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Biodegradation of Fluoranthene in an Aerated Biofilm Reactor with Biosurfactant Producing CSTR Staged in Series

Tshilidzi B. Lutsinge Nembudani*, Evans M. N. Chirwa

Water Utilisation and Environmental Engineering Division, Department of Chemical Engineering, University of Pretoria, Pretoria 0002, South Africa
blutsinge@gmail.com

One of the challenges faced during the bioremediation of Polycyclic Aromatic Hydrocarbons is the limited bioavailability of these compounds due to their hydrophobic nature. Polycyclic aromatic hydrocarbons (PAHs) known as part of the persistent organic pollutants (POPs) are organic compounds consisting of two or more fused benzene rings and are introduced into the environment through both natural and anthropogenic activities. In the past few decades, biosurfactants have gained attention because of their ability to dissolve hydrophobic compounds such as PAHs, and they exhibited some advantages such as biodegradability, low toxicity, ecological acceptability and ability to be produced from renewable and cheaper substrates. Biological remediation of PAHs offers numerous advantages over physical and chemical remediation technologies. In the biological process, toxic organics can be completely converted to carbon dioxide (CO_2) and water (H_2O), thereby living no trace of potentially toxic organic intermediates. Literature has delineated the presence of PAHs in surface water, industrial wastewater and municipal or domestic wastewater, leaving drinking water and water for irrigation purposes vulnerable to PAHs contamination. These mutagenic, carcinogenic and toxic compounds are also known to have potential risk to human health and the environment. Overcoming the bioavailability of PAHs has been previously proven to increase the biodegradation of these compounds using capable microorganisms. The current study aims at degrading fluoranthene in a two−stage process with suspended biomass and attached biomass. A continuous stirred tank reactor was used to produce biosurfactants, and the biofilm tank reactor was used to degrade Fluoranthene. 84.78 % Fluoranthene was successfully degraded from the reactors after the operation of the system.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) known as part of the persistent organic pollutants (POPs) are organic compounds consisting of two or more fused benzene rings (Isaac et al., 2015) and are introduced into the environment through both natural and anthropogenic activities (Kumari et al., 2018). PAHs are formed during pyrolysis or incomplete combustion reactions produced when materials containing carbon and hydrogen such as coal, oil and wood are heated at temperatures higher than 100 - 150 ^oC (Ribeiro et al., 2018;). Fluoranthene is a High Molecular Weight (HMW) four-ring PAH and is considered as one of the 16 priority pollutants by the United States Environmental Protection Agency (US-EPA). Exposure to PAHs has been associated with genotoxic and epigenetic effects, changes in DNA methylation, and potentially, gene expression (Lee et al., 2016). Several studies have been conducted experimenting on animals, and various forms of cancer, such as skin, lung, bladder, liver and stomach have been reported as results of PAH exposure (Zhang et al., 2016). Case studies focusing on fluoranthene exposure reported tumours and cancers in animals (Li et al., 2016). Fluoranthene as an HMW is generally resistant to environmental degradation due to its greater hydrophobicity (Gan et al., 2009) and tend to adsorb to soil particles, soil aggregates and the organic matter which results in its persistence in the environment. To overcome this challenge, biosurfactants are used to increase the bioavailability of fluoranthene in water. Biosurfactants are biological amphipathic compounds consisting of hydrophilic and hydrophobic moieties. Where the hydrophobic moiety is either a long chain fatty acid, hydroxy fatty acid, or α-alkyl-β-hydroxy fatty acid and the hydrophilic moiety can be, a carbohydrate, an amino acid, a

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cyclic peptide, a phosphate, carboxylic acid or alcohol, etc (El-Sheshtawy and Doheim, 2014). In the past few decades, biosurfactants (considered as “green technology” products) has gained attention because they exhibited some advantages such as biodegradability, low toxicity, ecological acceptability and ability to be produced from renewable and cheaper substrates (Paraszkiewicz et al., 2018). Microorganisms in natural environments are generally found in close proximity with surfaces and interfaces, in the form of multicellular aggregates attached combined with the slime they produce (Singh et al., 2006). Accumulation of these microorganisms on interfaces to form assemblages of single or multiple populations through extracellular polymeric substances (EPS), results in the formation of biofilms (Wimpenny et al., 2000). Biofilm formation and EPS are associated with higher bioavailability of PAHs and their increased degradation rate because of their microbial biomass, bacterial chemotaxis and the ability to immobilise compounds (Meliani and Bensoltane, 2014). Various microorganisms (or various nutritional conditions) will affect the type of biofilms produced and not all kinds of biofilms can degrade and tolerate PAHs (Rasamiravaka et al., 2015). Biofilms produced by Pseudomonas aeruginosa can synthesise biosurfactant compounds and tolerate aromatic hydrocarbon compounds (Meliani and Bensoltane, 2014). Studies have shown that PAHs can be removed from a conventional wastewater treatment plant in Varese Olona, Italy (Torretta 2012) and a typical Municipal Wastewater Treatment Facility in Guangzhou, China (Liu et al., 2017). Most of the emerging pollutants such as PAHs, including fluoranthene, are not removed in water treatment plants even those that uses biological processes. It is important to know the processes involved in the removal of such pollutants and the kinds of microorganisms capable of degrading compounds such as fluoranthene. Most of PAHs degradation studies have been done in batch systems and few on continuous flow systems. The removal of fluoranthene in a system simulating a wastewater treatment was investigated in this study. The aim of the study was to use a biofilm process, subsequent to a continuous stirred tank bio-reactor (CSTR) producing biosurfactants to successfully remove fluoranthene from water. The provision of biosurfactants prior to the biofilm system was to increase the bioavailability of fluoranthene which could also be advantageous in operating wastewater treatment that have challenges of PAHs adsorbing to sludge instead of being degraded.

2. Materials and methods

2.1 Microorganism and culturing

A previously isolated biosurfactant-producing/PAH-degrading culture identified as Pseudomonas Aeruginosa (Lutsinge and Chirwa, 2018) was obtained from storage facilities and used in this study. To prepare an inoculum from the stored cultures, 400 mL of nutrient broth was added to a 2 L Erlenmeyer flask and inoculated with 5 mL of pseudomonas aeruginosa for microorganisms’ cultivation. The media was incubated for 24 h in a rotary shaker with 160 rpm at 34 ºC.

2.2 Biosurfactant production

Prior to the dissolution and degradation of fluoranthene, the reactor system was prepared by producing biosurfactants and also cultivating cultures in the respective tanks. 500 mL of cultivated pseudomonas aeruginosa was centrifuged to harvest biomass for the purpose of biosurfactant production. In an 8 L tank, 6.4 L of mineral salt medium adopted from (George and Jayachandran, 2013) with a composition of (g/L): 0.1 g $MgSO_4.7H_2O$, 4.5 g $Na_2HPO_4.2H_2O$, 0.68 g KH_2PO_4, 4.5 g $NaNO_3$ and 0.5 g yeast extract was inoculated with the harvested biomass and supplemented with 100 mL of sunflower oil. The tank was continuously stirred to keep media aerated and completely mixed. Samples were taken from the tank periodically after 24 h for a period of 12 d to measure the surface tension.

Produced biosurfactants were partially purified using the extraction method adopted from (Soares da Silva *et al.*, 2017). Produced biosurfactants were centrifuged at 4 ºC, 6,000 rpm for 10 min. The cell-free supernatant pH was adjusted from 8 to 2 using 3M HCl. Biosurfactants were extracted thrice from the solution using an equal volume of chloroform:methanol (2:1v/v). The solvents were evaporated using rotary evaporation, Figure 2b, and solvents were collected from the receiving tank. After evaporation of solvents, the product was treated with NaOH to readjust the pH to 8.

2.3 Reactor setup

The reactor consisted of 8 L mixing tank where biosurfactants were produced followed by a Pyrex glass column (Figure 1) filled with 8 mm diameter ceramic beads with a liquid volume of 1.38 L and attachment surface area of 0.676 m^3. The flow into the Biofilm tube was measured to be 0.05 L/h, and the liquid retention time was calculated to be 27.6 h. The reactor was operated in an up-flow mode to ensure operation under fully submerged conditions. A recycle flow was created from the aeration tank to the biofilm tank to increase the substrate retention time in the biofilm tank, provide oxygen to the tank and minimise wastage of biomass with the effluents.

To evaluate the movement of the compound in the reactor, all variables with a compound initial concentration of 73 mg/L were used to simulate and construct the tracer study trends.

2.4 Fluoranthene degradation

In the biofilm tube, culture was grown over a period of 20 d to allow the biofilm to form on the ceramic beads used as media with a surface area of 0.676 m^3. To ensure the presence of oxygen in the biofilm tank, a recycle flow from the aerated tank was pumped into the biofilm tank. Samples were taken from sampling points 1, 2 and 3 (Figure 1) to evaluate the degradation of fluoranthene by its concentration. From the dissolution tank B (Figure 1), dissolved fluoranthene was measured from sampling point 1 and reported to be within the range of 70 mg/L and 75 mg/L during the first 12 h of active operation. For fluoranthene degradation, dissolved fluoranthene was pumped together with MSM into the biofilm tank at a flow rate of 0.05 L/h for a period of 12 h. After 12 h, the fluoranthene flow was stopped, and the MSM only was pumped into the biofilm tank at the same rate of 0.05 mg/L for a period of 13 d.

2.5 Analytical Methods

PAHs were measured using a Waters 2695 high-performance liquid chromatograph (HPLC) (Waters Corporation, Massachusetts, USA) separation module equipped with waters Photodiode Array Detector Model 2998. The PAHs were separated using a reverse phase mode and a Waters PAH C18 column (250 mm × 4.6 mm, 5 µm particle size) at a temperature of 25 ^oC and pressure of 4,000 psi at a detection wavelength of 254 nm.

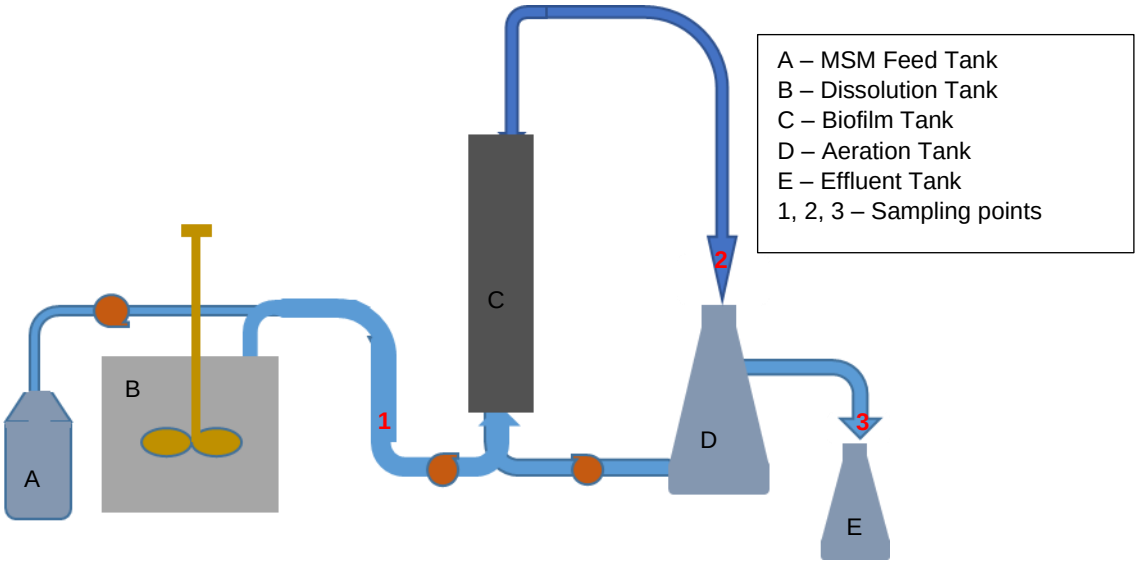

Figure 1: Reactor Setup

3. Results and discussions

3.1 Biosurfactant Production

Biosurfactants were successfully produced and were partially purified. The presence of foam in the biosurfactant producing tank was observed. This signified the presence of biosurfactants, and the foam came as a result when surfactants became concentrated at a gas-liquid interface leading to the formation of bubbles. From the measured surface tension, it was observed that in the first 5 d of incubation surface tension dropped significantly from 55 mN/m to 28 mN/m after 5 d a slight surface tension decrease was observed, meaning that the quantity of biosurfactants produced was sufficient to reach the possible lowest surface tension.

Fourier transform infrared spectroscopy (FTIR) is used to explain the chemical structure of unknown samples by identifying types of functional groups. These infrared absorption bands, identify specific molecular components and structures (Pornsunthorntawee et al., 2008). Partially purified biosurfactant was analysed and the FTIR spectrum indicated (Figure 3) –NH- (peptide group) broad absorbance peak centred on 3,264 cm^{-1}, C-H (alkane group) sharp peak around 2925 cm^{-1}, C-ON (amide group) strong stretching mode peak around 1713 cm^{-1}, -CH_3,CH_2- (aliphatic chains) medium weak multiple bands from around 1,457 to 1,418 cm^{-1} and C-N (amine

group) strong stretching mode peak around 1,084 cm^{-1}. A wide range of literature indicates these as characteristic of lipopeptide.

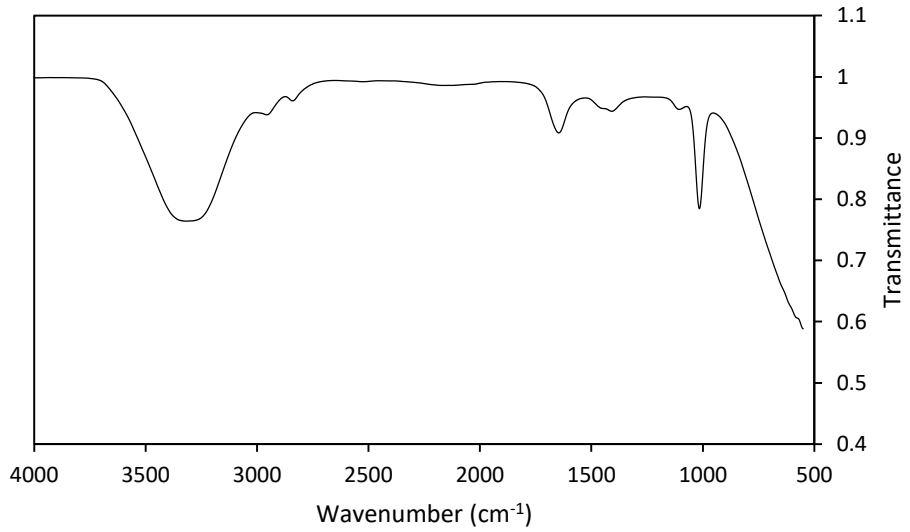

Figure 2: Fourier transform infrared (FTIR) absorption spectrum of biosurfactant produced by Pseudomonas aeruginosa strain

3.2 Fluoranthene degradation

Dissolution was successfully achieved in the dissolution tank and this was verified by analysing samples that were filtered before extraction of fluoranthene. An increase of fluoranthene concentration in filtered samples was observed. From the biofilm tank, a significant difference between the tracer trend (signifying abiotic system) and the measured fluoranthene concentration (Figure 3 and 4) signifies the removal of fluoranthene within the system. In an ideal reactor, a perfectly mixed continuous tank reactor is modelled. In reality, ideal systems rarely exist and there is often an observation of various behaviour from the expected exemplar or idealised reactor.

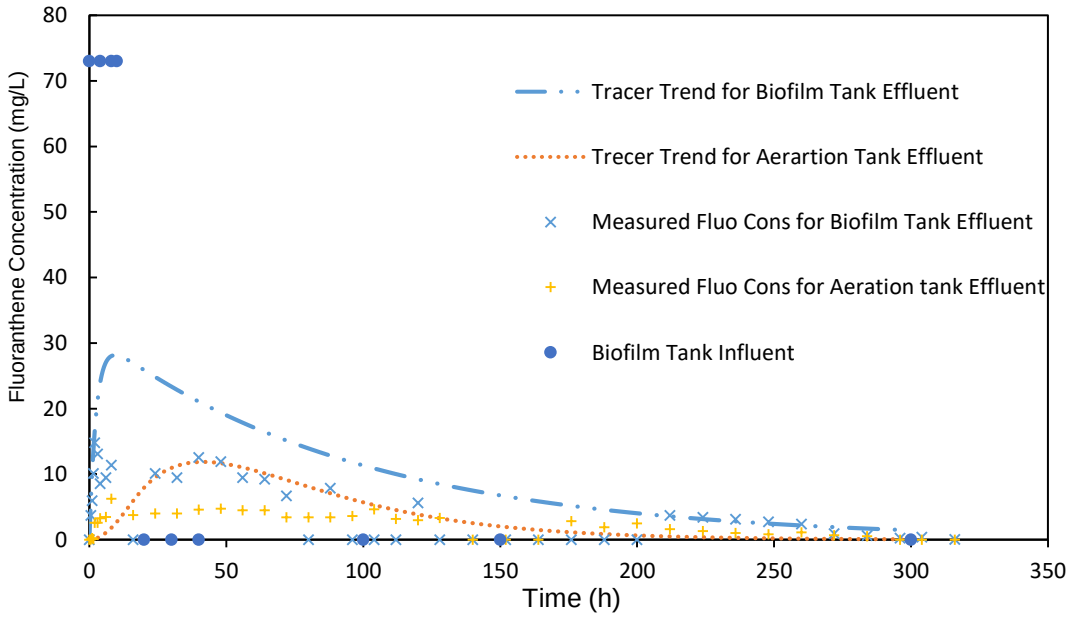

Figure 3: Fluorathene Degradation results from the biofilm and aeration tanks with influent concentration from dissolution tank.

Three concepts with an effect resulting in a non-ideal model include: the distribution of residence time in the system, the quality of mixing and the model used to describe the system. The model may underestimate or overestimate the real measured values, due to the inaccuracy in the residence time caused by factors, such as flow rate variations during the experimental runs and insufficient mixing.

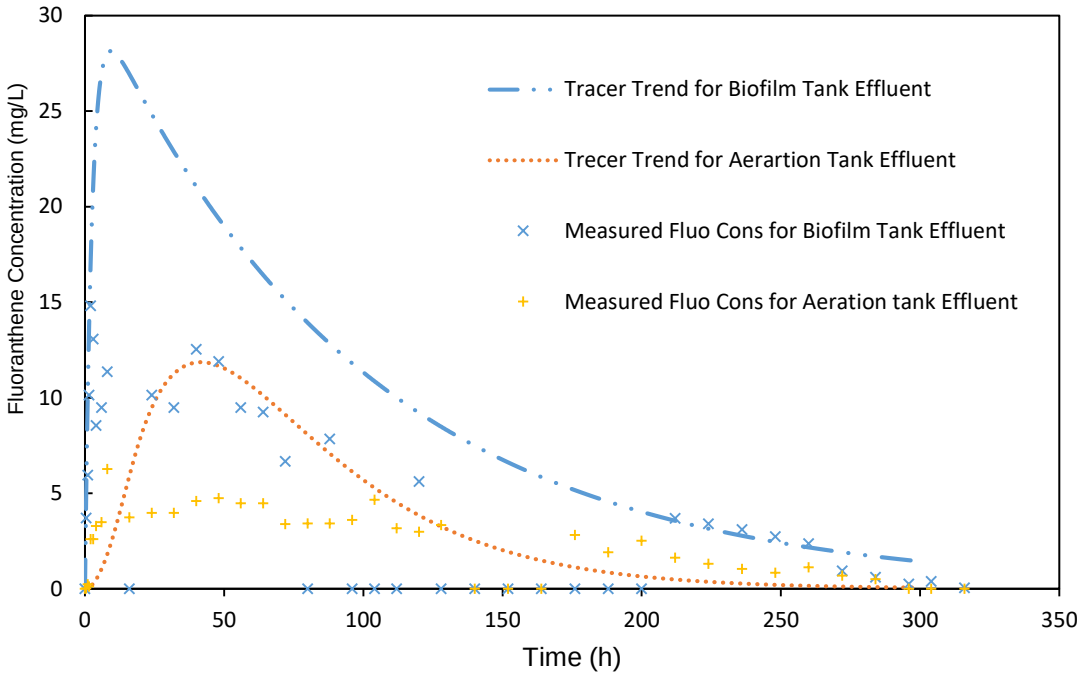

Figure 4: Fluorathene Degradation results from the biofilm and aeration tanks

From Figure 4, it was observed that during the first eight hours of the system's run, the tracer and degradation study data points are very close to each other. This indicates that during the first eight hours of the run, there was close to zero fluoranthene degradation in the system. This is attributed by introducing the toxic fluoranthene into the reactor. After the microorganisms adapted, they could use fluoranthene as their carbon and energy source and thereafter breaking it down using their metabolism, resulting in its degradation. The appearance of a gap between the two trends after 8 h of run validated that the microorganism could degrade fluoranthene. The highest influent concentration from the dissolution tank into the biofilm tank was 28.05 mg/L, and the highest effluent from the biofilm tank was 14.824 mg/L with an average effluent of 4.44 mg/L. The highest influent concentration from the biofilm tank into the aeration tank was 12.54 mg/L and the highest effluent from the aeration tank was 4.27 mg/L with an average effluent of 2.28 mg/L The fluoranthene removal in the system was calculated, considering the highest concentration to enter biofilm tank and the highest effluent from the aeration tank. Fluoranthene removal from the aqueous solution in the system was approximately 84.78%.

4. Conclusions

Pseudomonas Aeruginosa was successfully used to produce biosurfactants identified as part of the lipopeptide family. Produced biosurfactants successfully dissolved the hydrophobic fluoranthene and increased its availability to biological degradation. The same culture strain was used to degrade dissolved fluoranthene using a biofilm system with an aeration tank to increase the retention time within the system as well as providing oxygen through the recycle flow. Approximately 84.78 % of fluoranthene was degraded from the system after a period of 13 d. This signifies that the biofilm system designed is able to produce biosurfactants and degrade fluoranthene using the same culture named Pseudomonas aeruginosa.

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