



# The accelerated enzymatic biodegradation and COD removal of petroleum hydrocarbons in the SCR using active bacterial biomass capable of in-situ generating peroxidase and biosurfactants

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## HIGHLIGHTS

- An enzymatic biodegradation process was developed for TPH removal.
- A high amount of peroxidase was generated in-situ the SCR.
- Biomass produced a high concentration of biosurfactants at the presence of H<sub>2</sub>O<sub>2</sub>.
- Peroxidase efficiently mediated the TPH biodegradation in the SCR.
- Complete biodegradation of 4 g/L TPH obtained in the developed process.

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## ABSTRACT

The enzyme-accelerated biodegradation of total petroleum hydrocarbons (TPH) was investigated in a sequencing continuous-inflow reactor (SCR) at different operational parameters of H<sub>2</sub>O<sub>2</sub>/TPH ratio, initial TPH concentration and hydraulic retention time (HRT). The optimum H<sub>2</sub>O<sub>2</sub>/TPH mass ratio was determined to be 0.35 at which the complete TPH removal of inlet TPH concentrations up to 4 g/L at HRT of 24 h, corresponding to the loading rate of 4 kg TPH/m<sup>3</sup>.d, was attained. The average COD removal efficiency at this loading rate was 96.7%. With increasing the inlet TPH concentration from 1 to 2.5 g/L, the biomass bacterial activity as dehydrogenase activity (DHA) increased from 7.5 to 27.1 µg TF/g<sub>biomass</sub>.d and remained almost unchanged with further increase of TPH concentration. The peroxidase activity (PA) remained high between 382 and 410 U/g<sub>biomass</sub>. In addition, the complete removal of 1 g/L TPH (88.7% COD removal) was observed at HRT of as small as 4 h (corresponding to the loading rate of 6 kg TPH/m<sup>3</sup>.d) under optimum H<sub>2</sub>O<sub>2</sub>/TPH mass ratio. With the decrease of HRT from 24 h to 4 h at the constant TPH concentration of 1 g/L the value of DHA remained between 24.4 and 28.4 µg TF/g<sub>biomass</sub>.d while the PA value increased from 287.9 to 394.4 U/g<sub>biomass</sub>. Total production of biosurfactants was 131 mg/L (38 mg/L *rhamnolipid* and 93 mg/L *surfactin*) when the SCR was operated at TPH loading rate of 6 kg/m<sup>3</sup>.d.

Finally, the enhanced enzymatic biodegradation of TPH by using diverse microbial consortia capable of in-situ production of peroxidase and biosurfactant generation in the SCR is a very efficient and promising technique for accelerated biodegradation and COD removal of petroleum hydrocarbons.

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## 1. Introduction

The petroleum and gas industry is known as the main source of energy supply and basic of dynamic development around the world [1]. The high volumes of water with a mixture of various substances such as hydrocarbons, heavy metals, salts and radionu-

clides are usually produced during oil and gas extraction and processing operations [2]. Discharging water produced during the oil-processing activities into environment causes serious environmental, health and economic problems including productive disorders in aquatic life, water pollution and corrosion of instruments [3]. One of the main management strategies to deal with the produced water is discharging polluted streams into the sea or receiving waters. For protecting the environment, the efficient treatment of these waste streams, mainly for petroleum hydrocarbons, is

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required prior to discharging to the environment [4]. In order to minimize or avoid the adverse effects of produced water, a variety of treatment methods including electrochemical treatment [5], membrane filtration [6], biological treatment [2] and hybrid technologies [7] can be used for removal of the impurities. Due to the capability of hydrocarbons to be used as carbon and energy sources by microorganisms, biodegradation could be a promising process in decreasing the adverse effects of petroleum hydrocarbons in the environment [8]. Thus, the biodegradation petroleum hydrocarbon is an appropriate technology due to environmental friendliness, cost effectiveness, simple in design and operation and decomposition of organic pollutants instead of their accumulation in another phase [9].

Many biological treatment processes have been developed for biodegradation of total petroleum hydrocarbons (TPH) including single activated sludge [10], activated sludge coupled with immobilized biological aerated filter [8], continuously stirred tank bioreactor [11], rotating biological contactor [12], continuous upflow anoxic sludge-blanket/fixed-film hybrid bioreactor (UANsFB) [2], upflow anoxic fixed-bed bioreactor (UANFB) and a sequencing anoxic batch reactor (SANBR) [9], membrane bioreactor [13] and hybrid membrane-aerated biofilm reactor [14].

Based on the above-cited literature, TPH biodegradation has been studied using various bioreactors under aerobic [15], anoxic [9] and anaerobic [16] metabolic conditions. Although, the aerobic metabolism is the most efficient bioprocess used for complete biodegradation of petroleum hydrocarbons, the aeration of oily wastewater during biological process suffers from the several drawbacks mainly large bioreactor size, increasing maintenance and treatment costs (pumps and electric power) and stripping the light hydrocarbons [17]. In addition, the petroleum hydrocarbons are complex compounds with low bioavailability making their biodegradation limited. As a promising treatment alternative, the enzymatic biodegradation has been recently developed for treating the waste streams containing recalcitrant contaminants [17,18]. In this technique, bacteria participate in decomposition of organic and recalcitrant compounds by producing different intracellular and extracellular enzymes [17,18]. One of the main classes of enzymes used extensively in biodegradation of organic compounds is oxidoreductase enzymes. Peroxidase is a versatile oxidative enzyme that can generate free radicals and catalyze the carbon–carbon bonds in complex organic compounds such as azo dyes and hydrocarbon resulted in accelerating their biodegradation [17,18]. Biostimulation of bacteria by  $H_2O_2$  enforce them to generate peroxidase in order to protect the living cell [19]. Therefore, addition of  $H_2O_2$  to the bacterial media is an interesting method of choice for rapidly triggers the in-situ secretion of microbial peroxidase which can enhance the intracellular signaling pathways and control various biological functions [17]. In addition, due to hydrophobic nature of petroleum hydrocarbons, they have low bioavailability for microorganisms making slow their biodegradation. Therefore, the production of biosurfactants by bacteria can enhance further the bioavailability of hydrocarbons and thus facilitate their consumption as a carbon and energy source by bacteria [20].

Accordingly, this study aimed at accelerating the biodegradation of a TPH mixture using a bacterial consortium capable of in-situ producing peroxidase and biosurfactants in a sequencing continuous-inflow reactor (SCR). The SCR is a modification of the sequencing batch reactor which has several features including single-based bioreactor, simple design, construct and operation, high robustness, high flexibility in operation and high reliability in performance for treatment of toxic and inhibitory pollutants [21]. To the best of our knowledge, no detailed scientific studies have been reported on the enzymatic biodegradation of TPH by the  $H_2O_2$ -stimulated bacterial biomass capable of in-situ producing

peroxidase and biosurfactant in the SCR. The effect of  $H_2O_2$ /TPH ratio, inlet TPH concentration and hydraulic retention time (HRT) was investigated on the performance of SCR in enzymatic biodegradation and chemical oxidation demand (COD) removal of a mixture of petroleum hydrocarbons. In addition, peroxidase and dehydrogenase activities of biomass in the SCR operated under different TPH loading rates were measured. The concentrations of two main groups of biosurfactants, *surfactin* and *rhamnolipid*, were also determined at the optimum SCR operational conditions. Finally, the composition of petroleum hydrocarbons in the influent and the effluent of SCR operated under optimum conditions were analyzed by GC/FID to determine the types of the residual hydrocarbons.

## 2. Materials and methods

### 2.1. Wastewater preparation

The wastewater was prepared synthetically by adding the known volume of kerosene oil as a TPH source (sole source of carbon) and an aliquot of the nutrient stock solution to dechlorinated tap water. Kerosene taken from a local petroleum refinery was selected as a model of petroleum hydrocarbon source because it contains a mixture of hydrocarbons classified in three categories in order of abundance: alkanes > cyclic aliphatics > aromatics. The nutrient stock solution was composed of  $K_2HPO_4$  and  $KH_2PO_4$  as the source of phosphorous and pH buffer,  $NH_4Cl$  as the source of nitrogen and  $NaHCO_3$  as the alkalinity agent. All chemicals were purchased from Merck Co. To prepare a homogeneous influent, the suspension was continuously recycled from the bottom to top of the influent tank using a centrifuge pump (2800 rpm impeller speed). The pH in influent tank was adjusted at  $7.5 \pm 0.3$  during the whole period of SCR operation.

### 2.2. Experimental setup

Fig. 1 shows the configuration of lab-scale SCR experimental setup used in this study. The setup was consisted up a cylindrical glass container with an inner diameter of 25 cm and a total height of 40 cm as the SCR, which was fed from the bottom by a peristaltic pump (Watson-Marlow 101U/R). A custom-made triaxial perforated Plexiglas propeller was installed at the center of SCR to mix the bioreactor content. The mixer was rotated by an electromotor (14 W). The SCR was operated in a cyclic mode; each cycle consisted of reaction time (3 h), settling time (0.75 h) and decanting time (0.25 h). At the end of each cycle, treated effluent was discharged by a decant valve with an automatic time controlling program installed 10 cm above the bottom of the bioreactor. Hydrogen peroxide was injected continuously into the bioreactor during the phases of reaction and settling by the syringe pump and the amount of injected  $H_2O_2$  was adjusted through a programmable keypad.

### 2.3. Reactor inoculation, start-up and operation

After installation of the setup and water-tightness checkup, the SCR was inoculated with 1.5 L of acclimated biomass with peroxidase production ability that efficiently degraded TPH in an SBR [18] with total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of around 36 g/L and 25 g/L, respectively. During the start-up period, synthetic wastewater was injected continuously into the SCR at the bottom and the bioreactor was operated with  $H_2O_2$ /TPH mass ratio of 0.5, TPH concentration of 1 g/L and HRT of 24 h. The effect of  $H_2O_2$ /TPH mass ratio (0.3–0.5), inlet TPH concentration (1–5 g/L at HRT of 24 h) and HRT (4–24 h at TPH

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