Bioresource Technology 154 (2014) 122–130

Contents lists available at ScienceDirect

Bioresource Technology

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

Aging biofilm from a full-scale moving bed biofilm reactor: Characterization and enzymatic treatment study

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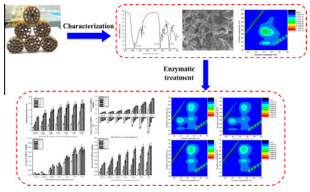
HIGHLIGHTS

- A organic-based aging biofilm with the PN/PS ratio of 20.17 was characterized.
- Four commercial proteases and amylases were tested.
- Better performances of proteases on MLSS and PN/MLSS removal and DOC/MLSS raising.
- Distinguishing mechanisms of the treating process were found for the two proteases.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 September 2013
Received in revised form 5 December 2013
Accepted 8 December 2013
Available online 14 December 2013

Keywords:
Aging biofilm
Moving bed biofilm reactor
Characterization of aging biofilm
Commercial proteases and amylases
Excitation emission matrix (EEM) fluorescence spectra

ABSTRACT

Effective removal of aging biofilm deserves to receive more attention. This study aimed to characterized aging biofilm from a full-scale moving bed biofilm reactor treating pharmaceutical wastewater and evaluate the hydrolysis effects of biofilm by different enzymatic treatments. Results from FTIR and biochemical composition analyses showed that it was a predominately organic-based biofilm with the ratio of total protein (PN) to polysaccharide (PS) of 20.17. A reticular structure of extracellular polymeric matrix (EPM) with filamentous bacteria as the skeleton was observed on the basal layer through SEM–EDS test. Among the four commercial proteases and amylases from Genencor®, proteases were shown to have better performances than amylases either on the removal of MLSS and PN/MLSS or on DOC (i.e., dissolved organic carbon)/MLSS raising of biofilm pellets. Difference of dynamic fluorescence characteristics of dissolved organic matters after treated by the two proteases indicated distinguishing mechanisms of the treating process.

1. Introduction

Biofilm process has been widely used in biological wastewater treatment nowadays, for its strong adaptability to shock loading of the influent, low sludge yield (about three-quarters of the activated sludge process) and high removal efficiency for organic pollutants and ammonia nitrogen of targeted wastewater (Gonzalez et al., 2009). A biofilm attached to bio-carriers is a structured community of mixed species enclosed in a self-produced extracellular polymeric matrix (EPM) which provides structural integrity, bacterial protection and intercellular communication, formation and maintenance of the microcolony, capturing and consumption of nutrients (Simões et al., 2010), and is of vital importance for the performance of biofilm processing system. Biofilm development on carriers is dynamic, beginning with adhesion followed by growth, maturity, senescence, and detachment and finally succeeded by new biofilm formation on denuded areas (Paul et al., 2012; Tang et al., 2011). A balance between biofilm adhesion, growth, and detachment is important in the generation and maintenance of a functional biofilm community. Once the biofilm fails to detach and update timely, aging

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http://dx.doi.org/10.1016/j.biortech.2013.12.031

biofilm (also known as slime) composed of microbial communities and adherent complex substances gradually deposits on the surface of carriers, resulting in a low efficiency or even collapse of the treating system (Bassin et al., 2012; Derlon et al., 2013). Therefore, effective removal of aging biofilm is an important issue in the field of attached-growth wastewater treatment. However, to the authors' knowledge, it has rarely been reported in literatures so far.

The moving bed biofilm reactor (MBBR) has emerged as a representative biofilm processing system with small carrier elements moving around in the aeration tank under operation (Accinelli et al., 2012; Salvetti et al., 2006). Aging biofilm appears at a certain stage along with the treating process and brings about severely adverse effects in many industrial wastewater treatment engineerings (Hu et al., 2013; Trojanowicz et al., 2011). Thus removal of these aging biofilms adhered on hundreds of cubic meters of carriers is of great necessity.

With respect to the removal of biofilms from the surface of supporting materials, studies have already been extensively developed, which were mainly focused in the fields of food industry, paper industry, and biomedicine (Simões et al., 2010). In the emerging reports, several kinds of biofilm control methods were presented, which could be divided into three categories: (1) physical methods such as sonication and hydrodynamics (Paul et al., 2012); (2) chemical methods, mostly common in the literatures, i.e., applying biocides (oxidizing and non oxidizing agents) to kill bacteria (Wunder et al., 2011) or surfactants to destroy the physical integrity of biofilm EPM for subsequent detachment (Seo and Bishop, 2007); (3) biological methods including the utilization of enzymes (Park et al., 2012), bacteriophages (Kim et al., 2012) and microbial interactions (e.g., quorum sensing) (Shrout and Nerenberg, 2012) for biofilm control. When applied for thick biofilms eradication, undesirable effects would be available through physical methods (Simões et al., 2010; Trojanowicz et al., 2011). Chemical agents have good removal effects and cost advantages, but they can inactivate some beneficial microbes against subsequent biofilm regeneration and are also potential sources of secondary pollutions, thus they have been restricted in use by a certain extent (Torres et al., 2012). Biological methods have been regarded as green countermeasures for biofilm control, especially the use of enzymes. Enzymes are biodegradable and of low toxicity, making them attractive biofilm control agents in different industries (Leroy et al., 2008; Simões et al., 2010). Furthermore, as proteins and polysaccharides occupy a major proportion in biofilm EPM, proteases (e.g., serine proteases, pepsin and trypsin) and glycosidases (e.g., amylase, dextranase and pectinase) were always chosen for biofilm removal (Marcato-Romain et al., 2012; Torres et al., 2012).

However, lots of studies were conducted either on young and mono-strain biofilms, e.g., *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Kim et al., 2012; Park et al., 2012), or based on sewage cultivation under laboratorial conditions (Marcato-Romain et al., 2012; Torres et al., 2012), which might be quite different from heterogeneous biofilms in industries in terms of the resistance to internal diffusion of the agents owing to three-dimensional structure configuration of biofilm EPM (Wunder et al., 2011) and the specificity of biofilms from different sources of wastewaters (Bassin et al., 2012). Consequently, the influence of enzymatic treatment on biofilm needs to be reevaluated when it comes to a particular industry.

A favorable strategy for biofilm control should begin with biofilm characterization, and then selection and assessment of control reagents (Simões et al., 2010). In authors' previous study, a high inorganic-type and calcium-accumulated biofilm from MBBR treating vitamin C wastewater was characterized (Hu et al., 2012) and chemical methods were applied for ex situ detachment of biofilm (Hu et al., 2013).

The present study focused on the influence of enzymatic treatment on a high organic-type aging biofilm from a full-scale MBBR treating pharmaceutical wastewater. It aimed to: (1) characterize the biofilm in terms of its functional group structure, surface morphology and biochemical composition so as to choose suitable enzymes; (2) evaluate enzymatic treatment effects of the biofilm; (3) reveal the mechanism of treating process and analyze the application prospect of enzymatic treatment for this type of aging biofilm.

2. Methods

2.1. Materials

Carriers were sampled from a full-scale MBBR in a pharmaceutical plant located in Zhejiang province, China, with the main products of vitamins, quinolones and macrolides. Wastewater with influent volume of 320–410 m^3/d was treated by air flotation, then by MBBR, and finally discharged into urban sewage pipe network. The influent for MBBR was: COD 5000–12,000 mg/L, ammonia nitrogen 200–400 mg/L, salinity 0.6–0.8%. The volume of MBBR tank was 2000 m^3 and the filling ratio of carriers was 30%, with sludge loading ranged from 1.8 to 2.2 kg COD/(kg sludge d). Carriers were cylindrical in shape with three concentric rings supported by interior plastic brackets, 25 mm in diameter and 12 mm in height, respectively, and the specific gravity of 0.98 g/cm^3 (Fig. 1(a)).

It was shown that the regeneration rate of biofilm on carriers was lower than 10% in three months after 425 days of operation, indicating emergence of aging biofilm (Hu et al., 2013) (Fig. 1(b)) and sampling began. Random carrier samples were collected from the MBBR tank by using a sieve and then placed in plastic basins at room temperature (25–30 °C) for subsequent use.

Four commercial enzymatic preparations containing proteases and amylases were selected. The proteases were PROTEX™ 15L and PROTEX®P, the former was an acid fungal endopeptidase from a genetically modified strain of *Trichoderma reesei* and the latter was a subtilisin from a genetically modified strain of *Bacillus subtilis*. The amylases were OPTIMALT®BBA and SPEZYME®XTRA, a barley 1,4-α-D-glucan maltohydrolase commonly referred to β-amylase, and a thermostable starch hydrolyzing α-amylase from a genetically modified strain of *Bacillus licheniformis*, respectively. The enzymes were all supplied by Genencor® (Genencor (Wuxi) Bio-Products Co., Ltd, China).

2.2. Preparation of enzyme solutions and determination of their activities

For each type of enzyme tested, seven different concentrations were prepared by dilutions of original commercial enzymatic preparations with MilliQ water (Millipore Elix®5, USA). Two buffers were used for stabilizing enzyme activity: citric acid buffer (pH 4.5) for PROTEX™ 15L, and phosphate buffer (pH 6.0) for PROTEX®P, OPTIMALT®BBA and SPEZYME®XTRA.

Protease activities were measured according to Leroy et al. (2008). The activities of PROTEX™ 15L and PROTEX®P were both expressed in protease units (PU). One PU was defined as the amount of enzyme required to produce a unit change of absorbance per minute at 25 °C. Amylase activities were determined by measuring the release of reducing sugars (glucose as the standard substance) (Leroy et al., 2008). The activities of OPTIMALT®BBA and SPEZYME®XTRA were both expressed in amylase units (AU). One AU was defined as the amount of enzyme capable of forming one reducing sugar equivalent per minute at 25 °C.