The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM)
ISSN: 2602-3199

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2022

Volume 21, Pages 311-315

IConTES 2022: International Conference on Technology, Engineering and Science

In-Situ Observation of Biofouling Growth in a Submerged Membrane Bioreactor using CLSM

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Abstract: Biofouling is one of the primary hurdles unavoidable in the operation of membrane bioreactors. The distribution and roles of cake layers in biofouling have been extensively investigated. Transparent exopolymer particles perform a variety of biological functions, which has a substantial impact on particle distribution as it is present ubiquitously in wastewater. Fouling was seen in this investigation to be the result of the deposition of particulate, colloidal or soluble material within the pores or on the membrane surface. The distribution of biofouling on the membrane surface and in the cake layer was investigated using a membrane bioreactor operating at a membrane flux of 10 L/m^{2}h, with the initial concentration of mixed liquor suspended solids of 10g/L treating actual sewage. Confocal laser scanning microscope and field emission scanning electron microscope were employed to divulge the mechanism of fouling in the membrane bioreactor. The trans-membrane pressure and membrane flux were observed throughout the membrane operation. As the fouling progressed, a membrane sample was taken and examined. During the membrane bioreactor operation process, the outcome of the fluorescent staining of the foulant was depicted. In the initial stage, humic-like substances contribute to membrane fouling. Highly concentrated protein-like compounds dominated the fouling behavior. Over time, a protein-controlling cake layer was formed. It can be concluded that in the long-term stage, protein was significantly linked with irreversible fouling.

Keywords: Biofouling, CLSM, FESEM, Sewage

Introduction

In 2021, the market for membrane bioreactors had a value of USD 3.3 billion (Matin et al., 2021). Global demand for water treatment solutions is the main factor driving the market. Apart from this, expanding environmentally friendly water and wastewater management technologies in various sectors, including chemical, pharmaceutical, power, food and beverage, and textile industries, is also boosting market expansion (Xiao et al., 2019). Membrane fouling, particularly biofouling, has grown to be a significant problem with MBR operation because it impairs membrane permeability and necessitates frequent chemical cleaning, reducing membrane lifetime (Aslam et al., 2018).

The emergence of a colony of microorganisms immersed in an organic polymer matrix on membrane surfaces is known as biofouling. There has been a significant amount of research done on the development and growth of

the biofouling layer, including mitigating membrane biofouling. Maintaining the MBR operating condition at optimal MLSS level (<10 g/L), SRT (20-40 d) and, temperature (15-30^{o}C) are just a few methods to control biofouling (L.Deng et al., 2016). Other methods include introducing a spontaneous electric field in MBR, which had delayed the deposition of EPS on the membrane surface (Yin et al., 2020b), and introducing more affordable enzyme extraction methods for enzymatic membrane biofouling control (Cui et al., 2021). Therefore, to comprehensively explore the fundamental growth processes of the biofouling layer on the membrane surface, CLSM and FESEM could be employed as techniques to validate the in-situ visualization of the dynamic behavior of EPS that greatly disturbs the MBR performance.

Method

Experimental Setup

The 100 L aerobic tank makes up the submerged membrane system initially containing MLSS at a concentration of approximately 10g/L as shown in Figure 1. The MBR system had been treating raw domestic wastewater for about 6 months. 10m^2 of a hollow fiber PVDF membrane was operated at a hydraulic retention time of 8 hours with a membrane flux of 10 L/m^2.h, and the dissolved oxygen concentration was maintained above 2 mg/L. while the conditions were fully hydrated, the membrane and fouling layer was carefully removed from the bioreactor to perform additional investigation. The operation of the MBR was examined weekly, and it was shut off as soon as the TMP was above 500 mmHg.

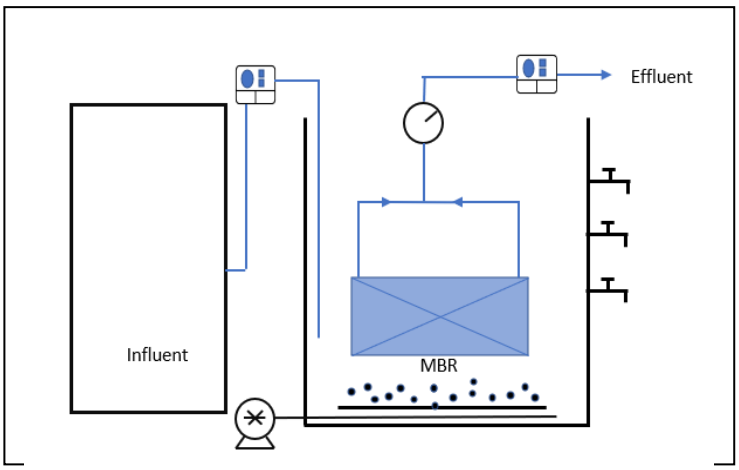

Figure 1. MBR setup in this study

FESEM Characterization

At various points during MBR operation, fouled membranes were taken out of the membrane bioreactor. All samples were dried for 24 hours at 105^{o}C and then coated with a gold sputter prior to FESEM analysis. The morphology was studied at a magnification of 100 um using a Hitachi SU8220.

CLSM Staining and Imaging

The foulants were stained with the following reagents: FITC, Con A conjugated with tetramethylrhodamine, SYTO 63 (Sigma Aldrich), Nile red, and calcofluor white. These reagents were produced following the method outlined by (Yang et al., 2007). Confocal laser scanning microscopy was used to examine the foulant structure (CLSM; Leica TCS SP8 Confocal Spectral Microscope Imaging System, Germany). Leica confocal software was used to analysed objective images at a 10x magnification. At 633 nm and 650–760 nm SYTO 63 fluorescence was observed (red). Con A conjugates were found between 550 to 590 nm (light blue). Wavelengths of Nile Red emission ranged from 630 to 700 nm (yellow). With 488 nm excitation and 500-540 nm emission, FITC was found (green). At 458 nm excitation and 460-500 nm emission, SYTOX Blue fluorescence intensity was determined (purple). Excitation at 405 nm and emission widths of 410-480 nm was used to measure the fluorescence of calcofluor white (blue). All images were scanned at a resolution of 100 µm x 100 µm, above the membrane surface.

Results and Discussion

The distribution of biofouling on the membrane surface and in the cake layer was investigated using a membrane bioreactor operating at a membrane flux of 10 L/m^{2}h, with the initial concentration of mixed liquor-suspended solids of 10g/L treating actual sewage. Figure 2 shows the depletion of membrane flux over time recorded in this study.

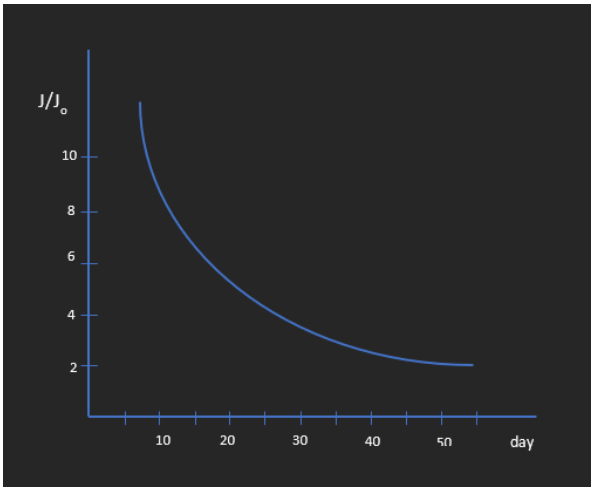

Figure 2. Membrane flux depletion in this study

CLSM images

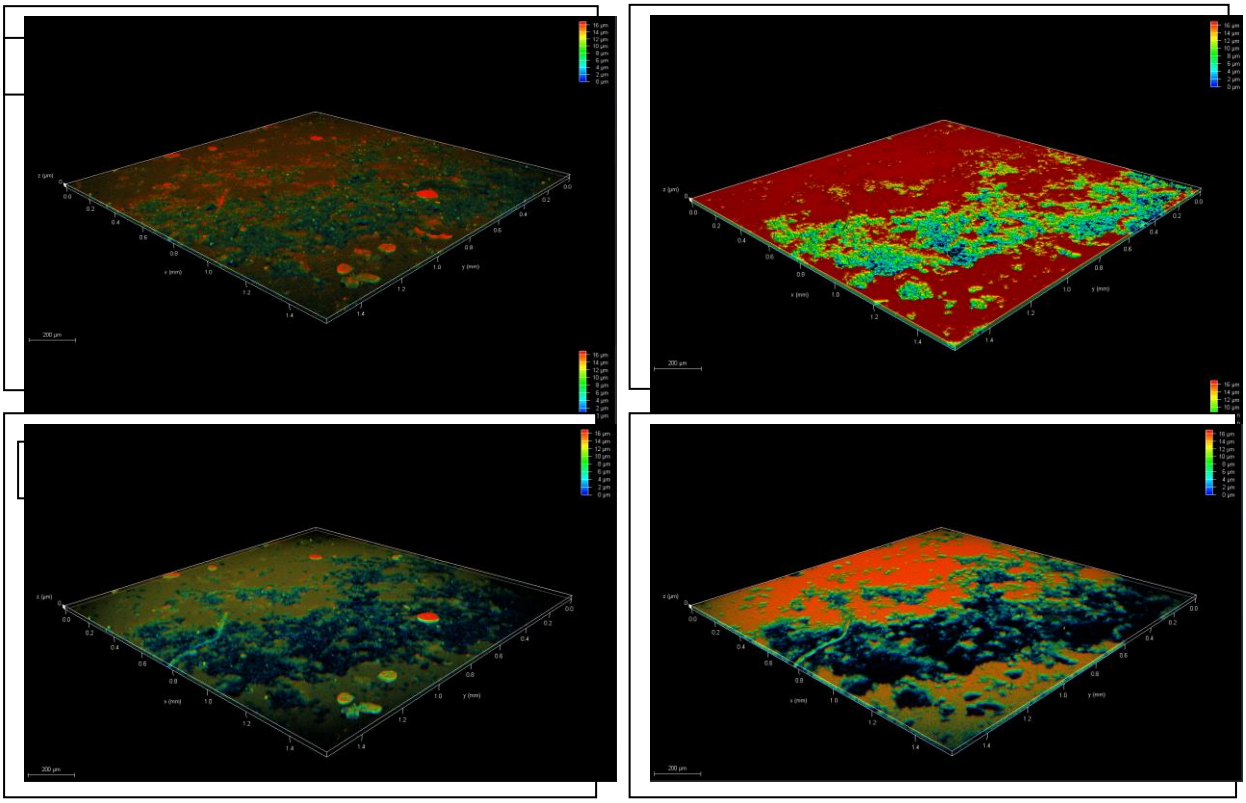

Figure 3. The biofouling 3D CLSM image is further split to denote 3D images of the constituents;(i) Polysaccharides, (ii) Protein, (iii) Lipids, (iv) Total cells.

The findings revealed that the developed biofouling layer was primarily composed of protein and polysaccharides, which are abundant in sewage. Other biological components discovered include lipids and total

cells. The protein accumulates primarily throughout the membrane structure, with a maximum depth of 16 um highlighted in red (Fig.3i), followed by polysaccharides (Fig. 3ii), lipids (Fig. 3iii), and total cells (Fig. 3iv). Protein and polysaccharides accumulation at the feed surface indicate severe membrane fouling observed and is consistent with the severe flux decline in the system.

Biofouling layers developed on the membrane surface pose a significant threat to the operation of MBR as the formation is influenced by the concentration of MLSS, the operating condition of MBR, and the concentration of pollutants. Reducing the fouling rate can be done by regulating membrane operational parameters, including cross-flow velocity and set-point flux. The growth of microorganisms on membranes should have spatial and temporal characteristics, therefore typical solutions to minimise biofouling such periodic backwashing or intermittent filtering cycles may not be sufficient to handle complicated biofouling processes.

In MBRs, EPS has typically been identified as the primary fouling component of the fouling layer, which reduces membrane flux and increases TMP (Sun et al., 2019). According to Chu and Li (2006), the EPS is made up of polysaccharides (PS), proteins (PN), extracellular DNA (eDNA) and metal ions, all of which aid in biofouling. It has also been claimed that the eDNA serves as a scaffold that provides structural integrity to the EPS matrix.

FESEM Images

The performance of MBR in terms of fouling control needs substantial improvements in order to make MBR competitive with the mature wastewater technologies. Figure 4 shows the deposited layer found on the membrane surface when fouling took place. It should be noted that there are many contributors to the development of biofouling, including bacterial cells adhere, grow and multiple (Fig 4i), and other slime layer and foulants (Fig 4ii).

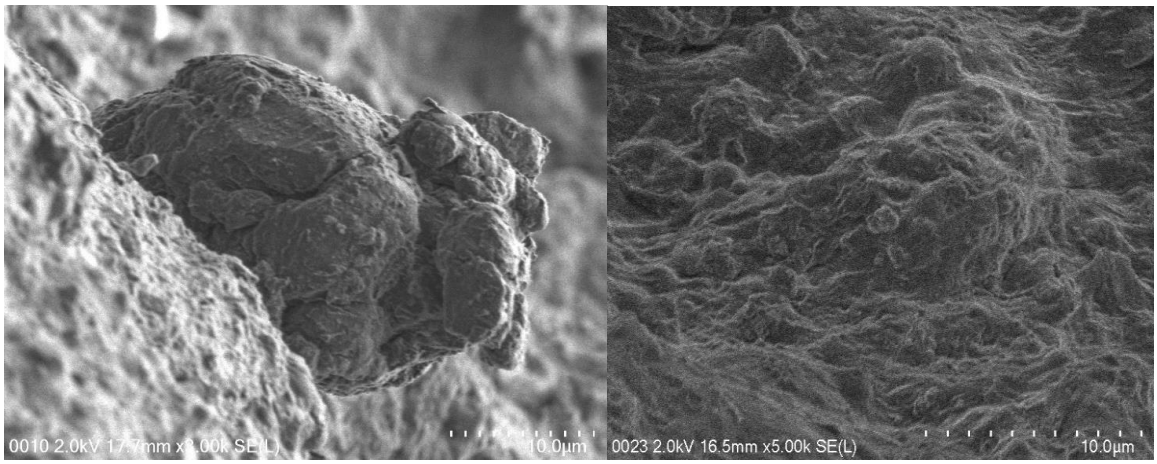

Figure 4. FESEM images; (i) bacterial cells, (ii) slime layer.

Conclusion

A membrane bioreactor treating actual sewage at a membrane flux of 10 L/m^{2}h was used in this study to investigate the distribution of biofouling on the membrane surface and in the cake layer. The CLSM results revealed that highly concentrated protein-like compounds dominated the fouling behavior. In the initial stage, humic-like substances contribute to membrane fouling, and with time, a protein-governing cake layer emerged.

Recommendations

This article will guide the scientist in comprehensively explore the fundamental growth processes of the biofouling layer on the membrane surface using CLSM and FESEM techniques to validate the in-situ visualization of the dynamic behavior that greatly disturbs the MBR performance.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on Technology, Engineering and Science (www.icontes.net) held in Antalya/Turkey on November 16-19, 2022.

* The authors gratefully acknowledge the support of SEGi University and Indah Water Konsortium (IWK) for the pilot plant study area, which was funded by the Fundamental Research Grant Scheme (FRGS/1/2019/TK02/SEGi/02/1) under the Ministry of Higher Education Malaysia (MOHE).

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To cite this article:

Mohamad-Idris, A.I., Mohammad, M., Abdul-Razak, P.R., & Mansor, H. (2022). In-situ observation of biofouling growth in submerged membrane bioreactor using CLSM. *The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 21*, 311-315.