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Texture and type of polymer fiber carrier determine bacterial colonization and biofilm properties in wastewater treatment

Tamás Felföldi [a,*], Laura Jurecska [a,b], Balázs Vajna [a], Katalin Barkács [b], Judit Makk [a], Gábor Cebe [a], Attila Szabó [a], Gyula Záray [b], Károly Márialigeti [a]

[a] Department of Microbiology, Institute of Biology, Eötvös Loránd University, Pázmány Péter stny. 1/c, H-1117 Budapest, Hungary
[b] Cooperative Research Centre for Environmental Sciences, Eötvös Loránd University, Pázmány Péter stny. 1/a, H-1117 Budapest, Hungary

HIGHLIGHTS

- Biofilm properties were studied in a cascade system treating municipal wastewater.
- Carrier type and wastewater matrix both affected biofilm structure and activity.
- In the first 2–3 weeks, bacterial taxonomic diversity of biofilm reached its maximum.
- Proteobacteria, Synergistetes, Bacteroidetes and Firmicutes dominated the biofilm.

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ABSTRACT

Effect of carrier texture and type on biofilm development and maturation were studied on an eight-reactors-containing pilot-scale cascade system which treated municipal wastewater under aerobic conditions and various system operational characteristics. Dissimilarities in the biofilm structures grown on four types of polymer fiber-based carriers placed in the reactors having different role during wastewater purification and compositional changes of bacterial communities during biofilm maturation were clearly observable. In the first 2–3 weeks, taxonomic diversity of biofilm bacterial communities increased and reached their maximum values. Carrier type and texture had comparable effect on the weight, activity and composition of biofilms as the wastewater matrix. Biofilm community grown on the carrier having the best colonization properties were dominated by Proteobacteria, Synergistetes, Bacteroidetes and Firmicutes. In the nascent 7-days-old biofilm, aerobic chemoorganotrophic genera, while in the mature 45-days-old biofilm anaerobes, such as sulfate-reducer and fermentative genera were the major members of the bacterial communities.

1. Introduction

Biological processes are fundamental in most wastewater treatment plants (WWTPs), since bacteria are the main drivers of organic matter and nutrients removal [1]. Furthermore, they may facilitate the biodegradation of contaminants that are present in the wastewater at very low concentration levels, such as pharmaceuticals and their metabolites [2,3]. Nonetheless, such microbe-driven purification processes are usually treated as a "black box" during WWTP operation, and treatment efficiency is monitored only by physicochemical parameters. Routine microbiological examinations are mainly restricted to light microscopic monitoring of activated sludge structure and to the continuous hygienic control with cultivation-based methods prior to purified water discharge into receiving waters [4].

Although general processes that take place at WWTPs, such as decomposition of organic compounds and other metabolic transformations, are well known [1,5], unexpected reduction of purification efficiency illustrates that even strictly controlled working conditions may result in shifts of microbial activities, since only a small fraction of biologically important factors are monitored during system operation. On the other hand, regular application of techniques suitable for deciphering the bacterial species that are actually present in individual reactors and their activities would significantly increase running costs of WWTPs. Therefore, studies focusing on the detailed understanding of microbiological processes in wastewater treatment are not only crucial for the

* Corresponding author. Tel.: +36 1 372 2500/8384; fax: +36 1 381 2178.
E-mail address: tamas.felfoldi@gmail.com (T. Felföldi).

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introduction of advanced technologies, but also in the case of routine applications with the short-term monitoring of continuously operating plant-scale or experimental model systems to enhance performance and stability [4]. Additionally, the introduction of next generation sequencing approaches (e.g. pyrosequencing) has opened a new dimension of microbial community analysis [6–8], since these methods are suitable for a much finer reconstruction of community structure and make possible the identification of the whole bacterial community present in WWTPs.

Active microbial biomass could be continuously maintained in two different forms at WWTPs, in a suspended phase, as activated sludge, or attached to a surface, as biofilm [9]. It has been shown that not even operational parameters but also the type of biofilm carrier may significantly influence the attachment of cells, mass of biofilm formed, biofilm structure, etc. [10–13]. These have a significant effect on the performance of individual biological reactors, and finally on the operational efficiency of the whole WWTP. Therefore, this study aimed to explore the role of environmental effects on WWTP microbial communities, namely factors involved in biofilm colonization, including features of the surface (carrier type and texture) and wastewater matrix (concentration of nitrogen compounds, dissolved oxygen, suspended solids, etc.). To reveal possible relationships of microbial and operational characteristics, besides the detailed analysis of wastewater composition, morphological (scanning electron microscopy), physiological (enzyme activity), genotypic (terminal restriction fragment analysis) and taxonomic (pyrosequencing) results of developing biofilm bacterial communities were combined in the case of a pilot scale municipal wastewater reactor cascade.

2. Materials and methods

2.1. Biofilm carriers

Four types of carriers were applied during the colonization experiments: three polypropylene (PP-A, PP-B and PP-C) and one polyester fiber (PES) carrier (distributed by Holges Tex Ltd., Hungary). PP-A and PP-B were both bulked continuous filaments manufactured and woven for carpet industrial applications, but had different color, PP-A was multicolored (yellow, light and dark brown) and PP-B was white. PP-C and PES were technical fibers. The most important characteristics of the applied carriers were determined according to the EN ISO 2062:1995 standard by the manufacturer and summarized in Table 1. according to the results of Jurecska et al. [10].

2.2. Experimental set-up and sampling

Two colonization experiments were carried out in a pilot-scale system consisting of eight cascade reactors operated in the Organica Ecotechnological Development Centre (Telki, Hungary). The cascade system worked with mechanically pretreated municipal wastewater, and each reactor tank had 2 m^3 volume. Each reactor contained PP-B type woven polypropylene fiber with a surface area of ~17 m^2 as carrier, which were covered with mature biofilm. Steel test frames with different uncolonized fiber types were placed only into the selected reactors as described below. The aeration of the cascade system was provided by membrane diffusers placed at the bottom of the reactors. A schematic illustration of experimental set-up is presented in Fig. 1, while some additional data are given in Benedek et al. [14]. The first colonization experiment took for 18 days during spring (S18 experiment, duration from 23rd May to 14th June in 2011), while the second was 45 days long in autumn (A45 experiment; duration from 19th Sept to 3rd Nov in 2011). Hydraulic retention time (HRT) values were 16 and 32 h, respectively. Based on the results of experiment S18, air flow rate was increased in the second part of cascade system to enhance nitrification during experiment A45. The air flow rates in the 5th, 6th, 7th, 8th reactor were 3.0, 2.0, 1.5 and 1.0 m^3/h during S18 and 4.5, 3.8, 3.0 and 2.0 during A45 experiment, respectively; which was checked continuously with a gas flow meter (*t*-mass 65F25, Endress & Hauser, Switzerland) in each reactor.

Test fibers with dimensions of 5 × 30 cm were fixed onto steel frames having at least 2 cm distance among the test pieces (Supplementary Fig. 1), and were placed into the 1st, 2nd, 4th, 6th and 8th reactors at the same time to study the bacterial colonization of the different carrier types. To reduce sample number, three reactors were omitted from the colonization studies, however, to overview the whole treatment process, physicochemical characteristics of the wastewater were recorded in all reactors. Selection was based on the preliminary results presented in Jurecska et al. [10], since the above mentioned reactors played different role during wastewater purification: the first two reactors were responsible for the main COD reduction, while nitrification was characteristic in the second part of the cascade system (see also Fig. 2). During the S18 experiment all types of carriers were investigated, while in the case of A45 experiment, only PP-B and PP-C carriers were tested. A separate frame with the same carrier size as specified above was prepared for each sampling.

Samples were taken on the days 3, 10, and 18 (S18 experiment) and on days 7, 14, 21, 29 and 45 (A45 experiment) after the installation of frames into the reactors.

2.3. Characterization of biomass weight, structure and enzyme activity

The biomass was removed from carriers with distilled water into aluminium pots until no more biomass wash-off was visually observable. The wash-off procedure was carried out under standard laboratory conditions. (The efficiency of the method was checked by comparing the mass of the uncolonized carriers and those of the air-dried, washed-off carriers. The weight of the carriers from which the biomass was removed was on average less than 15% higher than the weight of the carriers prior to colonization.) The weight of the biomass was measured after drying at 105 °C for 24 h to determine the dry mass of the biofilm. (The mass of the samples taken for enzyme activity measurements and molecular biologic investigations was taken into consideration during the calculation of dry biomass values.)

Table 1
General properties of the tested polymer fiber-based biofilm carriers based on the data presented by Jurecska et al. [10].

Fiber type	Filament diameter (μm)	Filament length in a test piece (m)	Filaments number in a test piece	Total surface area in a test piece[a] (m^2)	Wetting force (mN)	Contact angle (°)
PP-A	58	0.280	6600	3.4	−0.334	105.0
PP-B	56	0.275	6600	3.2	−0.170	97.3
PP-C	33	0.300	11,000	3.3	0.283	82.8
PES	22	0.295	8400	1.7	n.d.	n.d.

n.d., no data.
[a] Surface area values were calculated based on the diameter, length and number of filaments presuming a cylindrical geometry for all fiber types.