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KINETICS OF FLUORENE BIODEGRADATION BY A MIXED CULTURE

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ABSTRACT

The present study intended to determine the kinetics of biological degradation of fluorene by a mixed culture of microorganisms. Batch experiments were performed, being the inoculum an enriched mixed culture from a contaminated wastewater. Fluorene was quantified in the aqueous phase by solid phase micro-extraction (SPME), and analyzed in a GC-FID. The mixed culture was able to degrade fluorene and experimental results showed that biosorption had no influence on fluorene removal. The kinetic parameters, maximum reaction rate (k_{max}) and half saturation constant (K_s), respectively, 3.20×10^{-4} h^{-1} ± 0.93×10^{-4} h^{-1} and 126 μg L^{-1}, were calculated.

KEY WORDS

Polycyclic aromatic hydrocarbons, fluorene, biodegradation, biosorption, kinetics

1. Introduction

Polycyclic Aromatic Hydrocarbons (PAH's) are environmental contaminants, produced in human activities, that present toxic and carcinogenic characteristics. PAH's are composed of more than one aromatic ring and their low solubility in water leads to slow biodegradation rates and consequently environmental persistence in the air, water, soils and sediments [1]. Due to this, PAH's often appear in the environment in their solid state. Because the biological processes occur in aqueous phase, the biological degradation of solid PAH's are limited by their solubilization [2]. PAH's can be completely or partially mineralized by a limited number o microorganisms, which are able to adapt to their low availability by specific mechanisms, namely the production of exopoloysacharides to form biofilm directly on the solid substrate [3]. This proximity allows them to maximize the uptake in the mass transfer process. They can also produce biosurfactants that increase the mass transfer to the aqueous phase [5].

Fluorene is one of the most common PAH's found in the environment and it is listed as a priority pollutant by EPA. It has a two benzene ring structure coupled by a pentagonal ring (Figure 1). Some studies have reported that fluorene could not be biodegraded significantly by a mixed culture [6], or could only be degraded cometabolically [7].

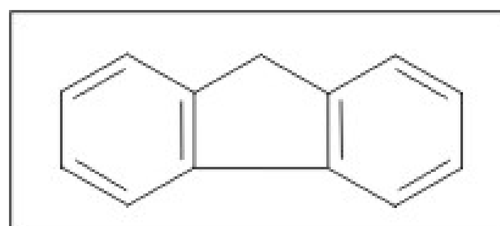

Figure 1 – Fluorene chemical structure.

Fluorene solubility in water, at 20 ºC, is 1.995 mg L^{-1}. Because the biodegradation depends on the amount of fluorene in the water phase, it becomes important to know at what rate it occurs. Although biodegradation is the most common process responsible for the removal of soluble PAH's, the adsorption to the biomass (biosorption) can occur. The process of biosorption is frequently reported in literature for toxic substances [8]. The values of PAH kinetics differ from each other depending on several factors like specific microorganisms, the tests conditions and the availability of the PAH to the cells [9]. Rodrigues [3], reported a rate of 12.9×10^{-3} h^{-1} for fluorene degradation. This study was carried out with solid phase fluorene, at a high concentration, and a high ratio of substrate to biomass.

Batch experiments for the determination of kinetic parameters are widely used, as reported by many authors [3, 4, 9-11]. The most important factor when performing batch experiments to determine the kinetic parameters of mixed cultures is the initial substrate to biomass ratio (C_0/X_0) [12]. If this ratio is high (much substrate to few biomass), growth will be favoured. The number of fast growing microorganisms will increase in proportion to slow growers, leading to a culture that is different from the initial one. In order to correctly assess the kinetic parameters of the initial culture we need to guarantee that the ecology of the culture will be maintained. To meet that goal, we need to ensure that no growth conditions will be used in the experiment, and the substrate will be only used for energy consumption. This can be

achieved using a low C_0/X_0 ratio. In this condition we can consider that no new biomass will be formed and the initial culture will be maintained during the experiment.

The present work intended to determine the kinetics of fluorene biodegradation without any mass transfer limitation. The kinetic parameters, k_{max} and K_s, were measured, and the effect of biosorption to the biomass on the biological removal of fluorene was evaluated.

2. Materials and methods

Chemicals

Fluorene (98 % pure) was purchased from Sigma Aldrich (Steinheim, Germany) and Ethanol (99.9 % pure) from Merck.

Inoculum and culture conditions

A mixed culture, obtained from a hydrocarbon contaminated wastewater, was cultivated for approximately four weeks in a mineral solution containing 8.8 g L^{-1} of $Na_2HPO_4.2H_2O$, 3.0 g L^{-1} of KH_2PO_4, 1.0 g L^{-1} of NH_4Cl, 0.5 g L^{-1} of NaCl, 0.25 g L^{-1} $MgSO_4$, and 2.5 mL of a trace element solution (23 mg L^{-1} of $MnCl_2.2H_2O$, 30 mg L^{-1} of $MnCl_4.H_2O$, 31 mg L^{-1} of H_3BO_3, 36 mg L^{-1} of $CoCl_2.6H_2O$, 10 mg L^{-1} of $CuCl_2.2H_2O$, 20 mg L^{-1} of $NiCl_2.6H_2O$, 30 mg L^{-1} of $Na_2MoO_4.2H_2O$, and 50 mg L^{-1} of $ZnCl_2$). Fluorene was added as the carbon source.

Biodegradation experiments

Batch degradation experiments were performed at room temperature (≈ 22 ºC) in 1 L Erlenmeyer flasks. A stock solution of fluorene was prepared in ethanol (2 g L^{-1}). A mineral buffer solution M9 (6.0 g L^{-1} of Na_2HPO_4, 5.0 g L^{-1} of KH_2PO_4, and 0.25 g L^{-1} of $MgSO_4.7H_2O$) was used. To each flask were transferred 300 mL of M9 buffer solution, with a fluorene concentration below the saturation level of 2 mg L^{-1}, prepared from the stock solution. Then the flasks were inoculated with the biomass, to obtain a concentration of approximately 277.5 mg L^{-1} of biomass, as volatile suspended solids (*VSS*). Samples were taken hourly.

Abiotic losses experiments

The biodegradation experiments procedure was also performed with inactive biomass, previously autoclaved at 121 ºC.

Fluorene extraction with SPME

15 mL samples were filtered with glass fiber filters, and extracted by solid phase micro-extraction (SPME), using a polydimethylsiloxane (PDMS) fiber. The fiber was exposed to the sample by direct contact with the liquid phase for 30 min. Extraction was carried out at room temperature [13].

Fluorene determination by CG/FID

Fluorene was analyzed in a gas chromatograph (GC), coupled with a flame ionization detector (GC-FID). The carrier gas was helium at a pressure of 50 kPa. The column was a Chrompack WCOT fused silica 25 m × 0.32 mm. The oven was set to an initial temperature of 60 ºC and a final temperature of 310 ºC with an increase of 10 ºC/min. The injector temperature was set to 250 ºC. The FID was set to 300 ºC.

Parameter estimation and biodegradation modeling

In order to determine the type of kinetics of the fluorene biodegradation, the values were fitted to a zero order model, to a first order model, and to a saturation type model. The saturation type model can be described by [2, 4]:

$$r_{bio} = \frac{dC}{dt} = \frac{-k_{max}\ X\, C}{K_s + C} \qquad (1)$$

In equation 1, r_{bio} is the biological degradation rate (mg L^{-1} h^{-1}); k_{max} is the maximum rate constant (h^{-1}); X is the system concentration of fluorene degrading biomass (mg L^{-1}); K_s is the half-saturation constant, that is the concentration when $k=k_{max}/2$ (mg L^{-1}); C is the concentration of fluorene (mg L^{-1}); and t is the time (h). This model describes the biodegradation of the bioavailable fluorene (water phase content). The kinetic parameters k_{max} and K_s can be estimated by a graphical method [9] that uses a linearization of equation 1 (at t = 0 h, $C = C_0$):

$$K_s\ \ln\frac{C}{C_0} + C - C_0 = k_{max}\ X\ t \qquad (2)$$

The value of k_{max} can also be calculated by considering the initial hours of the degradation curve (from 0 h to 6 h), where $K_s \ll C$, which yields a zero order reaction rate. Regarding to the fluorene concentration, which can be described by:

$$r_{bio} = -k_{max}\ X \qquad (3)$$

The calculation, using the saturation type biodegradation model (equation 1) solved by a 4th order Runge-Kutta method, was compared with the measured values.

3. Results and discussion

This study has focused on the determination of the kinetic parameters k_{max} and K_s for the biological degradation of fluorene. The effect of biosorption on fluorene removal was measured by inactivating the biomass and the C_0/X_0 ratio used was 2.21×10^{-3} mg/mg. Figure 2 presents the fluorene concentration evolution during the experiments.

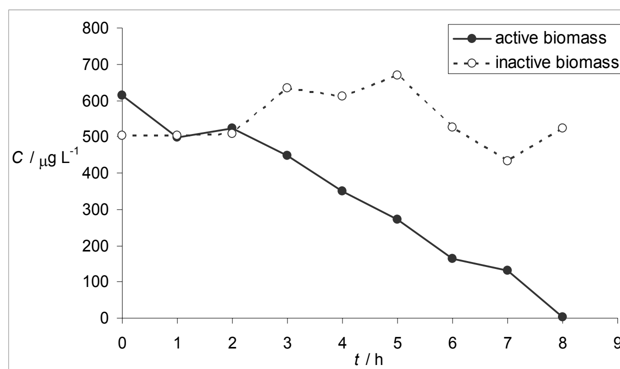

Figure 2 – Values from the fluorene biodegradation and biosorption experiments.

The results showed that practically all the fluorene was degraded by the culture in 8 h. It seems that biosorption is not responsible for the fluorene removal from the aqueous solution since the amount of fluorene in the inactive biomass experiment shows no overall decay. So, considering that fluorene is removed exclusively by biological degradation, the kinetic parameters k_{max} and K_s were estimated. The results obtained with the graphical method were $k_{max}= 3.86\times10^{-4}$ h^{-1} and K_s = 126 μg L^{-1}. Using the linearization of the first 6 h, k_{max} was 2.54×10^{-4} h^{-1}. An average of both values is 3.20×10^{-4} h^{-1}, with a standard deviation of 0.93×10^{-4} h^{-1}. Figure 3 presents a comparison between the experimental results and the curve calculated with the estimated parameters, using the saturation model described in equation 1. Other possible reaction orders were considered. In the case of limiting substrate availability, the decay could be of first order. A zero order reaction would occur only if the reaction rate was kept on maximum level until all the substrate was consumed. Figure 4 shows the fitting curves considering a zero order rate and a first order rate. The comparison of the correlation factor shows that the saturation type model can better describe the process. The low value of K_s (126 μg L^{-1}) indicates that the maximum reaction rate is maintained until a low concentration is obtained. This is a good indicator for the activity of the culture, meaning that the substrate is oxidized at the maximum rate until it reaches low concentrations.

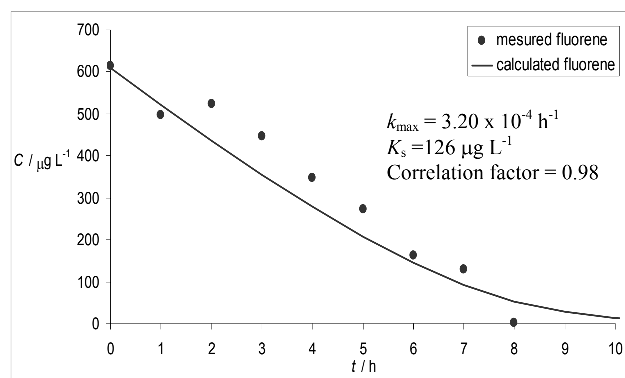

Figure 3 – Experimental and modeled results from saturation x kinetics for fluorene biodegradation.

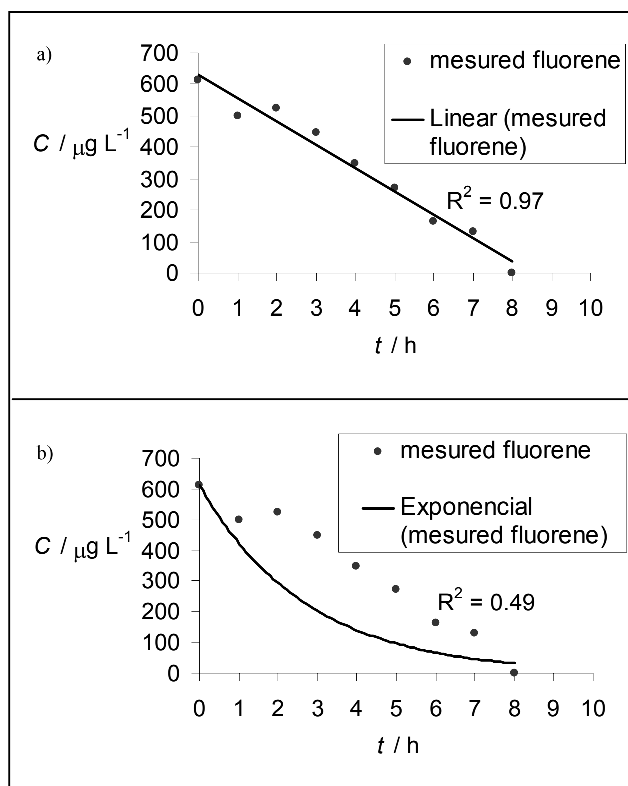

Figure 4 – Regression data from a) zero order and b) 1st order.

The fact that the culture was able to readily degrade fluorene was a positive aspect. In fact, some authors reported that practically no degradation occurred [6], or only cometabolism occurred [7] using mixed cultures. There is a large number of reasons for these different performances [14]. According to the latter, the history of the culture, the design of the experiment and the preparation of the inoculum can have a great influence on the variability of kinetic parameter estimates. The use of a culture long prepared to live in a PAH containing environment (hydrocarbon contaminated wastewater) could be a significant aspect in obtaining these results. The fact that this culture was then cultivated in lab with fluorene as a single carbon source could enhance the ability for degrading this compound.

4. Conclusion

The present studies demonstrate some aspects regarding the biological removal of fluorine. Among them, the following should be highlighted:

- The saturation type model showed to be the best kinetic model to describe the process;
- The abiotic losses of fluorene were not significant, including the biosorption process that did not affect the biological removal of fluorene and the biological degradation was the process responsible for the removal of fluorene;
- The degradation rate calculated was $k_{max} = 3.20\times10^{-4}$ $h^{-1} \pm 0.93\times10^{-4}$ h^{-1} with K_s = 126 μg L^{-1}. The low value of K_s indicates that the maximum removal rate is maintained until low concentrations are achieved;
- The culture showed a good ability to degrade fluorene as a single carbon source, a phenomenon not often reported in literature.

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