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Microbial biofouling potential of multispecies of batik dye wastewater

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Abstract

Biofouling is a serious and challenging problem in water treatment systems which hinder the efficiency of membrane filtration performance. The aim of this study was to investigate the biofouling propensity and biological treatment performance of a bacterial consortium in a biological membrane bioreactor for the treatment of dye wastewater. During bioreactor operation with the bacterial consortium, a significant relationship was revealed between transmembrane pressure (TMP) and extracellular polymeric substances (EPS). When tested for dye and chemical oxygen demand (COD) removal, SMBR showed increased removal performance with the operating time, possibly owing to the biofilm formation on membrane and the adaptation of sludge. Thus, it is expected that the results of this study will be valuable for further development of a suitable biofouling mitigation strategy for *batik* wastewater treatment in membrane bioreactor.

Keywords: Biofouling; biofilm, *Batik* wastewater; bacterial consortium; extracellular polymeric substances

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1. INTRODUCTION

Batik homemade textile industries discharge large amount of wastewater, which contains many types of dyes, solvents, salts and detergents[1]. Wastewater dye affects water transparency, gas solubility, and aesthetics of aquatic systems, and can be noxious to aquatic organisms[2]. Hence, it is vital to identify improved strategy to treat dye wastewater. MBRs have appeared as a leading technology in wastewater treatment[3]. However, MBRs are hindered by biofouling, which reduces membrane life, increases membrane cost, decreases filtration performance, and eventually augments extra cost for membrane changing. Accordingly, membrane biofilm problem is a foremost issue and it is difficult problem to control [4, 5].

Biofouling is related to the presence of microbial cells and their products on membrane surface, which blocks pores of membranes and affects filtration process. Different microbial cells cover itself in EPS, and make layers of gel which is called biofilm[6]. Moreover, biofilms are dynamic in nature, with structures that

comprise sequential developmental stages in which bacterial cells attach, and then develop a biofilm via a succession of steps [7]. The roles of microorganisms have already been studied in membrane biofouling [8].

EPS, which are produced by bacterial cells, are composed of a variety of components such as proteins, polysaccharides and lipids[9]. EPS are known to be major biofouling causing substances in MBRs[10]. Indeed, increases in EPS have been shown to cause reductions in membrane flux in MBRs [11, 12].

The aim of this work was to investigate the biofouling potential of a bacterial consortium from batik wastewater. Initially, experiments in submerged membrane bioreactor (SMBR) were carried out with the bacterial consortium to establish a relationship between EPS and transmembrane pressure as an indicator of membrane fouling. Also, for biological treatment potential dye and COD removal were studied.

2. MATERIALS AND METHODS

2.1 Sludge sample collection and preparation of bacterial suspension

Sludge sample collection and preparation of bacterial suspension was carried out according to the method described in our previous study [4].

2.2 Membrane reactor and operation

A laboratory scale membrane bioreactor system (Fig.1) was used in this study. The specifications for membrane and membrane modules are presented in Table 1. Simulated dye wastewater was used in this study. The composition is presented in Table 2 [3]. Bioreactor was fed nonstop and a peristaltic pump was used to collect permeate. Aeration was provided via air compressor. Other conditions for operation are presented in Table 3.

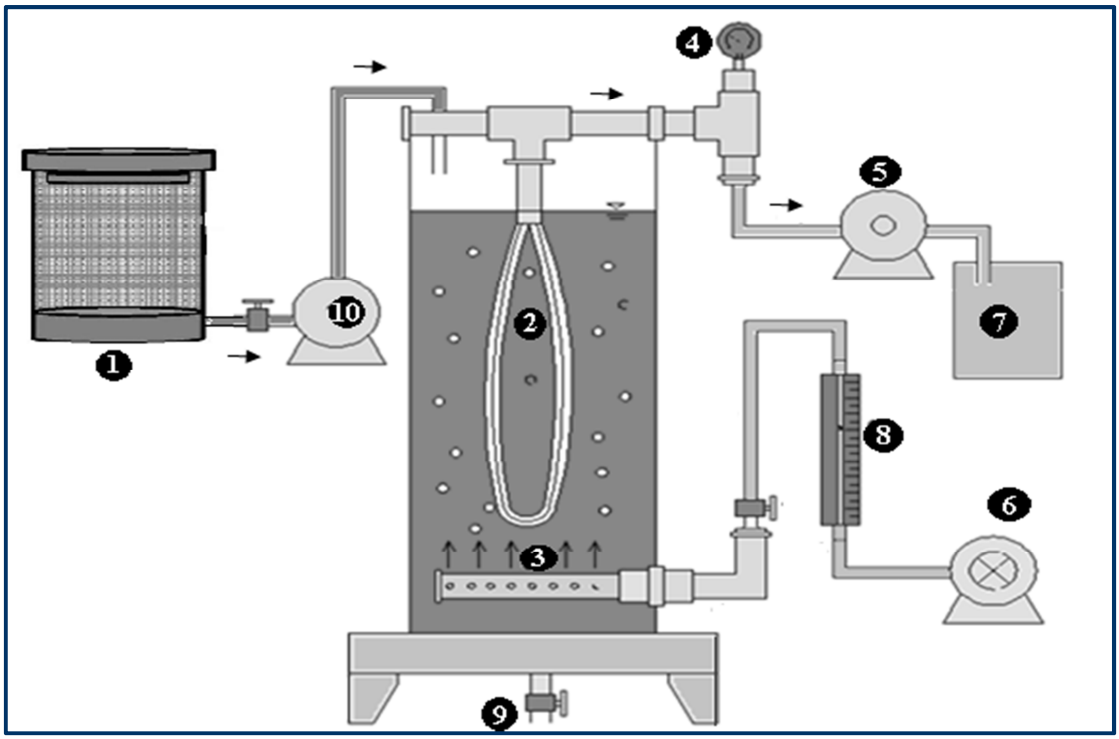


Fig. 1. The reactor setup: 1- Feed tank, 2- Hollow fibre membrane, 3- Air splitter, 4- Pressure gauge, 5- Peristaltic pump, 6- Air compressor, 7- Effluent storage tank, 8- Air flow meter, 9- Drain valve, 10- Feed pump.

Table 1. Membrane and membrane module specifications.

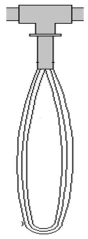
Membrane		**Module configuration**
Model	DL-F/K11-UF	
Material	Polysulfone	
Membrane type	Hollow fibre	
Pore size	0.03 µm	
Surface area	0.05 m^2	
Module		
Configuration	Loop-Shaped hollow fibre	
Fibre outer diameter	600 µm	
Fibre internal diameter	300 µm	
Sampling point	Middle	

Table 2. Composition of synthetic dye wastewater.

Composition	**Concentration (mg/L)**
Glucose	503
Peptone	1665
Yeast Extract	834
Urea	300
KH_2PO_4	167
NaCl	1665
$MgSo_4.\ 7H_2O$	34
Reactive Black 5	100 (± 0.4)

Table 3. Operating conditions used for reactor.

Conditions	
Working Volume (L)	2
TMP (kPa)	< 33
Constant flux (L/m^2 h)	5.2
Air flow rate (L/min)	1
pH	6.5-7.5
Organic loading rate (mg COD/L/day)	2333.33
HRT (h)	30
MLSS (mg/L)	4451 (±200)
SRT (day)	15

2.3 Isolation of mix bacterial cells from fouled membrane surface

The membrane containing biofilm was obtained and it was mixed with phosphate buffer saline. The loosely attached microbial cells were removed by performing gentle mixing. Sterile cotton buds were used to remove biofilm from membrane surface and sample was mixed with phosphate buffer saline. Then it was grown in nutrient broth medium and incubated at 30°C in shaking incubator.

2.4 Microtiter plate assay for biofouling

Microtiter plate assays were performed to investigate the biofouling potential of bacterial consortium. The isolated bacteria were grown in nutrient broth at 30°C for 24 h. Next, 3 µL of bacterial aliquots were inoculated in 96 well microtiter round bottom plates (Corning, Sigma) and incubated at 30°C for 72 h. The contents of each well were then removed by rinsing the plate three times with 150 µL of physiological saline. Further samples were subjected according to the method described by Peeters et al.[13]. It was subjected to microplate reader for quantification at 595 nm.

2.5 Production of EPS under batch conditions

For production of EPS, mix bacterial cells were grown in nutrient broth under shaking conditions at 250 rpm at at 25°C for 6 days. For obtaining capsular EPS in the pellet form, the mixed bacterial broth was centrifuges at 6000 *g* for 15 min (4°C) according to the method described by Zhang et al.[14]. Slime EPS was obtained in the supernatant after centrifugation.

2.6 Analysis of EPS

The concentration of EPS was determined as for polysaccharides and proteins. EPS is the main component of bacterial biofilm. The sum of proteins and polysaccharides was considered as total EPS in the bacterial biofilm[15]. The concentration of polysaccharides was determined by phenol sulphuric acid method[16] and concentration of protein in EPS was determined by Lowry's method[17].

2.7 Determination of dye and COD

Measurement of chemical oxygen demand (COD) was carried out according to standard methods for the examination of water and wastewater [18]. Amount of reactive black-5 was determined at 590 nm with a spectrophotometer (U-1800, HITACHI).

The % decolourization of reactive black 5 was calculated as follows:

$$\text{Decolorization } \% = \frac{A_0 - A}{A} * 100$$

Where A_0 is the influent dye concentration and A is the dye concentration in the effluent storage tank.

3. RESULTS AND DISCUSSIONS

3.1 Change of soluble EPS and its relationship with TMP

Fig. 2 shows the concentrations of soluble EPS in the MBR. In the first stage, the EPS increased slowly from 45.31 to 77.70 mg/L during day 1 to 4, indicating that the sludge induced the production of more soluble EPS. The concentration of EPS further increased to 88.26 mg/L at day 5, possibly owing to the adaptation of sludge. The increase in EPS from day 1 to 4 was smaller than that from day 5 to 8. This was likely because microorganisms were older in the mature stage than the initial stage and hence more EPS were accumulated in the reactor. The increase in soluble EPS can be attributed to deflocculation, but decrease in the same material to the entrapment during the flocculation process.

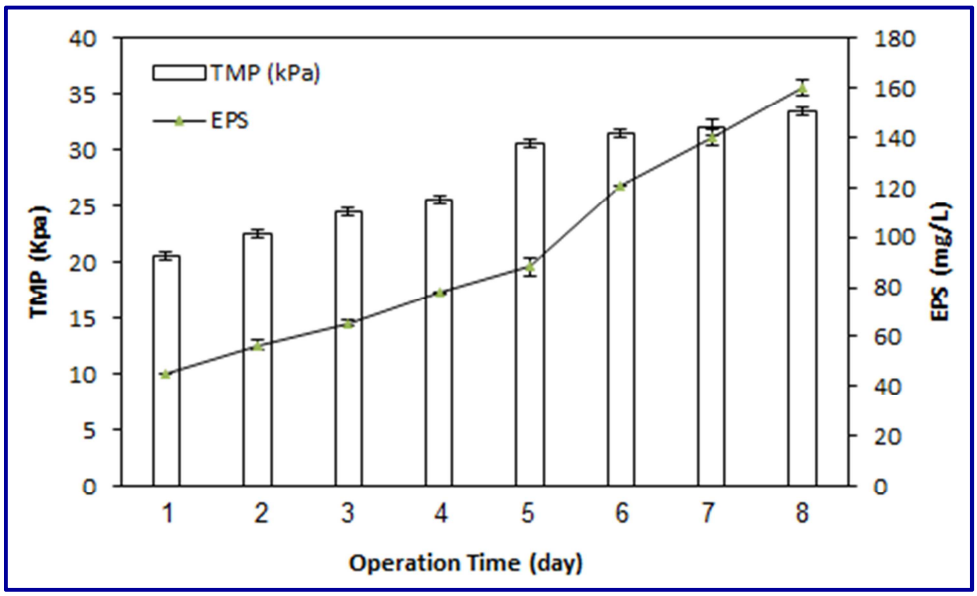


Fig. 2. Changes of EPS and TMP with operation time (bars indicate standard error)

The rate of transmembrane pressure (TMP) increase is a key aspect in assessing fouling in membrane bioreactor owing to its direct effect on the degree of fouling. Various transmembrane pressures with different soluble EPS concentrations were taken into account to establish a relationship. As shown in Fig. 3, the results indicated that the higher increase in TMP in response to EPS is an indicator of membrane fouling. There was a strong correlation (R^2= 0.9916) between soluble EPS and TMP (Fig. 3). Furthermore, the membrane filterability decreased as the soluble EPS increased[19]. These results indicate that increase in the EPS in soluble form will have negative impact on the filtration performance, therefore, it can be

explained that the soluble EPS accumulated on the membrane surface could form "gel-like" cake layers.

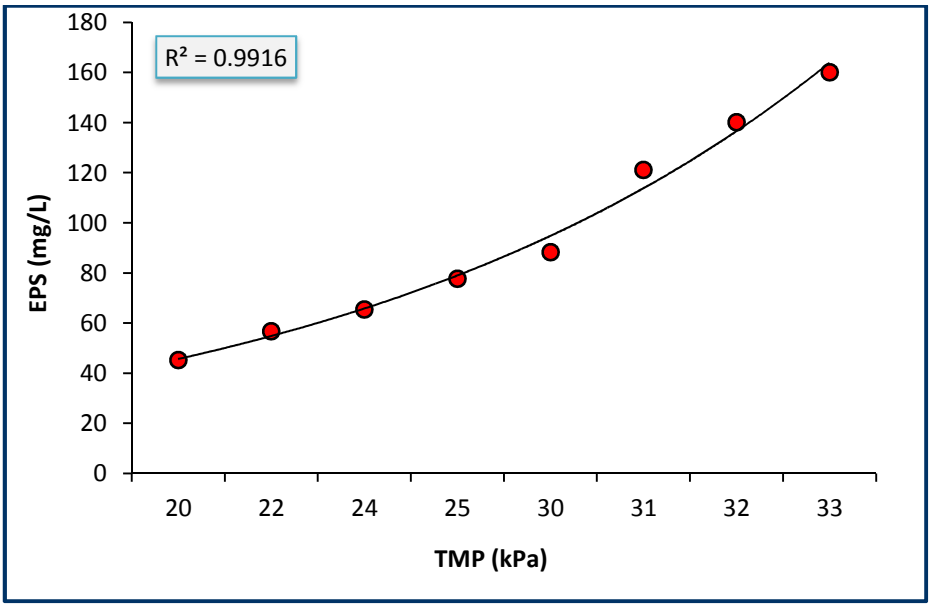


Fig. 3. Relationship of EPS with TMP

3.2 Performance of Biological treatment

The treatment performance of COD and dye removal were also investigated during eight days of operation. As shown in Fig. 4, the dye removal increased slowly from 94.74 to 95.44% during days 1 to 4, which may have been due to adaptation of the activated sludge in the reactor. The dye removal then increased to 98.35% at day 8, which could be due to biodegrading in bacterial biofilm and adsorption to sludge[3]. It was observed that COD removal efficiency was increased with time (Fig. 4). During the initial 4 days, the COD removal efficiency was low, increasing from 95.03 on day 1 to 95.98% on day 4. The removal of COD was increased to 97.77% on day 8 with biomass increase from 4451 to 46691 MLSS mg/L, which was higher than on day 4.

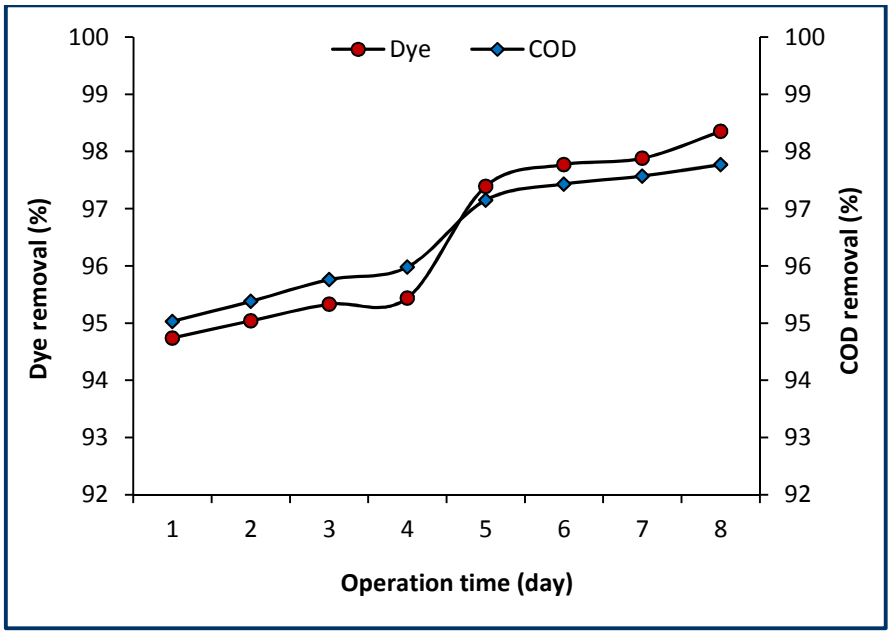


Fig. 4. Profile of removal performance of SMBR system for dye and COD

3.3 Biofouling potential of bacterial consortium

The microtiter plate assay is a standard technique of screening microorganisms for their ability to form a biofilm[20]. The absorbance of crystal violet dye bound to the biofilm cells was determined. Therefore, a larger absorbance indicates greater biofilm formation. The optical density of the biofilm produced by the bacterial consortium was 0.213 ± 0.046. This might have occurred owing to the complex interactions and competition among different strains comprising the consortium.

3.4 EPS production and biochemical characteristics

EPS is known to provide a matrix for microorganisms to grow in, which reduces membrane permeability. As a result, biofilms formed by microbial cells play an important role in membrane biofouling[21]. In this study, the potential of EPS production of bacterial consortium was examined. The EPS produced by the mixed bacterial cells are presented in the Fig. 5. The concentrations of EPS via mixed bacterial cells were increased after each day till six days. Also, it was exhibited that total EPS was increased with time after six days. The amount of EPS has been found to be related to membrane permeability[12] ; accordingly, a reduction in EPS would have a positive effect on membrane permeability.

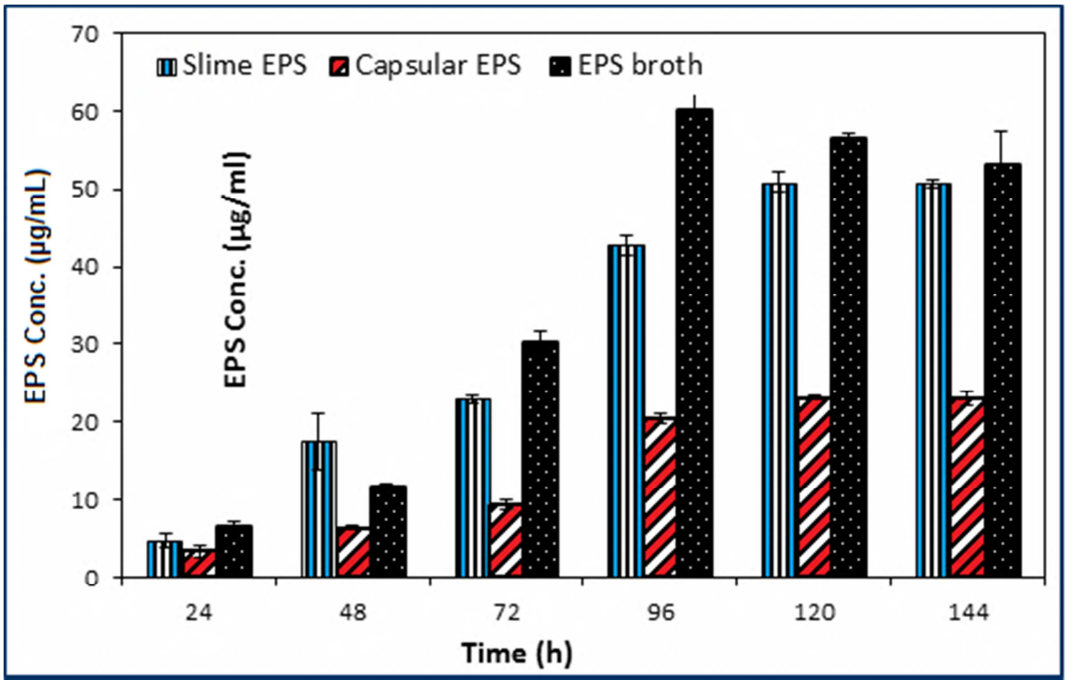


Fig. 5. Extracted EPS concentrations for bacterial consortium (bars indicate standard error)

This systematic study of biofilm formation and EPS characterization provides valuable insights, and therefore further advances our knowledge of biofilm formation and EPS behaviour during *batik* wastewater treatment. It is expected that the results of this study will be valuable for further development of a suitable biofouling mitigation strategy for *batik* wastewater treatment in MBRs. However, further work is needed to understand the microbial community structure on the membrane, which will facilitate the development of a protocol for early biofouling detection to hinder membrane biofouling.

4. CONCLUSIONS

In this study, the biofouling potential of bacterial consortium from the fouled membrane of *batik* wastewater was investigated. It was concluded that filtration tests for the bacterial consortium in the SMBR revealed a significant relationship between EPS and TMP, indicating a negative impact on membrane filterability. When tested for dye and COD removal, the MBR showed increased removal performance with operation time, which might have reflected in biodegradation in biofilm attached on membrane and adaptation of the activated sludge in reactor. The microtiter plat assay demonstrated the biofilm forming capacity of bacterial consortium. Batch tests of the production of extracellular polymeric substances (EPS) indicated that bacterial consortium produced a large amount of EPS which is the main culprit of biofouling.

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CONFLICT OF INTEREST

All authors declare no conflict of interest regarding this article.

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