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Characterization of the bacterial communities of aerobic granules in a 2-fluorophenol degrading process

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ABSTRACT

Aerobic granular sludge constitutes a novel technology for wastewater treatment. This study focused on the effect of 2-fluorophenol (2-FP) shock loadings on the microbial community diversity present in aerobic granules before and after inoculation with a bacterial strain able to degrade 2-FP, *Rhodococcus* sp. strain FP1. After bioaugmentation, apart from strain FP1, five culturable bacteria were isolated from the 2-FP degrading granules, belonging to the following genera: *Serratia*, *Chryseobacterium*, *Xanthomonas*, *Pimelobacter* and *Rhodococcus*. The latter two isolates are able to degrade 2-FP. Changes in the aerobic granules' bacterial communities related to 2-FP shock loadings were examined using denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene pool. Numerical analysis of the DGGE profiles showed high diversity with an even distribution of species. Based on cluster analysis of the DGGE profiles, the bacterial communities present in the aerobic granules changes were related to the sampling time and the 2-FP concentration fed.

1. Introduction

Fluorinated compounds, such as 2-fluorophenol, have been found to accumulate in the environment due to their widespread application as agrochemicals, pharmaceuticals and in other industrial processes [22,37]. Most fluoroaromatics are recalcitrant and cause acute toxicity to various life forms [22], although they occur discontinuously and at low concentrations.

Aerobic granulation is an innovative microbial self-immobilization strategy for biological wastewater treatment. Aerobic granules have a strong, dense, smooth and spherical structure, excellent settling properties, high biomass retention, ability to withstand at high organic loadings and tolerance to toxicity [2,10]. The aggregation of microorganisms into compact aerobic granules allows protection against predation and resistance to chemical toxicity [21,41,42]. Therefore, aerobic granular sludge has been successfully used to treat high strength toxic organic wastewater and synthetic wastewater containing pharmaceuticals, namely fluoroquinolones [2,5,25]. The granules have a diverse microbial community, a complex spatial structure, coordinated physiological functions and specific temporal changes [20,39,40,46,47,48]. The microbial diversity of aerobic granules, which has been scarcely studied, has been related to the structure of the aerobic granules and the composition of the culture media in which they were developed [2,25]. Most recently, aerobic granular sludge microbial diversity has been correlated with reactor characteristics and to fluctuations in operation conditions [14,47,48]. However, to our knowledge, the effect of toxic compounds on the microbial community composition dynamics of aerobic granular sludge was not reported yet. Thus, a better understanding of the microbial composition, structure and stability of the microbial community will help to further understand and optimize granule formation in the presence of toxic micropollutants.

It is known that culture-dependent methods have limitations for studying natural microbial community composition, because only a small part of bacteria in environmental samples are culturable under laboratory conditions [4,17,31]. DNA-based molecular techniques, which are culture-independent methods, such as denaturing gradient gel electrophoresis (DGGE), provide a more comprehensive, rapid and concise characterization of the bacterial population diversity in biological wastewater treatment systems. However, culture-dependent methods cannot be totally supplanted, as bacterial isolates are needed to better understand their physiology and function. Population diversity alone does not drive ecosystem stability; hence, gaining an understanding of the functions of microbial communities is important [8].

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The aim of this work was to investigate the dynamics of the microbial community present on aerobic granules subject to 2-FP shock loadings, before and after bioaugmentation of a granular sludge sequencing batch reactor (SBR) with a 2-FP degrading strain. Bacterial strains isolation and denaturing gradient gel electrophoresis (DGGE) analysis of the 16S rRNA gene were used for this purpose.

2. Materials and methods

2.1. Granular sludge SBR set-up

A laboratory scale sequencing batch reactor (SBR) with a working volume of 2.5 L was set up (110 cm height and an internal diameter of 6.5 cm) and was operated during 444 days [12] as described in Table 1. Briefly, the volume exchange ratio per cycle was ca. 40% and the settling time was chosen such that only particles with a settling velocity larger than 6 m h^{-1} were effectively retained in the reactor. This set up was previously reported to successfully conduct lab scale experiments with aerobic granular sludge [7,10,29,47]. The reactor was inoculated with granular sludge removing phosphate biologically (500 mL wet granules) from an aerobic granular sludge pilot plant treating sewage in The Netherlands (Epe wastewater treatment plant) and was bioaugmented with a specialized strain able to degrade 2-FP previously isolated in our laboratories [13], a *Rhodococcus* sp. strain FP1 (LMG 26251; DSM 45581), on day 210. Aeration was supplied at the bottom of the reactor at an airflow rate of 4 L min^{-1}. The experiment was conducted with no oxygen control (dissolved oxygen (DO) 100%) and the pH was maintained at 7.0 ± 0.8 by dosing 1 M NaOH or 1 M HCl.

The composition of the SBR influent media was as described in Duque et al. [12].

2.2. Analytical methods

Samples were collected from the influent (after 60 min influent feeding) and from the effluent of the SBR. The physico-chemical parameters were determined as follows: NH_4^+, NO_3^- and NO_2^- were measured by sequential injection analysis (SIA) as described by Segundo et al. [34] and Mesquita et al. [27], respectively; PO_4^- concentration of filtered samples was determined by flow injection analysis (FIA) according to Torres et al. [45]; 2-FP was analyzed by high performance liquid chromatography (HPLC) and fluoride concentration was measured with an ion-selective combination electrode as previously described [12].

The DO concentration in the reactor was measured online with a DO-sensor (InPro 6820, Mettler-Toledo) as percentage of the oxygen saturation concentration (100% = 9.1 mg L^{-1}) and the pH was monitored online using a pH-electrode (InPro 3030, Mettler-Toledo).

2.3. Sampling of aerobic granular sludge

Aerobic granular sludge samples (about 5 g of granules) were collected during the aeration phase in order to achieve a representative sample of the biomass present in the reactor. The granules were crushed, using a pottering tube and a pestle as described by Weissbrodt et al. [47]. The resulting bacterial suspensions were used for plating, bacterial identification and DNA extraction for DGGE analysis.

2.4. Isolation and identification of 2-FP degrading strains from the SBR

Granules characterization was done by plating serial dilutions of bacterial suspensions of crushed granules in saline solution (0.85% w/v NaCl) onto nutrient agar (NA) (LABM, UK). A volume of 0.1 mL of each dilution was spread onto the NA plates. Plates were incubated at 25 °C for 3 days. Based on size, morphology and pigmentation, different bacterial colonies were isolated from NA plates using the streak–plate procedure. The isolated strains recovered from the NA plates were tested for their capacity to degrade 2-FP, using 250 mL flasks containing 50 mL of sterile mineral salts medium (MM) [9] with 50 mg L^{-1} of 2-FP, as the sole carbon and energy source. In wastewaters pollutants can be found within the ppm range and *Rhodococcus* sp. strain FP1 has been shown to withstand up to 50 ppm of the compound in earlier studies [13]. Cultures were incubated on an orbital shaker at 100 rpm and 25 °C. When growth was observed, indicated by an increase in the optical density and by fluoride release, the culture was plated onto NA plates to verify its purity. DNA extraction and DNA sequencing analysis of the 2-FP degrading strains were performed as described by Duque et al. [12].

2.5. Aerobic granular sludge microbial community analysis

2.5.1. DNA extraction

The genomic DNA extraction of crushed aerobic granules samples was performed using the UltraClean Microbial DNA

Table 1
Summary of the operating conditions tested in the SBR and performance.

Operating conditions	Phase						
	Before bioaugmentation		After bioaugmentation				
	I	II	III	IV	V	VI	VII
Length of operation (days)	0–99	100–209	210–222	223–229	230–266	267–400	401–444
Cycle time (h)	3	3	12	12	8	4	4
Influent feeding (min)	60	60	60	60	60	60	60
Aeration (min)	112	112	652	652	412	172	172
Settling (min)	3	3	3	3	3	3	3
Effluent withdrawal (min)	5	5	5	5	5	5	5
HRT (h)	7.9	7.9	31.6	31.6	21.1	10.5	10.5
Influent $COD_{acetate}$ ($kg\,d^{-1}\,m^{-3}$)[a]	1.0	1.0	0.3	0.3	0.4	0.8	0.8
Inlet 2-FP concentration (mM)[b]	0	0.08	0.08	0.17	0.17	0.17	0.08
2-FP biodegraded (mM)[c]	NA	0	0.056 ± 0.009	0.104 ± 0.008	0.117 ± 0.010	0.106 ± 0.005	0.100 ± 0.009

NA: not applicable.
[a] $COD_{acetate}$: chemical oxygen demand based on acetate.
[b] 2-FP concentration inside the bioreactor.
[c] Values are means ± standard error of the mean (SEM).