

Study on the Performance and Stability of Algal-
bacterial Aerobic Granular Sludge in Wastewater
Treatment Using Continuous-flow Reactors

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Abstract

Aerobic granular sludge (AGS) is gaining more interest and becoming a promising technology in wastewater treatment since it possesses incomparable advantages over conventional activated sludge (AS) systems, such as excellent settleability, strong and dense microbial structure, and withstand to higher pollutants loading and toxicants. Previous works mainly focused on the essential factors like organic loading, shear force, hydraulic selection pressure and wastewater composition which influence the granulation process. Most of these studies were conducted in sequencing batch reactors (SBRs) which are regarded as the most successful ones. While from an engineer's viewpoint, continuous-flow reactors are more advantageous for large-scale application. During the operation of AGS, algae has been found to naturally grow in AGS systems which possess the ability to produce oxygen and at the same time bacteria can utilize the produced oxygen for organics degradation. This could provide a new opportunity for energy saving in wastewater treatment plants. Up to the present, however, little information is available on algal-bacterial granules, especially their stability in continuous-flow reactors. Therefore, this study aimed to explore various operation strategies to provide optimum reactor design and performance (in terms of pollutants removal and granules stability). The kinetics of reactions involved were also explored to assist the design and evaluation of the process, especially for future large-scale applications.

(1) One single reactor (R_1) and two identical reactors in series ($R_2=R_{2-1}+R_{2-2}$) were tested on algal-bacterial AGS for approximately 120 days' operation by seeding mature AGS at a ratio of 1:1 (general AGS/algal-bacterial AGS, w/w). Both R_1 and R_2 demonstrated almost similar organics and nutrients removal, and the two algal-bacterial AGS systems showed excellent performance even operated at double increased organic and nutrient loadings. Moreover, when double increased strength influent fed to R_1 , a better denitrification performance was achieved, in which total nitrogen (TN) removal increased from 29% to 80%. The two systems well maintained their granular stability, and all granules became algal-bacterial ones after 120 days' operation. Additionally, the mechanisms were proposed regarding the formation and enhanced stability of the new algal-bacterial granules in continuous-flow reactors.

(2) With the aim to provide a modest technique for kinetics development that could transform the sophisticated biological reaction mechanisms into an understandable approach, a novel kinetic development method was proposed. In this study, cycle test experimental data

were used as the basis for the determination of related reaction rates. With the purpose to check its validity and applicability, the developed model was tested by using the same reactor system (continuous-flow reactor) under different aeration strategies and a different reactor system (SBR) under different seed sludge, reactor dimension, and influent compositions. The proposed model was successfully applied to predict the reactor performance with good accuracy ($R^2 > 0.98$ and relative error $< 10\%$) for both tested reactor systems. Furthermore, the model was implemented in decision making on aeration strategy for optimum organics and nutrient removals along with energy requirement.

(3) Instead of aeration, effluent recirculation was employed to two continuous-flow reactor systems seeded with general AGS and algal-bacterial AGS, respectively. The results suggest that the algal-bacterial AGS possesses better overall performance and stability. A long-term operation of algal-bacterial AGS was also conducted by varying the effluent recirculation ratios, reflecting stable pollutants removal ability while deteriorated settleability and stability to some extent. More importantly, after being switched from no aeration to intermittent aeration, the algal-bacterial AGS could quickly adapt to the change in operation conditions, which was obviously indicated by its recovered removals of dissolved organic carbon (DOC) from 40% to 100%, TN from 61% to 98%, and total phosphorus (TP) from 14% to 64% along with improved sludge settleability and stability.

It is expected that results from this study could provide important and scientific data for the development of algal-bacterial AGS, especially for continuous-flow reactor systems.

Key words: Algal-bacterial aerobic granular sludge; Continuous-flow reactor; Stability; Kinetic modeling

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Abbreviations

AGS	Aerobic granular sludge
AOB	Ammonia oxidizing bacteria
AS	Activated sludge
ASM	Activated sludge model
CFR	Continuous-flow reactor
CMBR	Continuous-flow membrane bio-reactor
COD	Chemical oxygen demand
DO	Dissolved oxygen
DOC	Dissolved organic carbon
EBPR	Enhanced biological phosphorus removal
EPS	Extracellular polymeric substances
HRT	Hydraulic retention time
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
N	Nitrogen
NOB	Nitrate oxidizing bacteria
OLR	Organic loading rate
P	Phosphorus
PAO	Phosphorus accumulating organism
PHA	Poly-hydroxyalkanoate
PN	Proteins
PS	Polysaccharides
RMSE	Root-mean-square error
SBR	Sequencing batch reactor
SRT	Solids retention time
SVI	Sludge volume index
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus

TS

Total solids

VS

Volatile solids

Chapter 1 Introduction

1.1 An overview on aerobic granular sludge systems

The most common suspended growth process for domestic wastewater treatment is the activated sludge (AS) process [1]. Aside from its effective removal of organics, AS process has several drawbacks such as high energy requirement and large land area occupation for secondary settling tank(s) due to poor sludge settling ability. As a result, innovative technologies are demanding for wastewater treatment with good performance at low investment and operation costs.

Aerobic granular sludge (AGS) is a compact, dense aggregates composed of self-immobilized cells which is a novel and promising technology for wastewater treatment and can solve several problems in the AS process. The aerobic granules are recognized to have the attributes of dense and strong structure, excellent settling ability and the ability to treat toxic pollutants [2, 3].

In the past two decades, AGS systems have attracted the attentions of many researchers in wastewater treatment field. Previous studies explored the influence of various factors on aerobic granulation, such as shear force (aeration intensity), settling time, wastewater composition, and organic loading [4–8]. Most of these studies were carried out in sequencing batch reactors (SBRs), the most successful systems for AGS [9].

Aerobic granulation is a process which involves self-aggregation of bacteria forming compact structure with size bigger than 0.1 mm [10]. Wastewater characteristics and reactor operation conditions are crucial for the granulation process. In SBR operation, suitable settling time and hydraulic retention time (HRT) controlled by cycle time could provide hydraulic selection pressure for aerobic granulation, which is regarded as the most important factor [11]. The bigger and denser particles are retained in the reactor with smaller particles being discharged. However, if strong hydraulic selection pressure is used (short settling time and HRT), much biomass would be washed out. Organic loading rate (OLR) also plays a major role in the aerobic granulation process. High OLR can provide suitable conditions for fast-growing bacteria resulting in filamentous overgrowth and unstable reactor performance [12, 13], leading to instability of AGS [14].

From the above literature review, two major aspects have been focused in previous studies of AGS: (1) exploration of shortening granules formation, and (2) investigation on the operation strategy that is able to maintain granular stability. Liu and Tay [10] suggested that the factors affecting granules formation and long-term operation stability are different. A strong hydraulic selection pressure or a high OLR during granules' formation period is believed to shorten granules' formation. On the other hand, a lower OLR or a stronger aeration rate could maintain the long-term stability of aerobic granules.

1.2 Aerobic granular sludge in continuous-flow reactors

The study on aerobic granular sludge for pilot- or large- scale application is still very limited. In other words, most of AGS studies were conducted in SBRs. A typical SBR operation for an AGS system consists of feeding, idling, aeration, settling and effluent discharge. Each period contributes some essential condition for aerobic granulation. The above sequential processes in the SBR are usually controlled through a sophisticated control mechanism. The more complicated operation in SBR is a hindrance for its application for large-scale systems. In an engineering prospective, continuous-flow reactors (CFRs) are preferable over SBR systems due to its lower installation cost, and easier operation, control and maintenance. However, CFRs lack several important factors for granule development such as hydraulic selection pressure and alternation of feast-famine period. Hydraulic selection pressure is important for granules' formation to retain dense and bigger biomass, and discharge loose and filamentous biomass [10, 15]. As explained in a previous study [10], hydraulic selection pressure may be prerequisite for granules' formation that determines the length of granulation time. A previous study reported that aerobic granules developed in a CFR lost their stability faster than those in a SBR [16].

The absence of feast-famine condition in a continuous-flow membrane bio-reactor (CMBR) led to microbial competition with resultant accumulation of filaments in the reactor [17]. In addition, the absence of hydraulic selection pressure in a continuous-flow reactor is also regarded as the cause for filamentous accumulation and thus granules' disintegration [17, 18]. Aerobic granules cultivated in a column-type continuous-flow reactor with external settling tank gradually lost their stability during 140 days of operation due to the growth of filaments in the reactors [19–21].

Previous works indicate that the realization of granulation and maintenance of granular stability are the two major challenges for the large-scale application of AGS technology. The current bottleneck emphasizes the need for further study on granular stability in continuous-flow reactors as a base for practical application purposes.

1.3 Algal-bacterial symbiosis in wastewater treatment

Excess nutrients in water body can bring about environmental issues such as eutrophication that leads to algal bloom phenomenon. If the excess nutrient(s) or substance(s) enter wastewater treatment facilities in a proper quantity, with the help of sunlight exposure algae growth will occur in the wastewater treatment plants. Algae-activated sludge systems for wastewater treatment have aroused much interests in academic fields due to the ability of algae to produce oxygen [22] that is required for the aerobic degradation of organic substrates, and their ability to uptake and remove P and N simultaneously. This could open some new opportunity for energy saving in the wastewater treatment plant with optimum performance. Previous studies reported that the co-existence of algae with activated sludge could have good performance on nutrients uptake [23–25].

1.4 Algae growth in aerobic granular sludge

Symbiotic algal-bacterial granular sludge could be very attractive for municipal wastewater treatment. It has the advantages of algae-activated sludge system in addition to the extra benefits from AGS (excellent settling ability, able to withstand toxic pollutants, high removal efficiency), which could also become a possible solution for effective separation of algal-bacterial biomass from the wastewater.

Algae have been found naturally grown and co-existed with AGS in a SBR reactor treating 600 mg/L of chemical oxygen demand (COD) (with COD/NH₄-N/PO₄-P ratio of 60/10/1) under natural sunlight exposure [26, 27], yielding deteriorated N and P removal. In another report [28] algae-bacteria granular consortia was successfully developed by seeding specific algae (*Chlorella* and *Scenedesmus*) and aerobic granules, which didn't show better N and P removals than that from Huang et al. [26]. However, other report stated that algal-bacterial granular symbiosis seeded by activated algae inoculum composed of microalgae (*Chlorella*)-bacteria flocs treating low-strength wastewater in

sequencing batch mode showed good performance on N and P removals without introducing external aeration [29].

These previous works indicate that there is still limited information about bioactivity and stability of algal-bacterial granules, especially in continuous-flow systems which could be very useful for practical application of AGS technology. This suggests the need for further research on algal-bacterial granules' stability and bioactivity in a continuous-flow system.

1.5 Kinetic modeling of aerobic granular sludge

Mathematical kinetic models can be used as means to aid environmental engineers to evaluate the design, operation and optimization of a treatment system. The mathematical simulation can provide a fundamental information for reactor design and operation, which is essential for describing the treatment performance. Various kinetic models have been applied to study the performance of bioreactors, particularly on the COD and N removals. The popular and essential models that can be used to predict the individual substrate removal rate in a simple approach are zero-order, first-order and Grau second-order kinetic models [30–32]. While other popular models applied for describing aerobic biological treatment processes are generally adopted from the activated sludge models (ASMs) [33] including ASM1, ASM2, ASM2d, and ASM3 [34–36]. As a matter of fact, the actual reactions occurred in the biological process are complex. The original ASMs need to be modified to predict the closest reaction pathway to provide accurate results, and the modifications could be varying from one system to another. In addition, the ASM3 neglects nitrite as the intermediate product due to its assumption that both nitrification and denitrification happen as a single step process. Therefore, some modification has been tried to include nitrite which is essential in nitrification and denitrification processes [34, 37–40]. On the other hand, ASMs can be used effectively only if all the parameters are known. Even though most of the model parameters can be obtained from experiments, some of them still need to be obtained from model calibration. Calibration is still required even for the model parameters derived from literature to acquire good consistence between simulation and experimental results due to the differences in reactor configuration, influent characteristics, operation condition, and reaction mechanisms involved in the processes.

Previous researches demonstrate that the major hindrance in the model development for AGS process is the complex biological reaction mechanisms that could affect model development, modification or selection of model parameter values. Although the kinetic model of AGS has already been studied in detail with satisfactory simulation results [34], there is yet no information about kinetic parameters and mathematical models which could be used for continuous-flow reactor (CFR) based algal-bacterial AGS system. In the present work, organics and nutrient removals were estimated using the kinetics models developed from typical cycle tests on the algal-bacterial AGS.

This study tried to transform sophisticated reaction mechanisms involved into simplified individual substrate removal expression. The implemented model was able to show organics, N and P removals, in addition to both nitrite and nitrate accumulation in the system, and incorporation of aeration and non-aeration conditions in a typical cycle operation. Moreover, the model was implemented to study the effect of aeration/non-aeration duration on organics and nutrients removal regarding energy requirement to optimize the operation conditions that contribute to the maximum organics and nutrients removal. It is expected that the findings from this work could provide some simple and applicable means to predict reactor performance which benefits the optimization of reactor design and operation conditions for the algal-bacterial AGS system in large-scale applications.

1.6 Research objective and structure of the thesis

This study aims to explore various operation conditions for algal-bacterial AGS in continuous-flow reactors that could provide good pollutants removal and stable granules. The successful results of this thesis are expected to shed light on the long-term stable operation of algal-bacterial AGS in large-scale applications. To achieve these objectives, this thesis is divided into four chapters with the major points illustrated in Figure 1-1.

Chapter 1 introduced the recent achievements of AGS systems and its problems related during operation. The extensive research works on AGS were focused on the factors effecting granulation, and most importantly, very less information could be found on AGS operation in continuous-flow reactors. While during operation, occasionally algae growth occurs in the system, which may have effect on the reactor performance. With the aim of realization of successful large-scale applications of AGS (or algal-bacterial AGS),

mathematical models were claimed as a useful mean to assist the development of AGS. The objectives of this study were also arrived in this chapter.

Chapter 2 focused on the investigation on the stability of algal-bacterial AGS in two different reactor configurations (single and two series reactors) for approximately 120 days of operation. In the Stage I of operation (days 0 – 60) both systems were operated under the same influent concentrations. During the subsequent 60 days, the influent concentration of single reactor was elevated to twofold of the previous stage with the purpose to test the performance under a higher pollutant loading. Organics, N and P removals, biomass settleability and stability of the two systems were compared and discussed. In addition, the mechanisms regarding the formation and enhanced stability of the new algal-bacterial granules in continuous-flow reactors were proposed.

Chapter 3 introduced the novel approach to predict kinetics to study the reactor performance. Mathematical modeling could be useful for further investigation. The kinetic model was developed by using experimental data during cycle tests on the algal-bacterial AGS in a continuous-flow reactor, which was successfully applied to describe the reactor performance with good accuracy. More importantly, the model was also successful to be implemented for general AGS in a SBR reactor with good consistence. At last, the model was implemented to study the effect of aeration strategy for N and P removals with respect to energy consumption.

Chapter 4 investigated the potential and capability of algae co-existence in AGS systems that could realize low energy consumption for wastewater treatment by testing the performance of algal-bacterial AGS with effluent recirculation instead of aeration. The performance of general AGS and algal-bacterial AGS were compared and then a further test was conducted on algal-bacterial AGS. Eventually, to test the adaptability of algal-bacterial AGS under different operation strategies and to recover its performance and stability, the reactor operation was switched from non-aeration to intermittent aeration.

Chapter 5 summarized the major conclusions of the thesis. For better understanding on the continuous-flow algal-bacterial AGS, future studies were also proposed.

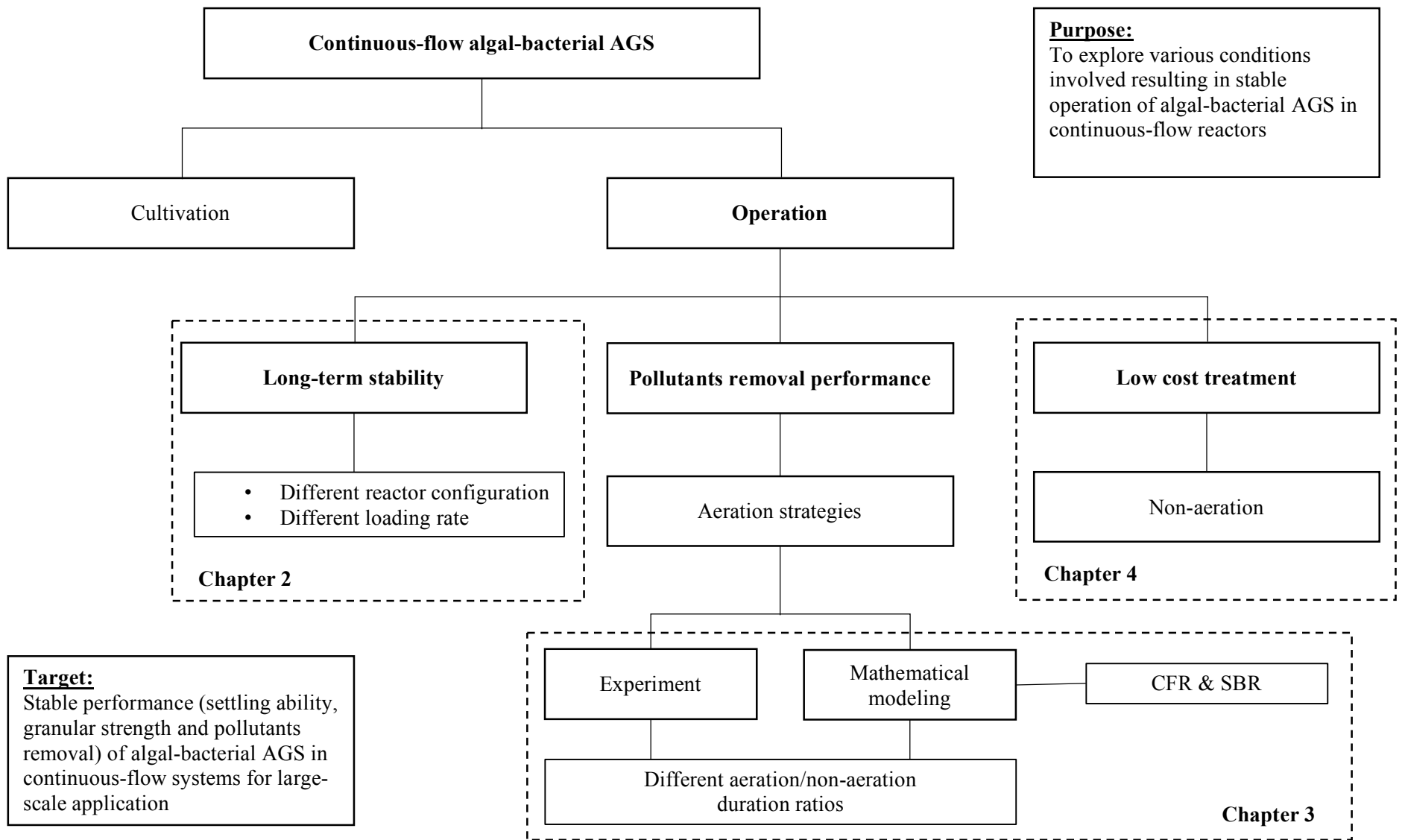


Figure 1-1. Structure of the thesis.

Chapter 2 Stability of algal-bacterial aerobic granules in continuous-flow reactors to treat varying strength of domestic wastewater

2.1 Introduction

My lab previous work [26] found that algae could naturally grow in the AGS system operated in SBR under natural sunlight exposure leading to decrease in N and P removals. While other researcher [28] successfully cultured algal-bacterial consortia by seeding mature AGS and microalgae (*Chlorella* (FACHB-31) and *Scenedesmus* (FACHB-416)). Up to present, however, very limited information is available on algal-bacterial granules, especially on their stability in continuous-flow reactors during long-term operation.

In a continuous-flow reactor, the reactor configuration such as single-, series-, or parallel-reactor are regarded to have different effects on its performance. This study investigated the stability of algal-bacterial AGS (in terms of organics and nutrients removal, granular settling performance and stability) operated under two different reactor configurations (1 single and 2 reactors in series) for approximately 120 days. Besides, the mechanisms of algal-bacterial AGS in the continuous-flow reactor regarding the formation and stability enhancement of new algal-bacterial granules were also discussed.

2.2 Materials and methods

2.2.1 Experimental set-up and operation conditions

One single reactor (R_1) and two reactors in series (R_2), made of acrylic plastic, were used in this study. R_2 consisted of two identical reactors (R_{2-1} and R_{2-2}) with automatically internal recirculation from R_{2-2} to R_{2-1} . Each system had the same total working volume of 1 L. The structural diagram of the two reactor systems is shown in Figure 2-1.

A mixture of mature general AGS and algal-bacterial AGS with a ratio of 1:1 (w/w) cultivated in SBRs in the laboratory was used for seed sludge. The general and algal-bacterial AGS were obtained using the same cultivation method as described by Huang et al. [26] with the main characteristics of synthetic wastewater for AGS cultivation as follows: 300 mg chemical oxygen demand (COD)/L (50% of which was contributed by sodium acetate and glucose, respectively), 10 mg PO_4 -P/L (KH_2PO_4) and 100 mg NH_4 -N/L (NH_4Cl). The initial mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were 5.0 g/L and 4.0 g/L (MLVSS/MLSS = 0.8), respectively in each reactor in this study.

The two systems were operated for two stages (stage I and stage II) about 120 days. A synthetic wastewater with sodium acetate as carbon source was used as influent. The components of synthetic wastewater were prepared according to the characteristics of domestic wastewater in Yogyakarta, Indonesia in stage I: 300 mg COD/L (sodium acetate), 10 mg PO₄-P/L (KH₂PO₄), 100 mg NH₄-N/L (NH₄Cl), 10 mg Ca²⁺/L (CaCl₂·2H₂O), 5 mg Mg²⁺/L (MgSO₄·7H₂O), and 5 mg Fe²⁺/L (FeSO₄·7H₂O) (COD/N/P ratio = 30:10:1).

All the reactors were operated continuously at room temperature (25 ± 2°C) and hydraulic retention time (HRT) of 6 h under alternative aeration (60 min) and non-aeration (30 min) conditions, respectively. Only room light was provided for these reactors with no light control throughout the experiments (light illumination intensity about 900 - 1100 lux with all lights on). During aeration, air was supplied by an air pump (AK-30, KOSHIN, Japan) from the bottom of reactors through air bubble diffusers at an air flow rate of 0.5 cm/s. The average dissolved oxygen (DO) concentration was 7 - 8 mg/L and 2 - 5 mg/L during aeration and no aeration periods, respectively. Reactor modification was done on day 50 by installing an internal separator in each reactor to achieve better retention of granules in the reactors. As no control was conducted on solids retention time (SRT) for the two reactor systems, SRT for both R₁ and R₂ was estimated to be 30 ~ 70 days according to the total biomass amount and the effluent condition (flowrate and solids concentration) during the 120 days' operation.

From day 60 on (stage II), as the performance of the two reactor systems became relatively stable, influent COD, NH₄-N, and PO₄-P concentrations to R₁ were increased to 600, 200 and 20 mg/L (COD:N:P ratio = 30:10:1) by using the same chemicals as above respectively to test the reactor stability when encountering higher strength wastewaters. The major difference in influent for the two reactor systems during stage I and stage II is shown in Table 2-1.

2.2.2 Analytical methods

Influent and effluent samples were collected once every 10 days, and sampling was done at 12:00 pm (at the end of non-aeration period) and then filtered through 0.45 µm membrane prior to analysis. Parameters related to reactor performance (PO₄-P, NH₄-N, NO₃-N, and NO₂-N concentrations) in addition to sludge volume index (SVI₅), MLSS and MLVSS were analyzed in accordance with standard methods [41]. Algae content in granules was estimated based on the extracted chlorophyll-a amount from the granules [41]. Dissolved organic carbon (DOC) was measured by TOC analyzer (TOC-V_{CSN}, SHIMADZU, Japan) equipped with an auto-sampler (ASI-V, SHIMADZU, Japan). DO meter (HQ40d, HACH, USA) was used for measuring DO level in the reactors. pH was monitored using a pH meter (Horiba, Japan). A

pocket digital lux meter (ANA-F11, Tokyo Photoelectric Co., Ltd., Japan) was used to measure the light intensity around the reactors.

A stereo microscope (STZ-40TBa, SHIMADZU, Japan) with a program Motic Image Plus 2.35 (Version 2.3.0) was used to measure the granular size, and Leica M205 C Microscope (Leica Microsystems, Switzerland) was used to observe granular morphology change.

The stability of granules was evaluated by the change of turbidity in sludge sample after shaking at 200 rpm for 10 minutes [42]. Standard buffer solution was used to replace distilled water in this work.

Extracellular polymeric substances (EPS) extraction from granules was carried out according to the heating method [43]. EPS was the sum of protein (PN) and polysaccharides (PS) in this study. PS content of the granules was measured using the phenol-sulfuric acid method [44], and PN content of the granules was determined using the Bradford method with bromine serum albumin (BSA) as standard [45].

All the determinations were performed in triplicate, and average values were used if there's no special indication.

2.2.3 Calculations

DOC, TN and TP removal efficiencies were calculated according to Cai et al. [5], while their removal capacities were calculated by using Eq. (2-1), which can be used to compare the reactor performance between the two reactor systems.

Removal capacity (X , mg/g-MLVSS/d) = $24 \times (C_{inf} - C_{eff}) / (MLVSS \times HRT)$ (2-1)
 where X (mg/g-VSS/d) is the removal capacity of DOC, TN or TP, and C_{inf} (mg/L) and C_{eff} (mg/L) are concentrations of DOC, TN or TP in the influent and effluent, respectively. MLVSS (g/L) is the MLVSS concentration in the reactor, HRT (h) is the hydraulic retention time of wastewater in the reactor, and 24 is the conversion unit from day to hour.

Increase in turbidity ($\Delta Turbidity$) used to indicate granular stability was calculated as follows:

$$\Delta Turbidity \text{ (NTU)} = Turb_1 - Turb_2 \quad (2-2)$$

where $Turb_1$ (NTU) and $Turb_2$ (NTU) are the granular sample turbidity before and after shaking, respectively. $\Delta Turbidity$ is defined herein as the change of turbidity in the supernatant before and after shaking test. A lower $\Delta Turbidity$ denotes a greater strength of granules.

2.2.4 Statistical analysis

A statistical analysis of variance using one-way analysis of variance (ANOVA) was conducted, not only to test the significance of change in granular stability and pollutants (DOC,

N and P) removal in each reactor during the two stages' operation, but also to check the significance of variance in granular stability and pollutants removal between the two reactor systems when being fed the same strength influent (like stage I). Significant difference was assumed at $p < 0.05$.

2.3 Results and discussion

2.3.1 Performance of the two reactor systems

To evaluate the performance of algal-bacterial AGS on organics and nutrients removal in the continuous-flow reactor systems, biomass growth and its settleability were recorded along with DOC, P, and N removals during the 120 days' operation.

(1) Biomass growth and settleability of algal-bacterial AGS

MLSS and SVI₅ were determined to evaluate biomass growth and granular settleability in the continuous-flow reactors.

As seen from Figure 2-2a, it is clearly that MLSS was averagely decreased from initial 5.0 g/L to 3.5 and 3.4 g/L on day 50 in R₁ and R₂, respectively. This observation is most probably attributable to the carry-out effect of effluent that flows from the algal-bacterial AGS systems during the aeration period, leading to the significant decrease in MLSS in both reactor systems. As it is known, a smaller SVI₅ value reflects better settleability. During the first stage of operation (stage I, days 0 – 60), the SVI₅ value averagely increased to some extent from initial 52 to 67, 67 and 62 mL/g in R₁, R₂₋₁ and R₂₋₂ on day 50, respectively, followed by detectable decrease in biomass concentration in both reactor systems (Figure 2-2a). Filamentous bacteria were also observed to grow in these two systems. This is possibly caused by different operation strategies (as SBR was used for seed granules cultivation while continuous-flow reactors were used for this test) and different wastewater characteristics used for seed granules cultivation and this study, respectively, thus the granules might need a certain period for adaptation and stabilization. Unlike SBR systems, continuous-flow reactors lack hydraulic selection pressure which is helpful to discharge sludge with worse settleability. Filamentous bacteria are reported to be a substantial element for granulation, which can serve as the backbone of granular sludge [46]. However, excessive amount of filamentous bacteria can bring about the worsening of granules and lead to sludge wash-out. Being similar to Chen et al. [18] and Corsino et al. [17], it is also necessary to pay close attention to filamentous accumulation in the continuous-flow algal-bacterial AGS system.

With the aim to retain algal-bacterial AGS in the reactor, a reverse funnel-shape internal separator was installed in each reactor on day 50, which seems to effectively retain algal-bacterial AGS in the reactor. After the installation of internal separator (stage II, days 60 – 120), the average biomass concentration steadily increased from 3.5 to 4.8 g/L in R₁ and from 3.4 to 4.3 g/L in R₂, respectively. Zhou et al. [47] noticed that the internal separator could optimize granular size distribution in SBR. Results from this work also indicate that the internal separator could achieve hydraulic selection pressure in continuous-flow reactors, thus favoring the selective discharge of biomass. Selective filamentous discharge successfully may help to gain better granular settleability [48]. As shown in Figure 2-2a, after installation of the internal separator, the SVI₅ values in all reactors gradually decreased, showing better granular settleability than their initial conditions (SVI₅ = 44, 49, and 46 ml/g on day 120 for sludge in R₁, R₂₋₁, and R₂₋₂, respectively).

Algae content in the granules was found to be relatively stable in granules from the reactors, varying from initial 1.64 mg/g to 1.69 mg/g, 1.65 mg/g, and 1.67 mg/g in AGS from R₁, R₂₋₁ and R₂₋₂, respectively on day 120 under the tested conditions.

(2) DOC removal

The changes in DOC removal capacity and MLVSS concentration in both systems (R₁ and R₂) are shown in Figure 2-2b. Steady increase in DOC removal capacity was observed in R₁ and R₂ during stage I, which remained relatively stable at ~150 mg DOC/g-MLVSS/d (or 0.5 kg COD/kg-MLVSS/d) in R₂. However, in R₁ this removal capacity was increased to ~290 mg DOC/g-MLVSS/d (or 0.9 kg COD/kg-MLVSS/d) and then remained relatively stable during stage II. As shown in Figure 2-2c, the average effluent DOCs from R₁ and R₂ were around 4.5 and 4.0 mg/L, achieving average DOC removal efficiency of 96% and 95%, respectively. During the 120 days' operation, no significant difference in DOC removal efficiency was detected between these two reactor systems ($p = 0.106$). Results show that these two continuous-flow systems can be used to effectively remove organics, even for the treatment of high strength domestic wastewater with high concentrations of organics and nutrients.

(3) N and P removals

Figure 2-3a shows the N profiles in the effluents from the three reactors during 120 days' operation. Seen from the results, algal-bacterial AGS in both R₁ and R₂ systems exhibited excellent performance in treating NH₄-N wastewater with NH₄-N removal rate > 99% from the very beginning of this test. Nitritation and nitrification efficiencies were also calculated according to Li et al. [27] (Figure 2-3b). Results reflect that both reactor systems demonstrated excellent nitritation efficiency (99-100%). That is, in both systems NH₄-N can be easily and

effectively converted into NO₂-N. However, better nitrataion (from NO₂-N to NO₃-N) was always noticed in R₂ than in R₁ during the whole operation, possibly due to its relatively lower DOC concentration (Figure 2-2c). In addition, as shown in Figure 2-3c denitrification process also occurred better in R₂ system during the first stage under the same operation conditions of R₁. The effective denitrification in R₂ might be resulted not only from the automatically internal recirculation of treated water (with high level of NO₃-N) from R₂₋₂ to R₂₋₁ (Figure 2-1b), but also from the high organics level in the influent of R₂₋₁ as both are prerequisite and beneficial for denitrification.

In this study, total nitrogen (TN) concentration was calculated as the sum of NH₄-N, NO₂-N and NO₃-N in the reactor. Results show that TN removal efficiency was averagely 29% and 76% for R₁ and R₂, respectively during stage I of this test. Interestingly, during stage II the nitrataion efficiency in R₁ was improved from 88% to 94%, even though this efficiency was still a little bit lower than that in R₂ (99%) (Figure 2-3b). Moreover, denitrification process advanced better in R₁ during stage II, resulting in increase of average TN removal efficiency up to 80%. This observation might be attributable to the following two aspects. Firstly, excessive organics concentration could function as carbon source essential for the growth of denitrifiers. Secondly, anaerobic ammonium oxidation (ANAMMOX) to some extent might also contribute to the enhanced denitrification in R₁ during stage II as its influent NH₄-N concentration was also doubled, thus the produced NO₂-N from nitritation could have more chance to react with NH₄-N, bettering the whole system's denitrification performance. Still, the real reason needs more detailed investigation.

In this work, the influent P in synthetic wastewater was prepared with KH₂PO₄, thus TP removal can be reflected by PO₄-P removal. Results show that the effluent PO₄-P concentrations from R₁ and R₂ were averagely 5.60 and 5.11 mg/L during stage I, which were 10.86 and 5.01 mg/L during stage II, respectively. Even though the effluent PO₄-P concentration from R₁ increased to around 11 mg/L during stage II (when influent PO₄-P concentration was doubled from 10 to 20 mg/L), its PO₄-P removal efficiency was almost similar. In this study, the P removal efficiency was about 44% and 49% for R₁ and R₂ during stage I, which relatively stabilized at 46% and 50% during stage II, respectively. Statistical analysis indicates that under the same influent condition (like stage I), R₂ performed slightly better in TP removal efficiency than R₁ ($p = 0.002$). For each reactor system, however, no significant difference in TP removal efficiency was found between the operation of stage I and stage II ($p = 0.119$ and 0.725 for R₁ and R₂, respectively). Results also show that algal-bacterial AGS in this work exhibited lower P removal efficiency when compared to the general AGS

operated in continuous-flow enhanced biological P removal (EBPR) systems [19–21]. Two aspects may contribute to the lower phosphorous removal in the algal-bacterial AGS systems in this work. On one hand, no intentional biomass discharge from the reactors was performed during the whole test period (or no SRT control) as the purpose of this work is to compare the stability of algal-bacterial AGS in the two reactor systems, which is closely associated with P removal efficiency in biological wastewater treatment systems. Biomass was lost or discharged mainly by the carry-out effect of effluent. On the other hand, the organic loading applied for the algal-bacterial AGS systems in this work is much higher than those for AGS EBPR processes [19–21], which is not beneficial for enhanced P removal. The investigation is still on-going with respect to further enhancement on nutrients (N and P) removal by optimizing the operation strategies and organics and nutrients loadings to the continuous-flow algal-bacterial AGS systems.

2.3.2 Characteristics of algal-bacterial granules

(1) Change in morphology

In this study, a mixture of mature AGS and algal-bacterial AGS was used for seed sludge which was to mimic the startup of a new algal-bacterial AGS system. At the beginning, the mature AGS was yellowish while the algal-bacterial AGS was green, and both AGS exhibited irregular, compact and dense structure. During the operation, fluffy outer surface was observed on the granules in both reactor systems, indicating the existence of filamentous bacteria. As discussed previously, filamentous bacteria may cause worsen settleability and decreased amount of biomass in the reactors. In this work, the installation of internal separator successfully provided selective discharge of sludge from the reactors, resulting in effective retaining of granules with larger size in the reactors. Restated, some filamentous bacteria were still observed in the reactors till the end of experiments, which seemed to have little negative effect on granular stability of algal-bacterial AGS.

(2) Granular size and distribution

Figure 2-4 shows the dynamic changes of granular size and its distribution. The average diameter of granules in R₁, R₂₋₁ and R₂₋₂ was determined to be 0.84, 0.89, and 1.31 mm respectively on day 1. During stage I (days 0-60), the granules in all reactor systems showed some increase in size to 1.09, 1.16 and 1.41 mm in R₁, R₂₋₁ and R₂₋₂ on day 60, respectively. The granular size of AGS in R₂₋₂ remained relatively stable, possibly due to the lower organic loading applied to R₂₋₂ (the second reactor in the series reactor system) compared to the other two reactors (R₁ and R₂₋₁). During the stage II, the granular size in all the tested reactors seemed

to be relatively stable most probably due to the existence of co-aggregation and compaction of AGS at the same time [49], which is partially indicated by the increased biomass concentration in R₁, R₂₋₁ and R₂₋₂ (Figure 2-2a) and their relatively stable granular diameters. In addition, very limited change in granular size distribution was observed for the granules in all the reactors after internal separator installation, especially during stage II. This indicates that the internal separator could not only retain biomass in the reactor but also maintain granular size distribution. In addition, the change of organics and nutrients concentrations in influent exerted limited effect on the granular size distribution as well.

(3) EPS and granular stability

EPS are secreted products from bacterial cells, which are regarded to have contribution to granular structure [50] and granulation process [51]. PS and PN are essential constituents of EPS and the characteristics of granules are usually related to PS/PN ratio [52] which may have contribution to granular stability. Thus, PS, PN and PS/PN ratio during the 120 days' operation were also measured and recorded in this study. An increased average EPS content was noticed in the granules from R₁ after switching from stage I (47.1 mg EPS/g-MLSS) to stage II (63.0 mg EPS/g-MLSS), while EPS content in the granules from R₂₋₁ and R₂₋₂ seemed to be relatively stable during the whole operation (averagely 54.5 mg EPS/g-MLSS and 44.6 mg EPS/g-MLSS in R₂₋₁ and R₂₋₂, respectively). The increase of EPS content in R₁ could be contributed by the increase of organic loading to R₁ from stage I to stage II, as higher organics feeding might stimulate biomass to produce more EPS. However, very limited change was detected in PS/PN ratio of granules from all the reactors during the 120 days' operation, indicating that the algal-bacterial granules could maintain their structural stability in continuous-flow reactors under the tested conditions.

On the other hand, during the whole operation, no significant difference was found on the change of $\Delta Turbidity$ for granule samples from the three reactors ($p = 0.064$, Fig. 5). As shown, the three reactors could quickly and positively respond to the changes in operation strategy (from SBR to continuous-flow) and influent wastewater characteristics, maintaining their granular stability throughout this test. To some extent, R₁ seems to have more potential for stable operation as its granules could keep their stability even when its influent organic and nutrient loadings were double increased during stage II. As the organic and nutrient loadings to R₂₋₁ and R₂₋₂ were different from each other due to the configuration of series reactor system itself, it's understandable that some difference in granular stability could be noticed among the granules from the three reactors. According to the results from the 120 days' operation, algal-bacterial AGS possesses excellent stability in continuous-flow reactor systems. Cai et al. [5]

examined the granular stability after feeding glucose/acetate and glucose/propionate wastewater into two SBRs and found that the $\Delta Turbidity$ for general AGS samples were 3.59 and 6.74 NTU, respectively. In this work, the $\Delta Turbidity$ values on day 120 were 1.67, 1.85, and 1.67 NTU for the algal-bacterial AGS samples from R₁, R₂₋₁ and R₂₋₂, respectively. This observation also indicates that algal-bacterial AGS could have better granular stability in continuous-flow reactor systems.

2.3.3 Preliminary analysis on mechanism involved

Based on the microscopic images and the phenomenon observed during the 120 days' operation, mechanisms involved in the development of new algal-bacterial AGS with higher stability are proposed as illustrated in Figure 2-6.

The seed sludge used in this study was a mixture of general AGS and algal-bacterial AGS where algae was initialed and grew naturally. Under the designed operation conditions (including uncontrolled room light with illumination intensity about 900-1100 lux when all lights were on), new algae appeared with fluffy outer surface that is similar to filamentous bacteria. Probably due to aeration and resultant shear force, the fluffy algae could entrap the suspended particles and form microbial co-aggregates, the small clusters for algal-bacterial AGS. More specifically, the filamentous algae might also provide a nucleus for these aggregates thus binding with smaller granules. The shear force from aeration may enhance effective collision and then strengthen the binding effect between nucleus and aggregates [49, 53]. Consequently, compact algal-bacterial granules were generated in the continuous-flow reactors.

Algal-bacterial granules usually grow up to a diameter of 1.0 – 1.5 mm with a solid or dense structure and clear appearance of filamentous algae inside the granule body, which serves as the backbone for the whole granule. Their cross-section images also confirm the role of algae as the nucleus or backbone of algal-bacterial granules. Algae functioned as nucleus and backbone along with the existence of EPS might contribute to the enhancement of granular stability to a great extent [52]. In addition, with the help from shear force created by aeration, the continuous-flow reactor systems used in this work could provide suitable conditions to sustain algal-bacterial AGS with better stability.

In this work, most of the naturally growing algae were found to be *Phormidium* sp. Future exploration will be conducted on the major functional algae species which provide the nucleus or binding sites. During the 120 days' operation, biomass wasn't intentionally disposed from the two systems as no control on SRT was applied in this study. Compared to general AGS,

less increase in MLVSS was detected in the algal-bacterial AGS systems (Figure 2-2), partially attributable to some loss of biomass (which was not retained by the internal separator) with lower settleability along with the discharge of effluent. On the other hand, the bacteria in the initial seed sludge may be mainly composed of slow growing bacteria, to some extent resulting in less increase in biomass concentration during the test period. In addition, some small animals eating biomass appeared gradually in the reactors (data not shown), which may also contribute to this phenomenon. Therefore, attention should also be paid to the changes in species of bacteria, algae and small animals, and their functions during the long-term operation of algal-bacterial AGS reactors.

2.4 Summary

Algal-bacterial AGS kept its stability during 120 days' operation in the tested two continuous-flow systems. Installation of internal separator successfully achieved effective retention of granules facilitating biomass growth and maintenance of granular stability. Results show that both systems exhibited almost similar efficiencies in overall organics and nutrients removal, implying that algal-bacterial AGS possesses excellent stability in continuous-flow reactors even operated at double increased organic and nutrient loadings. Future research is necessary to shed light on the mechanisms of stable operation of algal-bacterial AGS, and further optimization of continuous-flow systems could also pave the way for its application in practice.

Table 2-1. Characteristics of synthetic wastewater used in this study.

Reactor	Major parameters for influent	Stage I (Days 1-60)	Stage II (Days 60-120)
R ₁	COD (mg COD/L)	300	600
	NH ₄ -N (mg NH ₄ -N/L)	100	200
	PO ₄ -P (mg PO ₄ -P/L)	10	20
R ₂	COD (mg COD/L)	300	300
	NH ₄ -N (mg NH ₄ -N/L)	100	100
	PO ₄ -P (mg PO ₄ -P/L)	10	10

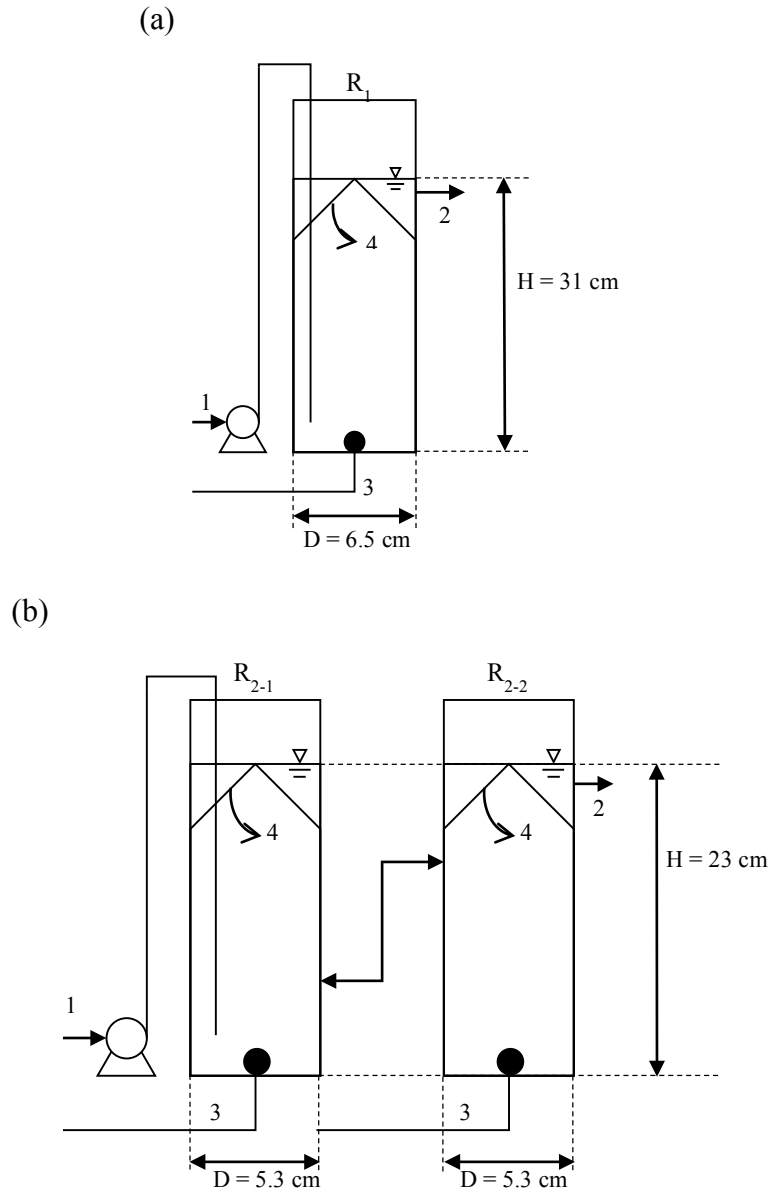


Figure 2-1. Structural diagram of the reactors: single reactor (R_1) (a) and series reactor (R_2) (b). 1-Influent, 2-Effluent, 3- Aeration line, 4-Internal separator for solid-liquid separation, 5- recirculation pipe.

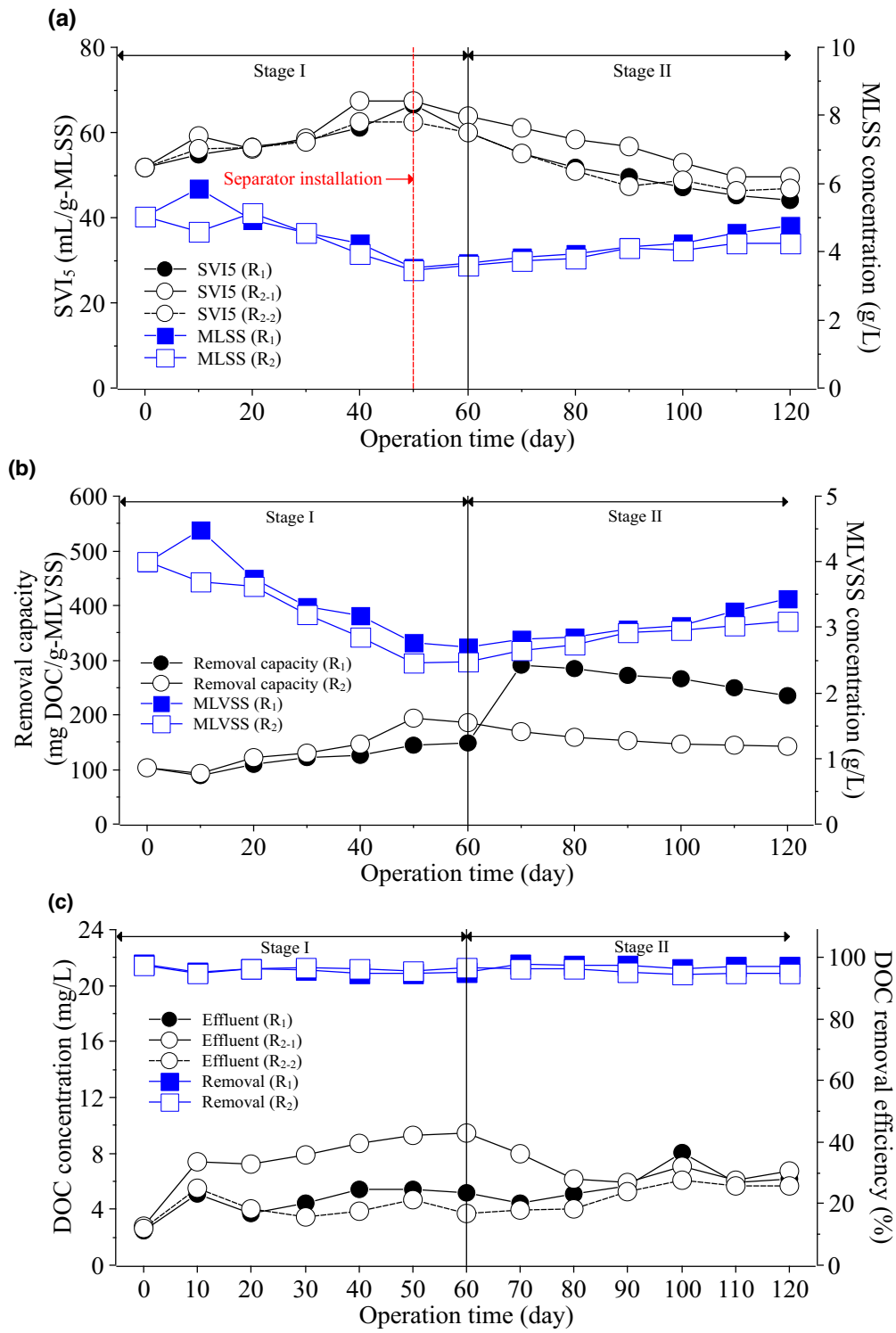


Figure 2-2. Changes in granular settleability and organics removal during 120 days' operation. SVI₅ and MLSS variations (a), DOC removal capacity and MLVSS variations (b), and effluent DOC and DOC removal efficiencies (d) in the two reactor systems.

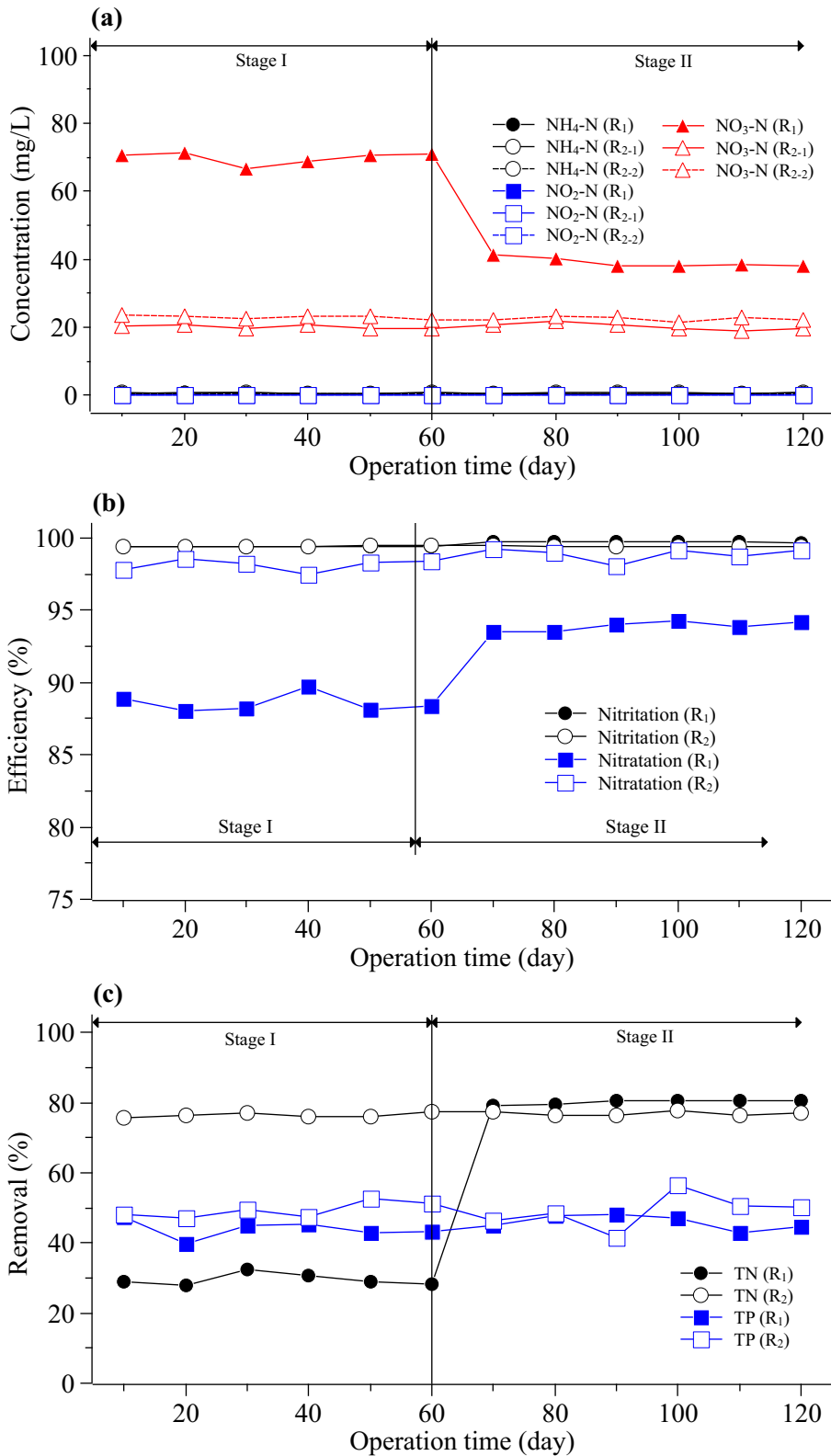


Figure 2-3. N and P removal profiles for the two reactor systems during 120 days' operation. N species (a), nitritation and nitrification efficiencies (b) according to Li et al. [27], and TP and TN removals (c).

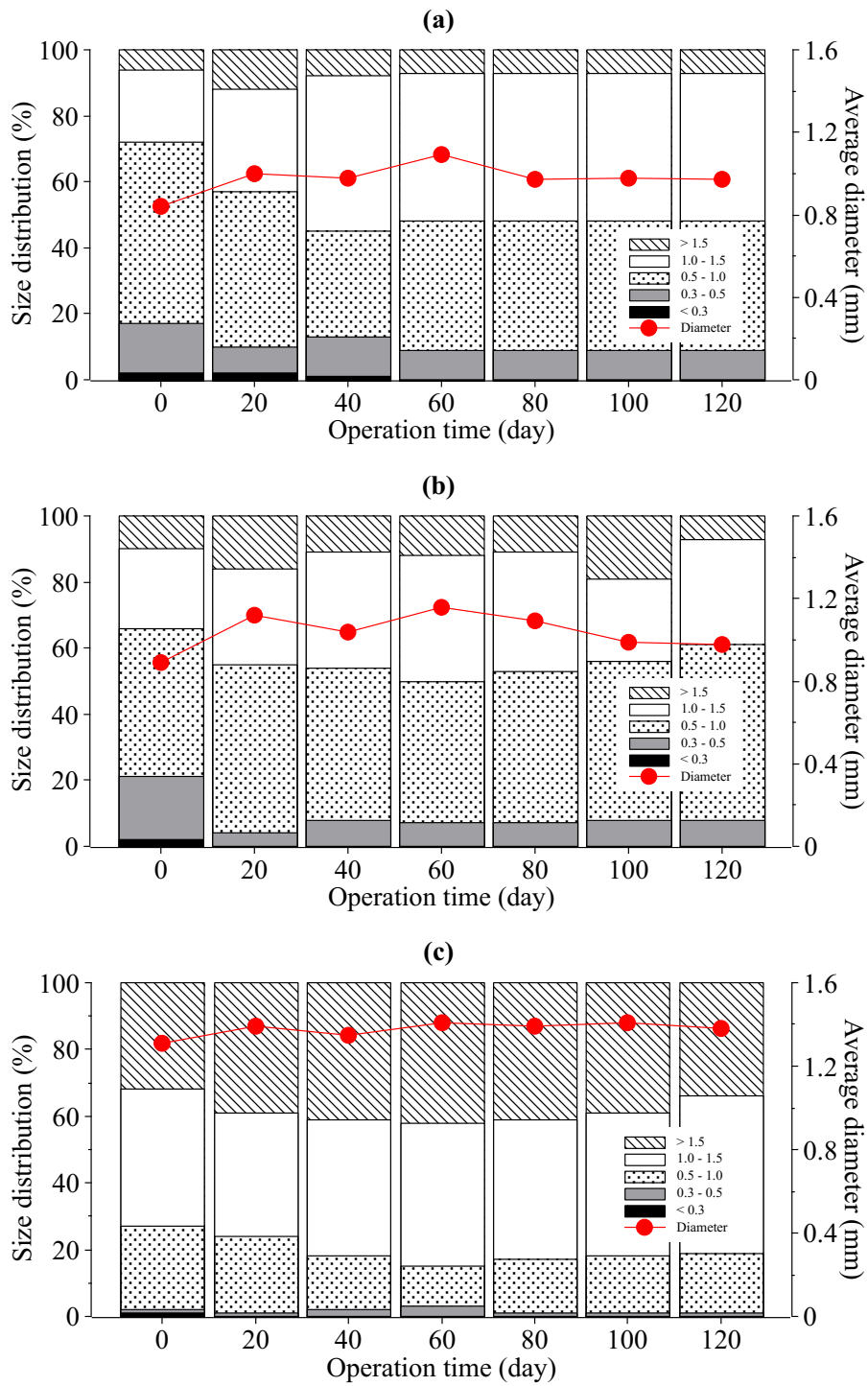


Figure 2-4. Dynamic changes of algal-bacterial granular size and distribution in R_1 (a), R_{2-1} (b), and R_{2-2} (c).

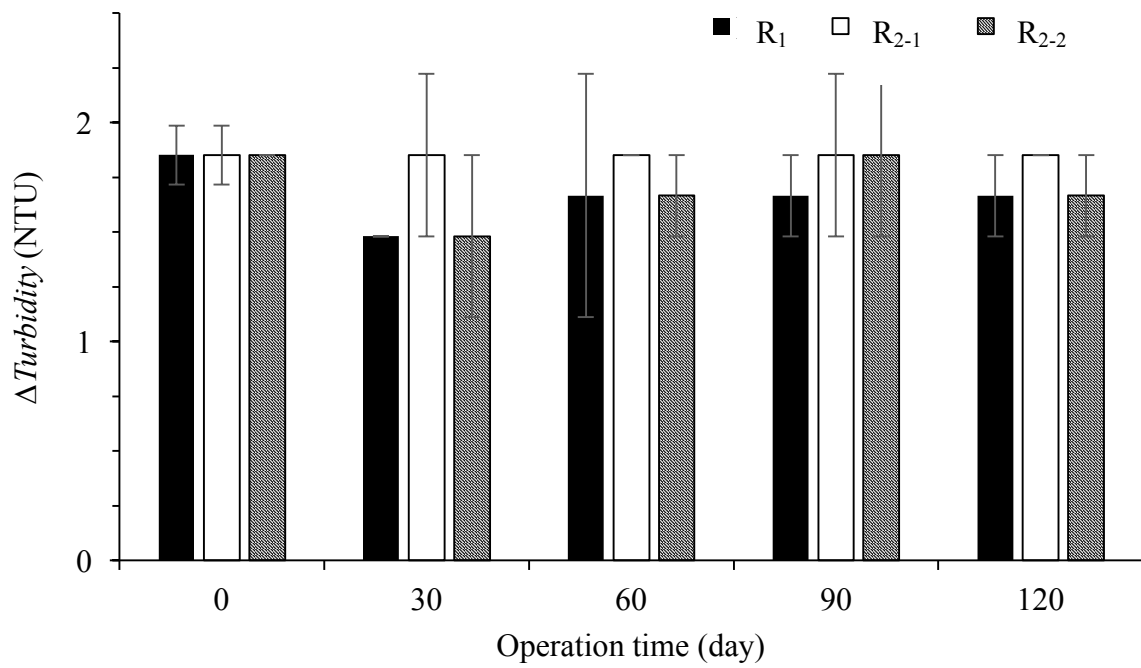


Figure 2-5. Changes in average granular strength of algal-bacterial AGS from the two reactor systems during 120 days' operation.

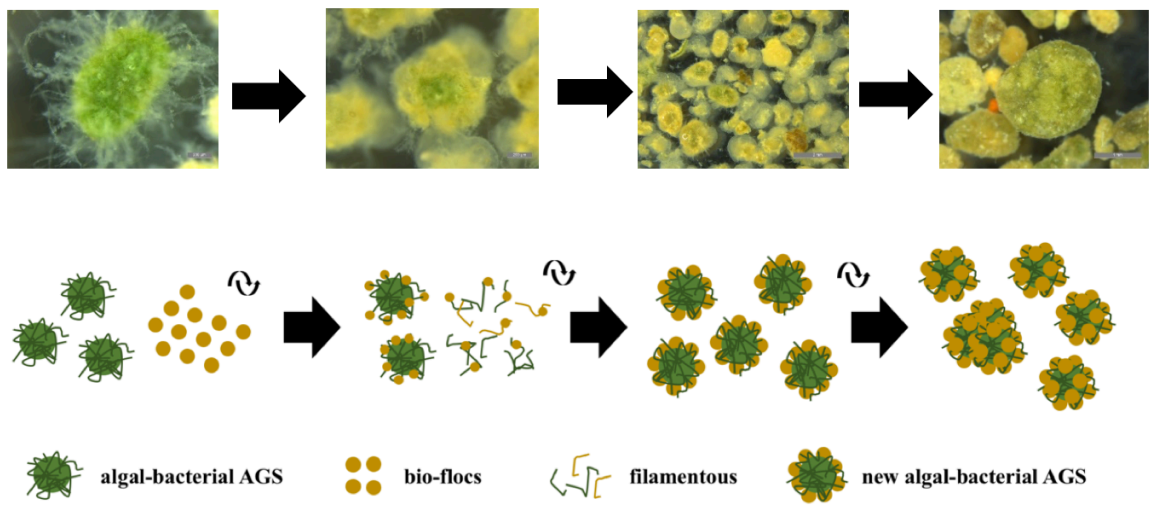


Figure 2-6. Schematic of proposed mechanisms of algal-bacterial AGS formation and enhancement.

Chapter 3 Novel approach for kinetics prediction on algal-bacterial aerobic granular sludge in a continuous-flow reactor and its application in decision on aeration strategy

3.1 Introduction

Mathematical kinetic model can be utilized as a useful means to aid environmental engineers to evaluate the design, operation and optimization of a treatment system. The mathematical simulation can provide fundamental information for reactor design and operation, which are essential for optimization of the whole treatment system. Various kinetic models have been applied to study the performance of bioreactors, particularly on COD and N removals. Previous research works demonstrate that the major hindrance in the model development for AGS process is its complex biological reaction mechanisms that could affect model development and modification or selection of the model parameter values. In addition, up to now, there is no mathematical models that can be used for algal-bacterial AGS systems. This work for the first time demonstrated a novel kinetic model development from typical cycle tests on the algal-bacterial AGS, which is able to transform sophisticated reaction mechanisms involved into some simplified individual substrate removal expressions with the incorporation of aeration and non-aeration conditions in a typical cycle operation. The developed model successfully predicted the organics, N and P removals in addition to both nitrite and nitrate accumulation in the system. Furthermore, the effect of aeration/non-aeration duration on optimum organics and nutrients removal with respect to energy requirement were explored by implementing this model. The results from this work are expected to be able to provide some simple and accurate means to predict reactor performance which benefits the reactor design and optimization of operation conditions for algal-bacterial AGS systems in practice.

3.2 Materials and methods

3.2.1 Reactor configuration, synthetic wastewater and seed sludge

A cylindrical continuous-flow reactor with a working volume of 1 L equipped with internal separator was used. The reactor operation conditions (HRT, aeration strategy, lighting, and air flow rate) were the same as those in Chapter 2. During aeration, air was supplied from the bottom of the reactor by an air pump (AK-40, KOHSIN, Japan). The average DO concentration during aeration and non-aeration period was 5–6 mg/L and below 1 mg/L, respectively. The operation of the reactor was conducted at room temperature ($25\pm 2^\circ\text{C}$). A synthetic wastewater

was fed into the reactor with the composition as described in Table 3-1. Algal-bacterial AGS cultivated from the previous study (Chapter 2) were used for seed sludge. The total suspended solids (TSS) concentration was maintained at approximately 3 g/L during the reactor operation. The volatile suspended solids to total suspended solids ratio (VSS/TSS) during the whole operation was almost constant at 65%. Algae content in the sludge was around 2.5 mg/g-TSS during the test period.

3.2.2 Cycle test experiments

On day 30 after the start-up, a batch experiment for the typical cycle operation was conducted. The cycle test experiment was performed to observe the bioactivity of the biomass (indicated by specific uptake rate) and as a basic approach for kinetic development.

Approximately 250 mL of mixed liquor was transferred to an Erlenmeyer flask and washed with distilled water for several times to remove background substrates. After the complete sedimentation of granules, fresh influent was introduced for the batch test for one cycle period (90 minutes) with aeration and non-aeration time of 60 and 30 min, respectively. Sampling was done every 10 minutes to measure the concentration evolution of substrates (DOC, NH₄-N, NO₂-N, NO₃-N and PO₄-P). Triplicate samples were analyzed with their average values being used for further calculations. The dynamic change in DO concentration during cycle test was also monitored (once in 1 minute) to confirm whether the DO concentration in the reactor was sufficient or not for the required biological process. The data of cycle test experiment were collected for the calculations of specific removal and accumulation rates and for the development of the proposed kinetic model.

The specific removal and accumulation rates of substrates during aeration and non-aeration periods could be calculated from the slope of the change in the specific substrate concentration versus duration of aeration or non-aeration period divided by VSS concentration in the reactor. A negative value of this specific rate indicates removal while a positive one denotes the occurrence of accumulation.

3.2.3 Kinetic models

This work presented different methods to predict kinetics by utilizing experimental data from cycle tests to describe overall treatment removal with a similar approach. The overall reactor performance was assumed to follow individual removal or accumulation occurred in the reactor during one cycle of operation, described by the evolution of individual substrate (COD, NH₄-N, NO₂-N, NO₃-N and PO₄-P) concentration. From the experimental results of cycle tests, the removal and accumulation of substrates were noticed to occur in one cycle.

Thus, each substrate removal and accumulation kinetics were discussed in the context of the real reactor operation.

(1) Substrate removal model

The substrate removal rate during aeration or non-aeration periods was determined using zero-order, first-order and modified Grau second-order kinetic models.

The expressions of zero-, first- and modified Grau second-order removal rates are given by Eqs. (3-1) to (3-3).

$$-\frac{dS}{dt} = q_r \times X_b \quad (3-1)$$

$$-\frac{dS}{dt} = K_1 \times S \quad (3-2)$$

$$-\frac{dS}{dt} = K_2 \times \left(\frac{S}{S_{inf}}\right)^2 \quad (3-3)$$

where q_r (mg/g-VSS/min), K_1 (1/min) and K_2 (mg/L/min) are the zero-, first- and second-order kinetic coefficients, and q_r also indicates the specific substrate removal rate. X_b (mg/L) is VSS concentration in the reactor, and S (mg/L) and S_{inf} (mg/L) are the substrate concentration in the reactor and the influent, respectively.

(2) Substrate accumulation model

Substrate accumulation rate was determined by using zero-order or modified first-order kinetic models. The expressions of zero- and modified first-order accumulation rates are given by Eqs. (3-4) and (3-5).

$$\frac{dS}{dt} = q_a \times X_b \quad (3-4)$$

$$\frac{dS}{dt} = K \times (a - S) \quad (3-5)$$

where q_a (mg/g-VSS/min) and K (1/min) are considered as zero- and first-order kinetic coefficient, respectively, and q_a is also known as specific substrate accumulation rate. a (mg/L) is the equilibrium substrate concentration in the reactor.

3.2.4 Applicability of the proposed method

With the purpose to check the applicability of the proposed kinetic development method by using the cycle test data, an additional test on general AGS in a sequencing batch reactor (SBR) was also conducted. The SBR with a working volume of 15 L (D×H=20 cm × 46 cm) seeded with general AGS was operated under 6 h of cycle time consisting of 3 min feeding, 60 min non-aeration, 290 min aeration, 3 min settling and 4 min discharge. The average TSS concentration and VSS/TSS ratio in the SBR during the whole operation period were approximately 2.8 g/L and 65%, respectively.

3.2.5 Model implementation

Scilab ver. 6.0.0 platform (Scilab Enterprises, France) was used for the simulation. The proposed model was applied to study different aeration strategies (Table 3-2) under the same cycle time of 90 min in a continuous-flow reactor. The effects of aeration scenarios on organics, N and P removal efficiency, and energy requirement were also discussed.

3.2.6 Analytical methods

Influent and effluent samples were collected once every day then filtered through 0.22 μm membrane prior to analysis. The concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{PO}_4\text{-P}$, TSS, and VSS were determined according to Standard Methods [41]. Organics concentration was determined as dissolved organic carbon (DOC) by TOC analyzer (TOC-V_{CSN}, SHIMADZU, Japan). DO concentration in the reactor was measured using DO meter (HQ40d, HACH, USA). Chlorophyll-a was measured to indicate algae content in the granules according to Standard Method [41].

3.2.7 Calculations

The overall pollutant removal efficiency was calculated according to Eq. (3-6).

$$\text{Removal (\%)} = 100 \times (1 - S_{eff}/S_{inf}) \quad (3-6)$$

in which S_{inf} (mg/L) and S_{eff} (mg/L) are the influent and effluent concentrations for the designated pollutant, respectively.

3.2.8 Model accuracy and statistical analysis

The coefficient of determination (R^2) and root-mean-square error (RMSE) were used to evaluate the goodness between the experimental and simulation data. A model with an R^2 of greater than 0.75 indicates a good model while those with R^2 of 0.25 or less are considered as not-usable, and those with R^2 between 0.25-0.75 are regarded as fair models [54]. At the same time, a model with a smaller RMSE indicates a better fit. In this study, one-way ANOVA was also conducted to test the significance of changes in DOC, TN and TP removals, and energy requirement under different aeration scenarios, and $p < 0.05$ was considered as statistically significant.

3.3 Results and discussion

3.3.1 Experimental results from cycle tests

In this study, the CFR was operated under an alternative aeration and non-aeration period of 60 min and 30 min, respectively. The change in DO concentration during the cycle operation

was monitored, which is imperative due to its particular implication for TN and TP removals. As illustrated in Figure 3-1, the DO level reached a relatively constant value at 5.23 mg/L during aeration and dropped to below 1 mg/L (near to anoxic/anaerobic condition) after 10 minutes of non-aeration period started. This DO level information during non-aeration period is vital to ensure that both the nitrification-denitrification process and P removal could be well realized in the tested reactor.

Based on the evolution of substrates concentration in the cycle test (Figure 3-1), substrate removal and accumulation rates were calculated (Table 3-3). The removal and accumulation rates could be used as an indicator of biomass bioactivity in the reactor [55, 56]. It was observed that the DOC uptake rate (1.920 mg/g-VSS/min) during aeration was higher than that in non-aeration period (0.159 mg/g-VSS/min), resulting in higher DOC removal during the aeration period (43% and 3% in the aeration and non-aeration period, respectively). A similar activity was observed on NH₄-N removal (45% and 11% during aeration and non-aeration period, respectively). A higher NH₄-N removal achieved during aeration period indicated that NH₄-N removal mainly occurred from nitrification by ammonia oxidizing bacteria (AOBs) and a very low DO level during non-aeration period might inhibit the activity of AOBs. During the aeration period, nitrite was produced from NH₄-N oxidization, which can further be oxidized into nitrate by nitrite oxidizing bacteria (NOBs) (indicated by nitrate accumulation). The nitrite accumulation was possibly due to a faster nitrite production rate than the nitrite oxidation rate. The accumulation of NO₂-N and NO₃-N could also suggest the occurrence of two-step nitrification (nitritation and nitrataion) during the aeration period. On the other hand, denitrification successfully happened during the non-aeration period (indicated by NO₃-N removal), while the NO₂-N accumulation during non-aeration period might be resulted from the byproducts of denitrification or from NH₄-N oxidation. The results suggest that the applied aeration and non-aeration period can successfully provide the desirable conditions for both nitrification and denitrification.

Phosphorus uptake was detectable during the aeration period while phosphorus release was observed during the non-aeration period. The increased TP concentration during the non-aeration period might be attributable to the phosphorus release by phosphorus accumulating organisms (PAOs) [57]. Under anaerobic conditions PAOs can release phosphorus to produce carbon storage compounds like poly-hydroxyalkanoates (PHAs), while under aerobic conditions PAOs consume organics or the stored PHAs are used for cell growth. A similar TP release phenomenon was reported for general AGS in a SBR during non-aeration period [5] and also detected in the cycle test experiment in the SBR in this study.

The cycle test results imply that the aeration and non-aeration periods could affect DOC, N and P removals. Most notably, the aeration operation benefits P removal and non-aeration operation could provide denitrification condition with resultant better TN removal.

3.3.2 Kinetic evaluation and model verification

Kinetic and mathematical modeling has been employed to predict the reactor performance. Three substrates removal models and two substrates accumulation models were applied to investigate the performance of the continuous-flow algal-bacterial AGS reactor in the present work. Since the actual reactor operation involves consecutive aeration and non-aeration periods, the overall kinetics applied would be a kinetic combination between the aeration and non-aeration periods. As for the model development, when the zero-order removal reaction rate was applied for DOC and $\text{NH}_4\text{-N}$, negative substrate concentrations was always obtained in the effluent either during aeration or non-aeration periods. Thus, the zero-order removal reaction rates for DOC and $\text{NH}_4\text{-N}$ were excluded from the combination attempt. All the simulation data were then plotted and compared with the experimental data (Figure 3-2). Finally, the combination of reaction kinetics got the best fit, indicating by the highest R^2 and minimum RMSE, which was selected for further model implementation (Table 3-3).

The results show that the reaction kinetics for $\text{NH}_4\text{-N}$ concentration followed the first-order removal rate (during both aeration and non-aeration) with R^2 and RMSE of 0.994 and 2.37, respectively. As for $\text{NO}_2\text{-N}$, the reaction kinetics has the best fit to the zero-order accumulation rate (aeration) and first-order accumulation rate (non-aeration) (R^2 and RMSE of 0.993 and 0.20, respectively). The best consistence between the simulation and experimental data of $\text{NO}_3\text{-N}$ concentration are given by the zero-order accumulation rate for both aeration and non-aeration periods (with R^2 and RMSE of 0.994 and 0.16, respectively). The $\text{PO}_4\text{-P}$ concentration evolution followed the zero-order kinetic model during uptake or removal (aeration) and release or accumulation (non-aeration) (R^2 and RMSE of 0.996 and 0.07, respectively). The overall reaction kinetics for DOC removal followed the first-order model during aeration and non-aeration periods (with R^2 and RMSE of 0.981 and 6.77, respectively).

Furthermore, the simulated effluent quality for 10 days' operation also fitted very well to the experimental data as shown in Figure 3-3a, giving the R^2 value for all substrates greater than 0.95. The above results indicate that the mathematical model developed from the cycle test data could accurately predict the performance of the continuous-flow reactor used in this study.

3.3.3 Validity and applicability of the proposed method

With the purpose to check the validity of the developed model in this study, aeration and non-aeration durations were changed from 60 min and 30 min to 45 min and 45 min, respectively while the total cycle duration was kept at 90 min for the subsequent 10 days' operation. Further analysis on the applicability of the proposed kinetic model was also conducted by applying the same procedure for a SBR reactor. The SBR has been continuously and stably operated for approximately 110 days, and the effluent sampling was done every ten days. The cycle test data used in this study was obtained on day 60. This test was intended to check the applicability of the proposed kinetic development method on different types of reactors, wastewater composition and seed sludge by using the cycle test experimental results. A summary of the selected kinetic parameters that provide the best fit of simulation to experimental data for both CFR and SBR operations are given in Table 3-5.

Figure 3-3b shows the measured and simulated results of the effluent quality parameters for 10 days of CFR operation when both aeration and non-aeration periods lasted 45 min. A very good consistence between the experimental and simulation data was obtained with respect to the concentrations and removal efficiencies of DOC, $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$, respectively. Most importantly, the simulated effluent concentrations and removal efficiencies of the effluent quality indicators from the SBR also fitted very well with the experimental data. Table 3-6 summarizes the simulated and measured effluent quality indicators from the CFR and the SBR, respectively.

Therefore, the above results prove that the developed mathematical model based on cycle test data is applicable for both types of reactors (batch and continuous-flow), varying seed AGS sludge and different influent characteristics.

3.3.4 Practical application

The obtained kinetic models were then implemented to study the effect of different aeration strategies (Table 3-2) on DOC, TN and TP removals taking energy consumption into consideration. The same influent characteristics as the CFR, cycle time of 90 minutes and reactor volume were used for the model input. The composition of the influent used for simulation in this section was 115 mg DOC/L, 100 mg $\text{NH}_4\text{-N/L}$, and 10 mg $\text{PO}_4\text{-P/L}$. Then, the effluent quality indicators (DOC, $\text{NH}_4\text{-N/L}$, and $\text{PO}_4\text{-P}$), their removal efficiency and energy consumption were estimated from the developed model.

The simulation results show that variation in aeration duration does not have contribution to DOC removal, while influences TN and TP removals (Figure 3-4a). It is noticeable that a

longer aeration duration could enhance TP removal but reduce TN removal. However, the change of TN removal by varying aeration duration was found not significant ($p = 0.161$). On the other hand, change in aeration duration has significant influence on TP removal ($p = 0.003$). Thus, the selection of optimum aeration duration would be dependent on TP removal.

The above results suggest that a longer aeration duration is beneficial for TP removal. In addition, it is known that extending aeration period requires higher energy consumption. However, the required energy for TP removal might decrease with the prolonging of aeration due to a higher TP removal efficiency achieved at a longer aeration duration. On the other hand, a longer aeration duration would contribute to a higher energy consumption for TN removal since a lower TN removal was achieved under a prolonging aeration duration (Figure 3-4b). Although a lower energy requirement for specific pollutant removal is desirable, the increase in total energy requirement should also be considered. From the above results, with the aim to get the optimum TP removal, it is suggested that the aeration duration should be longer than non-aeration. As shown, increase in aeration/non-aeration duration ratio greater than 2 does not contribute to a significant reduction of energy requirement for TP removal.

When considering all pollutants removal together with energy requirement, an aeration to non-aeration duration ratio of 2:1 is considered as the optimum aeration strategy. When TP removal is the major target regardless of energy requirement, the aeration to non-aeration duration ratio of 8:1 is regarded as the optimum condition.

3.4 Summary

This work presented that kinetic model for a sophisticated biological treatment process by applying algal-bacterial AGS and general AGS in CFR and SBR reactors respectively could be simplified by using experimental data from cycle tests. The proposed model was proven to accurately predict reactor performance under different aeration strategies. Moreover, the proposed kinetic model was also proven to be applicable for different types of reactors operated by using different influent compositions and seed AGS. Results from the aeration strategy study suggest that an aeration/non-aeration duration ratio of 2:1 could balance the optimum pollutants removal performance and energy consumption. The proposed kinetic development approach could be used as a helpful tool for process design and evaluation.

Table 3-1. Composition of synthetic wastewater used in this study.

Component	Concentration	Unit
CH ₃ COONa	300	mg COD/L
NH ₄ Cl	100	mg NH ₄ -N/L
KH ₂ PO ₄	10	mg PO ₄ -P/L
CaCl ₂ ·2H ₂ O	10	mg Ca ²⁺ /L
MgSO ₄ ·7H ₂ O	5	mg Mg ²⁺ /L
FeSO ₄ ·7H ₂ O	5	mg Fe ²⁺ /L

Table 3-2. Aeration scenarios in the simulation set-up.

	Set scenarios					
	1	2	3	4	5	6
Aeration / non-aeration ratio	1	2	3	4	5	8
Aeration duration (min)	45	60	68	72	75	80

Note: The total cycle duration was kept at 90 min.

Table 3-3. Water quality indicators: removal or accumulation rates.

Substrate	Unit	Removal or accumulation rate	
		Aeration	Non-aeration
DOC	mg DOC/g-VS/min	-0.640	-0.053
NH ₄ -N	mg NH ₄ -N/g-VS/min	-0.386	-0.105
NO ₂ -N	mg NO ₂ -N/g-VS/min	+0.007	+0.004
NO ₃ -N	mg NO ₃ -N/g-VS/min	+0.048	-0.014
PO ₄ -P	mg PO ₄ -P/g-VS/min	-0.014	+0.006

Note: The positive (+) values denote accumulation; The negative (-) ones denote removal.

Table 3-4. Combination of order of reactions used to test the related models.

Parameter	Order of reaction during consecutive aeration – non-aeration period					
	S-1	S-2	S-3	S-4	S-5	S-6
NH ₄ -N	1r-1r*	1r-2r	2r-1r	2r-2r	-	-
R ²	0.994	0.986	0.984	0.974	-	-
RMSE	2.37	3.72	4.44	5.82	-	-
NO ₂ -N	0a-0a	0a-1a	1a-0a	1a-1a	-	-
R ²	0.989	0.993	0.992	0.995	-	-
RMSE	0.10	0.08	0.20	0.22	-	-
NO ₃ -N	0a-0r	1a-0r	0a-1r	0a-2r	1a-1r	1a-2r
R ²	0.994	0.994	0.994	0.591	0.990	0.581
RMSE	0.16	0.16	0.16	1.48	0.35	1.57
PO ₄ -P	0r-0a	0r-1a	1r-0a	1r-1a	2r-0a	2r-1a
R ²	0.996	0.995	0.995	0.993	0.992	0.985
RMSE	0.07	0.06	0.07	0.07	0.10	0.12
DOC	1r-1r	1r-2r	2r-1r	2r-2r	-	-
R ²	0.981	0.977	0.911	0.899	-	-
RMSE	6.77	9.27	12.46	14.81	-	-

Note: 'S' denotes scenario; 'r' denotes removal; 'a' denotes accumulation. The number before 'r' or 'a' indicates the order of reaction, i.e. 1r-1r describes the first-order removal during aeration and the first-order removal during non-aeration.

Table 3-5. All the selected kinetic reactions for CFR and SBR.

	NH ₄ -N	NO ₂ -N	NO ₃ -N	PO ₄ -P	DOC
Aeration – Non-aeration					
CFR	1r-1r	0a-1a	0a-1r	0r-0a	1r-1r
Non-aeration – Aeration					
SBR	2r-1r	1a-1r	1r-1a	2a-1r	1r-2r

Note: ¹CFR - Continuous-flow Reactor; SBR - Sequencing Batch Reactor.

²'r' denotes removal; 'a' denotes accumulation. The number before 'r' or 'a' indicates the order of reaction, i.e. 1r-1r describes the first-order removal during aeration in CFR (or non-aeration in SBR) and the first-order removal during non-aeration in CFR (or aeration in SBR).

Table 3-6 Summary of reactors' performance from experimental and simulated results

	CFR-1			CFR-2			SBR		
	Exp	Sim	Δx	Exp	Sim	Δx	Exp	Sim	Δx
Effluent (mg/L)									
NH ₄ -N	29.06	31.29	0.08	31.44	33.47	0.06	0.21	1.65	6.83
NO ₂ -N	5.05	3.68	0.27	3.61	3.29	0.08	0.01	0.18	0.98
NO ₃ -N	20.41	18.82	0.08	12.05	11.38	0.06	28.05	25.29	0.10
PO ₄ -P	5.06	5.43	0.07	7.58	7.75	0.02	0.38	0.55	0.44
DOC	15.81	17.25	0.09	15.67	17.46	0.13	65.08	67.04	0.03
Removal (%)									
TN	49.42	50.58	0.01	55.76	54.77	0.02	43.12	46.12	0.07
TP	50.57	46.90	0.07	25.24	23.57	0.07	92.32	88.91	0.04
DOC	85.86	84.38	0.02	86.12	84.38	0.02	80.72	80.13	0.01

Note: ¹CFR - Continuous-flow Reactor; SBR = Sequencing Batch Reactor.

²CFR-1 = CFR under 60 min of aeration and 30 min of non-aeration; CFR-2 = CFR under 45 min of aeration and 45 min of non-aeration.

³The relative error $\Delta x = (\text{experimental value} - \text{simulation value})/\text{experimental value}$.

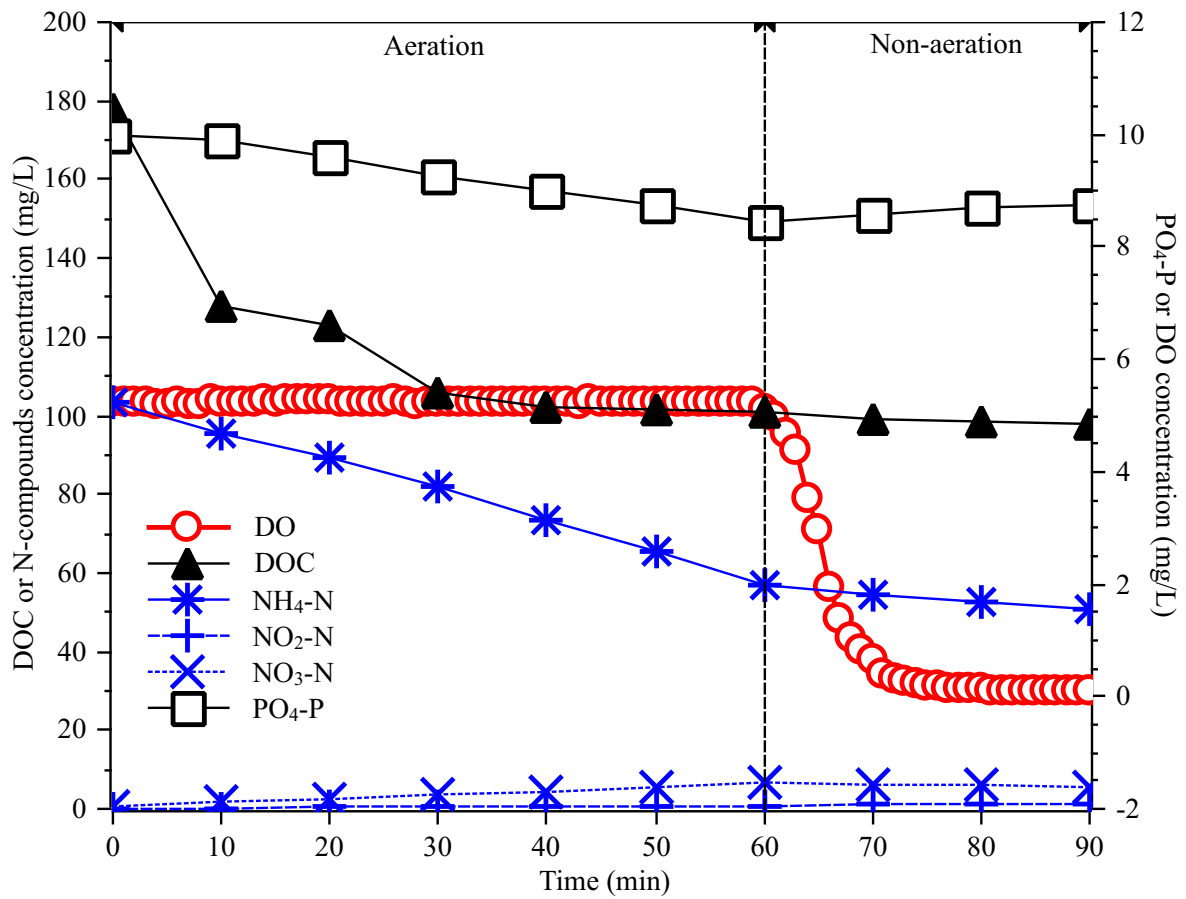


Figure 3-1. Profiles of DO and water quality indicators in the CFR reactor during a typical cycle test.

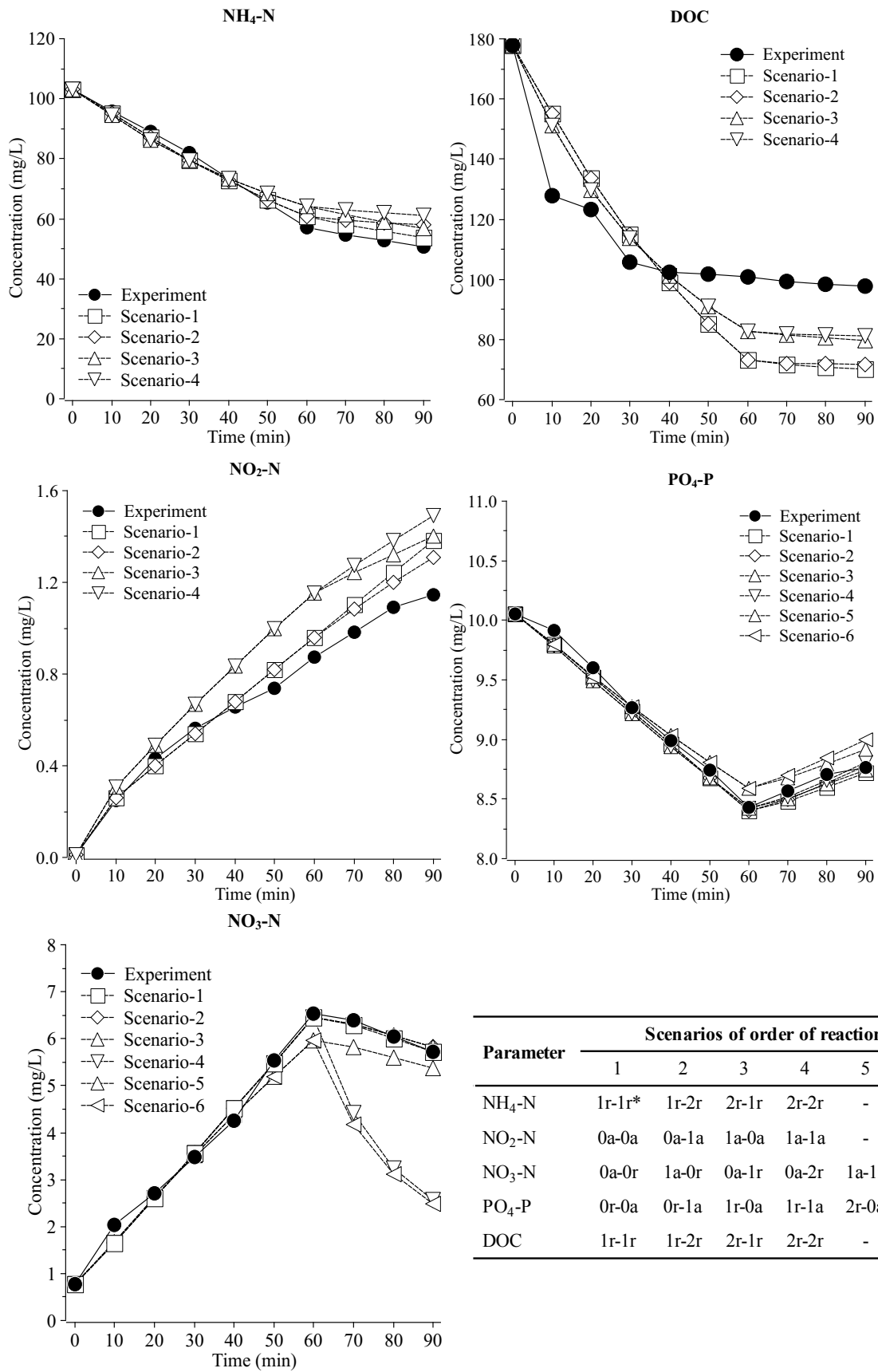
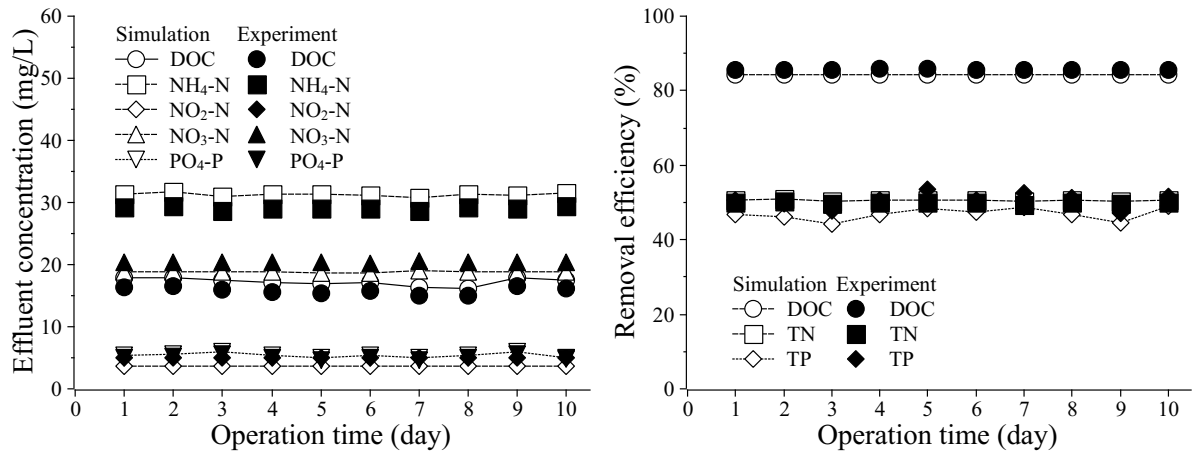


Figure 3-2. Experimental and simulated data for the determination of kinetic models.

(a) Aeration/non-aeration ratio = 2:1



(b) Aeration/non-aeration ratio = 1:1

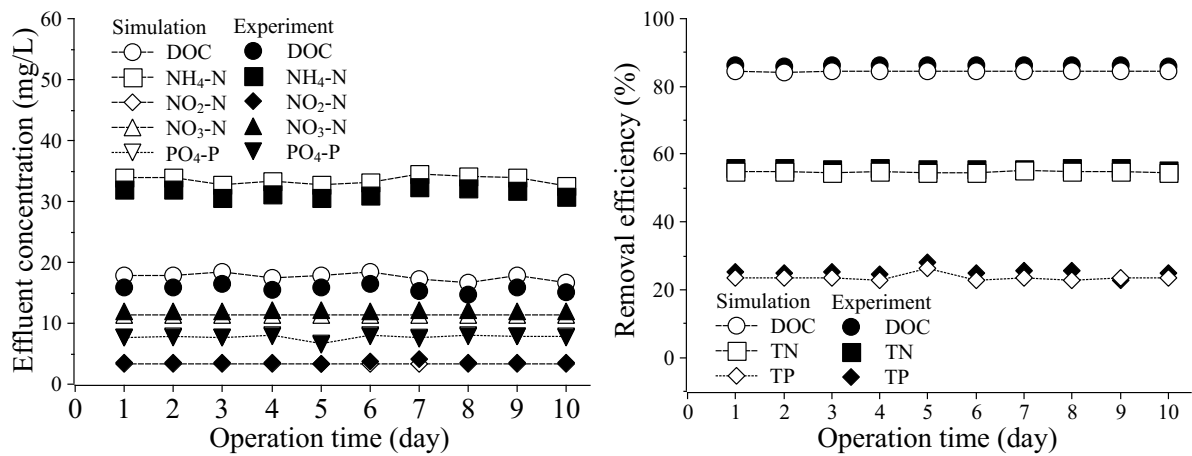


Figure 3-3. Comparison of reactor performance between the experimental results and model predictions under the aeration/non-aeration duration ratio of 2:1 (a) and 1:1 (b), respectively.

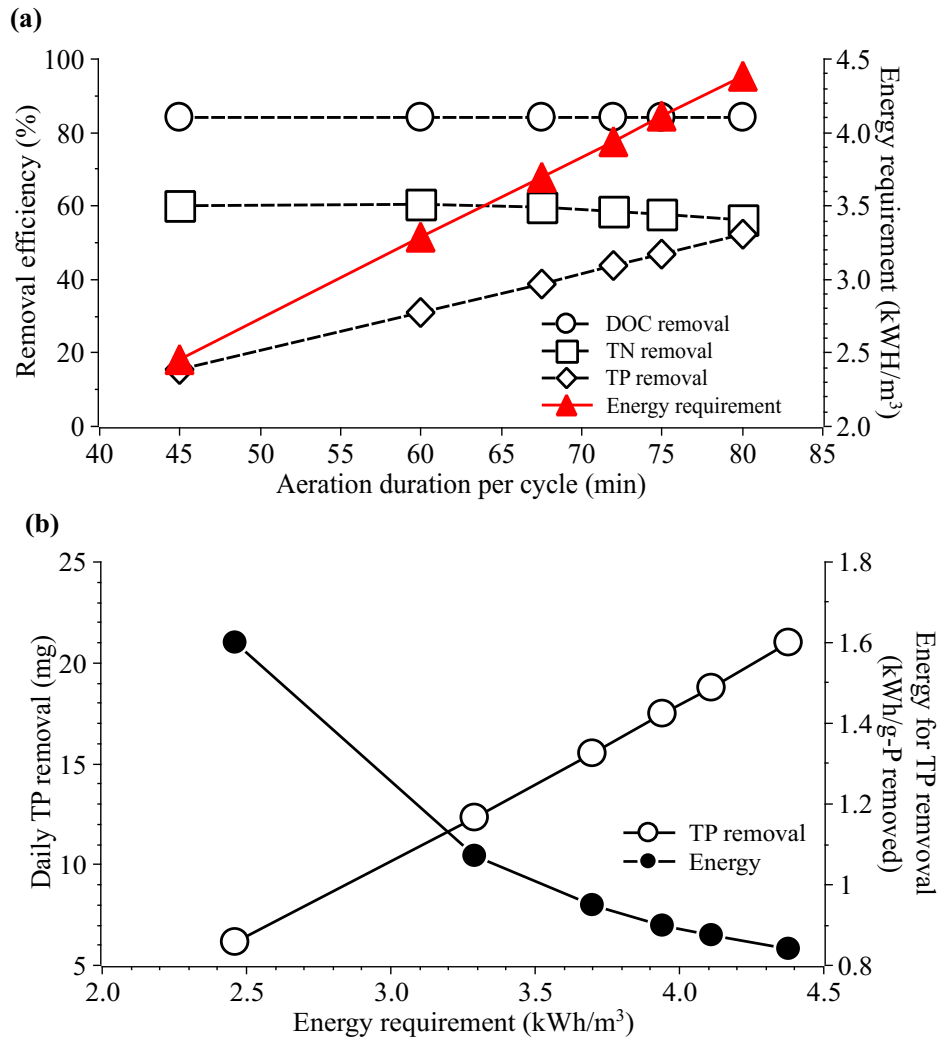


Figure 3-4. Effect of aeration duration on DOC, TN and TP removal efficiencies (a) and energy required for P removal (b).

Chapter 4 Stability of algal-bacterial aerobic granules in a continuous-flow reactor with effluent recirculation instead of aeration

4.1 Introduction

With the potential capability of nutrients uptake and oxygen production by algae [22, 23], the co-existence of algae and bacteria in wastewater treatment units is beneficial for enhanced nutrients removal efficiency at lower operational cost. Moreover, algae combined with AGS may become more attractive. It has the advantage of algae potential in addition to the extra benefits by AGS (excellent settling ability and higher removal efficiency). It is expected that oxygen production by algae could assist aerobic degradation by bacteria and reduce energy requirement for aeration during the aerobic biological process. Previous studies found that algae could naturally grow and co-exist with AGS in a SBR under natural sunlight exposure but yet resulted in lower TN and TP removals [26, 27]. Results from Chapter 2 demonstrated that a new algal-bacterial granular system could be established by seeding 50% of algal-bacterial AGS in a continuous-flow reactor system with intermittent aeration and non-aeration periods of 60 min and 30 min, respectively, and this system exhibited excellent granular stability as well. Still, very limited information is available in algal-bacterial AGS, especially in continuous-flow reactors with lower or without aeration to explore the potential benefit of algae co-existed.

This study aimed to investigate the performance of algal-bacterial AGS in a continuous-flow reactor with effluent recirculation instead of aeration to treat domestic wastewater. Organics and nutrients removal along with granules stability and settling ability was also recorded during the operation. The results from this work is expected to provide some basic information that could help to further apply this symbiotic algal-bacterial AGS system at lower energy consumption.

4.2 Materials and methods

4.2.1 Experimental set-up and operation conditions

The experiment was conducted in 4 stages of operation, namely stages 1 – 4. In the stage 1 (day 0 – 6), two continuous-flow reactors at a recycle ratio of 1:1 were used to compare the performance of general AGS (R1) and algal-bacterial AGS (R2). Each reactor had the same working volume of 1 L. After the operation of stage 1, R1 was stopped and only R2 was continued to operate for the left experimental stages. During stage 2 (day 7 – 18) and stage 3

(day 19 – 30), the R2 was operated at different recycle ratios of 5 and 10, respectively. All the operation from stages 1 – 3 were conducted without aeration. In the stage 4 (day 31 –42), the biomass from R2 were moved to R3 (with a working volume of 500 mL) due to insufficient biomass amount left from the stage 3. The R3 was operated under an intermittent aeration for 60 min and non-aeration for 30 min. All the reactors were operated under room temperature ($25\pm 2^\circ\text{C}$) and HRT of 6 h without light control throughout the experiment (900–1100 lux with all lights on). SRT during the whole period was maintained at 12 days. During aeration in the stage 4, air was supplied from the bottom of the reactor by an air pump (AK-40, KOHSIN, Japan) through air bubble diffuser at an air flow rate of 0.5 cm/s. All the reactors were equipped with an internal filter (with an average pore size of 0.3 mm) installed at the effluent port inside the reactor, which could function as retaining biomass larger than its pore size in the reactor. The detailed information about the operational conditions is presented in Table 4-1. During the operation, an additional mixing was provided by magnetic stirrer at 120 rpm. And the schematic diagram of the reactor is shown in Figure 4-1.

4.2.2 Synthetic wastewater and seed sludge

During all the stages of experiment (Stages 1 to 4), a synthetic wastewater with sodium acetate as carbon source was used as influent with its compositions as follows: 300 mg COD/L (sodium acetate), 10 mg $\text{PO}_4\text{-P/L}$ (KH_2PO_4), 100 mg $\text{NH}_4\text{-N/L}$ (NH_4Cl), 10 mg Ca^{2+}/L ($\text{CaCl}_2\cdot 2\text{H}_2\text{O}$), 5 mg Mg^{2+}/L ($\text{MgSO}_4\cdot 7\text{H}_2\text{O}$), and 5 mg Fe^{2+}/L ($\text{FeSO}_4\cdot 7\text{H}_2\text{O}$).

General AGS and algal-bacterial AGS were seeded for R1 and R2, respectively with an initial MLSS concentration in each reactor of approximately 2.5 g/L and MLVSS to MLSS ratio (MLVSS/MLSS) of 0.75. The initial algae content in the seed algal-bacterial AGS was 2.556 mg/g-TS. Both AGS and algal-bacterial AGS were cultivated in the laboratory in the continuous-flow reactor under alternative aeration (60 min) and non-aeration (30 min) condition with the same influent characteristics used in this study.

4.2.3 Analytical methods

The concentrations of water quality in the influent and effluent ($\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$) in addition to sludge volume index (SVI_5), MLSS and MLVSS were analyzed according to Standard Methods [41]. Dissolved organic carbon (DOC) was measured by TOC analyzer (TOC-V_{CSN}, SHIMADZU, Japan) equipped with auto-sampler (ASI-V, SHIMADZU, Japan). Chlorophyll-a amount was analyzed and used to estimate algae content in the granules [41]. Dissolved oxygen (DO) concentration in the reactors was measured using a DO meter (HQ40d, HACH, USA). A pH meter (Horiba, Japan) was used to monitor pH in the reactors.

Light intensity around the reactors was measured by a pocket digital lux meter (ANA-F11, Tokyo Photo-electric Co., Ltd., Japan). The granular size was measured by a stereo microscope (STZ-40TBa, SHIMADZU, Japan) connected to computer by software Motic Image Plus 2.35 (version 2.3.0). The stability of the granules was evaluated with the same method used in Chapter 2.

4.2.4 Calculations

In this work, TN concentrations in the influent and effluent were calculated as the sum of $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations, and TP removal was reflected by $\text{PO}_4\text{-P}$ removal since only KH_2PO_4 was used as the P source in the influent. Thus, DOC, TN and TP removal efficiencies were calculated according to Cai et al. [5] and the removal capacities were calculated according to Chapter 2. The parameter used to indicate granular stability (ΔTurb) was determined and calculated as in Chapter 2.

4.3 Results and discussion

The organics and nutrients concentrations in the influent and effluent were monitored to evaluate the performance of algal-bacterial AGS in the continuous-flow reactor under effluent recirculation without aeration. In addition, the granular strength, size and settling ability were also determined to evaluate the stability of the granules.

4.3.1 Granule characteristics

The experiment at Stage 1 aimed to test the performance and stability behavior of general AGS and algal-bacterial AGS operated in a continuous-flow reactor with effluent recirculation and no aeration. The following tests from Stage 2 and 3 were designed to investigate the effect of different recycle ratio, and the Stage 4 aimed to recover biomass settleability and performance, and then test the adaptability of the biomass to different operation conditions. During Stage 4, R2 was operated under an alternative aeration and non-aeration periods of 60 min and 30 min, respectively, with no effluent recirculation applied to the reactor.

During the test of Stage 1, both R1 and R2 were operated with effluent recirculation at a recirculation ratio of 1:1 (effluent/fresh influent, flowrate/flowrate). The average DO concentrations in R1 and R2 during the whole operation of no aeration was almost stable at 0.37 mg/L. With the aim to provide shear force to the granules, which is essential for granule formation and stability [6, 49, 50], an additional mixing by using magnetic stirrer with rotation speed of 120 rpm was provided. An obvious granular breakage into aggregates size was observed since the first day of Stage 1, as shown in Table 4-2, and more noticeable breakage

was seen on algal-bacterial AGS. Only after 6 days' operation, the average granule diameter of algal-bacterial AGS in R2 was sharply decreased from 0.78 mm to 0.38 mm. This result might be attributable to the consequence of using stirrer along with the low DO concentration in the reactor. As mentioned before, during the whole period of Stages 1 to 3, DO concentration was almost constant at 0.37 mg/L. In agreement with a previous report [15], granular instability under limited DO concentration also occurred. Low DO concentration in the reactor may cause the formation of anaerobic core in the granules leading to granules' breakage [7]. Under the stirring by the magnetic stirrer, it could amplify the influence on granular breakage. However, granule breakage didn't seem to influence granular settleability and stability (indicated by Δ Turbidity). SVI_5 of both AGS and algal-bacterial AGS was almost constant during this test period (averagely 54 mL/g and 52 mL/g for AGS and algal-bacterial AGS, respectively). Both AGS and algal-bacterial AGS showed little change in Δ Turb (from 2.1 to 2.8 NTU and 2.1 to 2.4 NTU, respectively). In general, algal-bacterial AGS exhibited better performance in terms of settleability and stability in the continuous-flow reactor system with effluent recirculation and non-aeration.

After Stage 1, R2 was continued to operate with different effluent recirculation ratios with the aim to check its effect on the reactor performance. A noticeable decrease in MLSS concentration and settleability were detected after increasing the recirculation ratio from 1:1 ($SVI_5 = 52$ mL/g) to 5:1 ($SVI_5 = 92$ mL/g) and 10:1 (114 mL/g) (Figure 4-2b). This is probably attributable to the failure of the installed 0.3 mm filter to retain granules which thus were washed out from the reactor. The average granule diameter decreased from 0.38 mm during Stage 1 to 0.25 mm in Stage 2 and to 0.24 mm in Stage 3, respectively. In addition, due to use of the lower biomass to treat the same strength of influent (higher organic loading), filamentous growth could easily occur in the reactor (Figure 4-2a). It may also contribute to the worsening of granular settleability and stability as shown in Figure 4-2(b-c). The granular strength decreased along with the operation of Stages 2 and 3 (indicated by increased Δ Turb). The results seem to suggest that a steady increasing algae concentration in the granules (Figure 4-2d) has limited contribution to the granular stability.

With the aim to recover granular performance as the granule properties worsened because of the operation from Stages 1 to 3, from day 42 on, the reactor operation was switched to alternative aeration and non-aeration as that before these tests. The operation conditions of Stage 4 appear to successfully recover the granular properties. SVI_5 was decreased from 114 mL/g (at the end of Stage 3) to 65 mL/g (after 12 days' operation of Stage 4), and then to 48 mL/g (after 24 days' operation of Stage 4). At the end of Stage 4 operation, a lower SVI_5 than

its initial condition was achieved, indicating a better granular settleability. Simultaneously, the granular strength was recovered almost to its initial condition. The biomass growth was also noticed in the reactor, reflecting an increased MLSS concentration and granular size as shown in Figure 4-2c. As the granules size grew to greater than 0.3 mm, the installed filter could successfully retain the biomass in the reactor, minimizing the washout of granules with a resultant increase in MLSS concentration in the reactor.

4.3.2 DOC, N and P removals

Influent and effluent samples were collected one every two days, and then filtered through 0.22 μm membrane prior to analysis. However, since there were almost similar results for each sampling time during every stage, the results would be summarized into average influent and effluent concentrations during the same stage operation.

During the operation of stage 1, R2 always showed better performance than R1 on DOC, TN and TP removals (Table 4-2), although both AGS and algal-bacterial AGS reflected poor DOC removal efficiency (32% and 40% in R1 and R2, respectively). This could be brought about by the very low DO concentration which is not suitable for the growth of aerobic bacteria. On the other hand, a better TN removal was achieved in the algal-bacterial AGS during Stage 1 (65% in R2 and 35% in R1, respectively). The low DO concentration in the reactor may inhibit AOBs growth resulting in a lower $\text{NH}_4\text{-N}$ utilization. However, the low DO concentration condition might contribute to an excellent denitrification process for both general AGS and algal-bacterial AGS indicated by the very low $\text{NO}_3\text{-N}$ concentration in their effluents (Table 4-2). Meanwhile, TP removal in R2 was also higher than in R1. The better TN and TP removals in R2 may be also contributed by the additional nutrients uptake by algae. Still, further research is necessary to shed light on the real mechanisms involved.

The experiments from Stage 2 to Stage 3 investigated the effect of effluent recirculation ratio on the performance of algal-bacterial AGS under no aeration. As seen in Figure 4-2a, during Stages 1 to 3, the biomass concentration in the reactor decreased noticeably, while the MLVSS/MLSS ratio was constant at 0.75 during the whole experiment period. Restated, during the operation of Stages 1 to 3, the organic and nutrient loading rates to the reactor was noticeably increasing due to the additional organics and nutrients from the recycled effluent and decreased biomass concentration in the reactor. Although the settleability and stability of algal-bacterial AGS was deteriorated along with the operation of Stages 1 to 3, the organics and nutrients removal rates were improved as shown in Figure 4-3a, resulting in stable removal efficiencies (Figure 4-3b). The algal-bacterial AGS could maintain their performance on

pollutants removal while their settleability and stability were worsened. The above results imply the stable performance (in terms of pollutants removal) of algal-bacterial AGS even being operated at higher organic and nutrient loading rates.

During Stage 4, the switching operation from no aeration to intermittent aeration could not only restore granular stability and settleability of algal-bacterial AGS, but also improve their DOC, TN and TP removal rates and removal efficiencies. The measured DO concentration in the reactor during aeration and non-aeration period was 6.2 mg/L and 0.37 mg/L, respectively. A higher DO concentration during aeration period may provide suitable conditions for bacteria who are mainly responsible for DOC, NH₄-N and PO₄-P removals. During the first 12 days' operation of Stage 4, an obvious improvement on DOC (from 40% to 100%) and TP (from 14% to 64%) removals was achieved with slight enhancement on TN removal efficiency detected during this period (from 61% to 67%). However, after prolonging the Stage 4 operation for another 12 days, the TN removal efficiency was improved to 98% and at the same time the DOC removal efficiency remained at around 100%.

Another factor relating to the improved granular performance was probably due to the seed AGS was cultivated under the same operation condition of Stage 4, thus it could have quick positive response to the change of operation conditions from Stages 1-3 to Stage 4.

4.4 Summary

Algal-bacterial AGS exhibited better overall performance in terms of granular settleability, stability and pollutants removal compared to general AGS when both were operated under completely no aeration (with effluent recirculation) condition. A higher effluent recirculation ratio applied may deteriorate the granular properties of algal-bacterial AGS while its pollutants removal could keep stable. Most importantly, the properties and performance of algal-bacterial AGS could be quickly recovered after the operation condition was switched from no aeration to intermittent aeration (the conditions before these tests).

Table 4-1. Operation strategies of the reactors.

	Stage 1	Stage 2	Stage 3	Stage 4
Days	0 – 6	7 – 18	19 – 30	31 – 55
Aeration	No	No	No	On-off (60-30 min)
Seed sludge	R1=AGS R2=AB-AGS	R2=AB-AGS	R2=AB-AGS	R3=AB-AGS
Recirculation ratio	1	5	10	–

AGS: aerobic granular sludge; AB-AGS: algal-bacterial AGS; Recirculation ratio= recirculated effluent/ fresh-influent (flowrate/flowrate).

Table 4-2. Performance of AGS and algal-bacterial AGS during operation of Stage 1.

	AGS (R1)		AB-AGS (R2)	
	Day 0	Day 6	Day 0	Day 6
Biomass properties				
Algae content, mg/g-VS	0	0	2.56	2.70
MLSS, g/L	2.5	2.5	2.5	2.5
MLSS/MLVSS	0.75	0.75	0.75	0.75
SVI ₅ , mL/g	54	54	52	52
Δ Turb, NTU	2.1	2.8	2.1	2.4
Average diameter, mm	0.45	0.37	0.78	0.38
Concentration in effluent, mg/L				
DOC	-	69.96	-	61.54
NH ₄ -N	-	66.63	-	41.02
NO ₂ -N	-	0	-	0
NO ₃ -N	-	0.18	-	0.13
PO ₄ -P	-	66.81	-	41.15
Removal efficiencies, %				
DOC	-	32	-	40
TN	-	35	-	60
TP	-	10	-	15

AGS: aerobic granular sludge; AB-AGS: algal-bacterial AGS.

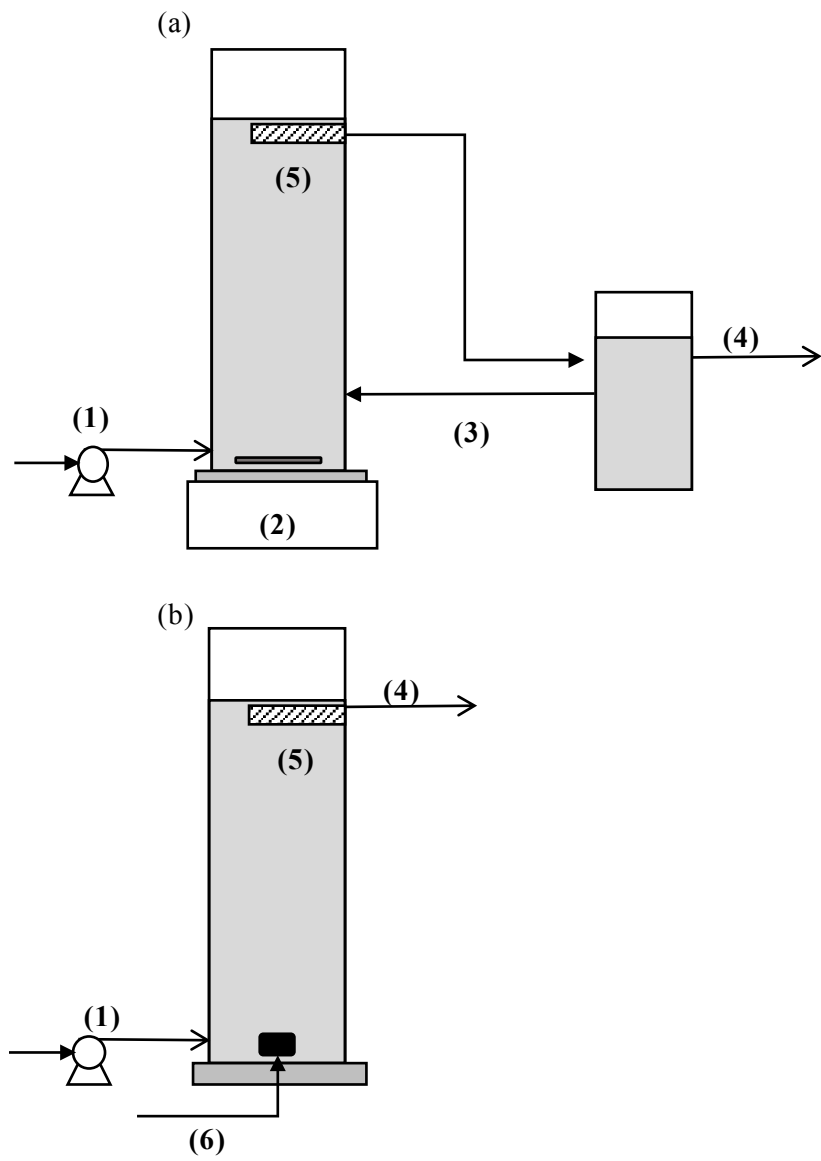


Figure 4-1. Schematic diagram of R1 and R2 (a) and R3 (b). Fresh influent (1); magnetic stirrer (2); effluent recirculation (3); effluent (4); internal filter (5); aeration line (6).

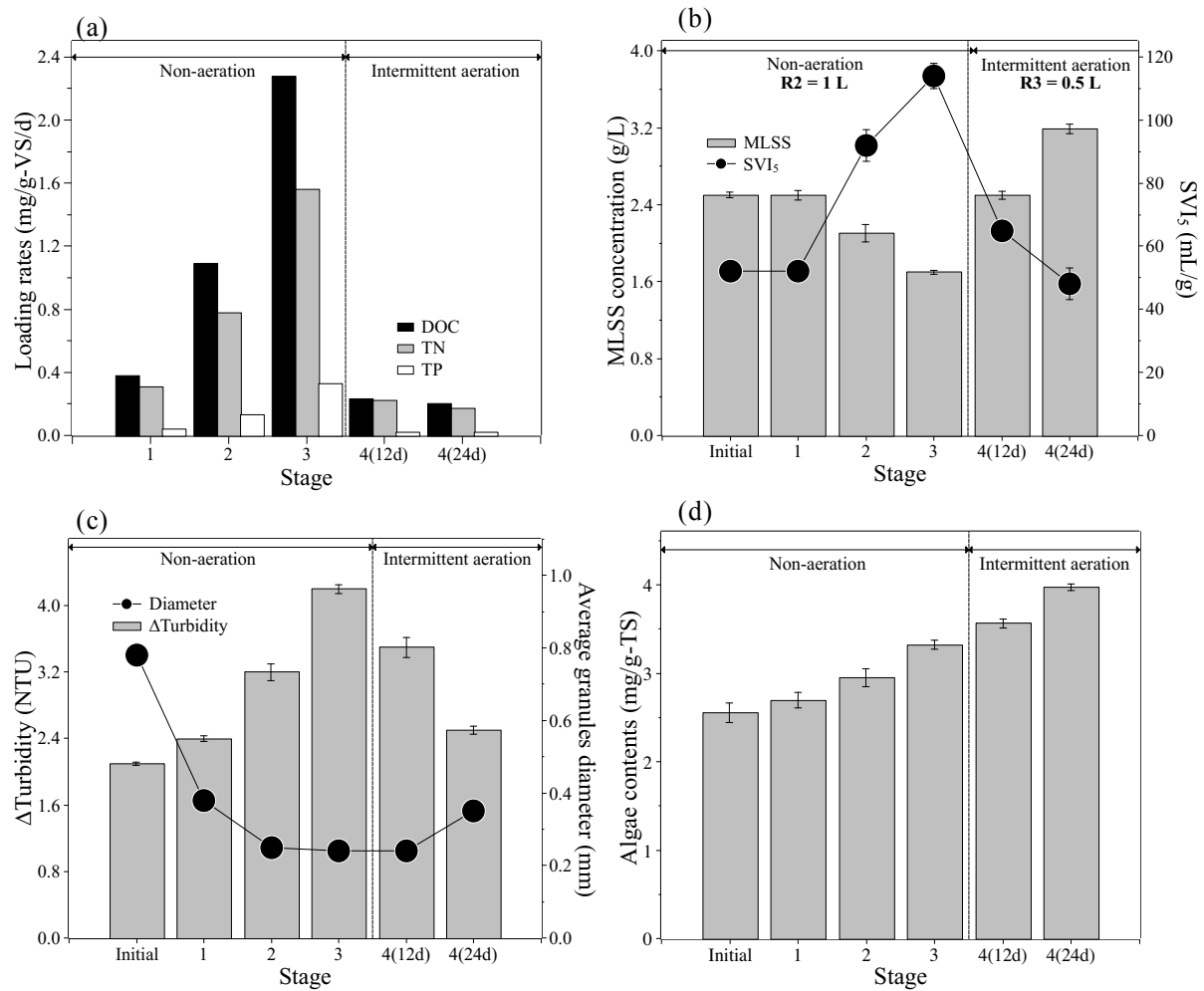


Figure 4-2. The profiles of loading rates (a), settling performance (b), granular stability (c) and algae content (d) of algal-bacterial AGS during the operation of Stages 1 to 4.

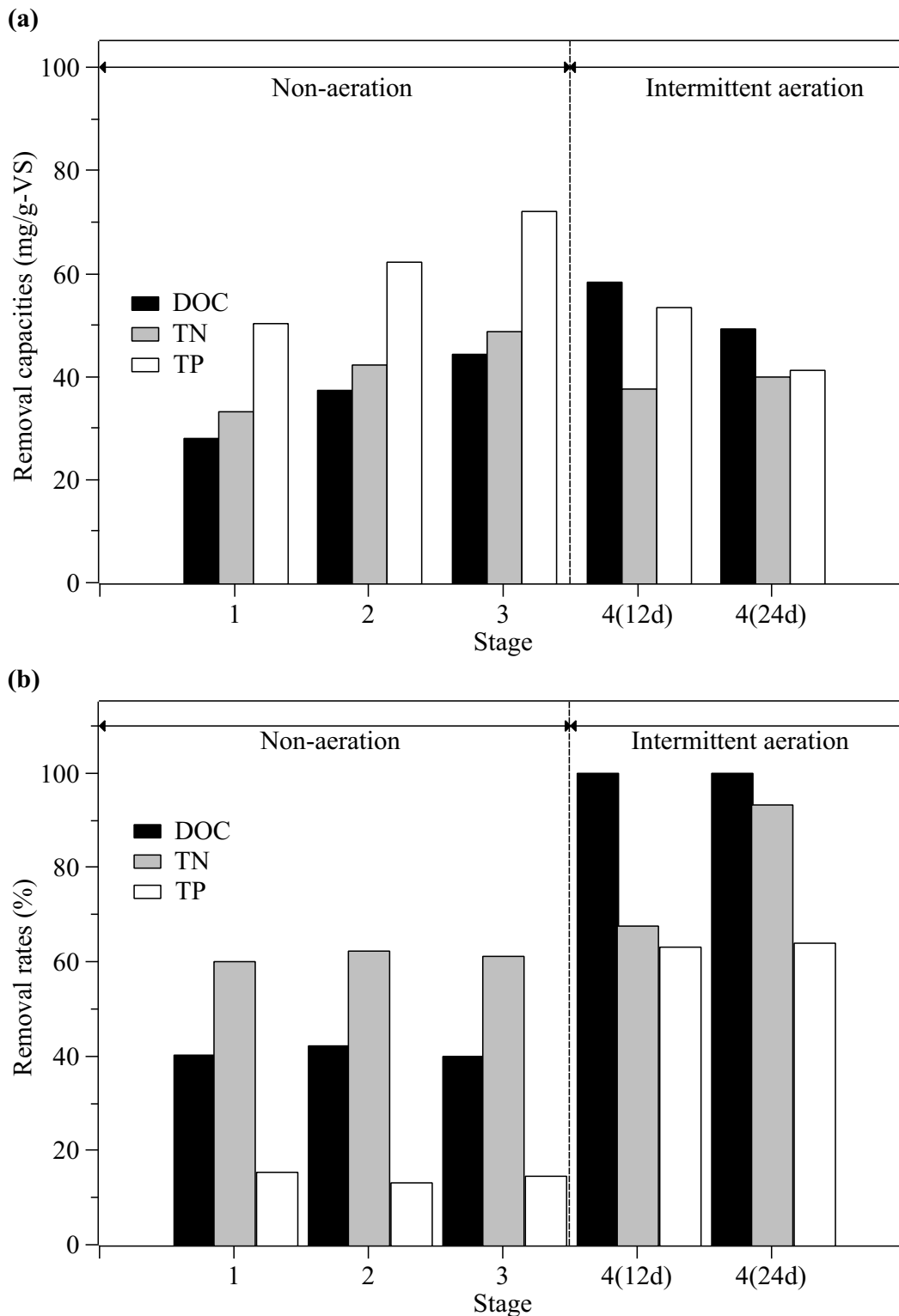


Figure 4-3. Changes in removal capacities (a) and rates (b) of algal-bacterial AGS from Stages 1 to 4 during the test.

Chapter 5 Conclusions and future research perspectives

5.1 Conclusions

This study explored various operation strategies for algal-bacterial AGS in continuous-flow reactors and analyzed the resulted performance in terms of pollutants removal and granular stability. Kinetic models and its further study on the effect of aeration strategy on pollutants removal and energy consumption were also conducted. The main results can be summarized as follows:

(1) Both single- and series- reactors systems exhibited similar efficiencies in DOC (~95%) and TP removal (~46%). Moreover, it kept stable even after double increased the organic loading rate in the single-reactor system. When operated under a smaller loading rate, series-reactor performed better TN removal (29 % and 76% in R₁ and R₂, respectively). However, after double increased organic and nutrient loadings, TN removal in the single-reactor system was improved up to 80%. This possibly due to the excessive organics concentration in the reactor could function as carbon source that is essential for denitrifiers.

(2) Algal-bacterial AGS demonstrated excellent stability (in terms of pollutants removal and granules strength) in the continuous-flow reactor even operated at double increased organic and nutrient loadings. And the new algal-bacterial AGS systems can be established by seeding 50% algal-bacterial AGS.

(3) The installed internal separator acted as hydraulic selection pressure thus could retain biomass with good settling ability resulting in increased granular settleability and stability.

(4) Kinetic reactions were successfully developed from cycle test experiments and proven applicable to simulate reactor performance for AGS in the SBR reactor and algal-bacterial AGS in the continuous-flow reactor with good accuracy (with $R^2 > 0.98$ and relative error $< 10\%$).

(4) Simulation study suggested that aeration to non-aeration duration ratio of 2 could provide desirable organics and nutrients removal and energy consumption.

(5) Algal-bacterial AGS exhibited better overall performance and stability over general AGS when operated under effluent recirculation and no aeration condition in the CFR system, achieving DOC, TN and TP removals of 40%, 60%, and 15% (algal-bacterial AGS) in comparison to 32%, 35%, and 10% (general AGS), respectively.

(6) For a longer-term operation under no aeration condition, algal-bacterial AGS could maintain good pollutant removal performance even at higher loading rates. More importantly, after being switched from no aeration to intermittent aeration, the algal-bacterial AGS could

quickly adapt to the change in operation conditions, which was obviously indicated by its recovered removals of DOC from 40% to 100%, TN from 61% to 98%, and TP from 14% to 64% along with the improved sludge settleability and stability.

It is expected that results from this study could provide important and scientific data for the development of algal-bacterial AGS, especially for the continuous-flow reactor systems.

5.2 Future research perspectives

In the present study, a symbiotic algal-bacterial AGS demonstrated excellent performance in the continuous-flow reactor. With the aim to optimize the reactor performance and minimize energy requirement, further explorations are still necessary, especially on the algae potential ability to produce oxygen that is expected to be able to assist aerobic process of bacterial degradation. The following aspects should be considered in the future research.

(1) Exploration on the reactor configuration that could provide required shear force without external mixing and aeration.

(2) Further examination on the change in microbial communities and species to better understand the effect of operation conditions in continuous-flow reactors

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Appendix

The following publications are related to this thesis.

1. **Johan Syafri Mahathir Ahmad**, Wei Cai, Ziwen Zhao, Zhenya Zhang, Kazuya Shimidzu, Zhongfang Lei, Duu-jong Lee, 2017, Stability of algal-bacterial granules in continuous-flow reactors to treat varying strength domestic wastewater. *Bioresource Technology*, 244, 225-233.
2. **Johan Syafri Mahathir Ahmad**, Ziwen Zhao, Haniif Prasetiawan, Wei Cai, Kazuya Shimizu, Zenya Zhang, Zhongfang Lei, Motoo Ustumi, 2018. Novel approach for kinetics prediction from cycle tests on algal-bacterial granular sludge in a continuous-flow reactor and its application in decision on aeration strategy for optimal organics and nutrients removal from wastewater. *Environmental Science & Technology* (under preparation).
3. **Johan Syafri Mahathir Ahmad**, Ziwen Zhao, Kazuya Shimizu, Zenya Zhang, Zhongfang Lei, Motoo Utsumi, 2018. Stability of algal-bacterial granules in a continuous-flow reactor with effluent recirculation instead of aeration to effectively treat domestic wastewater. *Bioresource Technology* (under preparation).