New BIOTECHNOLOGY xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

New BIOTECHNOLOGY

journal homepage: www.elsevier.com/locate/nbt

Full length Article

Influences of ammonium and phosphate stimulation on metalworking fluid biofilm reactor development and performance

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ARTICLE INFO

Keywords:
Biofilm
Biostimulation
Metalworking fluids
Bioreactor
Biodegradation

ABSTRACT

In this study, the effects of common wastewater stimulants, namely NH_4Cl and KH_2PO_4, on the development and performance of metalworking fluid biofilm bioreactors are presented. It is shown that biofilms flourished only when one of these components was present in limiting quantities. Biofilm yields significantly declined when both of the components were withheld from the bioreactors or when both components were provided in excess. Stimulations to the reactors using NH_4Cl significantly reduced the total carbon removal performance, while stimulations using KH_2PO_4 resulted in significant increases in performance. Chromatographic analyses showed that the NH_4Cl stimulation enhanced the removal of saturated fatty amides and diethylene glycol butyl ether from the metalworking fluid, but inhibited the removal of diisoproponolamine. Furthermore, NH_4Cl additions inhibited the oil/water separation carbon removal mechanism and resulted in the re-dispersion of recalcitrant organic material. The results from this study show that metalworking fluid practitioners should take care in choosing the nutrients used for stimulating bioreactor performance and microbe development. Incorrect stimulations with NH_4Cl may result in negative treatment performances due to the inhibition of amine utilisation and enhancing emulsion stability.

Application of fixed-film reactors for treating chemical wastes

When considering the design of microbial reactors, the mode of growth is an important parameter that must be taken into account [1]. Microbes such as bacteria are able to grow in both a planktonic form, or in a fixed-form that is associated with a solid surface through the formation of biofilms [1–4]. From an engineering perspective, physically fixed modes of growth within bioreactors offer the advantages of increased biomass concentration in a reactor, protection against hazardous waste, and inherent biomass retention [5–7].

The biological treatment of waste metalworking fluids is a cost-effective means of remediation [8]. However, both the presence of inhibitory components and the slow degradation kinetics associated with complex organic components hinder the effectiveness of the treatment process [9,10]. Since the application of biofilms to the treatment of hazardous wastes is a suitable means of compensating for these disadvantages, several studies have utilised fixed-film reactors for the treatment of metalworking fluids [11–14]. This mode of treatment is typically applied to effluents which have had their inhibitory components degraded with time and to formulations that contain biodegradable components. For formulations containing inhibitory, or non-biodegradable components, the biological treatment of metalworking fluids is applied after a pre-treatment step (which could be a physical or chemical treatment). Since the biological process is typically slower than other forms of treatment, it is more feasible for treating large volumes of metalworking fluids [8].

It is common practice to add nutrients to wastewater treatment systems to facilitate bioreactor development and improve treatment performance [15,16]. While there is an abundance of data on how biostimulation may influence biofilm development in industrial wastewater treatment reactors [17–20], there are a limited number of studies looking at how it may influence the development and performance of biofilm reactors treating metalworking fluids. Since different nutrient conditions may trigger different biofilm reactor responses [21], a study looking into the effects of stimulation on the development of an indigenous consortium biofilm in metalworking fluid reactors is warranted. In this study, the effects of KH_2PO_4 and NH_4Cl stimulation and limitation on the production of biofilm biomass and bioreactor performance are presented. These compounds were chosen since they are commonly added to wastewater systems to promote growth and development of biomass and to improve reactor performance. The novelty of the investigation lies in the combination of investigating how stimulation affects both biofilm development and reactor performance simultaneously and explaining the trends observed through an in-depth

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http://dx.doi.org/10.1016/j.nbt.2017.09.002
Received 13 November 2016; Received in revised form 27 August 2017; Accepted 11 September 2017
1871-6784/ © 2017 Published by Elsevier B.V.

chemical analysis of the metalworking fluid. The choice of metalworking fluid formulation and reactor system makes this possible and allows for a more in-depth understanding of the process to be obtained.

Materials and methods

Micro-organisms

A mixed consortium used for the industrial treatment of metalworking fluid wastes was supplied by Microbial Solutions Ltd. (a company specialised in the disposal of metalworking fluids). The mixed consortium was provided in freeze-dried form, and originated from an aged reactor treating spent metalworking fluid wastewaters. The inoculum used for this community was described by van der Gast and Thompson [22].

In order to resuscitate the freeze dried micro-organisms, 2 mL of phosphate buffered saline solution (PBS) was added to the vials in which they were contained. The vial was left to stand for two hours, before the contents were transferred to a 250 mL flask containing 95 mL of Luria–Bertani (LB) Media and 5 mL of 10% metalworking fluid solution (recipe for the metalworking fluid is given below). The flask was incubated in an orbital shaking incubator set at 28 °C and 120 rpm for 18 h. After incubation, the contents of the flask were centrifuged at 4100 rpm to collect the cell pellet and to discard the supernatant. The cell pellet was washed with deionised (DI) water twice, before being resuspended once more in DI water. This suspension served as the inoculum for all experiments described.

Metalworking fluid

An artificial, semi-synthetic concentrate was developed and used for all of the experimental work. The formulation used was modified from that given by Childers [23]. The formulation was developed since metalworking fluid wastewaters and commercial concentrates usually contain a mixture of organics which are difficult to identify and characterise. By developing an artificial proxy, observed trends can be investigated in further detail. To prepare the semi-synthetic concentrate, 13.5 g of Naphthenic Mineral Oil (base oil), 4.5 g of Sodium Sulfonate (emulsifier), 10 g of Tall Oil Fatty Amide (emulsifier), and 1.8 g of Diethylene Glycol Butyl Ether (coupler) were mixed together. 28 g of this mixture was added to 75 g of water to create the final semi-synthetic concentrate. All experiments described used a 2.5% (v/v) metalworking fluid concentration prepared by diluting this metalworking fluid using an artificial tap water [24]. The chosen components are common ingredients that are found within metalworking fluid formulations to add confidence that the results obtained will be applicable across different formulations [23]. To ensure that the developed consortium is capable of treating formulations containing biocides, a developed bioreactor was applied to formulations containing varying concentrations of sodium orthophenyl phenate (Na-OPP).

Reagents

Analytical grade inorganic salts ($CaSO_4{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, $NaNO_3$, NaCl, $FeSO_4{\cdot}7H_2O$, KH_2PO_4, K_2HPO_4, $(NH_4)_2SO_4$) Triton X-100, acetonitrile (99.9% purity), acetic acid and Benzoyl chloride were purchased from Sigma Aldrich.

Samples of naphthenic mineral oil were provided by Nynas Base Oils. Samples of sodium sulfonate were provided by Sonneborn. Tall oil fatty amides were provided by Colonial Chemical.

Bioreactor system

A schematic for the bioreactor system is provided in Fig. 1A and a photo of the set-up is provided in Fig. 1C. Briefly, air was passed through a 0.22 μm syringe filter to ensure sterility and through a humidifier made from a 50 mL centrifuge tube, before being bubbled into a 100 mL bioreactor (100 mL Duran bottles). The air was humidified so that evaporation was negligible during the course of the experiment. The air was pumped at 0.5 L/min. The reactor was submerged in a water bath kept at 27° C. Biofilms were grown on a 3 cm × 5 cm polyethylene matrix which is commercially sold as BioBlok 300. A picture of the matrix is given in Fig. 1B.

Bioreactor operation

In order to achieve a significantly measurable amount of biomass, the batch biofilm reactors were operated for 2 cycles. The first cycle lasted for 4 days and the second for 3 days (since nutrients are consumed faster in the second cycle). At the end of each cycle, the total carbon was measured, and the total carbon removal efficiency of the bioreactor was calculated using the following equation:

$$TC\ \ removal\ \ efficiency\ (\%) = \left(1 - \frac{TC_{cyc1} + TC_{cyc2}}{2{*}TC_{initial}}\right){*}100$$

TC_{cyc1} and TC_{cyc2} are the total carbon concentrations at the end of the first and second batch cycles respectively. TC_{in} is the initial total carbon concentration of the artificial metalworking fluid used for the experiments (4600 mg/L).

Biofilm dry weight and biofilm yield

Biofilm dry weight measurements were taken at the end of the second cycle of bioreactor operation. To measure the weight of the biofilm attached to the substratum, the matrix was taken out of the reactor and placed within a 50 mL centrifuge tube. The matrix was gently rinsed with deionised (DI) water twice and then the biofilm was dislodged into 20 mL of PBS solution through a combination of vigorous shaking and vortexing using a Vortex Genie 2. 0.2 mL of a 10% Triton X-100 solution was added to the centrifuge tube to re-emulsify the oils that had dislodged from the biofilm and the matrix. The tube containing the dislodged biofilm was centrifuged at 4100 rpm for 15 m, which resulted in a distinct biomass layer floating on the top of the tube and a cell pellet. The pellet was found to be of a negligible mass compared to the floating biomass and thus only the floating biomass was considered in dry-weight determinations. To determine the mass of the floating layer, the supernatant of the tube and the floating biomass were passed through a piece of Whatman filter paper (Qualitative Number 1). The filter paper was dried overnight at 60 °C and the difference in its weight before and after the biomass addition was taken as the dry-weight of the biofilm.

The biofilm yield can be calculated using the total carbon (TC) removed from the bioreactor over both cycles of operation, and the biofilm dry weight obtained:

$$Biofilm\ \ Yield\ \left(\frac{mg}{mg\ \ TOC\ \ removed}\right) = \frac{Dryweight}{(2{*}TC_{in} - TC_{cyc1} - TC_{cyc2}){*}\ 0.1}$$

TC_{cyc1} and TC_{cyc2} are the total carbon concentrations at the end of the first and second batch cycles respectively. $TC_{initial}$ is the initial total carbon concentration of the artificial metalworking fluid used for the experiments (4600 mg/L).

HPLC

HPLC analyses were used for the determination of the concentrations of amides, DGBE, DIPA within the reactors. All analyses were done on an Agilent 1120 compact HPLC system equipped with an Agilent C18 Eclipse Plus Column and a UV–vis detector. All analyses were done at 210 nm using an isocratic elution made up of a ratio of acetonitrile acidified with 0.2% acetic acid and DI water (for amides, the ratio was

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