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Treatment of aqueous effluents containing non-aqueous phase liquids in rotating biological contactor with algal bacterial biofilm

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HIGHLIGHTS

- A biofilm of oil degrading bacteria and sessile algae was developed in a RBC.
- Greater than 99% TPH removal could be achieved at HRT greater than 18 h.
- Algae facilitated operation at relatively high organic loading rate.
- In-situ generation of alkalinity prevented pH drop during oil biodegradation.
- Oil was sorbed on the biofilm and bulk of the aliphatic fraction was biodegraded.

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ABSTRACT

Rotating biological contactors (RBCs) have been widely used for treatment of effluents containing soluble organic compounds. This study reports treatment of aqueous effluent containing diesel oil (0.6%) as a model non-aqueous phase liquid (NAPL) in a RBC. The NAPL serves as the sole substrate for the bacterial culture, *Burkholderia cepacia* that is inoculated along with a sessile algal culture for biofilm formation. After biofilm formation in batch mode, the 3-stage reactor was operated in a flow through mode. Data was collected over the unsteady state and also for steady state operation over hydraulic retention time (HRT) range 12–24 h and total petroleum hydrocarbon (TPH) loading range 23.9–47.8 g TPH/m^2 d. Greater than 99% removal could be achieved for TPH loading up to 31.8 g TPH/m^2 d. The models available for predicting removal efficiency of soluble substrate at various HRT values could not be applied for this system. While the assumption of uniform distribution in each stage of the RBC is valid for soluble substrate, it is invalid for NAPLs as illustrated by the unsteady state profile. The stage-wise effluent TPH profiles obtained during unsteady-state operation was indicative of a plug flow reactor with dispersion (PFDR) with sorption and biodegradation occurring simultaneously. The diesel oil in the aqueous phase sorbed on the algal–bacterial biomass and bulk of the aliphatic fraction was biodegraded. The aromatic fraction of diesel accumulated on the biofilm. This system is very complex and new models need to be formulated for understanding and elucidating treatment of NAPL containing aqueous effluents in RBCs.

1. Introduction

A rotating biological contactor (RBC) is an attached growth bioreactor consisting of a series of discs covered with active microbial film that are partially submerged in the wastewater. Rotation alternately exposes the biofilm on the discs to air and wastewater for efficient oxygenation. Due to its various advantages, the RBC reactor has been widely used for the treatment of effluents containing soluble organic compounds. The advantages include: high treatment efficiency, low energy requirement, low manpower requirement and resistance towards shock loadings. Successful application of RBC has been reported for domestic sewage treatment and for treatment of wastewater containing soluble organics, such as, phenols [1] and complex wastewater containing phenolics, heterocyclics and polynuclear aromatic hydrocarbons (PAHs) in dissolved form [2].

In addition to the utilization of RBC for the treatment of soluble substrate, a few studies have proposed the use of RBC for treatment of effluents containing oil and other non-aqueous phase liquids (NAPLs). Tyagi et al. [3] reported the treatment of petroleum refinery wastewater containing low concentration of oil (27–125 mg/L) using a RBC equipped with discs lined with polyurethane foam (RBC–PUF) and inoculated with seed culture obtained from an activated sludge unit treating the same wastewater. In petroleum refineries and petrochemical complexes biological treatment is typically attempted after recovery of oil in oil–water separators

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(API separators, corrugated plate interceptors and tilted plate interceptors) and removal of oil in dissolved air flotation (DAF) units. If biological treatment is feasible directly after oil recovery where oil concentration ranges from 1000 to 10,000 mg/L [3–5], the DAF units that generates hazardous oily sludge may be eliminated. Treatment of wastewater containing higher concentration of fuel oil may be feasible if bioreactors are inoculated with oil degrading cultures. This was demonstrated using an achlorophyllous oil degrading algae, *Prototheca zopfii*, in a single stage lab-scale RBC where 60% degradation of model oil composed of n-alkanes (1% n-C14, 1% n-C15, 1% n-C16) was reported for batch mode operation [6]. Degradation of palm oil mill effluent containing oil and grease (O&G) and COD of 4800 mg/L and 46,945 mg/L, respectively, using *Saccharomyces cerevisiae* has also been demonstrated in RBC [7].

Use of algal–bacterial system in attached growth bioreactors can provide various benefits for treating oily wastewater even when the algae does not directly contribute to degradation. In oil polluted coastal environment, oil degrading bacterial cultures are found to adhere onto cyanobacterial mats and macro-algae [8,9]. Such a phenomenon can promote formation of algal–bacterial biofilm in bioreactors. Algae can facilitate growth of bacteria by enhancing oxygen transfer and can thus facilitate aerobic degradation of the hydrocarbons present in oil. Moreover, some algal and cyanobacterial cultures are also reported to play a direct role in hydrocarbon degradation [10,11]. Batch sorption studies with pure cultures of *Spirulina sp.* (cyanobacteria) and *Scenesdesmus abundans* (algae) have demonstrated high sorption capacity of lubricating oil and diesel on dead biomass [12]. Thus, presence of algae in the biofilm may facilitate sequestration of oil on the biofilm and may thus facilitate bacterial utilization of oil.

The rotating discs in a RBC causes mixing of the reactor content which is often considered sufficient for modeling the RBC as a completely mixed flow reactor (CMFR). Such a completely mixed assumption is widely used for oxygen transfer studies in RBC operated in batch mode prior to development of biofilm on the RBC discs [13]. The same assumption is also widely utilized for soluble substrate utilization in RBC in presence of a steady state biofilm, such that a RBC with multiple stages can be modeled as CMFRs in series (Eq. (1)). This assumption forms the basis for the steady state models proposed by Kornegay and Andrews [14], Clark et al. [15, Eq. (2)] and also more recent models for substrate utilization in RBC [16]. Clark's model has been shown to be applicable even for complex wastewater, such as, biomass gasifier wastewater where the organics in wastewater is composed of dissolved phenolics, heterocyclics and PAHs [2].

$$V\frac{dS_{e,i}}{dt} = QS_{o,i} - QS_{e,i} - \frac{\mu_{\max}}{Y_a}A_w X_a \frac{S_{e,i}}{K_s + S_{e,i}} \quad (1)$$

$$\frac{1}{R} = \left(\frac{K_s}{P}\right)\frac{1}{S_{e,i}} + \frac{1}{P} \quad (2)$$

where V (m^3) is volume of each stage of RBC, Q (m^3/d) is the flow rate, $S_{o,i}$ and $S_{e,i}$ (kg/m^3) is influent and effluent substrate concentration for the ith stage, respectively, Y_a is the yield coefficient of attached biomass; X_a is the mass of attached active biomass (kg/m^2), K_s (kg/m^3) is the Monod's half velocity constant and $\mu_{\max}$ (d^{-1}) is the max specific growth rate of attached biomass. At steady state Eq. (1) yields Eq. (2). The group ($\frac{\mu_{\max}}{Y_a} * X_a$) is referred as the area capacity constant, 'P' (kg m^{-2} d^{-1}), i.e., the maximum amount of substrate that can be removed per unit area of disc per day. The rate of substrate removed per unit area of disc is given by $Q(S_{o,i} - S_{e,i})/A_w$ which is referred as removal capacity constant, 'R' (kg m^{-2} d^{-1}). A straight line plot obtained for $1/R$ versus $1/S_{e,i}$, for each stage of the RBC indicates validity of Clark's model.

However, the complete mixing assumption may be invalid for influents containing substrate present in the form of oil or NAPL. Researchers reported non-uniform distribution of diesel in RBC reactors in the absence of biofilm (disc rotational speed 10 rpm) [17]. For water containing 0.025–2% (v/v) diesel, the saturation concentration of oxygen in water at equilibrium differed significantly in three RBC reactors of varying dimension (working volume 0.85 L, 4 L and 18 L). Diesel stained with oil red-O was first found to cover the surface of the discs and the remaining oil floated on the surface as a thin film or existed as dispersed droplets depending on the degree of turbulence in the reactor. Thus, even for studies in presence of the biofilm, uniform distribution of substrate and complete dispersion of NAPL in the aqueous phase may be an invalid assumption for disc rotational speed of 10 rpm.

Researchers recently demonstrated the effect of N:P ratio on degradation of diesel oil using an algal–bacterial biofilm in RBC [18]. This study reported treatment of effluent containing diesel oil (0.6%) as a model NAPL using a diesel degrading bacterial culture, *Burkholderia cepacia* and algal cultures. The prime objective of this paper is to illustrate the performance of this reactor as a function of one of the important design variables, hydraulic retention time (HRT). Moreover, a conceptual description of NAPL utilization in the algal–bacterial biofilm in RBC is also described based on data obtained from unsteady-state and pseudo-steady state period of reactor operation.

2. Materials and methods

2.1. Chemicals

All the chemicals used for preparation of the nutrient medium and the solvents used for extraction were purchased from S.D. Fine Chemicals Ltd. and Merck Ltd. (Mumbai, India), respectively. All chemicals were of high purity and were of analytical grade. Diesel oil obtained from a Petrol station in Mumbai was artificially weathered over 48 h in a fume hood at room temperature.

2.2. Microbial cultures and inocula preparation

In this study, experiments were carried out using the pure bacterial culture, *B. cepacia* (MTCC 5332, IMTECH, India) which was isolated using diesel oil as the sole source of carbon and energy. This culture is reported to enhance diesel bioavailability by inducing hydrophobic cell surfaces [19]. A consortium of fresh water algal culture obtained from the surface of rocks near Powai Lake (IIT Bombay, India) was used [18]. Initially the bacteria and algae were separately cultured in the laboratory. Since the bacterial medium originally used for isolation of *B. cepacia* [19] could not sustain algal growth, a nutrient medium was first formulated for supporting growth of both the algal and bacterial cultures [20]. It contained the following constituents (mg/L): di sodium EDTA (0.5), citric acid (3), $Na_2CO_3{\cdot}H_2O$ (20), $CaCl_2{\cdot}2H_2O$ (7), $MgSO_4{\cdot}7H_2O$ (370), KCl (500), ferric ammonium citrate (3), $K_2HPO_4{\cdot}3H_2O$ (21.75), KH_2PO_4 (8.5), Na_2HPO_4 (33.4), $NaNO_3$ (750) and trace elements. The trace elemental substances (TES, mg/L) in the medium included: $CoCl_2$ (0.0382), H_3BO_3 (0.0618), Na_2MoO_4 (0.0254), $CuCl_2{\cdot}2H_2O$ (0.0392), $ZnCl_2$ (0.1363), $NiCl_2$ (0.0130), $FeCl_2$ $4H_2O$ (0.7016), $MnCl_2$ $4H_2O$ (0.2807) and Na_2SO_4 (0.1420). This medium was characterized by a higher N:P ratio than the medium used for isolating *B. cepacia* [19] and relatively lower buffer capacity (the concentration of $K_2HPO_4{\cdot}3H_2O$, KH_2PO_4 and Na_2HPO_4 in the algal–bacterial medium was lowered, while the relative concentration of these salts was maintained constant in both media). KCl, Na_2CO_3 and citric acid were found to be essential for promoting growth of the algal cultures. Typical algal media (such as ASN III and BG11) has much