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ORIGINAL ARTICLE

Biodesulphurization of Crude-oil Using Immobilized Spores of *Rhizopus Nigricans*

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ABSTRACT

Oil biodesulphurization is an important step in combating pollution problems emanating from burning fossil fuels. *Rhizopus nigricans* was used as the biological agent during the biodesulphurization process. The organism was cultured in a broth medium containing 1.5% glucose, 0.05% yeast extract, 0.5g/litre KCl, 2g/litre NH_4NO_3, 1.5g/litre KH_2PO_4 and 1g/litre sodium metabisulphite ($Na_2S_2O_5$). *R. nigricans* was continuously cultured in this medium composition but with increasing concentrations of $Na_2S_2O_5$. The organism could not grow beyond 15g/litre concentration of $Na_2S_2O_5$. The organism was harvested and immobilized using the alginate entrapment method. The oil samples were digested and analyzed for sulphur. The analysis showed that when 50g of the immobilized spores of *R. nigricans* were used to treat 100ml of the oil samples, not less than 90.5% sulphur were metabolized. 92.18% and 90.9% sulphur were also metabolized at an optimum temperature of 35°C and 168 hours respectively

Key words: Biodesulphurization, Immobilized spores, *Rhizopus nigricans*, Bioreactor, Alginate entrapment, Metabolized.

Introduction

Crude oil is a complex mixture of large numbers of organic compounds. The combined carbon and hydrogen contents of a typical type of crude oil are about 97%. Sulphur compounds form the largest group of non-hydrocarbon compounds and are the most important and expensive for the refiner to deal with (Maurice, 2003). Upon combustion, the sulphur in fuels can contribute to air pollution in the form of particulate material and acidic gases such as sulphur dioxide (Speight, 1981). The problem with fossil fuel is that combustion products are harmful to the planet (Aribike *et al.*, 2008). Carbon dioxide emissions have been implicated in global warming. Nitrogen oxides and sulphur oxides emissions have been shown to be responsible for acid rain which destroys buildings, kills forests and poison lakes According to the Environmental Protection Agency (EPA) regulation, sulphur levels in transportation fuels, must be reduced by 90% or more below current levels (Al-Haj-Ihrahim and Morsi, 1992; Dave, 2000). The availability of low-sulphur crude has decreased over the last decade as a consequence of the increasing reserves of heavy crude (Grossman *et al.*, 2001). Refineries remove organic sulphur from derived fuels by hydrodesulphurization (HDS). Hydrodesulphurization is a process that converts organic sulphur to hydrogen sulphide gas by reacting crude-oil fractions with hydrogen at high pressures and temperatures depending upon the feed and level of desulphurization required (Speight, 1981). Biological desulphurization offers an attractive alternative to conventional treatments due to its mild operating conditions of temperature and pH, resulting in energy saving and greater sulphur removal potential (Dave,

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2000; Monticello, 2000). This work was designed to culture *R. nigricans* in a broth medium containing high concentrations of sodium metabisulphite which serves as the sulphur source and to investigate the ability of the organism in metabolizing sulphur contents in oil samples.

Materials and Methods

Collection of Materials

The crude oil was collected from Shell Petroleum Company, Warri, Delta State, Nigeria.

Micro-organism Maintenance and Culture Conditions

The micro-organism used was obtained from the stock culture bank in the department of Pure and Applied Biology of Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The strain was maintained under normal environmental conditions as a slant culture.

The medium for culturing the organism contained 1.5% glucose, 0.05% yeast extract, 0.5g/l KCl, 2g/l NH_4NO_3, 1.5g/l KH_2PO_4 and 1g/l sodium metabisulphite ($Na_2S_2O_5$). *R. nigricans* was cultured in separate broth medium containing different concentrations of the sodium metabisulphite at 1g, 3g, 5g, 10g and 15g while other medium components remained constant. The sodium metabisulphite concentrations used were 1g and 15g minimum and maximum concentrations respectively. The semi-defined medium was first prepared with 1g/l $Na_2S_2O_5$. 50ml of this medium was poured into fermentation vessel sterilized in an autoclave at 121^{o}C for 15 minutes (Fawole, 2001). The organism was inoculated into the medium and incubated at 25^{o}C. The organism was transferred at its exponential phase from the medium containing 1g/l $Na_2S_2O_5$ into another fresh medium of same composition but containing 3g/l $Na_2S_2O_5$, also prepared in duplicates. The cultures were also incubated at 25^{o}C for 4 days. This same procedure was repeated for media containing increasing concentrations of $Na_2S_2O_5$ while other salts concentrations remained constant until *R. nigricans* could no longer grow in the medium beyond 15g/l $Na_2S_2O_5$.

Harvest of spores

Spores of *R. nigricans* were harvested from the broth culture with sterilized forceps under an aseptic condition from the surface of the medium.

Immobilization of spores

Harvested spores from an actively growing culture were immobilized by mixing 80g of the harvested spores with a 1% w/v sterile alginate solution and gelled into beads by dropping the suspension into 15g $CaCl_2$/litre solution (Kirk *et al.*, 1993). The beads were allowed to stay in this cold condition for about 4hours to make it hardened and maintain the bead structure.

Aeration

Sterile oxygen from air was supplied to the immobilized spores of *R. nigricans* via an aerator connected to the fermentation vessel. 100ml of the crude oil sample was weighed into the bioreactor after which different weights of the immobilized spores of 10g, 50g and 100g were introduced accordingly for each experimental set-up.

Sulphur digestion

To investigate sulphur digestion, 2ml of the treated oil samples was taken from the bioreactor and weighed into a Kjedhal digestion flask. 20ml of concentrated H_2SO_4 and 10ml distilled water were added. The mixture was then placed on a digestion system and heated at 80^{o}C for 3 hours. The digested samples were homogenized to cool. The oil samples were filtered into a 100ml conical flask. The filtrate was filled to the 50ml level with distilled water.

Elemental Analysis

Elemental analysis was carried out to obtain the percentage concentration of sulphur in the oil samples.

The oil sample was injected through a nebulizer attached to a Flame Atomic Absorption Spectrophotometer (Model; Alpha 4, Serial Number; 4200) supplied by Chem. Tech. Analytical Lagos State, Nigeria. It was used to determine the percentage concentration of sulphur in parts per million according to the method Varma (1985). Statistical analysis of the result was done using the Duncan's multiple range test (Snedecor and Cochran, 1987).

Table 1: Effects of different concentrations of immobilized spores of *R. nigricans* on sulphur removal from crude oil.

Weight of spores (g)	Absorbance value	Sulphur concentration (ppm/g)	Sulphur removed (%)
10	1.812	0.235	3.29
50	0.122	0. 017	93.00
100	0.137	0.019	92.18

Table 2: Influence of different time durations on sulphur removal from crude-oil using immobilized spores of *R. nigricans*

Weight of spores (g)	Time (hrs)	Absorbance value	Sulphur Concn (ppm/g)	sulphur removed (%)
50	24	0.120	0.1660	31.70
50	48	0. 140	0.1940	20.20
50	72	0. 165	0. 0228	90.50
50	168	0. 159	0.0220	90.90

Table 3: Effects of different temperatures on sulphur removal from crude-oil using immobilized spores of *R. nigricans*

Temp. (ºC)	Weight of spores (g)	Absorbance value	Sulphur conc. (ppmg)	Sulphur removed (%)
35	50	0.138	0.019	92.18
40	50	0.180	0.025	89.70
50	50	0.197	0.027	88.90

Results and discussion

Immobilized spores of *R. nigricans* were able to metabolize the sulphur contents of crude-oil when cultured in broth medium containing 15g/l $Na_2S_2O_5$ maximum concentration after 3 days. The relative sulphur removed was calculated in parts per million per gram (ppm/g) for different concentrations of the immobilized spores used as shown in Table 1. There was a significant difference in the percentage sulphur removed when 10g and 50g of the immobilized spores were used as shown in Table 1. Also, there was a significant difference when 10g and 100g concentrations of the immobilized spores used, although there was no significant difference when 50g and 100g concentrations of the immobilized spores were used. Their percentage difference was just 0.82%. The influence of different time durations was also monitored during this work. In Table 2, the concentration of the immobilized spores of *R. nigricans* used was maintained at 50g, while the time for each experimental set-up was varied accordingly. After 1 to 2 days, there was a significant difference in the percentage sulphur removed compared to that removed after 3 to 7days as shown in Table 2.

The effects of different temperatures on the biodesulphurization process at 35ºC, 40ºC and 50ºC is as shown in Table 3. In the course of this work, as the temperature was varied, the concentration of the immobilized spores used remained constant. However, as the temperature increases, the percentage sulphur removed decreases, although there was no significant difference as shown in Table 3. Immobilization technology (Freeman and Aharonowitz, 1981) was applied using the alginate entrapment method to entrap the spores of *R. nigricans.* The most extensively used method in cell immobilization technology is the entrapment of microbial cells in polymer matrices (Ramakrishma and Prakashan, 1991). The polymer matrices used in this work to entrap the spores of *R. nigricans* was sodium alginate (Obgonna *et al*, 1989). The molecular size of the alginate with the physical properties of the beads (Martisen *et al*, 1989) could have influenced its performance during this work. Immobilized spores of *R. nigricans* were highly effective and showed a promising potential in reducing sulphur contents of crude-oil. The entrapment method operated under a mild condition and also prevented the spores of *R. nigricans* from diffusing into the medium yet allowing penetration of substrate. Entrapment of spores in alginate gel used in this work is preferable and better than other entrapment method used such as adsorption method previously used by Hattori and Furusaka (1961) in binding *Escherichia coli* on to ion exchange resin. The immobilized spores were able to metabolize sulphur in oil efficiently at a mild temperature of 35ºC. This removal seems to be probable because at higher temperatures, enzymatic activity and catalytic property of the organism can be disrupted (Shifflet and Krenzke, 2002). This is an advantage over hydrodesulphurization that operates under very high temperatures (Christopher, 2001). At this temperature, the crystal lattice of the immobilized spores was maintained (Ramakrishna and

Prakasham, 1991) and this makes them to be re-used in other subsequent experiments. In conclusion, immobilized spores of *R. nigricans* is another emerging biological agent of high promising potential that can be used in biodegrading sulphur contents of crude-oil at very mild environmental condition (Singleton, 1997).

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