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Study on growth characteristic and microbial desulfurization activity of the bacterial stain MP12

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ABSTRACT

The growth characteristic and interrelated microbial desulfurization activity of stain MP12 on both DBT and crude oil systems were investigated in order to desulfurize crude oil. The results showed that DMSO was the most suitable sulfur source for stain MP12 growth and expression of desulfurization activity, while initial 2-HBP had very significant influences on the growth and desulfurization activity. Moreover, a novel method using the production rate of sulfate was developed for rapid determination of desulfurization activity in crude oil system. Analysis of total 2-HBP and total sulfate molar balance suggested that the total 2-HBP buildup had a very good match with the total sulfate buildup during microbial desulfurization of crude oil. The desulfurization activity of high density cells could be increased sharply with an increase of agitation speed. In conclusion, the strain MP12 was able to effectively desulfurize recalcitrant organic sulfur compounds in both DBT and crude oil systems.

1. Introduction

The crude oil contains sulfur compounds, and the content of sulfur can vary from 0.03 to 7.89% (w/w) [13]. After carbon and hydrogen, sulfur is considered as the third most abundant element in crude oil, and preferentially associated with the higher molecular mass components. When crude oil is refined the sulfur concentrates into the high molecular mass fractions. During the combustion of these oil fractions, the sulfur is converted to sulfur oxides as a byproduct released into the atmosphere [22], while these emissions may lead to serious environmental problems such as acid rain [19]. To reduce sulfur-related air pollution, governments have regulated the progressive annual decreases in sulfur content of fuels and have mobilized worldwide to resolve the problems associated with sulfur oxides emissions [6]. In order to meet these requirements sulfur must be removed from fuels during the refining process by effective and affordable approaches. Hydrodesulfurization (HDS) is the most common technology used by refineries to remove sulfur from crude oil and intermediate fractions. However, HDS has several disadvantages, in that it is energy intensive, costly to install and to operate, and does not work well on refractory organic sulfur compounds [21]. Organosulfur compounds in the lower-boiling fractions of petroleum can be removed readily by HDS, while middle-distillate fractions are considerably more difficult to remove by HDS. Thiophenic sulfur is usually the most abundant form of sulfur in petroleum, of which dibenzothiophene (DBT) and its alkylated derivatives (DBTs) are the most common organosulfur compounds typically found in crude oil and fractions, and also accepted as the representatives of refractory organosulfur compounds [13]. Their resistance to HDS and inherent chemical limitations associated with HDS make some possible alternatives to this technology into the crude oil industry.

Biodesulfurization (BDS) had been studied as a potential alternative for the removal of organic sulfur from fuels that cannot be removed by HDS [3]. According to the past BDS studies, microorganisms have different mechanisms for removing or using the sulfur atom from DBT, which has been used as a model compound in most cases, degradation of molecule with a cleavage of C—C bonds or a sulfur-specific cleavage of the C—S bonds [28]. The latter is preferable and ideal for desulfurization because the carbon skeleton remains intact and the heating value of fuels remains constant [25]. An important route for achieving this is the 4S pathway via which the sulfur in DBT can be removed as a result of sequen-

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tial biochemical reactions involving different enzymes, in which DBT is ultimately converted into 2-hydroxybiphenyl (HBP) and sulfate [24]. With DBT as the model compound, research has been focused on numerous microbial strains isolated from natural environments. For example, the most studied microbial desulfurization strain, *Rhodococcus erytropolis* IGTS8, was able to completely desulfurize DBT and some substituted DBTs in model oil system, but unable to significantly reduce the sulfur content in crude oil [12]. Yu et al. [32] investigated the microbial desulfurization of crude oil by *Rhodococccus erythropolis* XP, and found that the total sulfur content of Fushun crude oil was decreased from 0.321 to 0.122 wt% Sulfur and that of Sudanese crude oil was decreased from 0.124 to 0.0656 wt%, when the crude oils were treated for 72 h in shake-flasks with 1:20 of oil to water volume ratio and 16 g DCW/L of resting cells in the water phase. Lee et al. [16] reported the isolation of an *Arthrobacter* strain that could specifically cleave sulfur from sterically hindered DBTs. Okada et al. studied the desulfurization of alkylated DBTs in a model diesel oil. They found the desulfurization rates decreased significantly for heavier, more-substituted DBTs. Both the resting and growing cells of *Gordonia alkanivorans* RIPI90A utilized DBT at a rate of 182 μmol/L h when the cell growth at OD_{660} was between 15 and 25 [20]. A bacterial strain *Microbacterium* sp. ZD-M2 was isolated from sludge in China, which could totally desulfurize DBT within 58 h in model oil system [18]. The mixtures of different age cells belonging to a single species or different organisms may improve the effectiveness of desulfurization [17,5]. Some researchers also focused on creating genetically modified bacterial strains with higher specific activity and broader range of refractory compounds, higher hydrocarbon tolerance and resistance to heat [30,2]. After taking all of these points into consideration, although there are many studies done on the microbial desulfurization of simple model oils such as hexadecane to understand the nature of desulfurization, there are only a couple of studies on microbial desulfurization of actual crude oils. Therefore, more researches are needed to design or isolate a new strain with high desulfurizing potency in genuine whole crude oil system, instead of model oil system.

In this work, strain MP12 was chosen to desulfurize organic sulfur in crude oil. The growth characteristic and desulfurization activity were investigated in both DBT and crude oil systems. Firstly, biomass growth was measured in media with different sulfur sources, together with the desulfurizing capability developed by the cells, and then effects of initial 2-HBP concentration on growth characteristic and desulfurization activity were evaluated in DBT system. Moreover, in crude oil system, a new method was developed to use sulfate production rate to express desulfurization activity for improving the simplicity and convenience of conventional measurement method, and effects of cell density and agitation speed on desulfurization activity were also evaluated.

2. Materials and methods

2.1. Biocatalyst

The microorganism *Rhodococcus* sp. MP12 used as a desulfurizing strain in this study was isolated from oil field soil by repeated cultivation in a selective medium with DBT as the sole sulfur source in Shaanxi, China. The soil samples collected from the top and subsurface layers of each site were mixed and dealt with as one sample during microbial enrichment and screening. Approximately 6 g of soil was added to each of vessel contained 500 mL sterilized distilled water for growth of liquid cultures at 30 °C, then take 1 mL liquid culture into the selective plate medium with DBT as a sole sulfur source to cultivate the single colony. Colony forming units were enumerated using nutrient agar. Strain MP12 was finally screened out for candidate of desulfurization strain. Preliminary experiments had proved that this MP12 possesses the ability to efficiently convert DBT to 2-hydroxy-biphenyl (2-HBP) and sulfate. The MP12 was identified by microscopic observation, measuring various biochemical parameters using the API Coryne bacterial identification system (bioMerieux, Hazelwood, Mo.), and the result showed that it belongs to the genus *Rhodococcus*. The strain MP12 was maintained at 4 °C on Luria-Bertani (LB) agar plates for experiment.

2.2. Chemicals

Medium components, DBT and 2-HBP were purchased from Sigma–Aldrich (Milwaukee, WI). Tris hydroxymethyl aminomethane (TRIS), ethanol, $MgSO_4$, and MOPS-Na were obtained from VWR International. All chemicals were analytical grade, available commercially, and used without further purification. Deionizer water was used to prepare all media and solutions.

2.3. Experimental media

In order to obtain both high biocatalyst biomass and high desulfurization activity, LB medium was used for the first activation of the biocatalysts cells, then cell growth was done in adjusted medium according to Abin-Fuentes, et al. [1], which contained carbon source (30.0 g/L glucose), nitrogen source (6.0 g/L NH_4Cl), and sulfur source (1 mM DBT, 1 mM $MgSO_4$ or 730 μL/L DMSO, depending on the experiment design, respectively). The growth medium was prepared with phosphate buffer with the initial pH 7.2.

2.4. Resting cells preparation for desulfurization

Strain MP12 was cultivated in 400 mL of growth medium with different sulfur sources in a 2 L shake flask with 250 rpm at 30 °C in order to profile the growth characteristic and desulfurization activity. When study desulfurization activity, cultures were sampled according to experiment design at different time points, and centrifuged at 5000 rpm and 4 °C for 5 min. Biocatalyst pellets were resuspended to the experimental cell density in 10 g/L glucose and 10 mM phosphate buffer of pH 7.2 in DBT system; in crude oil system, 10 mM phosphate buffer was replaced by 10 mM MOPS-Na buffer.

2.5. Analytical methods

2.5.1. Cell density

Cell density was determined by converting the optical density value obtained in a spectrophotometer (Shimadzu, Model UV 1603) at 600 nm in grams dry cell weight per litter (g DCW/L). A linear correlation between culture $OD_{600\,nm}$ and dry cell weight of *Rhodococcus* sp. MP12 was obtained in the $OD_{600\,nm}$ range of 0.35–1.45, and the equation as follows:

$$\text{Drycellweight}(g/L) = (0.0743OD_{600}) + 0.231$$

It was found that 0.305 g dry cell weight of culture was equivalent to 1.0 $OD_{600\,nm}$ unit.

2.5.2. 2-HBP and sulfate analysis

In this study, the specific desulfurization activity in DBT system was expressed as the amount of 2-HBP produced per gram dry cells weight per hour (μmole HBP/g DCW/h). 2-HBP produced in DBT system was quantified by Gibbs assay, since Gibbs reagent reacts with aromatic hydroxyl groups, such as 2-HBP, to form a blue-