FATE OF PROTOZOA DURING THE START-UP OF A SEQUENCING BATCH BIOFILM REACTOR USED FOR THE DEGRADATION OF HYDROCARBONS

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ABSTRACT

Large amounts of oily sludges are retained in patrol stations by gravity interceptors in order to prevent their discharge in surface waters. Those residues are mainly composed of a mixture of hydrocarbons, water and inert solids. Therefore, their final disposal represents a significant environmental problem. This work concerns the study of microfauna biomass during the start-up of a SBBR designed for the treatment of hydrocarbon slurries retained in an API oil separator. The findings of this study suggest that protozoal colonization of the SBBR followed the same pattern of activated sludge systems, the free swimming and crawling ciliates, and also fagellates being the dominant protozoa group during the star-up period of the reactor. When the plant performance dropped, the number of flagellated protozoa rapidly increased, and a concomitant decrease in the ciliated population was observed.

KEYWORDS

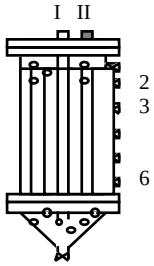
Protozoa; Sequencing Batch Biofilm Reactor; hydrocarbons.

INTRODUCTION

The hydrocarbons still cause great public concern due to their persistence, toxicity, mutagenicity and carcinogenicity (Hutzinger and Veerkamp, 1981). Gravity interceptors are used in patrol stations in order to reduce the hydrocarbon release into natural waters (*e.g.:* API, CPI separators). The retained "oily sludge" (a mixture of organic compounds, water and sand) has to be removed periodically from the interceptors. Then, the final disposal of such sludges is the problem. Biological processes can eliminate the aromatic compounds if favourable environmental conditions are provided (Liu and Sulfita, 1993). In that regard, Sequencing Batch Biofilm Reactors (SBBR) have been used to remove specific organic compounds present in different industrial effluents (Irvine and Ketchum, 1989). The ability of protozoa to graze free swimming bacteria and control floc formation in treatment plants is well known. More recently, attention has been paid to the changes in microfauna composition and their monitoring potential in SBR used for biological nutrient removal (Cybis and Horan, 1995; Rodrigues *et al.*, 1998). However, there is a lack of information concerning the distribution of suspended microfauna biomass in SBBR used for the degradation of toxic pollutants. Consequently, the aim of this research was to study the succession of protozoa species during the start-up of a SBBR used for the degradation of hydrocarbons.

MATERIALS AND METHODS

The SBBR system consisted of an 8,8 L reactor. Cylindrical PVC tubes were used as support material. The SBBR was filled up with a mixture of hydrocarbon slurries (previously filtered through a mesh with porosity of 1 mm) and water, in the proportion of 1:10 (v/v). The reactor was operated at room temperature. Each cycle of the SBBR consisted of 4 periods: Fill, Reaction, Settle and Draw. The duration of the Reaction phase was 5 hours, continuously aerated. The settle period was 1 hour and Fill and Draw were almost instantaneous. The volume exchange in each cycle was 50% of the total volume. The reactor was inoculated with a *Pseudomonas putida* strain ATCC 175414 (DSM 548). The HRT was 12 days. Figure 1 presents the schematic diagram of the SBBR used in the present study.



Legend:
I – Air inflow
II – activated carbon trap
2,3,6 – Sampling points

Figure 1 – Schematic diagram of the SBBR.

The composition of the hydrocarbon slurries retained in the API oil separator is presented in Table 1.

Table 1 – Average composition of the oily sludge.

TSS (mg/L)	VSS (mg/L)	Total COD (mg/L)	Soluble COD (mg/L)	Oil and grease (%)
660 ± 57	60 ± 28	22400 ± 9051	8550 ± 1485	1.248 ± 0.003

SBBR monitoring was carried out by measurements of COD, solids and oil and grease. The analytical procedures followed the guidelines prescribed by APHA (1989). Protozoa were identified and enumerated as described in Madoni (1994).

RESULTS

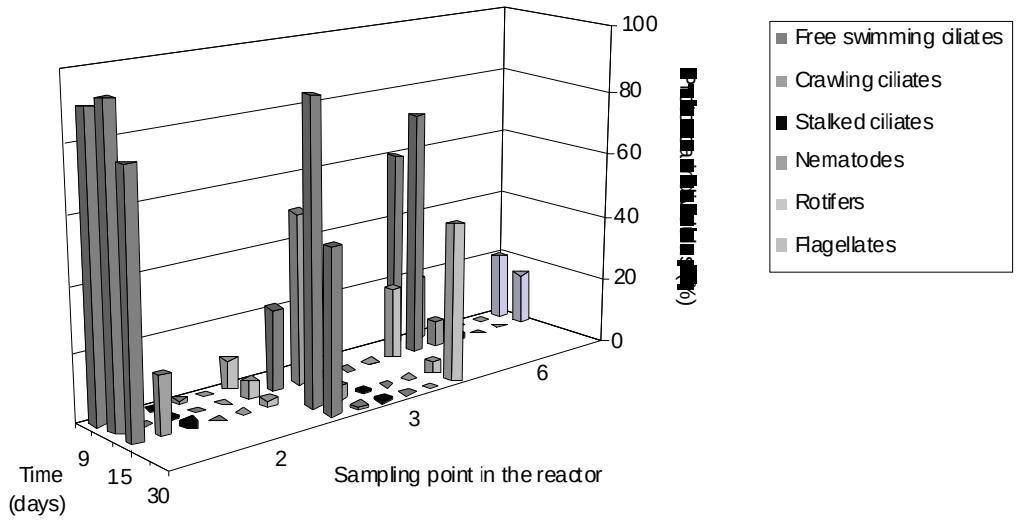
After 30 days of SBBR operation, a significant fraction of contaminants was eliminated with an efficiency of 96% as total solids, 61% as total suspended solids, 58% as total COD and 85% as oil and grease. Nevertheless, there was still 400 mg/L of oil and grease present in the wastewater (after discharge).
During the whole period, the microfauna suspended in the liquid phase was monitored by microscopic observations and identified according to their morphology, size and mobility. The observations revealed a high density of protozoan organisms comprising free-swimming, crawling and attached ciliates, as well as flagellated protozoa. Rotifers and nematodes were also detected. The main protozoa and metazoa species observed are summarized in Table 2.

Table 2 – Protozoa and metazoa species observed during SBBR operation.

Functional group	Functional sub-group	Observed species
Ciliated protozoa	Stalked	*Vorticella* sp. *Epistylis* sp.
	Free swimming	*Uronema* sp. *Colpidium* sp.
	Crawling	*Aspidisca* sp. *Chilodonella* sp.
Flagellated protozoa		*Peranema* sp.
Metazoa	Rotifers	
	Nematodes	

The evolution of the protozoan and metazoan populations during the first 30 days of SBBR operation is depicted in Figure 2.

Figure 2 – Succession of protozoa during the start-up of the SBBR in different sampling points.



DISCUSSION

The microscopic examinations of the microfauna during the start-up period of the SBBR, for a total of 120 cycles (30 days of operation), revealed a numerous and diverse protozoan population. The free swimming ciliates were the dominant protozoa species in the system, followed by crawling ciliates and also flagellated protozoa. In general, the microfauna distribution profile is identical in all the sampling points of the SBBR, as can be seen in Figure 2, indicating that the environmental conditions should be similar along the reactor's height. The high number of free swimming ciliates during the first days of operation should be related to the high concentration of bacteria suspended in the liquid phase. With floc formation, their number decreased. The number of free swimming ciliates reached the maximum value on day 26 (sampling point number 3), 25260 individuals per mL. On day 30, a drop on the free swimming ciliates population and a concomitant growth of flagellated protozoa was observed. A possible explanation was the build-up of recalcitrant contaminants in the system, an alarm level being attained then. Indeed, flagellated protozoa are often observed in treatment systems operating under unfavourable environmental conditions (Cybis and Horan, 1995). The density of nematodes and rotifers was much lower than the protozoan population, most probably due to the higher generation time of metazoans.

CONCLUSIONS

The protozoal colonization of a SBBR treating hydrocarbon slurries seems to follow the relative predominance diagram of a conventional activated sludge system (Curds, 1973), free swimming and crawling ciliates, as well as flagellates being the most observed protozoa groups during the start-up of the reactor. The results also revealed that a sudden growth of flagellated protozoa (and a concomitant decrease in the free swimming forms) may be an indicator of a toxic level in the treatment plant.

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