Abstract

Bacteria of the Comamonas genus are often present in various engineered and natural ecosystems, in particular, the aromatic compounds-contaminated and denitrifying environments. Intriguingly, they are usually found to be associated with surface or interface as multicellular aggregates known as biofilms. Extensive previous research efforts have been focused on the versatile catabolic capabilities of Comamonas; in contrast, little is known about their biofilm lifestyle and biofilm-mediated environmental processes. The objective of this study was to understand the biofilm lifestyle of Comamonas in environmental processes and the influence of environmental conditions. Specifically, using C. testosteroni as a model organism, this thesis work focuses on elucidating the influences of (i) a model aromatic pollutant 3-chloroaniline (3-CA), (ii) nitrate, and (iii) extracellular biomacromolecules in wastewater on the biofilm lifestyle of Comamonas and their environmental implications.

Bioaugmentation of C. testosteroni has been frequently proposed in wastewater and soil treatment to remove toxic aromatic compounds. The performance of bioaugmentation is affected by a number of biological and environmental factors including the interaction between the target pollutant and the augmented bacterial cells. The first part of this thesis work explored the influence of a toxic aromatic pollutant 3-CA on the biofilm lifestyle of C. testosteroni capable of degrading the aromatic compound toward a better understanding of cell-pollutant interaction in bioaugmentation. The results showed that the exposure to 3-CA greatly reduced the retention of C. testosteroni cells in packed-bed bioreactors, which could be attributed to the altered bacterial motility and cell surface hydrophobicity. Through an integrated genomic and transcriptomic analysis, we found that exposure to 3-CA reduced the intracellular c-di-GMP level by downregulating the expression of genes encoding c-di-GMP synthases and induced massive cell dispersal from the biofilms. As the reduced c-di-GMP caused biofilm dispersal, we attempted to develop robust biofilm processes by elevating c-di-GMP level. To achieve this objective, the C. testosteroni strain was constructed which constitutively expressing a c-di-GMP synthase YedQ. Under batch growth conditions with a high surface to volume ratio, an elevated c-di-GMP concentration in C. testosteroni significantly increased the contribution of
biofilms in 3-CA biodegradation. In continuous submerged biofilm reactors, *C. testosteroni* with an elevated c-di-GMP level exhibited an enhanced 3-CA biodegradation and a decreased cell detachment rate.

In microbial communities driving domestic wastewater treatment, bacteria of the *Comamonas* genus were often reported to be among the most abundant microorganisms. In addition, a high abundance of *Comamonas* was also found in nitrate-removing microbial communities presented in river wetlands, soil, and constructed wetlands. However, why they are there and what roles they may play remain largely unknown. In this thesis work, we demonstrated the involvement of *Comamonas* biofilms in denitrification under bulk aerobic conditions and elucidated the influence of nitrate respiration on its biofilm lifestyle. Our results showed that *C. testosteroni* could use nitrate as sole electron acceptor for anaerobic growth. Under bulk aerobic condition, biofilms of *C. testosteroni* were capable of reducing nitrate and, intriguingly, nitrate reduction significantly enhanced viability of the biofilm-cells and reduced cell detachment from the biofilms. Nitrate respiration was further shown to play an essential role in maintaining high cell viability in the biofilms. RNA-seq analysis, quantitative polymerase chain reaction (qPCR), and liquid chromatography-mass spectrometry (LC-MS/MS) revealed a higher level of bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) in cells respiring on nitrate than those grown aerobically (1.3×10^{-4} fmol/cell vs. 7.9×10^{-6} fmol/cell; P < 0.01). C-di-GMP is one universal signaling molecule that regulates the biofilm mode of life and a higher c-di-GMP concentration reduces cell detachment from biofilms. Taken together, this study reveals that nitrate reduction occurs in mature biofilms of *C. testosteroni* under bulk aerobic conditions and the respiratory reduction of nitrate is beneficial to the biofilm lifestyle by providing more metabolic energy to maintain high viability and a higher level of c-di-GMP to reduce cell detachment.

Wastewater bacteria of the *Comamonas* genus were often found to form cell aggregates in activated sludge-based wastewater treatment. However, the isolates rarely form flocs in synthetic wastewater, a chemically defined medium that is formulated based on key physicochemical characteristics of real wastewater, *e.g.*, pH, ionic strength, chemical oxygen demand (COD), and total nitrogen/phosphorus (NH₄-N and PO₄-P). The discrepancy observed for bacterial floc formation in real wastewater and synthetic wastewater suggests an important role of neglected,
unknown components, *i.e.*, “dark matter”, in real wastewater. In this study, using *C. testosteroni* as a model organism, we examined the effect of microfiltered (pore size of 0.22 µm) and ultrafiltered (molecular weight cut-off (MWCO) of 225 kDa) wastewater on bacterial floc formation. Combing the floc formation observations with the Fourier Transform Infrared Spectroscopy (FTIR) analyses and enzymatic treatment, we report for the first time that high molecular weight fraction of the soluble EPS, in particular, proteins and extracellular DNA, are the major floc-forming inducers in wastewater. The underlying mechanism was further characterized by using the DLVO theory. In the presence of soluble EPS, cell surface was found to become more hydrophobic and the energy barrier disappeared, leading to irreversible attachment and floc formation. Therefore, the physicochemical effect of soluble EPS in real wastewater should not be neglected in wastewater biofilm processes which facilitated the initial biofilm formation.