Use of *Bdellovibrio bacteriovirus* as Biological Cleaning Method for MBR Systems

Hilal Yılmaz, Merve Akay Çelik, Çisel Şengezer, and Melek Özkan

***Abstract*—**Membrane bioreactors (MBRs) are becoming more preferable over conventional wastewater treatment processes because of several advantages including higher effluent quality and good disinfection capability. In MBR systems, one of the most important problems during operation is membrane fouling. One of the reasons of fouling is wastewater microorganisms forming biofilm layer on the membrane. Physical and chemical cleaning methods are frequently used for cleaning the membranes. In this study, a biological approach including the function of *Bdellovibrio bacteriovorus*, which is a predator bacteria for other gram negative bacteria, was investigated for removal of biofilm layer formed on membrane surface. The microfiltration Poly(ether)sulphone (PES) membrane with a pore size of 0.05 mm was used for treatment of activated sludge with a MLSS value of 3000 mg/L. For removal of biofilm layer on the surface, the membrane was treated with Bdellovibrio cells after filtration. Cleaning of plugged membrane in this predator Bdellovibrio solution caused an increase in flux by 3.2 L/m2.h as compared to the control membrane. The presented novel biological cleaning method can be considered for future use in mitigation of MBR fouling.

***Keywords*—**Biological cleaning method, Bdellovibrio, membrane bioreactors

I. Introduction

WASTEWATER may be defined as a combination of the liquid (or water) carrying wastes from residences, institutions, commercial and industrial establishments, together with such groundwater, surface water and storm water as may be present [1]. Without treatment, domestic and industrial waste water discharges to sewers and the contaminated rainwater and other pollutants in rainwater run-off from urban areas draining to sewers would cause significant unfavourable impacts on the water environment. There are many different technologies for wastewater treatment including physico-chemical and/or microbial ones [1].

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Membrane bioreactors (MBRs) are now broadly favourable wastewater treatment technologies that combines membrane processes and suspended growth bioreactors [2]. Because of their high solid retention capacity, the MBR has many advantages over traditional wastewater treatment process, including small footprint, high quality effluent, less surplus sludge production and high treatment efficiency [3].The main drawback of MBR technology in comparison to conventional systems is membrane biofouling caused by microbial attachment to membrane surface, which leads to decreased membrane flux and increased filtration pressure and subsequently increased operation cost due to frequent cleaning and replacement of the clogged membranes [4]. So far, extensive research has been carried to investigate the possible methods to prevent or reduce membrane biofouling. The traditional methods that developed for reducing microbial attachment and membrane biofouling are mainly based on physico-chemical principles, such as modification of the solid surface, optimization of operation conditions, regular physical and chemical cleaning and may not be effective and energy efficient [4].

Since the biofouling is caused by various types of microorganism and their excreted products which develop into a complex and difficult to control problem, the conventional physical cleaning processes such as back-washing and back-pulsing are no longer effective [2]. Chemical cleaning also has limited success in solving fouling problem. Frequent chemical cleaning causes reduction on membrane lifetime also can impair permeate quality and is associated with undesirable wastestreams [2]. Moreover, microorganisms can adapt to these cleaning procedures by changing their physiological responses through gene regulation and metabolism [2], [5], [6]. Since there is still no accurate solution for fouling problem, it is important to find out a new environmentally friendly and effective solution for reducing fouling problem. Biological cleaning idea brings a new perspective to this problem. The most important advantages of biological-based cleaning strategies are higher efficiency, lower toxicity, more sustainability and less bacterial resistance over other control approaches [7]. Quorum quenching (QQ), enzymatic disruption (ED), energy uncoupling (EU), cell wall hydrolysis and the use of microbial predation and bacteriophages are the most commonly tested biological cleaning strategies for biofilm control and fouling problems [2].

Bdellovibrio bacteriovorus is a Gram-negative aerobic predatory bacterium that requires prey cells to invade and utilize as substrate for growth and reproduction. This bacterium preys upon a wide variety of other Gram-negative

bacteria. Although the main role of *B. bacteriovorus* in nature is not completely become known there is evidence that these bacteria play a role in the microbial ecology of natural environments, controlling the population size of bacteriain ecosystems. The effect of Bdellovibrio predatory activity against pathogenic microorganisms has been reported [8]. And some previous researches has revealed that *Bdellovibrio* can consume *E. coli* prey in simple biofilms, in some cases destroying the biofilms altogether, and thus have great potential to aid in a variety of fields in which biofilm formation is a critical concern [9]. However, biofilms in MBRs' membranes consist of multiple bacterial species, and there is no work on *Bdellovibrio* activity on plugged membranes and sludge bacteria. In this study, a biological approach including the predatory function of *B.bacteriovorus* was investigated for removal of biofilm layer formed on membrane surface.

II. Materials And Methods

A. Growing Bdellovibrio in E.coli

Bdellovibrio bacteriovirus was produced in HM buffer which contains *Escherichia coli* cells as prey [10]. HM buffer was prepared as 10 mM Hepes containing 1mM $CaCl_2$ and 0.1mM $MgCl_2$ as the final concentration and the buffer pH was fixed at 7.2. *B. bacteriovorus* were grown in suspensions of prey for 3 days at 29 ◦C at 180 rpm. In three days period, turbidity of solution decreased from OD_{600} of 0.3 to about 0.1 as prey cells are degraded by *B. bacteriovirus* activity.

B. Biofilm Degredation Experiments

These experiments were performed in order to investigate the effect of *B. bacteriovirus* on *E. coli* biofilms. 200µL *E. coli* was added to the wells of 96 welled plates, and then plates were incubated for biofilm formation. After one day incubation, *E. coli* cell soluion was removed from the wells and *B. bacteriovirus* was added. After plates were incubated for 6 hours with *B. bacteriovirus*, remaned biofilms in the wells were stained with (1%) crystal violet and then dissolved in ethanol:acetone solution (70:30). Intensity of violet colour was measureed at 570 nm wavelength by using plate reader spectrophotometer for determination of biofilm thickness.

C. Membrane Experiments with Dead end Reactor

Dead end Reactor was used for investigating the effect of *B.bacteriovirus* treatment on plugged membrane flux. MP005 PES membranes were used for microfiltration with dead end reactor. For each experiment, activated sludge with MLSS of 3000-3500 mg/L and COD of 730-780 mg/L was filtered through two membranes under 1.5 bar pressure. One of the membranes was treated with filtered *B.bacteriovirus* solution for three hours at 100 rpm incubation at the room temperature after each the filtration process. The other membrane was used as control and cleaned only with water (not cleaned with *B. bacteriovirus)*. After cleaning, activated sludge fluxes of two membranes were compared.

III. Results And Discussion

B. bacteriovorus is Gram-negative bacterium preying on other Gram-negative Bacteria. Biofilm formation is caused by adherence of bacteria to surfaces and excretion of glue-like extracellular polymeric substance that protects and anchors them to materials. In this study, in order to test the lytic action and biofilm degradation capacity of *B. bacteriovorus*, its activity on *E. coli* biofilm was analyzed firstly. Figure 1 shows effect of *B. bacteriovirus* activity on biofilm removal. Density of the violet colour in the wells shows that biofilm formed by *E. coli* cells were removed more efficiently by incubation in *B. bacteriovirus* solution as compared to control.

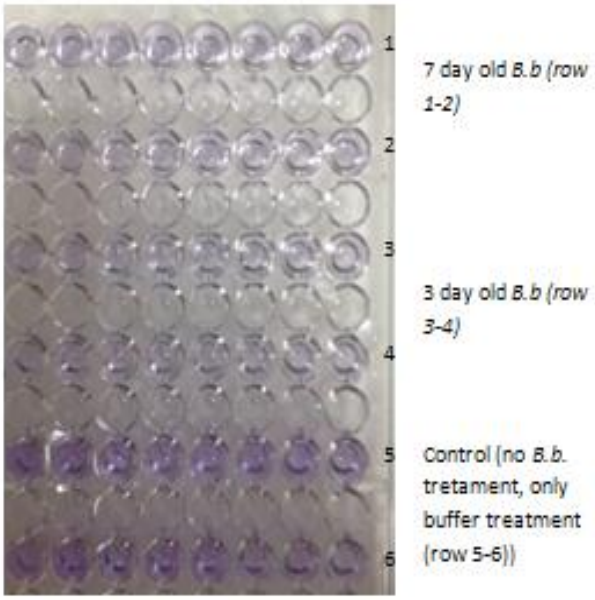
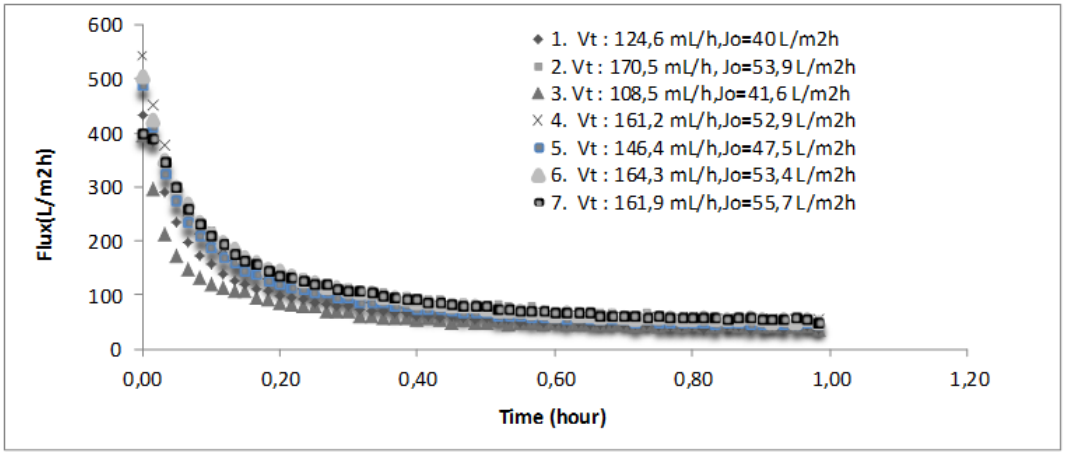
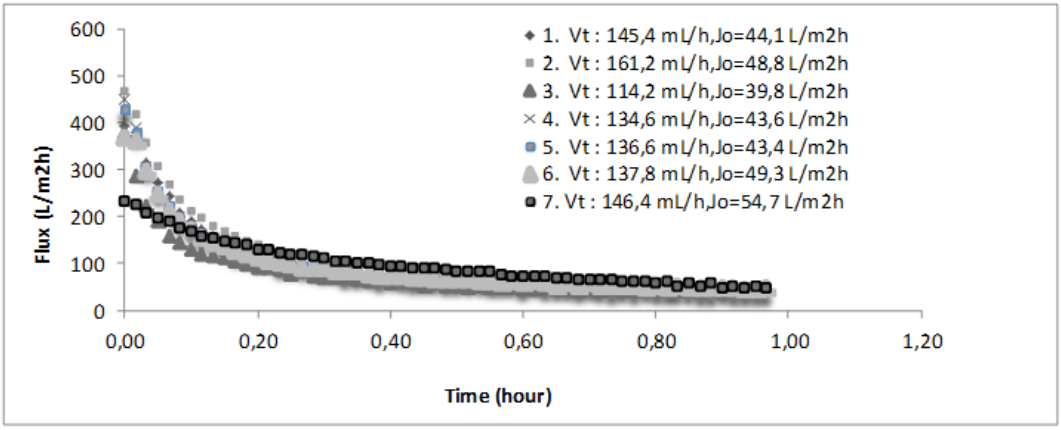


Fig. 1 Removal of *E. coli* biofilm by *B. bacteriovorus*

Absorbance of 3 day old *B. bacteriovorus* treated biofilms (row 3-4 in Fig. 1) gave an absorbance value of 0,07 ± 0,008, whereas buffer treated biofilm thickness corresponded to OD_{575} of 0,238 ± 0,18 (control rows, 5 and 6 in Fig.1). This result shows that 3 days *B. bacteriovorus* efficiently degrades *E. coli* biofilm. For cleaning of plugged membranes in following experiments, 3 day old *B. bacteriovorus* solution was used.



A)



B)

Figure 2. Flux of the membrane cleaned with *B. bacteriovirus* (A) and control membrane (cleaned only with buffer, not cleaned with *B. bacteriovirus*) (B)

Figure 2 shows fluxes of *B. bacteriovorus* treated membrane (A) and control membrane (B) used in dead-end system. 7 serial filtration was performed in each membrane, and plugged membranes were cleaned with appropriate solution after each filtration. Results shows that the membrane cleaned with the predator *B. bacteriovorus* are more efficient in filtration. The flux (Jo values shown on right part of graphs) of the *B.bacteriovirus* treated membrane (Fig. 2 A) is approximately 3.2 L/m^2.h higher than that of the control membrane. The results is not surprising since it is known that *B. bacteriovirus* efficiently degrades biofilms formed by many gram negative bacteria including *Acinetobacter*, *Aeromonas*, *Bordetella*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Serratia*, *Shigella* and *Vibrio* [8]. Most of those bacteria are also found in wastewater sludges and they may contribute pluging of the MBR. It is also reported that most of the bacteria colonizing membrane surfaces belong to different Proteobacteria group, [11] which are known to be Gram-negative.

IV. Conclusion

In this study, predator bacterium *B. bacteriovirus* was first used as cleaning method for plugged membranes used in Membrane Bioreactor System. Treatment of the membrane with this predator bacterium caused approximately 3.2 L/m^2.h improvement in membrane flux. Being more environmentally friendly as compared to chemical cleaning methods, the method described in this study has a potential to be used as biological cleaning method for large scale MBR systems.

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