P2M2: Physical and physiological properties of membrane-aerated and membrane-supported biofilms - DTU Orbit (14/08/2019)

Autotrophic nitrogen removal has become the process of choice to treat nitrogen-rich wastewaters due to its significantly lower operation costs. This technology makes use of stratified biofilm or bioaggregate structures to enrich aerobic and anaerobic ammonium oxidizing bacteria that catalyse the conversion of ammonium to nitrogen gas via nitrite in a single reactor. Recent work on membrane-aerated biofilm reactors (MABRs) has shown that this concept can be taken even further by growing these biocatalysts on aeration membranes, hereby significantly lowering aeration costs and greenhouse gas emissions without compromising performance. Preliminary experimental work manifested the difficulty of reducing the activity of Nitrite Oxidizing Bacteria (NOB), which lowered the removal efficiency of the system. Advanced molecular biology tools were used to confirm that periodic aeration of MABRs can serve as a control strategy to outcompete NOB and stimulate the metabolism of anaerobic ammonium oxidizing bacteria. Furthermore, it could be observed that the accurate control of the oxygen load, unique for MABRs, allowed the construction of a highly stratified biofilm structure with aerobic ammonium oxidizing bacteria (AOB) growing on the membrane surface, and anaerobic ammonium oxidizing bacteria (AnAOB) distributed in a very thin stratum by the liquid phase. AOB and AnAOB communities, both dominated by fast-growing genera, were relatively more diverse than observed in other conventional biofilm reactors performing the same process. Our results suggested that the detachment of large amounts of biofilm could seriously impair reactor performance due to the washout of AnAOB, growing in the outer anaerobic regions of the biofilm. It has been suggested that the excretion of extracellular polymeric substances (EPS) can enhance biofilm strength under certain conditions. Despite their crucial importance, there is currently no agreement within the scientific community on a protocol that optimizes EPS recovery from microbial samples without significantly compromising the viability of the embedded bacterial cells. Thus, we performed a rigorous benchmarking study on the effect of a wide range of published EPS extraction techniques on cell lysis and biopolymer extraction yields. According to our results, ultrasonic treatments could retrieve a larger amount of EPS from most studied samples, were less biased by molecular interactions, and did not have a significant impact on cell integrity. Further experimental work partially rejected the possibility of enhancing the strength of a model autotrophic MABR biofilm by either modifying the shear stress or the oxygen supply rates under cultivation. Overall, the biofilm layers closer to the biofilm-liquid interface displayed relatively lower cohesion forces against shear stresses, but still higher than observed in other conventional biofilms grown under similar environmental conditions. None of the biofilms tested detached completely from the substratum and proved to have very high adhesion strengths. Microscopic observations confirmed that this adhesion layer was dominated by very compact cell structures encapsulated in a dense layer of protein and carbohydrate. Observations at various scales were further used to confirm that a higher level of shear in the bulk liquid made the biofilms thinner, denser (less porous), more homogeneous, and with a higher content of volatile material than the rest of assayed conditions. The factors impacting oxygen transfer with and without biofilm, unclear until now, were successfully identified using sophisticated microprofiling investigations under undisturbed reactor operation. It could be concluded that conventional methods to characterise oxygen transfer rates in clean water underestimated those observed when a biofilm was present considerably. Higher degrees of bacterial activity at the biofilm base catalysed oxygen transfer. This behaviour was described with the addition of two terms (depending on system characteristics and reactor loading conditions) to existing model structures. In conclusion, we presented control strategies to engineer the microbial communities catalysing autotrophic nitrogen removal in MABRs, proposed methods to minimise the risk and effect of bacterial sloughing, and developed novel strategies to characterize, optimize, and better regulate oxygen transfer. Overall, the present work should serve to better design reactors supporting a cleaner, more robust, and cost-effective nitrogen removal. Furthermore, the large dataset of structural biofilm data reported here should facilitate the calibration of process models for the implementation of advanced process control.

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