Bioresource Technology 247 (2018) 591–598

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Insight into the microbial community and its succession of a coupling anaerobic-aerobic biofilm on semi-suspended bio-carriers

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ARTICLE INFO

Keywords:
Coupling biofilm
Semi-suspended bio-carrier
Microbial community
16S metagenomic analysis
Multi-habitat
3D printing technique

ABSTRACT

This work aims at establishing a coupling anaerobic-aerobic biofilm within a single bioreactor and revealing its microbial community and succession. By using a semi-suspended bio-carrier fabricated with 3D printing technique, an obvious DO gradient was gradually created within the biofilm, which demonstrated that a coupling anaerobic-aerobic biofilm was successfully established on the surface of bio-carriers. The results of metagenomic analysis revealed that the microbial community on the bio-carriers experienced a continuous succession in its structure and dominant species along with the operational time. The formed coupling biofilm created suitable micro multi-habitats for the co-existence of these microorganisms, including nitrifying and denitrifying bacteria, which were beneficial to the removing of organic pollutants and converting nutrients. Along with the succession, the microbial community was gradually dominated by several functional microorganisms. Overall, the results presented an approach to improve the microbial biodiversity by constructing a new structure and floating status of bio-carriers.

1. Introduction

Biofilm is a self-aggregated microbial community, which contains lots of functional microorganisms, and is capable of degrading organic pollutants and converting nutrients. Comparing with a conventional activated sludge (CAS) process, a biofilm process has many advantages, including higher biological volumetric conversion rate, greater tolerance to shock loads and toxins, higher biomass density, and less production of excess sludge, thus, biofilm processes have been considered as a modified method for CAS in some wastewater treatment plants (WWTPs) (Luostarinen et al., 2006). In biofilm, large amounts of microorganisms are immobilized, which constitute a stable and robust microbial ecosystem with a special community structure (Lu et al., 2014). The formed biofilm can extend the sludge retention time (SRT) for promoting the growth of those microorganisms having a long generation cycle, and on the other hand, the aged and detached biofilm may be preyed on by protozoa, metazoan and oligochaeta (Derlon et al., 2013; Hendrickx et al., 2011; Li et al., 2013), which can largely limit the production of excess sludge.

In terms of a microbial ecosystem, a common viewpoint has been widely accepted, that is, not an individual microbial species, but a whole system, actually realizes the function of degrading organic pollutants and converting nutrients. Generally, higher biodiversity of an ecosystem, greater stability and more effective performance will be realized (Torresi et al., 2016), therefore, necessary measures should be taken for promoting the biodiversity of an artificial ecosystem (such as a bioreactor) according to this principle. In an actual ecosystem, these environmental conditions, including nutrients, dissolved oxygen (DO), growing space, and the availability of removing metabolites is essential and prerequisite (Tang et al., 2014). However, to each biofilm process, apart from necessary nutrients, the growing space may be the second important factor for the microorganisms to survive. In this meaning, a reasonably constructed bio-carrier is of primary importance for its providing the growing space for most suspended microorganisms to attach on to form a layer of biofilm, and to grow to a mature microbial ecosystem. Only with an integrated microbial community with lots of functional microorganisms, can a biofilm reactor achieve the environmental function of decomposing organic pollutants and converting nutrients (Mohanty et al., 2016; Tang et al., 2016). Traditional bio-carriers are generally fixed in bioreactors, and have long been used in the engineering fields for treating both domestic and industrial wastewater. Their merits have been verified by so many successfully operating engineering projects, but the drawbacks are also very obvious, including the clogging of bioreactor and low mass transfer efficiency. In recent decade, a new kind of bio-carrier, called suspended bio-carrier, was invented and widely studied (Barwal and Chaudhary, 2014). With

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http://dx.doi.org/10.1016/j.biortech.2017.09.147
Received 28 August 2017; Received in revised form 20 September 2017; Accepted 21 September 2017
Available online 22 September 2017
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this kind of bio-carrier, a moving bed biofilm reactor (MBBR) was constituted, which had attracted lots of interests in the fields of theoretical investigation and engineering applications (Nogueira et al., 2015; Young et al., 2016). Comparing with traditional fixed bio-carriers, suspended bio-carriers can move freely in a biofilm reactor, which totally overcome the shortcoming of clogging, and obviously improve the mass transferring within a biofilm reactor. However, due to the abrasion among bio-carriers caused by drastic hydrodynamic conditions, the formation of biofilm on suspended bio-carriers is very difficult, which generally prolongs the start-up of an MBBR.

In a bio-process for treating both industrial and domestic waste water, a combining anaerobic-aerobic condition is needed for totally converting N- and P- containing substances. Based on such a mechanism, an alternative aerobic and anaerobic condition in time or space has been commonly adopted in practical engineering fields, which has been used to develop many practical bio-processes, including so-called the Anoxic/Oxic (A/O) process, Anaerobic-Anoxic-Oxic (A^2/O) process, and Sequencing Batch Reactor (SBR), and they have been successfully used in numerous engineering projects. However, in a previous investigation (Tang et al., 2014), an interesting phenomenon was found, namely, a coupling aerobic-anaerobic environment could be formed in a single bioreactor by utilizing the DO gradient created by mass transferring resistance. The formed coupling conditions composed a multi-habitat environment and greatly promoted the biodiversity within the bioreactor, which enhanced the efficiency of pollutants removal.

Comprehensively considering both the advantages and disadvantages of the traditional fixed and the suspended bio-carrier, a novel semi-suspended bio-carrier was designed in the authors' laboratory and fabricated with 3D printing technique (Tang et al., 2017). This bio-carrier was designed to be a spindle shape to reduce hydraulic resistance, whose smaller end was fixed and the larger end could move freely in a biofilm reactor. With such a configuration, the bio-carrier can be evenly installed in the bioreactor and avoid stacking in the dead zone; on the other hand, it is fixed on one end and the other end can move freely in water, which greatly improves the mass transferring and avoids of collusion to stimulate the formation of biofilm on the surface, therefore, all of the drawbacks of both the traditional fixed and suspended bio-carriers are expected to be totally overcome, and more importantly, an obvious and stable DO gradient was found to form within the biofilm on the bio-carrier (Tang et al., 2017), which might be a new approach to improve the biodiversity of a bioreactor. To the best of our knowledge, arranging bio-carriers in a semi-suspended status is a totally new idea, on which, the growing pattern of biofilm and the contained microbial communities are quite different from that on the traditional fixed or suspended bio-carriers, and may have a novel effect on the microbial community of the related bioreactor. In this regard, it is very essential to have a full understanding of the microbial community on this novel bio-carrier and further reveal its succession along with time. For this purpose, the present investigation carried out an experiment that continuously operated for 100 days in a biofilm reactor packed with this new kind of semi-suspended bio-carrier, which aimed at revealing the composition of the microbial community and its succession on this novel bio-carrier.

2. Methods and materials

2.1. Start-up of the experiment and the basic operational parameters

The used semi-suspended bio-carrier was fabricated by 3D printing technique, whose shape and structure details are shown in the Supporting Information (SI). After inoculating the original seed sludge (1500 mg/L MLSS) from the secondary sedimentation tank of a local WWTP (Lijiao municipal wastewater treatment plant, located in Haizhu district, Guangzhou, China), a rectangular bioreactor packed with these novel bio-carriers was started to carry out all experiments. Other experimental details are described in SI.

2.2. Measurement of the water quality indexes

Water samples were taken from the bioreactor and measured for the concentration of each water index in both the influent and effluent of every day. Regular water quality indexes, including COD, TN and TP, were chosen as the parameters to evaluate the performance of the bioreactor, and NH_3-N, NO_2^--N, and NO_3^--N were also measured simultaneously to evaluate their variation in the bioreactor. All of these water quality indexes were analyzed according to the standard methods (APHA et al., 2005), and they were measured in triplicate with the average value as the final result.

In the present experiment, two DO probes were installed in zone "A" and "B" separately, and the DO value in these two zones was measured simultaneously every 10 s by the connected DO meters. The DO profile within the biofilm was measured by a microelectrode system (including an oxygen probe (Unisense, OX25, Denmark) and a three-dimensional microelectrode propeller (Unisense, MM33-Z8140, Denmark)), whose stepping accuracy was 10 μm. All the measured DO data were transmitted to a connected computer and processed automatically with the average value as the final result.

2.3. Collection of biofilm samples

All the biofilm samples for analyzing the microbial community were collected at the middle line of each semi-suspended bio-carrier. To reveal the succession of the microbial community during the operational period, the biofilm samples were taken simultaneously from the same position of zone "B" and "A" after the reactor being operated for 19, 26, 36, 47, and 61 days, respectively. For making a comparison, the inoculated sludge was also sampled and labeled as "S0", which represented the original community in the bioreactor, and the other samples from different time points, representing the microbial community at different succession stages, which were marked as S19B/A, S26B/A, S36B/A, S47B/A, and S61B/A (the number represented for the day of sampling, and "A" and "B" stood for the corresponding zone that collected samples from), respectively.

2.4. DNA extraction

The biofilm samples collected from both zones at different date were cut into pieces with a sterilized cutter and totally mixed. The total DNA was extracted by using E.Z.N.A.™ Soil DNA Kit (Omega, Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. DNA samples were conducted a quantitative analysis by Qubit double-stranded DNA High Speed Assay Kit with the Qubit 2.0 fluorometer. And then, the extracted DNA was amplified using the bacterial specific primers forward primer Nobar_341F and reverse primer Miseq_805R annealing to the V3–V4 region of the 16S bacterial gene. Two polymerized chain reactions (called PCR) were performed by using Master Mix (Genebase, China). The PCR protocol was illustrated as follows: 94 °C for 5 min followed by 25 cycles (denaturing at 94 °C for 30 s, annealing at 55 °C for 20 s, extension at 72 °C for 30 s) and a final extension step at 72 °C for 8 min (Pitta et al., 2016).

Based on the requirement of DNA sequencing analysis, the quantification PCR products with balanced mixture was utilized by using a sequencing analysis again. Due to the relationship between the paired-end reads and overlap, DNA subsequences were spliced coupled reads, separated by a barcode and filtrated with quality control. Finally, 16S rRNA gene, the purified products were sent for sequencing by using the Miseq platform (Miseq, Illumina Inc, USA). The software preprocessing and information of sequences (PRINSEQ) were used for parsing and processing the acquired information after sequencing.

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