COMPOSITION AND ACTIVITY OF A DENITRIFYING BIOFILM ALONG AN ANOXIC RBC REACTOR

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ABSTRACT

The variation of the biofilm characteristics of an anoxic rotating biological contactor, formed under a carbon/nitrogen ratio of 2, were studied. The biofilm activity was assessed in terms of the substrate removal ability and it showed a decrease along the reactor. Parameters as thickness, dry weight, density and specific components (proteins and polysaccharides) were also quantified. These properties were determined in biofilm portions from different parts of the RBC reactor. They show a different trend of variation along the bioreactor.

KEYWORDS

RBC reactor; biofilm activity; biofilm components; anoxic denitrification

INTRODUCTION

Biological processes for nitrogen removal are important in wastewater treatment for removal of nitrogen compounds that otherwise result in eutrophication, algal blooms and oxygen depletion (Okabe *et al*., 1997). In such biological processes, rotating biological contactors have been widely used. The principal reasons are the operational simplicity, robustness and low energy consumption. To optimise the RBC performance, is essential the formation of a stable biofilm. Wastewater biofilms are very complex systems consisting of microbial cells and colonies embedded in a polymeric matrix which structure and composition is a function of biofilm age and environmental conditions (Lazarova and Manem, 1995). Thus, it is essentially to know the composition and the activity of the biofilm. Biofilm composition can be described by measuring specific constituents as exopolysaccharides and proteins. The physical properties can be estimated by the thickness, density or mass. The specific activity of a biological film can be calculated from the amount of substrate removed per unit of dry weight. The rates and extent of transport of nutrients or metabolites within or through the biofilm are largely dependent on the density and properties of the internal structure (Christensen *et al*., 1989). Understanding the properties of the biofilm and their interrelationships is crucial to elucidating the behaviour of the biofilm (Zahid and Ganczarczyk, 1994).
The objective of the present work is to study the variations of the properties of a denitrifying biofilm, formed under a carbon/nitrogen ratio of 2, along a rotating biological contactor.

MATERIALS AND METHODS

Experimental apparatus

The experimental set-up was an anoxic RBC system consisting of 13 poly-metilmethacrylate disks (diameter = 23.4 cm) and a hood in a single stage, having a total volume of 15.5 l. The rotational speed was 2 rpm and the temperature was maintained around 26ºC by means of a water bath. The reactor was operated with a hydraulic retention time of 2 hours and fed with a synthetic medium containing 50 mg $N\text{-}NO^{3-}$/l, using citrate as the carbon source.

Operational conditions and substrate composition

The reactor was inoculated with 600 ml of a culture of *Alcaligenes denitrificans.* For the initial formation and accumulation of the biofilm, the reactor was operated in a batch mode for a week. After this period, it was fed with a synthetic wastewater: 408.1.mg/l $C_6H_5Na_3O_7 \cdot 2H_2O$, 360.93 mg/l KNO_3, 930 mg/l K_2HPO_4, 180 mg/l KH_2PO_4, 0.242 mg/l $NaMoO_4 \cdot 2H_2O$, 0.56 mg/l $FeSO_4 \cdot 7H_2O$, 0.81 mg/l $MnCl_2 \cdot 2H_2O$, 0.515 mg/l $CaCl_2 \cdot 2H_2O$ and 4092 mg/l $MgSO_4 \cdot 7H_2O$.

Experimental procedure

During the course of continuous operation, the parameters such as pH, temperature, dissolved oxygen (DO), biomass concentration, nitrate concentration, nitrite concentration and citrate concentration, were measured. For the determination of nitrite, nitrate and citrate ions the sample was filtered through a 0.2 µm membrane filter in order to remove the interference of suspended particles.

Analytical Methods

Citrate and nitrate concentrations were measured by HPLC (Jasco) in an organic acids column (Chrompack, 300 mm x 6.5 mm). The total solids (TS), total volatile solids, pH and DO were determined in accordance with the Standard Methods of Analysis (APHA, 1992). Extraction of extracellular polymeric substances (EPS) was done according the method of Azeredo *et al.* (1998). The total protein content was determined by the Lowry modified method, using the protein assay kit SIGMA P5656 with a standard of BSA (bovine serum albumine). The polysaccharides were estimated colorimetrically by means of the phenol-sulphuric acid method of Dubois *et al.* (1956), using glucose as standard. The biofilm thickness was measured with a nonius measuring rule. The density was calculated in terms of dry mass per unit of wet volume and the procedure was as follows. A known portion of biofilm was inserted into a 10 ml graduated cylinder partially filled with distilled water. The liquid volume displaced was measured. The mass of biofilm was converted in total solids.

Activity tests:

The assessment of biofilm activity was performed by inoculating 150 ml of the feeding medium, in a 250 ml Schott flask, with a known amount of biofilm. The biofilm samples to be used as inocula, were removed from the 1st, 7th and 13th disks, in order to evaluate the activity along the reactor. All the assays were duplicated. The flasks were incubated in an orbital shaker, at 26ºC and 150 rpm. The consumption of substrate (nitrate and citrate) was followed along the time. The specific activity was expressed as mg substrate per mg dry weight.

RESULTS AND DISCUSSION

Table 1 shows some properties relevant for the characterisation of the *Alcaligenes denitrificans* biofilm formed under a carbon/nitrogen ratio of 2

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Table 1. Characteristics of the denitrifying biofilm along the RBC reactor, formed under a C/N ratio of 2.

	Thickness (mm)	Density (g/l)	VS/TS (g/g)	WW/DW	mg Prot./g TS	mg Polys./g TS
Disk 1	2.21	15.73	0.16	66.1	2.62	11.66
Disk 7	1.44	29.64	0.17	34.2	1.71	7.92
Disk 13	0.94	41.39	0.19	27.4	3.8	10.78

WW/DW – Wet weight/Dry weight; Polys.- Polysaccharides; Prot. - Proteins; VS – Volatile solids; TS – total solids.

The activity of the biofilm portions removed from different parts of the reactor (disk 1, 7 and 13) is expressed as substrate uptake rate (Figure 1).

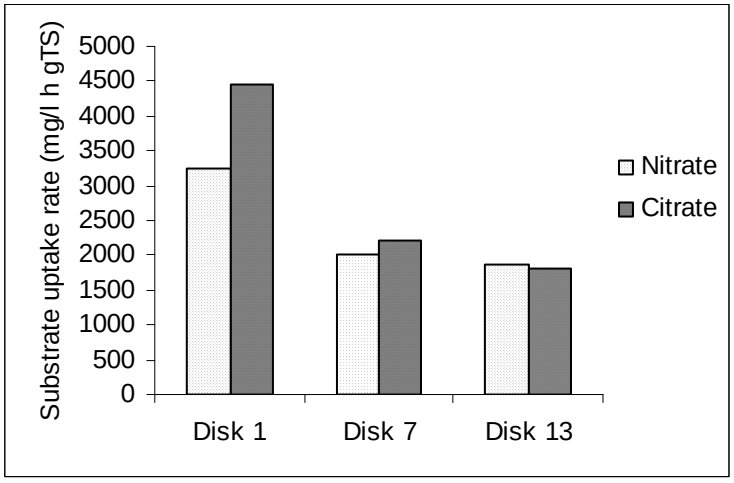

Figure 1. Maximum specific activity rate of substrate consumption.

It must be stressed that the ratio VS/TS in Table 1 is an estimation of the mass of cells per dry weight of the biofilm. The volatile solids were determined after the extraction of the polymeric matrix and so only the cells are expected to remain.

As can be seen in Table 1, the biofilm thickness decreases along the reactor together with the degree of hydration. However, the density and the cellular mass (VS/TS) follow an opposite tendency. This is a coherent result taking into account that a lower thickness and a less hydrated biofilm with a higher number of cells is expected to have a higher density.

Nevertheless, the activity is not directly related with the cellular mass, since it decreases along the reactor as can be seen in Figure 1. The higher number of adhered cells in the final part of the reactor can be due to the tendency that bacteria have to accumulate near the substratum in thin biofilms. In thicker biofilms, the moving boundary of the biofilm expands whenever bacteria grow or generate themselves (Zhang and Bishop, 1994).

It has been reported that thick biofilms are usually less active than the thin ones, on account of mass transfer limitations. In the present case, is the opposite situation that is verified. One reason is a higher concentration of nutrients in the inlet part of the reactor and probably also the structure of the biofilm. A more hydrated biofilm can have water channels favoring the transport of nutrients.

The higher protein content in the polymeric matrix of the biofilm in disk 13 might be due to an increase in natural cell lysis. In this part of the reactor the biomass was dark brown, which can be an indication of some cellular degradation.

REFERENCES

APHA (1992). Standard Methods for the Examination of Water and Wastewater. Greenberg, A. E., Clescer, L. S., Eaton, A. P. and Franson, M. H. A. (eds) 18th ed., APHA, Washington D. C., USA.

Azeredo, J., Oliveira, R. and Lazarova, V. (1998). A new method for extraction of exopolymers from activated sludges. Wat. Sci. Tech. 4-5, 367-370.

Christensen, F. R. *et al*. (1989). Biofilm structure - an important and neglected parameter in wastewater treatment. *Wat. Sci. Tech.* **21**, 805.

Dubois, M. Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956). Colorimetric method for determination of suggars and related substances. *Analyt. Chem.* **28**, 350-355.

Lazarova, V. and Manem, J. (1995). Biofilm characterisation and activity analysis in water and wastewater treatment. *Wat. Res.* **29**, 2227-2245.

Okabe, S., Hirata, K. and Watanabe, Y. (1997). Significance of the spatial distribution of microbial species in mixed-populations biofilms. *Biofouling* **11(2)**, 119-136.

Zahid, W. M. and Ganczarczyk, J J. (1994). Structure of RBC biofilms: *Water Environment Research* **66**, 100-106.

Zhang, T. C. and Bishop, P. L. (1994). Structure, activity and composition of biofilms. *Wat. Sci. Tech.* **7**, 335-344.