Chapter 2
Research Questions and Thesis Overview

2.1 Introduction

This chapter introduces the specific research questions based on the literature review in the previous chapter. These questions encompass the objectives of this PhD study, which focuses on the formation, characterization, and mathematical modeling of the aerobic granular sludge and associated systems. Each of the Chaps. 3–12 will address one of the specific research questions.

2.2 How Can the Dynamic Aerobic Granulation Process be Quantitatively Characterized?

Aerobic granulation is a very complex phenomenon of microbial immobilization. There are numerous internal interactions among process variables, such as growth, storage and endogenous respiration, and sludge characteristics, including biomass detachment, oxygen transfer and diffusion. All of them have significant effects on the overall reactor performance. Therefore, the empirical models are generally descriptive and are not able to simulate the aerobic granulation process in other reactors or cultivated under different conditions. In this work, aerobic granules are cultivated in SBRs fed with both soybean-processing and fatty-acids-rich wastewaters and the aerobic granulation process in terms of mean radius profiles is quantitatively characterized based on experimental observations and formation mechanism analysis in Chap. 3. A new mathematical model incorporating microbial growth, oxygen transfer, substrate diffusion, increased granule size, and biomass detachment is formulated to describe the aerobic granulation process of activated sludge, on the basis of a mixed-culture biofilm model and a simultaneous storage and growth model. The model evaluation results of three different case studies demonstrate that the developed model is applicable to describing the

B.-J. Ni, *Formation, Characterization and Mathematical Modeling of the Aerobic Granular Sludge*, Springer Theses, DOI: 10.1007/978-3-642-31281-6_2,

aerobic granulation process appropriately. With this model, the aerobic granulation process in terms of mean radius profiles could be quantitatively described.

2.3 How Can Autotrophic and Heterotrophic Growth and Competition in Aerobic Granular Sludge be Determined?

The microbial analysis on the aerobic granules reported in Chap. 3 strongly suggests that the architecture of aerobic granules is relevant to their microbial ecology. They consist of two main different microbial groups, i.e., autotrophic and heterotrophic bacteria. Both autotrophs and heterotrophs coexist and interact in aerobic granules. Usually, aerobic granules display considerable heterogeneity, with respect to the microorganisms themselves and their physicochemical microenvironment. The presence of organic matters in wastewater creates competition between them for DO and space in the aerobic granules. Such interspecies competition and mass transfer result in the stratification of microbial species in granules. In Chap. 4, the growth of autotrophs and heterotrophs and their activity in aerobic granules is explored in-depth using experimental and modeling approaches. The fractions of the active biomass (autotrophs and heterotrophs) and inert biomass are determined. It is found that biomass content increases with the increasing SRT, but active biomass ratio decreases. The heterotrophs consume more oxygen than the autotrophs. The autotrophs are mainly located on the outer layers of granules for DO consumption, whereas the heterotrophs occupy the granule center or on the outer layers.

2.4 Can a Thermodynamic Analysis on the Microbial Synthesis of the Aerobic Granular Process be Achieved?

Accurate microbiological modeling requires evaluation of the effects of biological reactions on all important chemical and biological species in the aerobic granular sludge system. The ASM requires a large number of empirical parameters, which are difficult to determine. Moreover, many important chemical and biological species are lumped into one assumed model component or one empirical process. To sort out these problems, a thermodynamic analysis of the biological synthesis in sludge could be performed and the stoichiometrics could be more accurately estimated with the cell yield derived from thermodynamic considerations of the flows of energy and electrons in the catabolic and anabolic pathways. In Chap. 5, the thermodynamic analysis on the microbial synthesis of the aerobic granular process described in Chap. 3 is performed using the bioenergetic methodology established by McCarty after integrating it with a modified ASM1. This approach is able to approximately describe the treatment of soybean wastewater in an SBR in terms of substrate utilization, biomass growth, and the elector acceptor consumption. Such an attempt provides useful information for accurate modeling of the complex aerobic granular process.

2.5 Do Simultaneous Microbial Storage and Growth in Aerobic Granules Occur?

In recent years, many studies refer the production of storage polymers (X_{STO}) by activated sludge when exposed to a transient carbon supply. An aerobic-granule-based SBR is repeatedly subjected to feast and famine conditions. As a result, simultaneous growth and storage processes always occur. The measurements of aerobic granule reactors in Chaps. 3 and 4 indicate that the microorganisms are exposed to significant concentrations of the external substrate only for a relatively short period of time under these dynamic conditions. In excess of external carbon substrate, the uptake is driven to simultaneous growth of biomass and polymer storage, and after substrate exhaustion, stored polymer can be used as energy and carbon sources as well. In Chap. 6, the internal storage mechanisms and electron flow from the external substrate occurring in aerobic granule sludge are explored with storage experiments under different initial conditions. The simultaneous growth and storage processes in aerobic granules are modeled. The results show that simultaneous microbial storage and growth in aerobic granules occur under both aerobic and anoxic conditions.

2.6 How do the Aerobic Granules Produce Extracellular Polymeric Substances?

Aerobic granules are composed of numerous microorganisms, immobilized in EPS and/or matrices constituting polymers of proteins, polysaccharides, humic acids, and lipids. The presence of EPS, a complex high-molecular-weight mixture of polymers in the aerobic granular sludge cultivated in Chaps. 3 and 4, is confirmed and observed using electron microscopy techniques. EPS are sticky solid materials secreted by cells, and they are involved in adhesion phenomena, formation of the matrix structure, controlling the microbial physiology, and the long-term stability of the granules. In Chap. 7, the EPS produced by mixed microbial community are characterized using gel permeating chromatography (GPC) and 3-dimensional excitation emission matrix (EEM) fluorescence spectroscopy measurement, and a novel and convenient approach is also developed to evaluate the EPS production kinetics. Results show that EPS increase rapidly with the substrate consumption, but decrease slightly after the external substrate is completely consumed. Electrons from the external substrate are distributed in the following order: new biomass synthesis of 61 %, oxygen for respiration of 21 %, and EPS of 18 %.

2.7 Can the Formation of Soluble Microbial Products be Fractionized and Determined?

In addition to making different biomass components (active cells, X_{STO}, and EPS), bacteria in aerobic granules also convert a fraction of organic substrate into SMP, which account for the bulk of soluble organic carbon in reactor effluents. Their presence is of particular interest in terms of achieving discharge consent levels for biological wastewater treatment plants. SMP have been found to have a very wide range of MW distribution and different structures/functions. If SMP could be accurately fractionized and quantitatively identified, it is possible to examine how refractory the individual compounds are and to determine which type of SMP is the most difficult to remove from aerobic granular reactor effluents. In Chap. 8, the subfractions of the SMP, i.e., utilization-associated products (UAP) and biomass-associated products (BAP) in terms of formation sequence, MW and chemical nature are characterized, and a new approach for determinating SMP, UAP and BAP, and their production kinetics is developed on the basis of the approach proposed in Chap. 7. The UAP, produced in the substrate utilization process, are found to be carbonaceous compounds with an MW lower than 290 kDa and are quantified separately from BAP. The BAP are mainly cellular macromolecules with an MW in a range of 290–5000 kDa, and could be further classified into the growth-associated BAP (GBAP) with an MW of 1,000 kDa, which are produced in the microbial growth phase, and the endogeny-associated BAP (EBAP) with an MW of 4,500 kDa, which are generated in the endogenous phase.

2.8 How Can a Comprehensive Microbial Products Model (X_{STO}, EPS, and SMP) for Aerobic Granular Sludge be Developed?

The results from Chaps. 6 and 7 indicate that X_{STO} and EPS, as two types of polymers, are produced respectively inside and outside the cell by microorganisms. They could be produced simultaneously. Chapter 8 shows that SMP have been classified into two groups on the basis of the bacterial phase from which they are derived: UAP derived from the original substrate in microbial growth and BAP generated in the endogenous phase. EPS, X_{STO}, and SMP are important sinks for electrons and carbon derived from the original substrate. Phenomena involved with multiple carbon and electron sinks should have significant implications on the performance of aerobic granule systems. Thus, a comprehensive model about these microbial products in aerobic granules is a matter of great interest to improve our understanding about aerobic granular sludge and thereby enhance the efficiency of such system through the optimization of operational parameters. In Chap. 9, the latest representations for the production and consumption of EPS, X_{STO}, and SMP (from Chaps. 6–8) are incorporated into a more comprehensive model to describe the microbial products formation in aerobic granules. The concepts behind this modeling approach quantify the interconnections of EPS, SMP and X_{STO}, and offer a new integrated framework to describe these concepts with mathematical expressions and complete mass balances. The model is able to capture the experimental observations accurately for the soluble and solid components in aerobic granules exposed to dynamic feast and famine conditions. In addition, the model illustrates that X_{STO}, EPS, and the biomass components have different behaviors during feast and famine periods.

2.9 Do the Heterotrophs Utilize the Microbial Products of Autotrophs in Aerobic Granular Sludge for Growth?

Results in Chaps. 4 and 9 indicate that aerobic granular sludge is inherently multicomponent as there are electron donors and acceptors, active biomass, EPS, residual inert material, and SMP produced under usual metabolic conditions. In an autotrophic system with the growth of autotrophs, inorganic carbon is converted into organic carbon in cell mass and EPS, and SMP are produced from substrate metabolism. The heterotrophs might utilize the SMP produced by autotrophs for growth, resulting in an existence of heterotrophs and autotrophs. Coexistence of a high level of heterotrophs with autotrophs has been often found in autotrophic nitrifying biofilms cultured without an external organic carbon supply. These microbial products, not contained in the feed solution, are likely to be the energy and carbon sources for the growth of heterotrophs. In Chap. 10, the formation mechanism, component characterization, and mathematical modeling of the microbial products of autotrophs and their exchange with heterotrophs in the nitrifying granular sludge are investigated systemically. Results show that the oxygen diffusion limitation causes anoxic microenvironments in the nitrite oxidizing granules and allows a sequential utilization of nitrate as electron acceptor and microbial products as electron donor for heterotrophic denitrification.

2.10 Is It Feasible to Cultivate Aerobic Granules for the Treatment of Low-Strength Municipal Wastewater in a Pilot-Scale SBR?

The results from Chaps. 3–10 indicate that many factors are involved in the granulation of activated sludge. Contributing factors include carbon source, hydrodynamic shear force, feast–famine regime, feeding strategy, dissolved oxygen, reactor configuration, volume exchange ratio and settling time. It shall be noticed that most of studies concerning aerobic granules have been focused on well-controlled lab-scale reactors with high- or middle-strength synthetic wastewaters.

Operating parameters and experience harvested from Chaps. 3–10 may be applicable to the utilization of aerobic granule process for the treatment of municipal wastewater, which is usually characterized as a low strength wastewater. In China, with the recent rapid economic growth and urbanization, a large number of municipal wastewater treatment plants are being constructed. The increasing population of China results in serious shortage of land area. Thus, aerobic granular process might be the promising technique for municipal wastewater treatment. Chapter 11 reports the cultivation of aerobic granules with an excellent settling ability in a pilot-scale SBR for the treatment of low-strength municipal wastewater for the first time. The volume exchange ratio and settling time are found to be two key factors in the granulation of activated sludge grown on such a low-concentration wastewater in an SBR. The mathematical model developed in Chap. 4 is applied to describe this pilot-scale granular reactor performance successfully.

2.11 Can Aerobic Granular Sludge be Used as Inoculum to Shorten the Startup Period of Anaerobic Ammonium Oxidation Process?

Anaerobic ammonium oxidation (anammox) is a promising new process to treat high-strength nitrogenous wastewater. Due to the slow growth rate of anammox bacteria, efficient biomass retention is essential for reactor operation. The results reported in previous chapters indicate that aerobic granules have a high microbial diversity (Chap. 4) and compact structure (Chap. 3) with very good settling properties (Chap. 11), resulting in an efficient means of biomass retention (Chap. 9). These properties including interspecies competition (Chap. 4) and mass transfer (Chap. 6) result in the stratification of microbial species (Chap. 4) with anoxic zones in the interior of the granules (Chap. 10), which may be suitable to harbor anammox bacteria. In Chap. 12, the feasibility to start up the anammox process by seeding the reactor with aerobic granular sludge is investigated. The anammox granules are successfully cultivated in an upflow anaerobic sludge blanket (UASB) reactor by seeding with aerobic granular sludge. The average total nitrogen removal efficiency exceeds 94 % after the formation of the granules. The heterogeneous composition of the seeding material contributes to a considerable reduce of the startup period to less than 160 days at a loading rate of 0.064 kg N kg VSS^{-1} d^{-1}. The ASM1 extended with a two-step denitrification consideration is incorporated with the anammox process to formulate a new platform to simulate the reactor performance well. Simulation results indicate that the optimum granule diameter for maximum N-removal in the case should be between 1.0 and 1.3 mm and that the optimum N loading rate should be 0.8 kg N m^{-3} d^{-1}.

http://www.springer.com/978-3-642-31280-9

Formation, characterization and mathematical modeling
of the aerobic granular sludge
Ni, B.-J.
2013, XVI, 340 p., Hardcover
ISBN: 978-3-642-31280-9