Characterization of Natural Biofilm in Wastewater for Enhanced Growth Rate of Rhodococcus Zopfii Bacteria

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ABSTRACT

We demonstrated characteristic of biofilm that naturally formed in wastewater to enhance growth rate of bacteria. Rhodococcus Zopfii bacterium was selected in this study and it was isolated from municipal sludge. Biofilm surface contained pores with different diameters in the range of 1.972 to 15.180 µm. After adding Rhodococcus Zopfii bacterium in the system, all the pores were covered and filled with Rhodococcus bacterium in three months. This results show that natural biofilm has ability to play a role as a house to Rhodococcus Zopfii bacterium to growth in aerobic condition. Based on FTIR result, O-H, C=H, C=O and N-H bonds were identified as main functional groups on the surface of biofilm. These functional groups may be functional as a source of nutrients to Rhodococcus bacterium to growth. Functional groups that contained carbon (C=C and C=O) can be a source of energy to Rhodococcus Zopfii bacterium. The experimental findings could provide fundamental knowledge on the characteristic of natural biofilm for the enhanced growth rate of bacteria to degrade Polycyclic Aromatic Hydrocarbons (PAHs) presence in aerobic wastewater.

INTRODUCTION

Toxic contaminants have been found in municipal and industrial waste water treatment plant due to intensive usage of chemicals from industrial activities and residential [1–4]. Polycyclic Aromatic Hydrocarbons (PAHs) have been detected in municipal and industrial waste water, and widely known as toxic and carcinogen contaminant to human health. It is very harmful to human health and environment (e.g., lung cancer), birth defects, reproductive anomalies and and chromosome mutation [1, 5]. Due to this reason, intensive studies have been done to remove PAHs in waste water treatment plant.

Recently, PAHs (e.g., Anthracene, phenanthere, pyrene) have been removed by using aerobic and anaerobic bacteria in waste treatment plant [6–8]. However, in typical wastewater treatment system, these bacteria were in suspension condition and easily wash out during the discharge of wastewater effluent from the aeration reactor. Due to this reason, degradation of PAHs by aerobic bacteria was inhibited and PAHs were remained in the effluent [9-10]. To solve this problem, bacteria in aeration reactor must be sustained in the reactor to enhance degradation of PAHs.

Literatures have shown that bacteria has ability to attach and growth on the surface through formation of biofilm [11]. Biofilm provide protection of bacterial cell against a UV light radiation, metal toxicity, pH and osmotic changes dehydration, host immune responses, antimicrobial agent and disinfectants, and source of carbon [12–16]. These findings indicates that biofilm plays an important role as a house for bacteria to growth. However, detail study on the characteristic of biofilm to enhance attachment and growth of bacteria on its surface remained unclear [17]. The aim of this study to characterize surface morphology of biofilm and identify potential chemical functional groups that promote attachment and growth of bacteria on its surface.

MATERIAL AND METHODS

2.1 Biofilm Development system:
Municipal wastewater was used to form natural biofilm in this study. The municipal wastewater was collected from an aeration tank in municipal wastewater treatment at Mawar College, Universiti Teknologi MARA. The sources of wastewater were from student hostels, faculties in Science and Technology Tower, cafeteria, and offices buildings. Biokube was bought from Biokube Malaysia Sdn. Bhd. and it was made from HDPE material. Biokube was putted into the aerobic reactor contained municipal wastewater for three months to promote formation of biofilm on its surface.

2.2 Attachment of Rhodococcus Zopfii on biofilm study:
Municipal wastewater sample (municipal wastewater was collected from aeration tank at Mawar College, Universiti Teknologi MARA) contained biofilm was transfer to the 100 mL of scotch bottle. The samples were autoclaved with sterilizer model Hirayama at temperature 121°C for 20 minutes. At room temperature (25°C) 1 mL of concentrated Rhodococcus bacterium was poured into autoclaved submerged aerated biofilm and mixed them for 24 hr. Samples were wrapped using aluminum foil and place on magnetic stirrer. Oxygen was supply by induce aeration using 0.5cm magnetic bar.

2.3 Bacteria culture:
Rhodococcus was selected as a model for this experiment due to its ability to degrade selected PAHs. Bacterial strain was isolated from municipal wastewater sludge from the Wastewater Treatment Plant at Mawar College, Universiti Teknologi MARA, Shah Alam done by Othman, 2010 [18]. A single colony of stock Rhodococcus bacteria from nutrient agar were growth in 50 mL of centrifuge tubes nutrient broth at temperature 30°C on a rotary shaker (150 rpm) for 7 days. The Rhodococcus bacterium was concentrated using centrifuge at 5000 rpm for 5 minutes. The remaining supernatant in the centrifuge tube was removed and 1 mL of Rhodococcus bacteria was spiked into submerged aerated biofilm system. The sample was leave at room temperature for 24 hours prior to analytical testing.

2.4 Fourier Transform Infrared Spectrometry (FTIR) Analysis:
Fourier Transform Infrared Spectrometry (FTIR) (Perkin Elmer, Model Spectrum RX) analysis was conducted to identify potential functional group on the surface of biofilm. Samples of biofilm were extracted from the layer of biofilm on the surface of biokube using forceps. Approximately 1.0 g of biofilm was transferred onto filter paper and dried it for a few days. Dried samples of biofilm were transferred into universe bottle (9 mL) and tightly cap to prevent any contamination before the FTIR analysis. The samples were placed onto the Universal Diamond Attenuated Total Reflectance (ATR) top plate and in direct contact with the Attenuated Total Reflectance (ATR) crystal. The FTIR analysis used 16 scans at a resolution of 4cm⁻¹ and range of measurement was between 400 - 4000 cm⁻¹.

2.5 Environmental Scanning Electron Microscopy (ESEM) Analysis:
Environmental Scanning Electron Microscopy (ESEM) analysis was conducted to investigate surface morphology of biofilm before and after Rhodococcus bacteria attached on the surface of biofilm. ESEM set up with LFD detection for wet samples (Quanta 450 FEG) with 10 kV voltage and magnification of 1000x, 5000x and 10000x, respectively. Samples were prepared by approximately 1.0g of biofilm surface after autoclave place on the double sided conductive adhesive tape. The biofilm samples were vacuum for 5 min to avoid charging on image results.

RESULTS AND DISCUSSION

![Image of ESEM results]
Fig. 1: ESEM micrograph of biofilm (a) Before 24 hours, magnification 1000x, (b) After 24 hours, magnification 5000x and (c) after 24 hours, 10,000 magnification

Figure 1(a) and (b) show surface morphology of biofilm without Rhodococcus bacterium after autoclave. ESEM analysis demonstrates that surface of biofilm was rough and contained pores with different diameter (1.972 - 15.180 μm). Figure (c) reveals that all the pores on the surface of biofilm were fill up with Rhodococcus bacterium. This result indicates that surface morphology of biofilm has ability to attract Rhodococcus bacterium to attach on its surface, particularly at the pores areas and the bacteria can be sustained in the reactor. This finding reveals the morphology of natural biofilm in this system promotes attachment of Rhodococcus bacterium on its surface.

3.2 FTIR:

FTIR analysis was conducted to detect functional groups on the surface of biofilm. Figure 2 shows detection of functional groups at wavelength 400 cm⁻¹ to 4000 cm⁻¹. Detection of functional group for alcohol/phenol (O-H) ,alkenes (C=C) and amide (C=O) were detected at 3293.87 cm⁻¹, 2124.19, cm⁻¹ and 1635.16 cm⁻¹ respectively. This result is consistent with the experimental results reported in previous studies [19-21]. However, the absorption at 1635.15 cm⁻¹ was attributed to N-H bending vibration of amines [21]. This results indicate that surface of biofilm rich with carbon and potentially play a role as a source of food for Rhodococcus bacteria to growth. From this experimental results, surface of biofilm may significantly enhance growth rate of Rhodococcus bacteria on its surface. Thus, degradation kinetic of PAHs can be enhanced.

Conclusions:
This study investigated surface morphology of natural biofilm form in aerobic condition and identified chemical functional groups that can promote attachment and growth of Rhodococcus bacteria. Rough and pores surface of biofilm promote attachment of Rhodococcus bacteria on its surface. C=C and C=N were dominant functional groups that can be sources of carbon for Rhodococcus bacteria to growth on the surface of biofilm. The experimental findings could provide basic knowledge on the characteristic of natural biofilm for the enhanced growth rate of bacteria to degrade PAHs presence in aerobic waste water

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REFERENCES


