Influence of nematodes grazing bacteria on biomass growth in a biotrickling filter treating chlorobenzenes

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ABSTRACT. Biotrickling filters can be applied for the treatment of volatile organic compounds in contaminated air streams but the clogging of high-performance biotrickling filters limit their implementation in industry. This work was aimed to evaluate the influence of nematodes on biomass accumulation and the performance of a biotrickling filter treating chlorobenzene-containing waste gas.
Microscopical investigations of microbial community growing on packing material of a 40-L laboratory biotrickling filter showed that up to a mass loading rate of 4.55 kg m^{-3} d^{-1}, there was a large diversity of organisms. When a nematode inhibitor, ivermectin, was added to the trickling liquid at a concentration of 0.2 mg l^{-1}, the removal efficiencies were maintained at 85-90 % whereas the biomass accumulation rate increased from 0.010 to 0.019 kg m^{-2} d^{-1}, two days after adding the inhibitor. This result indicated that nematodes were effectively reducing the biomass accumulation rate and the application of organic solvent-resistant nematodes can therefore be an efficient mean to reduce the frequency of backwashing.

1 INTRODUCTION

Biotrickling filters are used successfully for the purification of industrial waste gases containing specific pollutants such as chlorobenzenes. The waste gas is introduced through a packed column that is continuously wetted by recycled liquid phase. After inoculation with the mixed microbial culture, air containing chlorobenzenes was added to the system. The microbial degradation starts and the biofilm forms on the packing surface.
The excessive biomass development on the support material in the biotrickling filters, especially at higher inlet concentrations, can cause pressure drop and clogging problems. Clogging in the biotrickling filters is a complex phenomenon determined by diverse factors such as the characteristic of the pollutants, their microbial degradation rates and the morphology of the formed biofilm and the characteristics of the packing material. several options have been proposed for controlling the biomass accumulation for long-term operation in the highly loaded biofilters including: limitation of moisture (García-Peña *et al.*, 2001), nutrient limitation (Allan *et al.*, 2002; Holubar *et al.*, 1999;

Moe and Irvine, 2001) , addition of growth-limiting concentrations of NaCl (Schonduve *et al.*, 1996), insertion of periods with starvation (Cox and Deshusses, 2002). Alternatively, the inert packing material can be cleaned by regular backwashing with water or with various chemicals (Alonso *et al.*, 1997; Sorial *et al.*, 1995; Weber, Hartmans, 1996) or by physical removal of the accumulated biomass (Laurenzis *et al.*, 1998). However, all these methods suffer various drawbacks.

Recently, the processes that reduce the amount of immobilized biomass on the packing increase in importance. It was observed that the addition of protozoa or nematodes to the biotrickling filter resulted in a decrease of the biomass accumulation rate and an increase of pollutant mineralization (Selivanovskaya *et al*., 1997, Cox and Deshusses, 1999). Nematodes are among the primary grazers of bacteria. The development and activity of nematodes inside the biofilm also results in a higher diffusion of oxygen and nutrients and generally, increase microbial activity.

In this study, we investigated the performance of biotrickling filter and the nematode impact on biomass growth and biotrickling elimination capacity degrading an artificial waste gas containing monochlorobenzene (CB) and dichlorobenzene (*o*-DCB). To assess the efficiency of nematodes, the biomass growth on the packing material was followed with and without an inhibitor of nematodes, ivermectin, which has an anthelmintic effect.

2 MATERIALS AND METHODS

Experiments were performed in a laboratory scale down flow co-current biotrickling filter (BTF) operated at room temperature as described in our previous work (Seignez *et al*., 2002). The BTF column was made of glass, with 40 l total volume, 1.27 m height and 0.2 m internal diameter. The column was filled with three cylindrical elements with a height and diameter of 0.3 m and 0.19 m, respectively. The packing material was made of structured PVC (Biodek® FB 10.12, Munters Euroform, Aachen, Germany) with a specific packing area of 240 m^2 m^{-3} and a porosity of 96%.

A large part of the liquid was recirculated through the reactor with a flow rate of 20 l h^{-1} and the pH of the liquid was controlled to be about 7. The mineral salt medium of (Seignez *et al*., 2002) used to replace the trickling liquid contained nitrate as nitrogen source. The ivermectin (0.2 mg l^{-1}) in the experiment of nematode inhibition was added to the nutrient medium.

The empty bed residence time (EBRT) was maintained at 1.9 min and the rate of nutrient medium addition was 0.47 l h^{-1} at 1.8 kg m^{-3} d^{-1}. The waste gas was produced by injection of a mixture of pure CB and *o*-DCB (mass ratio CB: *o*-DCB = 4.2:1) in the air flow up to a maximal concentration of 8.9 g_{CB} m^{-3} and 2.8 $g_{o\text{-DCB}}$ m^{-3}. The biotrickling filter was inoculated with an adapted culture (Seignez et al. 2001) of bacteria cultivated in a batch reactor with CB and *o*-DCB as sole source of carbon and energy and during the biotrickling filter operation, no precautions were taken to prevent colonization with other organisms.

The outlet gas samples were analyzed for their chlorobenzene content by means of an online total hydrocarbon analyzer (Kull Instruments, Oftrigen, Switzerland) equipped with a flame ionization detector. Wet biomass growth was evaluated by continuous weighing of the biotrickling filter, by weighting the packing material elements after drip for 30 min. The biomass dry weight was determined by drying of wet biomass at 105 °C for 24 hours. The microfauna and the nematode vitality were observed by an optical microscope. The identification of nematode was performed on the basis of scanning electronic microscope pictures.

3 RESULTS AND DISCUSSION

3.1 *Biotrickling filter performances*

The evolution of total load and corresponding removal efficiency during the biofiltration experiment of 90 days is shown in Figure 1. In the course of colonization of 14 days, a small loading rate about 1.1 kg m^{-3} $d^{-1}$was applied to obtain the maximal removal efficiency at a given load. From day 15 to 27, the loading rate was increased up to about 2.0 kg m^{-3} d^{-1} which followed by removal efficiency increase up to about 65 %. From 27 to 44 days, the load was decreased to 1.1 kg $m^{-3}d^{-1}$ in order to enhance the chlorobenzenes conversion. In the course of experimentation, up to day 87 the load was stepwise increased to 7.30 kg m^{-3} d^{-1}. During this period the removal efficiency remained constantly high. By this strategy, we were able to increase the load rate without substantial decrease in the removal efficiency. A stable removal efficiency was obtained during whole working period with a maximum of about 90%.

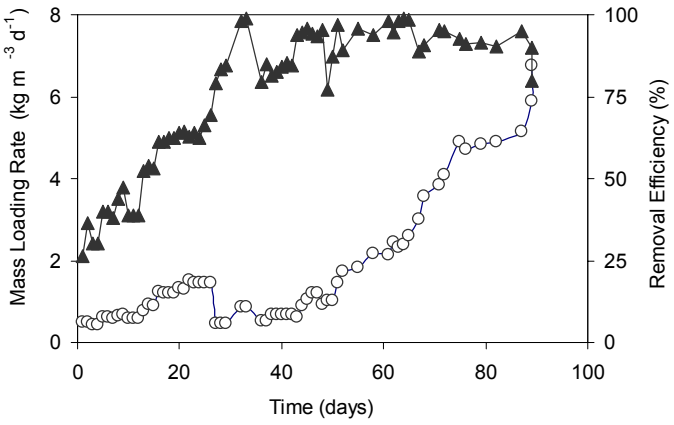

Figure 1. Time course of organic mass Loading Rate (○) and removal efficiency (▲) during the experimental period.

Further load increase to 9.5 kg m^{-3} d^{-1} during last three days of the experimentation resulted in the immediate fall of removal efficiency below 80 %.

3.2 *Mass transfer*

The oxygen and chlorobenzene mass transfer rate were studied to determine transport of which component is the rate limiting in the system.

The overall liquid mass transfer coefficient (K_La) is convenient as it represents the interfacial area per unit volume of the filter (a) and the liquid phase and gas phase mass transfer coefficients (k_L and k_G).

The oxygen-liquid mass transfer is important to get optimal performance of pollutant degradation in biofilm. If oxygen became a limiting factor, the microorganisms are not able to degrade the chlorobenzenes properly. The mass transfer coefficient for oxygen was measured in the absence of biomass. With the gas flow rate of 15 L min^{-1} after 15 min the oxygen concentration in the liquid phase attain 95 % and average oxygen K_La obtained was 11.1 h^{-1}. The maximal concentration measurements of CB and DCB in the liquid phase were 82 % for a load of 1.3 kg m^{-3} d^{-1} using 18.4 L h^{-1} flow rate and

not taking into account the repartition between the two phases according to Henry 's law.

Knowing the diffusivity of chlorobenzene ($D_{COV} = 0.87\times10^{-9}$ m^2 s^{-1}) and oxygen ($D_{O2} = 2.35\times10^{-9}$ m^2 s^{-1}) and $k_La_{(O2)}$ we can calculate the $k_La_{(COV)}$ of chlorobenzene:

$$k_La_{(COV)} = k_La_{(O2)} \frac{\sqrt{D\,\mathrm{cov}}}{\sqrt{Do_2}}$$

the chlorobenzene mass transfer coefficient calculated to be 7 h^{-1}.

The values between 6 and 10 h^{-1} have been established previously in our laboratory for the same biotrickling filter, but filled with another support (Seignez *et al*., 2002), while Pedersen and Arvin (1977) found chlorobenzene mass transfer coefficient being 2-10 h^{-1} depending on the specific system.

3.3 *Microfauna*

Both *Procaryotes*: bacteria and *Eucaryotes*: mycetes, protozoan and metazoan were detected in the biofilm. The bacterial consortium consists of different types of bacilli and cocci (Figure 2).

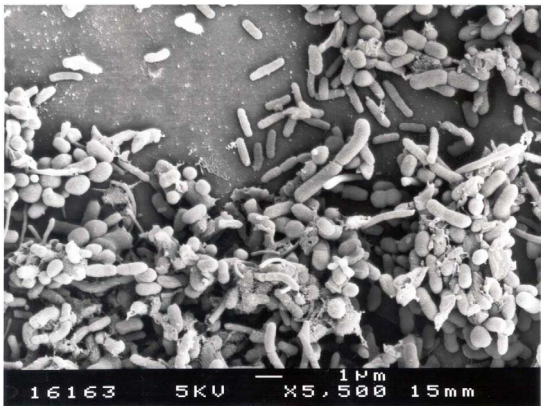

Figure 2. SEM picture of bacteria in the biofilm formed on the biotrickling packing.

In our study special attention has been paid to a particular specie of metazoan, nematodes. They were found in the biofilm together with protozoan and bacteria using optical and scanning electronic microscope (Figure 3).

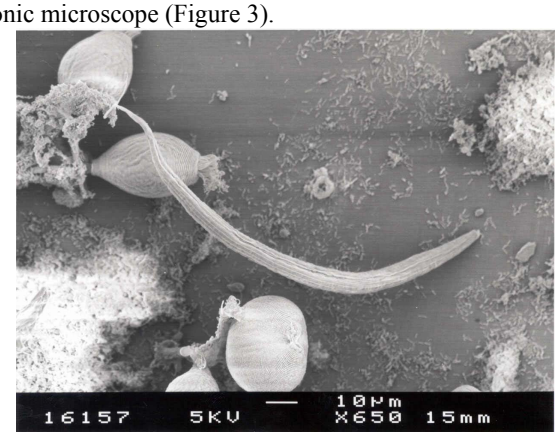

Figure 3. SEM picture of nematode and protozoa in biofilm.

The identification of nematodes, based on SEM pictures, has been made by P. Arpin from Musée National d'Histoire Naturelle, Paris (Figure 4). These nematodes belonged to the diplogasterideae, i.e. more precisely to *Diplogaster nudicapitatus*. Up to a mass loading rate of 4.5 kg m^{-3} d^{-1}, the diversity of microfauna in the biofilm was large including bacteria, fungi, protozoa, and nematodes. At higher mass loading rates, the protozoa disappeared and at loading of above 6.1 kg m^{-3} d^{-1} the nematodes also died off.

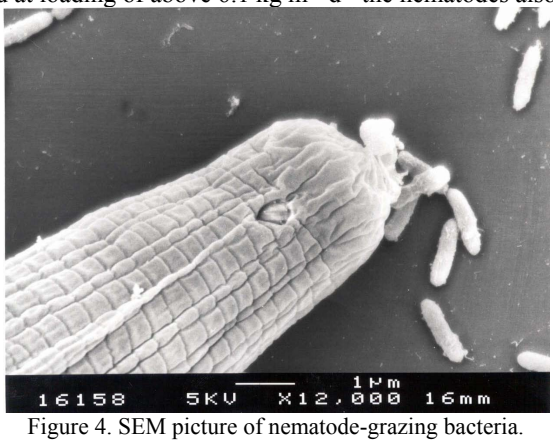

Figure 4. SEM picture of nematode-grazing bacteria.

3.4 *Nematode impact on biotrickling performance*

In the course of continuous biofiltration, due to rapid biomass accumulation in the packed bed, interfacial area for mass transfer decrease, the pressure drop and the pollutant removal decline and the biofilter gets clogged. The prolonged continuous biofiltration results in formation of biomass not actively involved in pollutant degradation (Zuber, 1995; Huub *et al*., 1999).

To investigate the influence of the nematodes on biomass accumulation, in the presence and in the absence of nematodes the experiments were carried out at the mass loading rate of 2.3 kg m^{-3} d^{-1}. To inhibit the nematode activity, ivermectin, disolved in the medium was added to the biotrickling filter at a concentration of 0.2 mg l^{-1}.

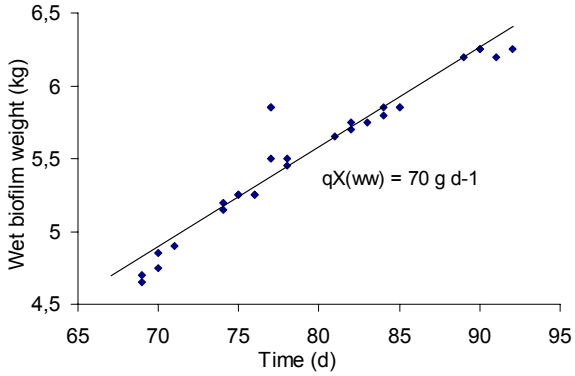

Figure 5. The biomass weight in biotrickling filter without nematode inhibition.

Without the nematode inhibitor, between day 68 and 92, the biomass increased at a constant rate of 70 g d^{-1} and a high removal efficiency of 85-90% was maintained

(Figure 5). In the presence of inhibitor the biomass growth and nematode mortality was measured during six days. The activity of ivermectin was not instantaneous, only 20-33% of the nematodes were dead after two days of the inhibitor addition, and 69-76% of the nematodes were inactive during the following four days. During the first period, the biomass growth rate did not change significantly (75 g d-1) because 70 to 80 % of nematodes were still active and grazed bacteria in the biofilm.

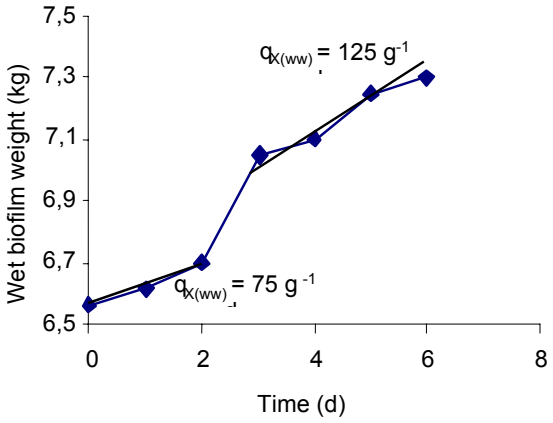

Figure 6. The biofilm weight in biotrickling filter with ivermectin nematode inhibitor.

After 3 days of inhibition when only 24-31 % of nematodes present were active, the biomass growth rate increase significantly and attained 125 g d-1 (Figure 6). The high removal efficiency of 85-90% was also maintained in these conditions.

To confirm these results, CB and DCB degradation experiments were performed in laboratory flasks without and with the addition of ivermectin (0.2 mg l^{-1}) to inactivate nematodes. The experiments were made using 0.8 g l^{-1} of the biofilm formed on the biotrickling filter and a CB and DCB concentration of 8 and 1.9 µg l^{-1}, respectively.

It was found that after about six hours, the chlorobenzenes were completely degraded in both samples and there was no difference between CB and DCB degradation rate in experiments with and without active nematodes (ivermectin addition). So, we concluded that there was no influence of nematodes on the chlorobenzenes degradation.

4 CONCLUSIONS

The influence of nematode presence on biomass accumulation in the biofilm was investigated in a biotrickling filter treating chlorobenzene-containing waste gas. It was demonstrated that the nematodes-grazing activity can help to solve the clogging problems in the biotrickling filters. The biomass accumulation rate was almost twice higher when nematodes were inhibited, showing that application of organic solvent resistant nematodes can be an efficient mean to control biomass increase and to extend operational periods without backwashing.

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