Application of quorum quenching to improve performance of membrane bioreactors: A comprehensive mini-review

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Abstract. Membrane bioreactors (MBRs) have been widely used for wastewater treatment, presenting however a major disadvantage, the membrane fouling problem. The sludge microorganisms produce soluble microbial products (SMP) and extracellular polymeric substances (EPS) that lead to a formation of a sticky biofilm layer. This biofilm layer causes membrane biofouling since the activated sludge substances attach to the membrane. Quorum quenching (QQ) constitutes an innovative method that effectively reduces membrane fouling with its research being evolved rapidly. Quorum quenching is the disruption of the inter-species and intra-species signaling pathways. The QQ application results in minimization of membrane biofilm formation, SMP and EPS production, and therefore membrane fouling reduction. This review work summarized all the cutting-edge research and patents of the application of the QQ method for membrane fouling reduction, the methods for screening QQ activity and biofouling mitigation, and finally described the challenges of the QQ application in real scale MBR units. This mini-review may be valuable and appropriate for further development work.

Keywords: Quorum quenching; Quorum sensing; Membrane biofouling; Membrane bioreactors; Biofilm mitigation

1. Introduction

Membrane bioreactors (MBRs) present important advantages compared to other conventional wastewater treatment processes, such as reduced sludge production, high effluent quality, including the eliminated release of antibiotic-resistant bacteria and a smaller environmental footprint (Manaia et al., 2018; Banti et al., 2020). In combination with the great potential they offer for water reuse, their application has been rapidly increased during the last years (Xiao et al. 2019). However, membrane biofouling remains their basic drawback, which prevents their broader application and increases the operational costs of the system. Membrane fouling is mainly attributed to the interactions between the membrane and the activated sludge suspension, which contains SMP, EPS and colloids (Banti et al., 2018). These components, also called foulants, have highly adhesive properties and are adhering to the membrane surface creating a biofilm cake layer or attached inside the membrane pores blocking them. Membrane biofouling results in a sharp reduction of the filtrate flow and increase of the trans-membrane pressure (TMP).

Several methods have been investigated to mitigate membrane fouling, with not much success. These methods ignore fundamental aspects of the activated sludge microbial ecology, leading to their low success. In contrary, the use of microorganisms that mitigate the foulant-production from the main biofilm/EPS/SMP producing bacteria via the inhibition of quorum sensing (QS) has shown promising results. Specifically, bacteria use the QS to sense the population size of closely related bacteria in their environment, which might lead to mutualistic or competitive interactions. QS regulates several phenotypes including biofilm formation or the production of SMP and EPS. Bacteria use several small molecules, such as the N-acyl-homoserine lactones (AHLs), the auto-inducing peptides (AIPs) and the auto-inducer type-2 (AI-2), which are auto-inducers and function as QS signals. The AHLs are amphipathic molecules, which are used mainly by gram-negative bacteria, while the AIPs are being utilized mainly by gram-positive bacteria. The AI-2 signaling systems are present in both gram-positive and gram-negative bacteria.

In the present review, special emphasis is placed on the most recent research regarding the application of QQ to mitigate membrane biofouling.

2. QQ applications for membrane fouling reduction

Generally, QQ microorganisms have been considered much more effective compared to QQ enzymes, as even one single bacterial strain can possess several types of

QQ-enzymes from a category and therefore enables higher degradation than the addition of one type of enzyme at high concentration.

Oh et al. (2012) used a genetic modified strain of QQ *E.coli* and a strain isolated from activated sludge of a municipal wastewater treatment plan (*Rhodococcus* sp. BH4). Both strains induced a delay on TMP increase. However, *Rhodococcus* sp. BH4 was considered as a better choice, since the genetic modified *E.coli* would require the addition of antibiotic, to retain the plasmid. *Rhodococcus* sp. BH4 was able to degrade AHLs intracellularly. The addition of encapsulated *Rhodococcus* sp. BH4 in the membrane tank and the application of high recirculation rate resulted to a long delay of TMP increase (Jahangir et al., 2012). Therefore, the configuration of the membrane, along with the efficient mixing and distribution of mixed liquor through recirculation can play a crucial role in the effectiveness of the encapsulated QQ-strain.

Cheong et al. (2013) isolated and used *Pseudomonas* sp. 1A1 to degrade AHLs that had the extra advantage of simultaneous producing extracellular QQ enzymes. In this case, *Pseudomonas* sp. 1A1 was encapsulated in a porous medium that contributed substantially to the anti-biofouling activity. Kim et al. (2013) used and encapsulated the strain *Rhodococcus* sp. BH4 in alginate beads and their effectiveness were found similar to other porous media.

Later, Kim et al. (2015) coated the alginate beads that contained *Rhodococcus* sp. BH4 with polysulfonate to increase their mechanical strength and stability. This method showed an excellent anti-biofouling capacity. However, as this process uses phase inversion with an organic solvent toxic to microorganisms, reduces the encapsulated bacteria, thus the beads require additional cultivation steps.

Apart from bacteria, fungal strains have also been used to mitigate biofouling of a membrane, according to Lee et al. (2016)**,** who used *Candida albicans* strain that was able to interfere with AI-2 mediated communication and prevent membrane biofouling. This strain, which was immobilized in alginate beads, was producing farnesoyl that interfered with AI-2 mediated signalling and decreased the TMP increase rate. The *Rhodococcus* sp. BH4 strain immobilized in alginate beads was further explored Lee et al. (2016b) in a real-scale MBR minimizing successively biofouling. Yu et al. (2016) explored the enrichment of a QQ bacterial consortium from activated sludge, consisted mainly of *Rhodococcus erythropolis* W2 and *Rhodococcus* sp. BH4, by gamma-caprolactone (GCL), aiming to stimulate the growth of AHL-degrading bacteria and mitigate biofouling. Finally, the results demonstrated that lower concentration of AHL and EPS (mg/g VSS) as well as a lower rate of TMP increase, and therefore membrane biofouling reduction. Lee et al. (2016b) used QQ-cylinders to immobilize *Rhodococcus* sp. BH4 and enhanced anti-fouling properties were detected compared to QQ-alginate beads. Moreover, it was found that QQ activity was highly dependent on the specific surface area of the QQ immobilization medium regardless of the shape of the medium.

The use of rotary microbial carrier frame for *Rhodococcus* sp. BH4 immobilization resulted to another successful encapsulation of QQ bacteria (Ergön-Can et al., 2017). In this method, QQ were entrapped into a polycarbonate frame covered with a microfiltration membrane. As a result, TMP was decreased by about 65%, and the reduction of biofouling was attributed to the QQ efficiency as well as to the shear forces caused by the QQ medium.

Kampouris et al. (2018) isolated four bacterial stains from the activated sludge of a municipal wastewater treatment plant with only one strain (*Lactobacillus* sp. SBR04MA) showing high degradation potential of AHLs. The strain was immobilized in alginate beads and its effectiveness was examined in a pilot-scale MBR. However, the beads were degraded in only seven days after their addition, gradually releasing *Lactobacillus* sp. SBR04MA cells in the mixed liquor. The cell release affected the sludge bacterial community and led to the reduction of SMP and EPS concentration in the mixed liquor, minimization of the TMP rate and mitigation of membrane fouling. Gül et al. (2018) investigated Gram-positive *Bacillus* sp. T5 and Gram-negative *Delftia* sp. T6 QQ microorganisms that successfully mitigated membrane fouling at 85% and 76%, respectively. Additionally it was found that the use of Gram-negative QQ microorganisms induced a Gram-positive microbial community and vice versa and therefore their combination was considered fundamental to improve further the MBR performance. A few studies have investigated the effect of interference in AI-2 mediated communication. Lee et al. (2017) showed the disruption of AI-2 by an indigenous activated sludge bacterium *Acinetobacter* sp. DKY-1. This specific strain was able to degrade 4,5-dihydroxy-2,3-pentanedione (DPD), an AI-2 signaling molecule.

According to the research work of Zhang et al. (2019)**,** where UV irradiation was used to quench microbial QS in MBRs compared to *Rhodococcus* sp. BH4, it was revealed that continuous or intermittent UV photolysis, could achieve similar or better anti-fouling properties than bacterial QQ. UV photolysis hampered biofilm growth inactivating the signal molecules DPD and AHLs. A novel patterned QQ membrane was investigated by Lee et al. (2019)**,** on the surface of which the AHL-degrading enzyme acylase was immobilized. The membrane was modified using a patterning process of an oxidized multiwall carbon nanotube. It was found that the patterned QQ membrane presented a high specific surface area able to retain 32% more enzyme and increased effective membrane area (~1.6 times) that compensated the membrane resistance usually caused by the enzyme immobilization. Finally, this QQ membrane lengthened the time between chemical cleanings (~2.2 times) compared to the control non-patterned membrane.

According to another research work (Lee et al. 2020), the QQ strain (*Rhodococcus* sp. BH4) was entrapped in mesoporous silica (<1 mm) that has been considered more cost-efficient than alginate. This improved QQ medium removed efficiently quorum sensing signaling molecules, with a long-life span due to its non-biodegradability nature and due to its small size did not clog the hollow fiber membrane module.

Liu et al. (2021) used a QQ consortium comprising of AHLs degrading strains *Enterobacter cloaca*, *Microbacterium* sp., *Pseudomonas* sp. and *Rhodococcus* sp. BH4 immobilized in alginate-powdered activated carbon beads. They found that the QQ beads reduced membrane biofouling, extending at 4.5 times the MBR operating period compared to the Control-MBR without QQ addition. The EPS and N-AHL concentration were also reduced in the biocake.

In general, the use of QQ microorganisms to inhibit QS-mediated signalling is a promising anti-biofouling method, because microbial cells can produce enzymes or metabolites, while the feed with untreated wastewater in MBR generally provides the necessary nutrients for their growth. However, further research is necessary to optimize biofouling mitigation using the QQ method. There are many unknown quorum quenching microorganisms in the environment, which potentially may exhibit characteristics such as faster degradation rate or higher potential of survival in MBRs treating municipal or industrial wastewater.

3. Conclusions

The present review indicates the potential of the wide use of QQ microorganisms, as a novel biological method for membrane biofouling mitigation. QQ works with the interruption of QS, mainly via the degradation of auto-inducer signals. The QQ bacteria minimize biofilm formation and SMP/EPS production without leading to excessive mortality of the main fouling related bacteria. Consequently the wastewater microbial community retains the full optimum capacity for removing wastewater pollutants. Further research on optimum encapsulation media and isolation of novel, more efficient QQ microorganisms will result to reduced operation costs, allowing more wide spread use of the MBR systems.

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