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ORIGINAL ARTICLE

Microbial Desulphurization of Crude oil Using Immobilized Spores of *Aspergillus flavus*

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ABSTRACT

The ability of the immobilized spores of *Aspergillus flavus* cultured in increasing concentrations of sodium-metabisulphite to remove sulphur from crude oil was investigated. When 50g of immobilized spores of *A. flavus* was added to crude oil for one, two, three and seven days, the amount of sulphur removed were 27.2%, 45.2%, 90.4% and 91.7% respectively. When 50g of immobilized spores of *A. flavus* was introduced into the crude oil for seven days at 35ºC, 40ºC and 45ºC, the amount of sulphur removed were 63.2%, 55.3% and 10.5% respectively. Lastly, when 10g, 50g and 100g of immobilized spores of *A. flavus* were added to crude oil for seven days, the amount of sulphur removed were 49.6%, 94.7% and 53.9% respectively. Hence, *A. flavus* can be used to reduce sulphur contents in crude oil to a minimal level.

Key words: Microbial Desulphurization, Immobilized spores, Metabisulphite, Bio-desulphurization.

Introduction

Crude oil is a complex mixture of a large number of organic compounds including sulphur compounds. Most products of crude oil contain sulphur, especially the low grade petroleum fuel. When they are burnt, they produce sulphurdioxide [19,3]. The total sulphur content of crude oil varies from reservoir to reservoir. Sulphur compounds form the largest group of non-hydrocarbon compounds and are by far the most important and expensive for the refiner to deal with [17]. Sulphur in crude oil plays a critical role in atmospheric pollution as it occurs in the form of acidic gases such as sulphurdioxide which may be harmful to health [19]. Environmental pollution and acid rain caused by the release of sulphurdioxide on combustion of sulphur-containing fossil fuels has brought about regulations for production of low sulphur in fuels. The level of sulphur in fuels is regulated to reduce sulphur related air pollution. To meet these regulations, sulphur must be removed from fuels during refining process. Organic sulphur in crude oil is removed by refineries using hydrodesulphurization – HDS [10,20]. The fact that Hydrodesulphurization can only remove the bulk of inorganic sulphur and simple organic sulphur but inadequate to produce low sulphur fuels due to the fact that the complex polycyclic sulphur compound present in petroleum and coal is not removed by the process (HDS) accounts for the introduction of microbial desulphurization [13,15]. Microbial desulphurization is a process where sulphur content of crude oil is exposed to micro-organisms that can specifically break carbon-sulphur bond, thereby releasing sulphur in a water soluble, organic form. Actions of micro-organisms on crude oil component transform contaminants into non-toxic compounds [16,4,8,9]. Various components of crude oil can be

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used as a source of carbon and energy by micro-organisms [5,3]. *Rhodococcus spp.* and *Arthrobacter sulphureus* are bacteria used in desulphurization of fossil fuel [12]. They are isolated from oil contaminated sludge/soil. They are used for reducing the sulphur content of diesel samples, thereby indicating their potential for use as biocatalysts in desulphurization of fossil fuels [14]. Immobilization method is used to entrap the spores of fungi so that it can be used for biodesulphurization. Immobilization makes organisms remain in their natural state.

Materials and methods

Collection of Samples:

The experiment was carried out in the Research Laboratory of Department of Pure and Applied Biology, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria. Crude oil was collected from an oil producing company (Shell Petroleum Company Warri, Delta State, Nigeria). The material comprises oily wastes such as drill cutting, tank bottom sludge and pure crude oil.

Training of Organisms:

The organism used for this research work was an isolate of *A. flavus.* The organism was originally isolated from an oil contaminated environment. To train *A. flavus,* minimal salt broth was prepared by weighing 2.0g NH_4NO_3, 1.5g KH_2PO_4, 0.5g KCl, 1.5% glucose. 0.05g yeast extracts into 1000ml distil water; sodium metabisulphite ($Na_2S_2O_5$) was added at different concentrations of 1g/l, 3g/l, 5g/l, 10g/l and 15g/l. The broth was poured into bottles and sterilized in an autoclave. The organism was cultured in the minimal salt broth with the increasing concentration of sodium metabisulphite being the source of energy. The culture was incubated at room temperature for 72 hours. Growing spores of *A. flavus* were harvested and blended to form a uniform suspension.

Spore Immobilization:

The spores of *A. flavus* was immobilized by mixing 80g of the harvested spores in 1% (w/v) sterile alginate solution and then gelled into beads by dropping the suspension into a cold 15g/l $CaCl_2$ solution.

Procedure for Sulphur Removal:

The immobilized spore of *A. flavus* was used to desulphurize crude oil under three conditions. These include different time duration, temperature and different concentration of immobilized spores. The desulphurized crude oil was subjected to ultra violet visible spectrophotometric analysis.

Quantification of Sulphur:

Biodesulphurized crude oil sample (2ml) was weighed in a conical flask and added to 10ml of concentrated HCl contained in Kjedahl digestion flask. Distilled water (20ml) was then added. The contents was well shaken to hydrolyse and then allowed to stay for 3 hours. The content was filtered with No.1 Whatman filter paper. The filtrate was kept for analysis. The filtrate (5ml) was poured into a test tube. Distilled water (15ml) and 2ml of conditioning reagent were then added. The test tube was covered and allowed to stand for few hours. A spatula full of $BaCl_2$ was then added. The turbidity was read with ultra violet visible spectrophotometer [1].

Results and discussion

Sodium metabisulphite "trained" *A. flavus* was used to desulphurize crude oil under three different conditions. These include different time durations, temperatures and concentrations of immobilized spores. Table 1 shows the effect of different time duration on sulphur removal from crude oil using *A. flavus*. When 100ml of crude oil sample was treated with 50g of the immobilized spores of *A. flavus* for one and two days, the amount of sulphur removed were 27.2% and 45.2% respectively (Table 1). When the experiment was run for three and seven days, the amount of sulphur removed were 90.4% and 91.7% respectively (Table 1). These two values show no significant difference from each other but significantly different from the values obtained for control ($pr < 0.0001$).

Table 2 shows the effect of different temperatures on sulphur removal from crude oil using *A. flavus.* When 50g of immobilized spores of *A. flavus* was introduced into the crude oil for 3 days at 35ºC and 40ºC, the amount of sulphur removed were 63.2% and 55.3% respectively (Table 2). The difference between the two values is insignificant ($pr < 0.0001$). At 45ºC, the sulphur level in the crude oil was reduced by 10.53% (Table 2). There is a significant difference between the two values obtained at 40ºC and 45ºC ($Pr<0.0001$). The values obtained from the three different temperatures are significantly different from the control value ($Pr<0.0001$).

Table 3 reveals the effect of different amount of immobilized spores of *A. flavus* on sulphur removal. When the crude oil sample was treated with 10g and 50g of immobilized spores of *A. flavus* for three days, the amount of sulphur removed were 49.6%

and 94.7% respectively (Table 3). The statistical analysis of the results shows a significant difference between the two values obtained (Pr<0.0005). In this research work, the spores of *A. flavus* were entrapped using sodium alginate. This was done to immobilize the spores of *A. flavus.* The immobilized spores of *A. flavus* were used for the biodesulphurization process. The entrapment of spores in alginate used in this work is preferable and better than other entrapment methods such as adoption used by Hattori and Furusaka [11] used in binding *Escherichia coli* spores on to an ion exchange resin [11]. This is due to the fact that the entrapment method operates under a mild condition and also because of the simplicity of the procedure used. When 50g of immobilized spores of *A. flavus* was used to treat 100ml of crude oil sample for three days and seven days, the amount of sulphur removed were 90.4% and 91.7% respectively (Table 1).

Furthermore, another parameter determined is the ability of *A. flavus* to biodesulphurize crude oil at different temperatures. Previous work on biodesulphurization was achieved at temperature as high as 50°C using thermophilic bacteria such as *Mycobacterium phlei WU-F1* and *Mycobacterium phlei WU-0103* [7]. In this study, *A. flavus* was able to biodesulphurize crude oil at 35°C. When 50g of immobilizerd spores of *A. flavus* was used to treat 100ml of crude oil at 35°C, the amount of sulphur removed was 63.2% (Table 2). Oldfield *et al*, [18] reported that at mild temperature of 35°C, elemental removal seems probable because at higher temperature, enzymatic activity and catalytic property of the organism can be disrupted [18]. When the temperature was increased to 40°C and 45°C, *A. flavus* was able to reduce the sulphur level of the oil by 55.3% and 10.5% (Table 2) respectively. It can be deduced from this result that the higher the temperature, the lower the amount of sulphur removed.

Table 1: Effect of different time durations on sulphur removal from crude oil using immobilized spores of *A. flavus*

Duration of Experiment (Day)	Weight of Spores (g)	Absorbance Value	% Sulphur removed
1	50	0.190	27.2
2	50	0.185	45.2
3	50	0.159	90.4
7	50	0.137	91.7

Table 2: Effect of different temperatures on sulphur removal from crude oil using immobilized spores of *A. flavus*

Temperature (°C)	Weight of Spores (g)	Absorbance Value	% Sulphur removed
35	50	0.160	63.2
40	50	0.162	55.3
45	50	1.819	10.5

Table 3: Effect of different concentrations of immobilized spores on sulphur removal from crude oil using immobilized spores of *A. flavus*

Weight of Spores	Duration of Experiment (Day)	Absorbance Value	% Sulphur removed
10	3	0.172	49.6
50	3	0.130	94.7
100	3	0.165	53.9

Grossman *et al*, [10] reported that desulphurization using *Rhodococcus sp. strain ECRD-1* reduced the sulphur content of the oil by 30%. Moreover, when 50g and 100g immobilized spores of *A. flavus* were used to treat 100ml of crude oil, the amounts of sulphur removed were 94.7% and 54.0% respectively (Table 3). These amounts of sulphur removed from crude oil were high compared to the total sulphur contents removed in the oil as reported by Grossman, 2001. This research work demonstrates contribution to knowledge by revealing the ability of *A. flavus* as a biological agent for the removal of sulphur from crude oil. It revealed the use of immobilization technology in trapping micro-organisms yet maintaining their catalytic properties without any cell washed out.

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