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Zymomonas mobilis biofilm formations on different types of carriers

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Abstract

Zymomonas mobilis biofilm have been proposed to enhance the bioethanol production from agricultural derived materials. *Z. mobilis* biofilm reactor has been prospected to be used for a large scale bioethanol production. The cost effective carrier for *Z. mobilis* biofilm reactor was searched. This study investigated the biofilm forming abilities of *Z. mobilis* strain TISTR 551 and ZM4 on biotic (loofah and corn silk) or abiotic carriers (flatted sheet polyvinyl chloride, PVC). Biofilm formation was visualized for 3 consecutive days under the bright-field microscope. Only *Z. mobilis* TISTR551 represented the biofilm forming ability on corn silk under the microscopic observation, while no biofilm formation on loofah and PVC was observed. The mature biofilm was developed on day 3. The biofilm formation was also quantitatively analyzed based on the weight differentiation of the carrier and the carrier with the bacterial attachment. The net biomass weight of TISTR 551 and ZM4 on corn silk carrier was 0.6 ±0.1 g and 0.33± 0.1g respectively. Therefore, corn silk illustrates its potential to be used as a cost effective biocarrier for *Z. mobilis* biofilm.

Keywords : *Zymomonas mobilis, biofilm*

1. Introduction

Nowadays, alternative energy has been emerging and producing in the industrial scale level to serve the high energy demand. Bioethanol production from lignocellulosic material has been considered to be a potential bioenergy source. *Z. mobilis* is a gram negative facultative anaerobic bacteria, can grow at high concentrations of sugar, and demonstrates high ethanol production though Entner-Doudoroff pathway using glucose as a substrate. *Z. mobilis* in form of free cells either wild type or engineered strains have been widely studied on the ethanol production from lignocellulosic materials [1]. However, the process does not completely optimize due to the high concentration of carbon sources and the availabilities of toxic substances (acetic acid, furfural, organic acid and aldehyde) in the pretreated lignocellulosic materials that impeded the bacterial growth and metabolism. This consequently caused the dramatic reduction in ethanol production through the growth effect [1, 2]. However, the toxic tolerance of *Z. mobilis* was found

to be simply enhanced by the development of *Z. mobilis* biofilm. *Z. mobilis* biofilm has been applied in the production of valuable products in the presence of toxic compounds for some prospects including ethanol production from lignocellulosic material [3, 4].

Biofilms are defined as microbial cell layers which are embedded in an exopolysaccharide metrix that is self-produced and attach to the solid supports. Microbial biofilms have been emerged as potential biocatalyst due to its natural immobilization, stability, remaining viable cells for many cycles of operations, productive for several months, cost effective, less affected by inhibitory compounds and facilitating product recovery [5-8]. In order to apply *Z. mobilis* biofilm reactor for the production of bioethanol from lignocellulosic materials or other sources, we need to search for the suitable biofilm carrier.

Carriers for cell immobilization can be classified into 2 categories; synthetic carrier and natural carrier. Natural carriers are tremendously available as they are cost effective, maintain viable cells for several cycles of operations, easily separated in downstream, process. Chitosan, sawdust, rice husk, rice straw, sorghum baggase and palm pressed fiber have been used as the natural carriersfor many applications [9-11]. Therefore, this research aims to compare the biofilm formation efficiencies on both biotic (loofah and corn silk) and abiotic carrier (polyvinyl chloride). The biofilm forming ability of *Z. mobilis* on these carriers were evaluated based on microscopic observation of biofilm development and dry weight basis.

Material and Method

2.1 Bacterial strains and cultivation

Z. mobilis strain ZM4 (a type strain) and TISTR551 from Thailand Institute of Scientific and Technological Research (TISTR) were used to study on the biofilm forming abilities. They were grown in yeast peptone glucose (YPG) medium (peptone 10 g, yeast extract 10 g and glucose 20 g/L, pH 6.4) at 30°C for approximately 24 hours until the optical density at 600 nm (OD_{600}) reached about 1.0. Biofilms of both strains were developed in the biofilm medium (1 L) containing 20 g glucose, 5 g yeast extract, 5g $(NH_4)_2SO_4$, 0.6g KH_2PO_4, 0.4g $Na_2HPO_4.12H_2O$, 0.2g $MgSO_4.7H_2O$ and 0.01g $CaCl_2$in 10 fold dilution at pH 6.4.

2.2 Preparation of carriers

Loofah and corn silk were cut into small pieces (approximately 2-3 mm). The fine particles were removed by repeatedly suspended in the water and discarded the unsettled particles. Then, the materials were washed with 1M NaOH using a funnel until the filtered liquid turned clear, followed by 0.25M HCl. The materials were repeatedly washed again with 0.25M NaOH until clear liquid was obtained. Finally, the materials were neutralized with tap water until pH 7.0 using litmus paper test. The treated carriers were oven dried at 60°C for 1-2 days to obtain completely dried carriers. The non treated carrier was prepared without acid and base pretreatment only oven dried the washed particles.

Polyvinyl chloride (PVC) was cut into small coupons with the size of approximately 2-3mm^2. Plastic material was sterilized by submerging in 0.1% (v/v) sodium hypochlorite

(NaOHCl) solution overnight. The coupons were submerged in steriled distilled water for 2 days in order to get rid of the excess NaOHCl.

2.3 Biofilm development on carriers

The total 2 g of treated loofah and corn silk were placed in 20 ml of the biofilm medium. The carriers and the medium were autoclaved. The sterile plastic coupons (2 g) were placed in the sterile biofilm medium prior inoculation.

Biofilm media containing loofah, corn silk and plastic carriers were then inoculated with 10% (v/v) of *Z. mobilis* ZM4 and TISTR 551 overnight cultures with the optical density 600 nm approximately 1.0. The biofilm developments were observed under the bright-field microscope and a digital camera (dino-eye model AM423x) on day 0 and day 3. The media were replaced every single day. In addition, the weight of the biofilms or the bacterial attachments on all carriers were monitored on day 3 based on the dry weight basis.

3. Results and Discussion

Z. mobilis have been previously reported to form biofilm on both biotic (DEAE cellulose), abiotic surfaces (glass bead) and plastic composite material (polypropylene with 25% w/w of agricultural materials) [4, 12, 13]. Biotic carriers have been found to be more benefit over abiotic carriers in terms of cost effectiveness, wide availability, less effected by inhibitory compounds [8, 14, 15]. Biotic carrier like DEAE cellulose is still a high cost carrier, and the detachment from biotic carrier is observable when it gets through many cycles of repeated batch process [13]. From our result, corn silk was illustrated as a potential biocarrier when compared to loofah. *Z. mobilis* TISTR 551 had higher ability to form biofilm than ZM4 under microscopic observation and dry weight analysis (Figure 3 and Table 1). The mature biofilm of TISTR 551 was observable on day 3 in which the bacterial cells were embedded in the biofilm matrix (Figure 3b). The principle of immobilization on the natural carrier is normally based on the passive adhesion to the biotic surface [9]. The biofilm forming on the biotic surface provides future benefit for adhesion technique with its simplicity and reducing mass transfer problem. Other bacterial immobilization techniques entrap cells in the insufficient space for living cells and low mass transfer efficiency [8].

In our experiment, the biotic carriers (loofah and corn silk) were treated with acid and base prior the bacterial immobilization in order to deform the rigid structure of the lignocellulosic carriers. This increased the opportunity for cell adsorption between the crystalline structures of the carriers. Without the acid and base treatment, the biotic carriers illustrated more rigidity with all fibrous structure (figure 1) that might impeded the bacterial attachment or biofilm development (figure 2 and 3). ZM4 biofilm was not detectable on non treated corn silk, while TISTR 551 appeared to be less (non published data). The treated loofah still represented more rigidity than treated corn silk, therefore the biofilm development was appeared only on the surface of treated corn silk. Biotic carriers have been reported to be a potential carrier since some bacteria preferred to attach as a potential source of substrate [16]. This could lead to the speculation that treated corn silk carrier provided the structure that was easily to get access through the carrier as substrate source. This also induced the bacterial attachment more on this biotic surface. The high biofilm density on corn silk was probably enhanced cell growth and

nutritional benefit to *Z. mobilis* biofilm. *Z. mobilis* had also been reported to produce cellulase enzyme in which could be a reason to get access to treated corn silk as nutrient source as well [17]. PVC coupon represented no biofilm formation toward 3 days cultivation in biofilm medium.

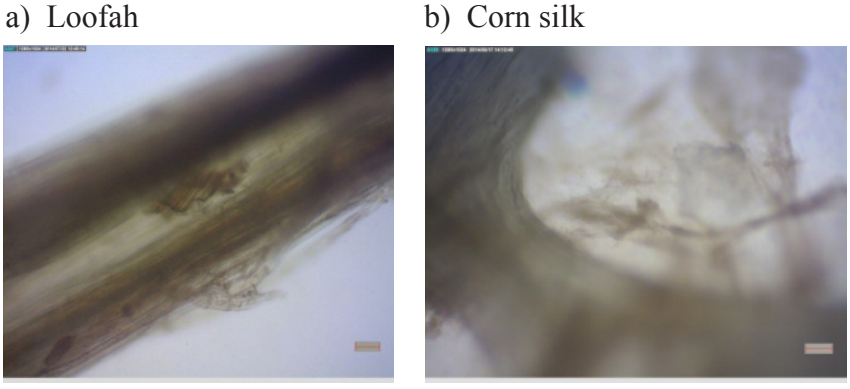

Figure 1. Non treated loofah (a) and non treated corn silk (b) under the bright-field microscope. The pictures were captured with the magnification of 300x, and the bar represents 0.1 mm.

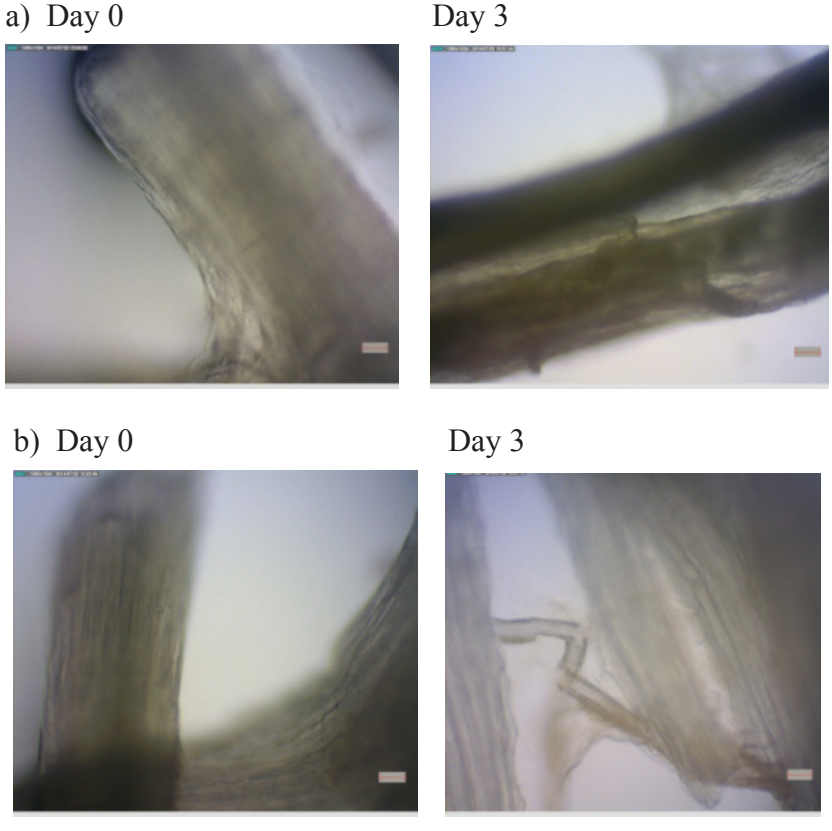

Figure 2. Biofilm formations of *Z. mobilis* ZM4 (a) and *Z. mobilis* TISTR 551 (b) on treated loofah. The pictures were captured by the bright-field microscope with the magnification of 300x, and the bar represented 0.1 mm.

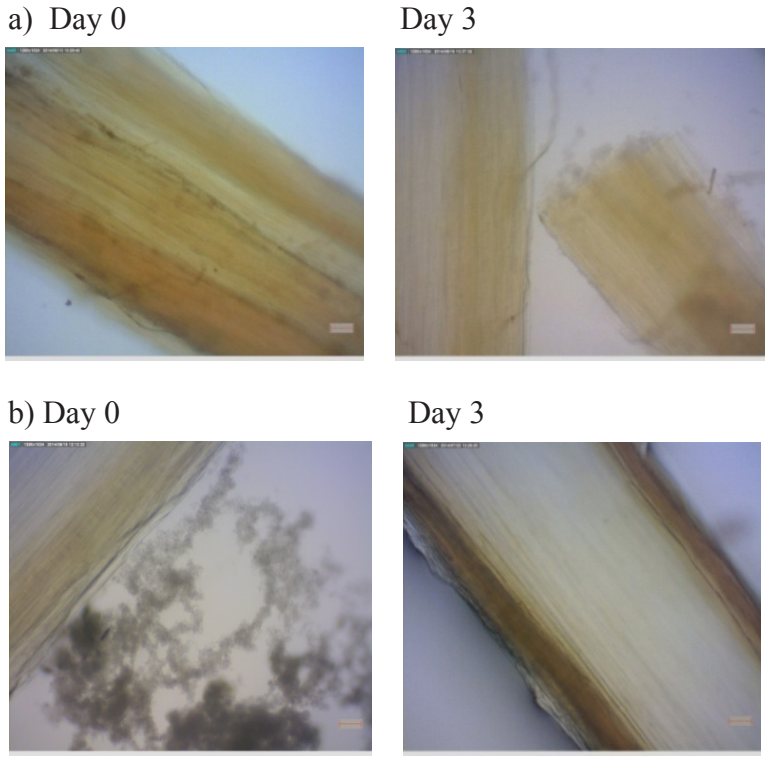

Figure 3. Biofilm formations of *Z. mobilis* ZM4 (a) and *Z. mobilis* TISTR551 (b) on treated corn silk. The pictures were captured by the bright-field microscope with the magnification of 300x and the bar represented 0.1 mm

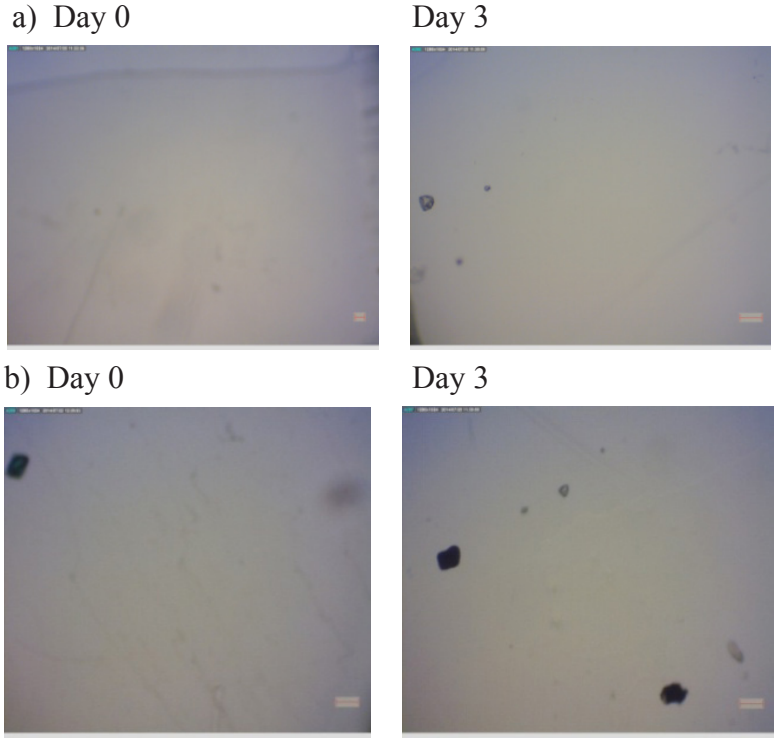

Figure 4. Biofilm formation of *Z. mobilis* ZM4 (a) and *Z. mobilis* TISTR 551 (b) on on plastic surface (PVC)**.** The pictures were captured by the bright-field microscope with the magnification of 300x and the bar represented 0.1 mm.

Table 1. Dry weight of bacterial attachment referred to the biofilm of *Z. mobilis* strain ZM4 and TISTR 551 on treated corn silk

Strain	Dry weight (g)
Zm4	0.33±0.1
551	0.6±0.1

4. Conclusion

In this study, treated corn silk was illustrated as a potential biotic carrier for *Z. mobilis* ZM4 and *Z. mobilis* TISTR 551 biofilm formation. Corn silk is also widely availability as a agricultural waste in which can be considered as a potential biofilm carrier in the biofilm reactor.

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