution
D	Normality of $Na_2S_2O_3$ solution
SOB	Sulfur oxidizing bacteria
FBR	Fluidized bed bioreactor
CSTR	Continuous stirred tank reactor
DIST	Distillery
H_2S	Hydrogen sulfide
SEM	Scanning electron microscope

described in literature [3, 4]. *Thiobacillus sp*. is obligate autotrophic, acidophilic, sulfur oxidizing bacterium and belongs to *Thiobacilli* genera. It exhibits sulfide-oxidizing capacity in a wide range of pH and had a potentiality to be used in biological sulfide treatment. It converts inorganic sulfur into its reduced forms and also grows and oxidizes reduced sulfur compounds. Conversion path of sulfide to elemental sulfur by *Thiobacillus sp*. is almost equal to stoichiometry [5, 6] and the produced elemental sulfur is further oxidized to sulfuric acid with sulfite and sulfate as intermediate products.

The sulfur oxidizing bacterium *Thiobacillus* sp. was first isolated in 1922 from soil [6]. The relevant bacteria are the gram negative, aerobic, rod shaped, motile cells, grow well at 20-30^{0}C and a pH of around 2 to 5. The growth of *Thiobacillus sp*., a chemoautotrophic bacterium, is dependent on CO_2 as a carbon source with energy obtained by the oxidation of inorganic sulfur compounds. Various researchers have studied the oxidation of sulfide in reactors like CSTR, packed bed reactor using isolated strains [7, 8, 9].

The two most important bioconversions of sulfide oxidation system are;

$$2HS^- + O_2 \rightarrow 2S^o + 2OH^- \quad (1)$$

$$2HS^- + 4O_2 \rightarrow 2SO_4^{2-} + 2H^+ \quad (2)$$

The formation of sulfur is preferred because it is insoluble and can be easily recovered from the water stream. Since, formation of sulfur is the preferred step in the sulfide oxidation, there is a great need to avoid the direct contact between elemental sulfur and free *Thiobacillus* sp. in the reactor. Results from earlier studies in our laboratory showed that, the CSTR with immobilized beads will results in breaking

of the beads and further sulfate formation in the CSTR was increased. Hence, in the current study, the *Thiobacillus sp*. are isolated from aerobic sludge of effluent treatment plant treating sulfate-containing wastewater and used for sulfide oxidation in a batch fluidized bed bioreactor with Ca-alginate immobilized cells.

2. Materials and Methods

2.1 Collection of Microbial Source

Isolation of sulfide oxidizing bacteria is done from aerobic sludge collected from dairy and distillery industry wastewater treatment plant. The aerobic sludge samples of aforesaid industries were collected and screened for the removal of big particles. Then the sludge is kept in aerobic conditions by continuous aeration in order to prevent growth of any anaerobic bacteria for a period of 7 days at temperature of $30+2^0$C.

2.2 Isolation of *Thiobacillus sp*

The sludge is then kept for activation by mixing sulfide oxidizing bacteria (SOB), *Thiobacillus* enrichment media [10] having following composition NH_4Cl, 1.0 gm; K_2HPO_4, 0.6 gm; $CaCl_2.2H_2O$, 0.2 gm; $FeCl_3.H_2O$, 0.02 gm; $Na_2S_2O_3.5H_2O$, 10 gm; $CaCl_2.2H_2O$, 100mg; $ZnSO_4.7H_2O$, 88 mg; $CuSO_4.5H_2O$, 40 mg; $MnSO_4$, 15 mg; $Na_2B_4O_7$, 10 mg; distilled water, 1000 ml; pH, 5.

The sludge is kept for aeration with the above said medium for a period of 7 days and after 7 days of activation period, media is replaced by fresh media. The aforesaid process is repeated for five transformations, to ensure the suppression of growth of any anaerobic bacteria in the sludge and to activate only sulfide oxidizing bacteria.

After acclimatizing the sludge to *Thiobacillus* media for a period of one month, it is used as source for the isolation of SOB of Thiobacillus sp. The media, which is used for the activation of SOB, has also been used for the isolation studies with 15% of agar and sterilized at 120^0C and 15 lbs pressure for a period of 20 minutes. Lukewarm media is poured into Petri plates and are streaked with loop full of previously activated aerobic sludge. The plates are incubated at $30+2^0$C in dark to eliminate any carbon fixing photosynthetic contaminants for a period of 7 days. After seven days of incubation fresh media is prepared and the 2 to 3 colonies, grown in previous step is inoculated and kept in incubator and this process is repeated for 3 times. The colonies from the above plates are inoculated into liquid broth of 100 ml. The flasks are kept in an orbital shaker at 250 rpm for a period of 7days. After seven days, fresh media is prepared and inoculated with (20% v/v) into freshly prepared media under same conditions as mentioned earlier. This process is repeated for 4 times in order to get pure cultures of *Thiobacillus sp.* Later the cultures are named as IICT-SOB-DAIRY-201 for the cultures isolated from dairy effluent treatment plant sludge and IICT-SOB-DIST-210 for the cultures isolated from distillery spent wash treatment plant sludge.

2.3 Enumeration and Characterization

Five Days old cultures of the two isolated strains are studied under scanning electron microscope for morphological characteristics like size and shape and also gram stain. Standard plate count method is used for the colony count at different serial dilution ranging from 10^{-1} to 10^{-10}.

2.4 Immobilization of *Thiobacillus sp*

The cultures grown in 100 ml of thiosulfate media is centrifuged at 7500 rpm for 10 minutes. After centrifugation the bacterial cells are separated by decanting the supernatant aseptically and stored in a vial for further use at 4 ^{0}C. The bacterial cell pellet is washed thrice with sterile double distilled water. The cells are then added to 4% sodium alginate solution and dropped into 4 % $CaCl_2$ solution using peristaltic pump to get approximately 2 mm diameter beads. The beads are then activated in buffer solution for 5 hours [10] and the whole process is carried out in aseptic conditions to prevent any possibility of contamination.

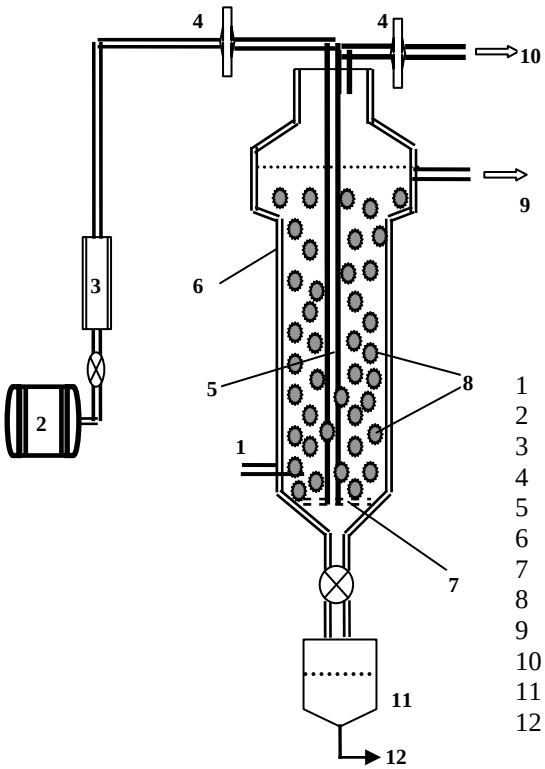
2.5 Sulfide Oxidation Activity Test in Batch Fluidized Bed Reactor with Immobilized Cells of IICT-SOB-DAIRY-201

Test for the sulfide oxidation activity for the isolated SOB IICT-SOB-DAIRY-201 is carried out in a batch fluidized bed reactor operated at 150 mg/l of initial sulfide concentration using immobilized cells of *Thiobacillus sp.* Isolated *Thiobacillus* cultures are maintained on a maintenance media having composition of $Na_2S_2O_3.5H_2O$, 10 gm; $MgCl_2.6H_2O$, 0.1 gm, NH_4Cl, 01 gm; $CaCl_2$, 0.1 gm; KH_2PO_4, 3 gm in one liter doubled distilled water and at pH 5 using 1N HCl solution.

2.6 Batch Fluidized Bed Reactor Operation

Fluidized bed reactor made up of glass has been used in the present experiments. The schematic setup of batch fluidized bed bioreactor is shown in Fig. 1. The various operational conditions for the batch fluidized bed bioreactor using immobilized cells of *Thiobacillus* sp. are shown in table 1. Synthetic media is prepared using sodium sulfide flakes are used as sulfide source. 10 ml of trace elements solution defined previously is added to each reactor as micronutrient source.

Beads along with the synthetic media are transferred to the reactor and the reactor is maintained in fluidized state through out the experiment by continuous supplying of sterile air using air pumps. The samples are drawn at 24 hours frequency and analyzed for various parameters.



1 : Feed inlet
2 : Air pump
3 : Rotameter
4 : Air filter
5 : Draft tube
6 : FBR
7 : Perforated tube
8 : Immobilized beads
9 : Treated effluent outlet
10 : Air outlet
11 : Sulfur settler
12 : Drain

Fig. 1. Schematic setup of batch fluidized bed bioreactor with immobilized cells of *Thiobacillus* sp.

2.7 Analytical Methods

Standards methods [12] are used for the analysis of the sulfide, pH, sulfate and temperature in the liquid media. Sulfide estimation: To 5 ml of sample 3 drops of 0.025 N standard Iodine solution, 2-3 drops of starch indicator and 2-3 drops of 6 N HCl were added in a 200 ml conical flask. Titrated against 0.025 N Na_2SO_3 till sample turns into colorless from typical blue colour. Sulfide was calculated using Eq. (3)

$$Mg / L / S^2 = \frac{\left[\left(A \times B\right) - \left(C \times D\right)\right] \times 16000}{ML \ of \ \ sample} \tag{3}$$

Sulfate estimation: 10 ml sample was taken into 250 ml Erlenmeyer flask and added exactly 5 ml conditioning reagent and stirred along with the addition of 5 gm of Barium chloride crystals. The turbidity of the solution was measured at 420 nm in spectrophotometer (Perkin Elmer, Lambda 25, USA). The amount of SO_4^{2-}

concentration in μg was calculated from the standard graph of sulfate. The pH was measured by multi parameter instrument (Denver, Model 20, USA)

Table. 1. Fluidized bed reactor specifications and operational parameters.

Unit/component	Dimension
Height of the FBR	54 cm
Internal Diameter of the FBR	6 cm
Draft tube height	52 cm
Draft tube diameter	6 mm
Total volume of the reactor	1500 ml
working volume of the reactor	1200 ml
Packed bed height	16 cm
Fluidized bed height	48 cm
Minimum fluidization air flow rate	$1.44x10^{-4}$ m^3/hr
Air filter, PTFE, GELMAN	0.2 μ M
Rotameter, Engineers India ltd	0.5 - 5 m^3/hr
Air pump,	$0.5x10^{-4}$ to $4x10^{-4}$ m^3/hr
Temperature	30 ± 5^0C
Initial pH	6.2
Average immobilized beads size	~2 mm

3. Results and discussion

3.1 Enumeration and Characterization

Standard plate count method is used for the colony count at different serial dilutions ranging from 10^{-1} to 10^{-10} and the cell count is in the range of $7x10^5$ and $4x10^7$ cells/ml for IICT-SOB-DAIRY-201 and IICT-SOB-DIST-210 respectively. The results of gram staining for both the isolated SOB from their native sources are negative.

Figure 2 shows the Scanning Electron Microscope (SEM) image of the isolated *Thiobacillus sp.* (IICT-SOB-DAIRY-201) at 50 K magnification

3.2 Sulfide Oxidation Studies in Batch FBR

Figure 3 shows the sulfide oxidation by isolated IICT-SOB-DAIRY-201 of Sulfide oxidizing bacteria in batch fluidized bed bioreactor during 168 hours of operation. Initially sulfide concentration in the fluidized bed bioreactor at zero hours of reactor operation is 150 mg/l and at the end of 168 hours it is oxidized completely. This shows that the sulfide is completely utilized by the isolated IICT-SOB-DAIRY-201 Sulfide oxidizing bacteria (SOB).

Fig. 2. Scanning electron microscope (SEM) image of isolated *Thiobacillus* sp. (IICT-SOB-Dairy-201)

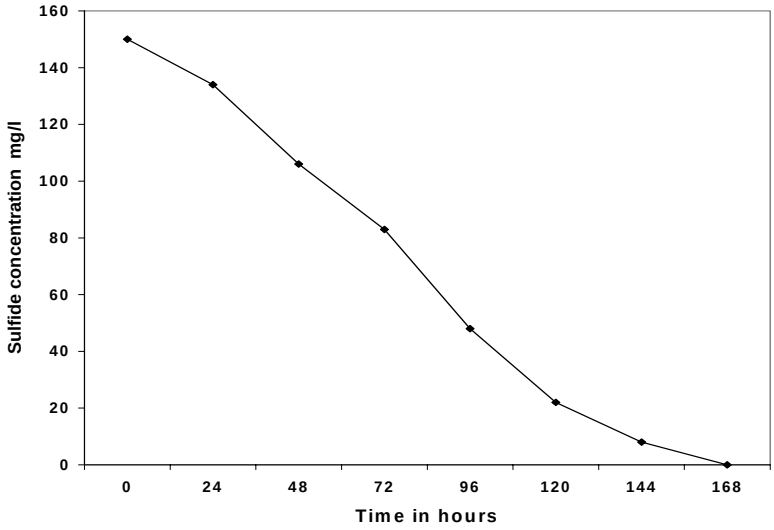


Fig. 3. Sulfide oxidation in batch fluidized bed bioreactor using isolated IICT-SOB-DAIRY-201.

Figure 4 shows the sulfate formation by isolated strains of SOB IICT-SOB-DAIRY-201 in batch fluidized bed bioreactor during 168 hours of operation. From the figure 2 it is evident that at the end of 168 hours of reactor operation, the sulfate formation is 38 mg/l, but during the operation of reactor, at 72 to 144 hors of reactor operation the sulfate formation is in the range of 59 to 45 mg/l, which is higher than the sulfate formation at 168 hrs of reactor operation. It is envisaged from this study that as the sulfide concentration in the reactor depletes the microbes start feeding on sulfate as their energy source. According to the biological sulfur cycle, upon sulfide oxidation [5, 6] elemental sulfur will form immediately. This can be observed in the reactor in the form of pale yellow crystals. As the sulfide in the reactor decreases the microbes consumes elemental sulfur and sulfate for their survival [8, 9].

Figure 5 shows the change in pH with time during the sulfide oxidation in batch fluidized bed bioreactor. Initially at the starting of reactor operation the pH is 5 and at the end of 168 hours it is 3.1. The decrease in pH is because of the formation of oxidative products of sulfide like sulfate, thiosulfate and sulfuric acid [5].

4. Conclusions

The study indicates that isolation and enrichment of SOB's from different sources is highly essential for enhanced performance of the fluidized bed bioreactor using immobilized cells of *Thiobacillus sp*. In batch reactor when the sulfide concentration decreases more sulfates will be formed and ultimately the sulfate will be oxidized and the system turns to acidic phase.

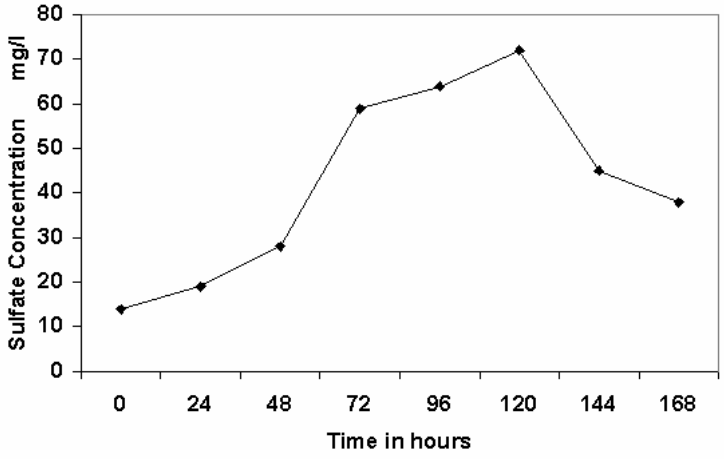


Fig. 4. Formation of sulfate in batch fluidized bed bioreactor using IICT-SOB-DAIRY-201.

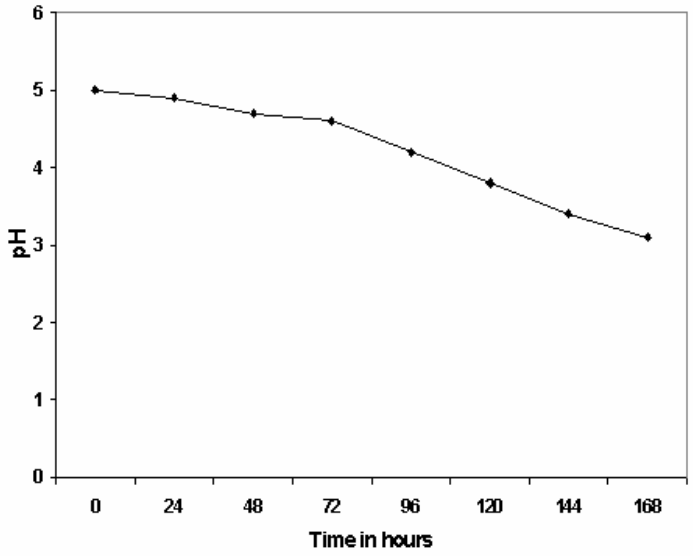


Fig. 5. Change in pH in batch fluidized bed bioreactor with isolated IICT-SOB-DAIRY-201.

Further work is in progress to assess the performance of this system and to generate sulfur as end product using isolated cultures. The results from the study also indicate the possible isolation of *Thiobacillus* cultures from native source and their application in the full-scale reactor.

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