Degradation of Hydrocarbons in a Rotating Biological Contactor with Biodrum

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Abstract

A novel rotating biological contactor (RBC) with biodrum was designed to remove hydrocarbons in wastewater from industrial discharges and its performance was investigated. The biodrum, a cylindrical mesh drum filled with random packing of polyurethane foam cube retaining petroleum-degrading achlorophyllous micro-alga *Prototheca zopfii* cells, was approximately 40% submergence in the culture. The amount of algal cells captured in the 10-mm-cube pieces was greater in pieces of smaller pore size under the experimental conditions. A mixture of n-alkanes (C_{14}, C_{15} and C_{16}) was used as a model oil and the influent hydrocarbons was removed by immobilized cells in the biodrum. The single stage RBC system was operated at 25℃ and at pH 7.0 in a batch mode. The removal rate for *n*-alkanes in the RBC with biodrum system was significantly increased with compared to those by the RBC with polycarbonate biodisk.

Key words: achlorophyllous micro-alga, biological treatment, immobilization, *Prototheca*, rotating biological contactors

1. Introduction

The development of efficient remediation processes for persistent chemicals in the environment including petroleum hydrocarbons is increasingly important *in situ* treatment for accidental release of petroleum and/or in wastewater treatment from industrial discharges. One possible scheme to achieve this goal is biodegradation. Research has been conducted to remove organic compounds such as benzene, toluene, phenol and halogenated hydrocarbons using aerobic or anaerobic microorganisms in laboratory experiments (Lewandowski & DeFilippi, 1998). So far, several types of bioreactor for the biodegradation of hydrocarbons have been proposed. CSTR (continuously stirred tank reactor) (Lee et al., 1993) and bubble-column bioreactors (airlift bioreactor) containing immobilized cells were developed for the treatment of liquid phase pollutants (Suzuki et al., 1998; Yamaguchi et al., 1999a). However, the common shortcoming of these bioreactors was that fair amount of pollutant was

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discharged to the environment by air injection for volatile organic compounds.

The rotating biological contactor (RBC), a fixed biological film (biofilm) reactor system, in general, consists of a series of plastic circular disks (biodisk) mounted on a horizontal shaft and rotated perpendicular to the direction of the waste flow. In a conventional RBC unit, approximately, 40–45% of the total disk surface area is submerged in the wastewater to be treated. As the disks rotate, the microorganisms in the biofilm are alternatively immersed in the wastewater, and the biodisk is rotated at a speed that allows adequate attached biofilm development. Transfer of oxygen is achieved by the exposure and renewal of air–water interfaces as the contactor rotates and the wastewater lifted out by the rotating device trickles back down into the sump. This cyclic immersion of the biofilm also provides the opportunity for the adsorption and uptake of organics from the wastewater.

Many types of proprietary RBC systems have been developed (Casey, 1997) and widely used in the aerobic treatment of wastewater. In practice, the disks are arranged in groups and several reactors are used in series. The advantage of RBCs is their relative low energy consumption, simple operation and maintenance, and successive treatment of the influent contaminants. As an alternative approach to treat hydrocarbons in bioreactors, the RBC appears to be a good choice because of these reasons. Successive applications of the RBC system have been found in aerobic treatment processes such as decolorization (Yin et al., 1989), Fe oxidation (Nikolov et al., 1986), removing pathogenic bacteria from domestic sewage (Sagy & Kott., 1990) and nitrification (Oga et al., 1991).

In a previous study (Yamaguchi et al., 1999b), biological degradation of hydrocarbons in the RBC system using a petroleum-degrading achlorophyllous microalga *Prototheca zopfii* cells was investigated. The RBC reactor with the biofilm developed on the polycarbonate discs was effective to significantly reduce abiotic attenuation of hydrocarbons by vaporization during operation with compared to the case of the bubble-column system. The additional facility such as bioscrubbers, trickling filters and biofilters to recover volatile hydrocarbons (Yeom & Yoo, 1999) might not be needed to combine with this type of bioreactor.

The purpose of the present investigation was to study the efficiency of a new type of RBC in which a cylindrical mesh drum filled with immobilized biomass in polyurethane foam pieces (biodrum) and compare the results with that by the biodegradation rates of a hydrocarbon mixture in the RBC with biodisk.

2. Materials and Methods

2.1. Microorganism and cultivation

The algal strain *Prototheca zopfii* Kruger ATCC 30253 was used. The volumetric

biodegradation rates observed in free cells were comparable to those reported in other systems of marine petroleum-degrading microorganisms, such as *Bacillus* sp. and *Pseudomonas* sp (Suzuki et al., 1998). Pre-culture was carried out in flasks containing Sabouraud Dextrose Broth (Difco, USA) medium on a reciprocal shaker at 25℃ at pH 6.8-7.0. After pre-culture for 48 h, cells in stationary-phase were harvested by centrifugation at $1000 \times g$ for 5 min, washed twice with sterilized water, and inoculated in a modified Bristol medium (Watanabe, 1960) supplemented with $FeCl_3$ (2.0 mg/L), yeast extract (0.25 g/L), glycine (0.25 g/L) and thiamine hydrochloride (0.001 g/L). The basal medium contained (per 0.1 dm^3): KNO_3, 100 mg; $MgSO_4 \cdot 7H_2O$, 25 mg; K_2HPO_4, 7.5 mg; KH_2PO_4, 17.5 mg; NaCl, 2.5 mg; $CaCl_2 \cdot 2H_2O$, 1 mg; trace element solution, 0.2 ml.

A mixture of *n*-tetradecane, *n*-pentadecane and *n*-hexadecane, each added at the level of 0.1 % (v/v), was used as a model paraffinic oil. Pure hydrocarbons were products of the Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The pH was adjusted at 7.0. Thiamine and *n*-alkanes were sterilized by filtration and added after autoclaving. Stationary phase cells were used for inoculation. The volumetric biodegradation rates observed in free cells were comparable to those reported in other systems of marine petroleum-degrading microorganisms, such as *Bacillus* sp. and *Pseudomonas* sp (Suzuki et al., 1998). Biomass concentration measurements were carried out by counting the algal cells with a hemocytometer.

2.2. Biodegradation in RBCs

A schematic view and dimensions of the RBC system which was made of stainless steel are shown in Fig. 1. The bioreactor was constructed from a reactor tank, a cylindrical mesh drum filled with random packing of 10-mm-cube polyurethane form (PUF) pieces, and a motor. Three reactors can be used in series. The single stage RBC system was operated at 25℃ and at pH 7.0 in a batch mode.

Another type of RBC manufactured is depicted in Fig. 2. This bioreactor was constructed from a reactor tank made of stainless steel, five rotating disks and a motor. The disks were made of polycarbonate and their diameter was 0.8 dm. The total disk surface area available for microbial growth in the reactor was 5.0×10^{-2} m^2. The submergence of the disks was about 30% and the rotational speed of the disks was 30 rpm.

2.3. Cell immobilization

P. zopfii cells were immobilized naturally by physical entrapment within the open pore network of 10-mm-side PUF cubes (INOAC Co., Nagoya, Japan) at 25℃. To find the most suitable cube which has greatest affinity for algal cells, seven kinds of PUF cubes having different chemical structure and varying density or pore size were tested (Table 1). ER-1 was a copolymer of polyether and polycarbonate,

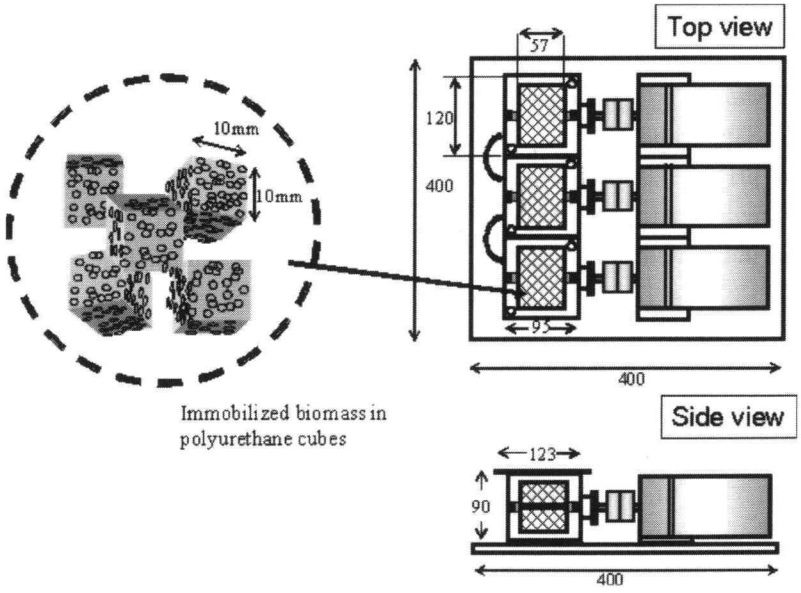

Fig. 1 Schematic diagram of the three rotating biological contactors with a cylindrical mesh drum filled with random packing of polyurethane cubes in series.

while others were polyurethane.

In order to evaluate the affinity of polyurethane foam to aqueous solution, following test (Honda et al., 1991) was performed to measure adsorbed water: After a 10-mm-side polyurethane foam piece was immersed in distillated water for 1 h, the piece was put on the wire cloth for 20 min to remove adhered water on the surface of the piece. Adsorbed water in the polyurethane foam piece was then harvested by centrifugation at 2000 rpm for 10 min.

2.4 Hydrocarbon analysis

The residual hydrocarbons in the bioreactor were extracted by hexane, and the extract was analyzed by GC (GC-8A; Shimazu). The operating conditions for GC were as described previously (Suzuki et al., 1998).

3. Results and Discussion

3.1. Affinity between Polyurethane Foam Cubes and Algal Cells

The biodegradation rates observed in the RBC with biodrum would depend heavily on the physical properties of the support particles. The most commonly used matrix for algae and cyanobacteria calcium alginate beads was not suitable for *P. zopfii* because of the hydrophobicity of the outermost surface of the algal cells and the nature of substrates, hydrocarbons to be degraded (Suzuki et al., 1998). There-

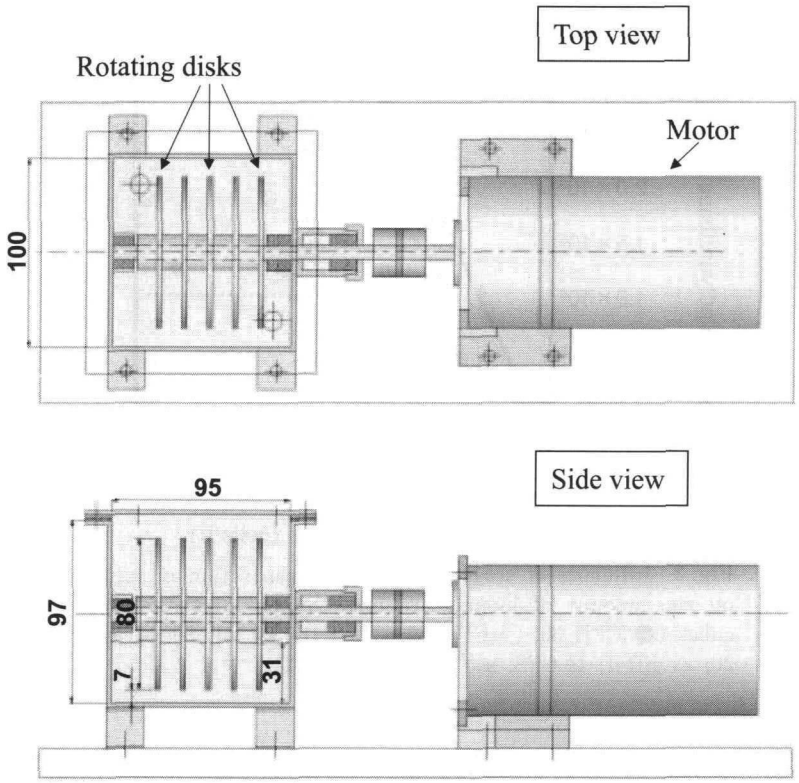

Fig. 2 Schematic diagram of the single stage rotating biological contactor with circular disks (biodisk).

Table 1 Physical characteristics of polyurethane form (PUF) cubes (INOAC Co. Nagoya, Japan).

trade name	density (kg/m^3)	cells (numbers/25 mm of PUF)
MF-30	30±5	30±4
MF-80	80±5	70 ≤
CFH-30	30±3	30±4
CFH-50	30±3	45 ≤
CF-17	30±3	17±3
CFS	75±10	60 ≤
ER-1	35±3	20

fore, *P. zopfii* cells were immobilized naturally by physical entrapment within the open pore network of 8-mm-side polyurethane foam cubes (average pore size=0.83 mm) and the particles were successfully used as support particles in a bubble-column type bioreactor (Suzuki et al., 1998). The observed dependence of the amount

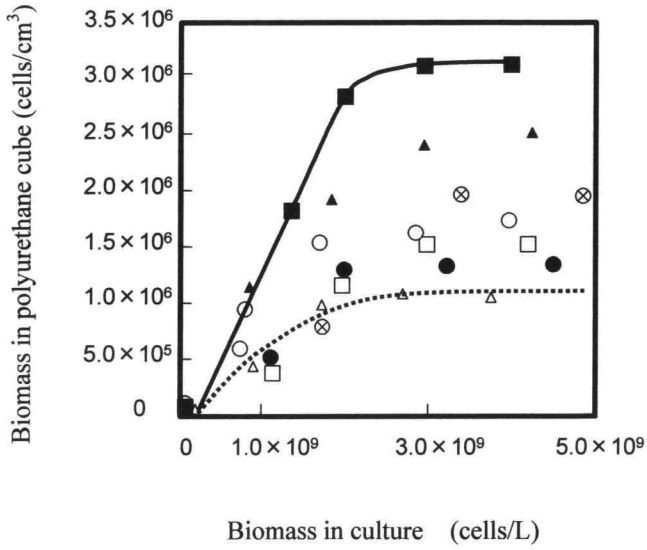

Fig. 3 Influence of seeded microbial concentration in culture on the amount of biomass retained by polyurethane foam cubes (● CFH-30; ○ CFH-50; □ CF-17; ■ MF-80; ▲ MF-30; △ ER-1; ⊗ CFS; see Table 1) at 25℃.

of biomass retained by PUF pieces on the microbial concentrations in culture at 25℃ is shown in Fig. 3. In general, the amount of biomass retained is increasing with increasing the microbial concentrations in culture and then coming to a saturated value, as "adsorption-isotherm" curve. The greatest affinity for the algal cells was observed for MF-80-PUF, while the least affinity was found for ER-1-PUF. The microbial retainment process is due to the fact that the seeded algal cells were captured into the cubes and/or trapped among the cubes by the liquid flow (Xing et al., 1992).

On the basis of the data in Fig. 3, scatter plot between the maximum amount of biomass immobilized within the cubes and density of PUF cubes is shown in Fig. 4. The amount of biomass retained by PUF pieces can be seen to increase approximately linearly with increasing density of PUF pieces. Since density of cubes is inverse proportion to average pore size, the results can be recognized that the amount of captured cells was greater in cubes of smaller pore size, regardless of other factors. This means that the suspended microbes were more easily retained by cubes of smaller pore size than by those of larger pore size. It was found that the specific capture rate of suspended microbes was greater in porous support particles of smaller pore size and that detachment had occurred in particles of larger pore size (Xing et al., 1992).

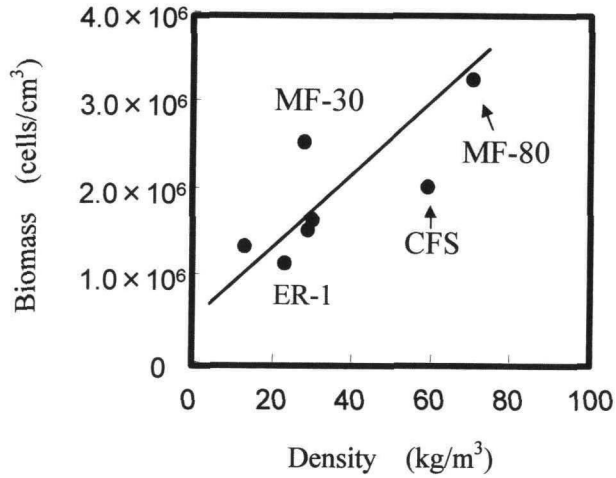

Fig. 4 Relationship between the maximum amount of biomass retained and density of polyurethane foam cubes.

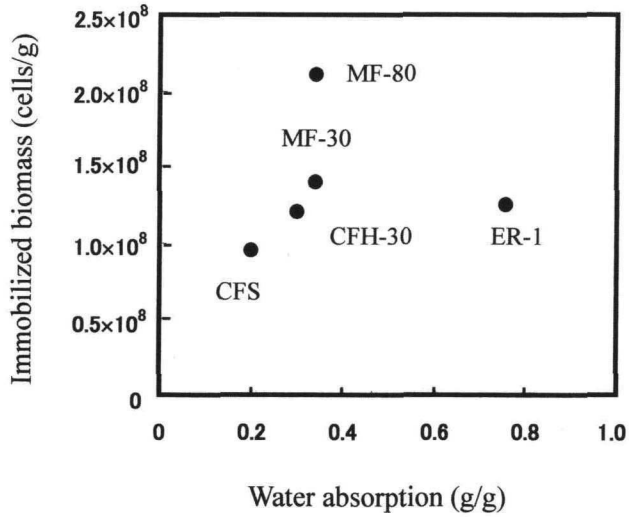

Fig. 5 Relationship between immobilized algal cells and water absorption to the polyurethane foam cubes.

For one of other important factors which governs immobilization process of microbes to polyurethane foam piece, the result of affinity test of water to the piece was shown in Fig. 5. It seems that there was no distinct correlation between the amount of immobilized microbes and water absorption in the experimental range, although the chemical structure of ER-1 was different from others. Thus, it was revealed that pore size of the particle had a gross effect on microbial attachement.

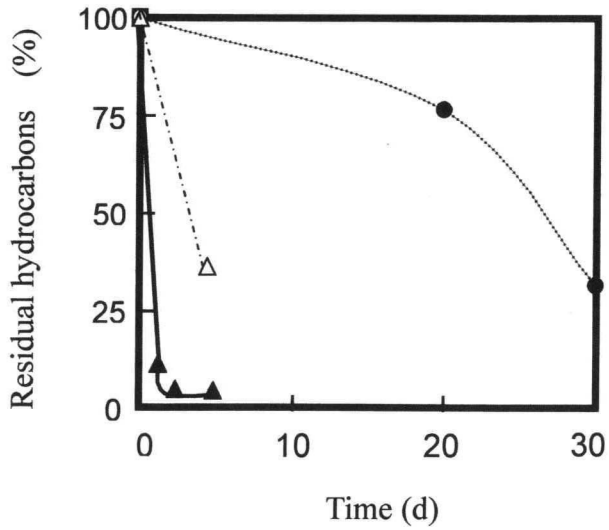

Fig. 6 Degradation of hydrocarbon mixture by *P. zopfii* in the single stage rotating biological contactor with biodrum (▲: with bimass; △: no biomass) and a rotating biological contactor with biodisk (●) at 25℃.

From above results, MF-80 was found to be most suitable for retaining *P. zopfii* cells and this PUF was incorporated into the RBC with a drum as porous support particles.

3.2. Biodegradation in RBC with Biodrum

The biodegradation profile of the hydrocarbon mixture in the single stage RBC with biodrum including immobilized algal cells in MF-80-PUF cubes in batch is shown in Fig. 6, together with the data from the RBC with biodisk. Initial concentrations of both biomass and hydrocarbons (initial total concentration of hydrocarbon mixture was 3% (v/v)) were adjusted to be equivalent in the two bioreactor systems (Yamaguchi et al., 1999b). The time courses of the amount of volatilized hydrocarbons in both systems can be seen from the data of control experiments (without biomass). About 95% of hydrocarbons were removed during just two days of operation in the RBC with biodrum. The remove rate of a mixture of *n*-alkanes (C_{14}, C_{15} and C_{16}) was much faster (about 20-fold increase) than that of the RBC with biodisk where only 65% of hydrocarbons were removed during 30 days of operation. According to the inherent biodegradation rate of hydrocarbons observed for free-living cells of *P. zopfii* (Suzuki et al., 1998), it can be estimated that most of hydrocarbons removed in the RBC with biodrum in two days of cultivation were trapped hydrocarbons in polyurethane foam by very strong hydrophobic interaction between the hydrocarbons used and the polyurethane form.

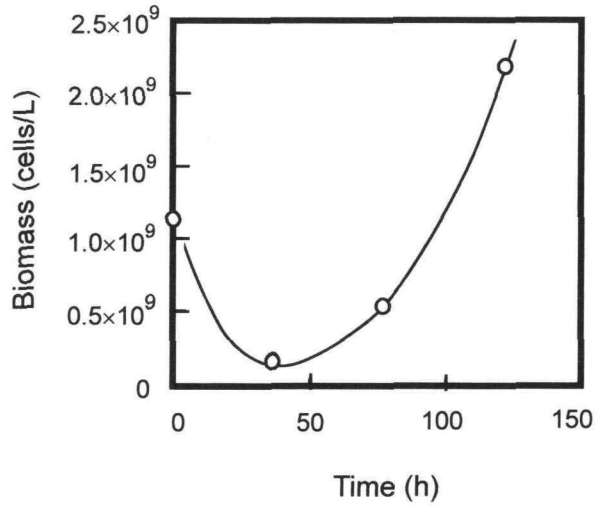

Fig. 7 Time-course variation of biomass concentration in the polyurethane foam cubes in the single stage rotating biological contactor with biodrum (Fig.1) at 25℃.

The time-course variation of biomass concentration in the polyurethane foam cubes was then observed (Fig.7). During the experiments, in the first two days, the amount of biomass was on the decrease due to poisonous effect of hydrocarbons in their higher concentrations, and thereafter growth associated biodegradation of hydrocarbons was confirmed from the increase of the number of algal cells. Thus, hydrophobic interaction between the substrates and the foam cubes resulted in a marked increase of the removing rate for hydrocarbons.

4. Conclusions

Laboratory scale rotating biological contactor with biodrum system was constructed and utilized to treat a mixture of *n*-alkanes as a model hydrocarbons using *P. zopfii* in a batch operation. The biodrum was filled with random packing of polyurethane foam pieces. For polyurethane foam, the affinity of seven kinds of cubes with density or pore size for *P. zopfii* cells were tested and the best one was used for the bioreactor system. The amount of algal cells immobilized in polyurethane pieces was greater in pieces of smaller pore size. The bioreactor system was successfully applied to the biodegradation of a mixture of n-alkans (C_{14}, C_{15} and C_{16}) and the usefulness of an alternative RBC with biodrum was confirmed.

Acknowledgement

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