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THE STUDY OF FILAMENTOUS FOAMING CONTROL IN ACTIVATED SLUDGE PROCESS

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M. Phil

The Hong Kong Polytechnic University

2013

THE HONG KONG POLYTECHNIC UNIVERSITY

Department of Applied Biology and Chemical Technology

The Study of Filamentous Foaming Control in Activated Sludge Process

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A thesis submitted in partial fulfillment of

the requirements for the degree of

Master of Philosophy

May 2011

CERTIFICATE OF ORIGINALITY

I hereby declare that this thesis entitled “The Study of Filamentous Foaming Control in Activated Sludge Process” is original and has not been accepted for the award of any other degree of diploma. It does not contain any material, partly or wholly, published or written by others, except those references quoted in the text.

YANG Yang

Abstract

Abstract of Thesis Entitled:

The Study of Filamentous Foaming Control in Activated Sludge Process submitted by YANG Yang for the degree of M.Phil. at The Hong Kong Polytechnic University in October 2013.

In Hong Kong, communal municipal sewage treatment plants have been extensively surveyed and were found to have severe occasional foaming problems. Foaming problems and associated sludge bulking problems in secondary sedimentary tanks have continuously led to operating and control problems in plants, and have adversely affected process efficiency.

The predominant foaming and bulking problems have been identified to be primarily caused by the excessive growth of filamentous microorganisms, which are the predominant microbial species in the activated sludge process of the sewage treatment system. Based on morphological and physiological studies, the causative microorganism in foaming sludge was determined to be *Gordonia amarae*, a recently discovered filamentous bacterial genus. Several bacterial physiological and morphological evidences have helped in the specific identification of this newly discovered and taxonomized genus.

The bacterial growth kinetics of this novel filament, *G. amarae*, were studied and used as a theoretical foundation to develop an effective operational strategy in controlling filamentous foaming. In the kinetics study, the food to microorganism ratio, which is a critical process operating parameter, is an

important factor for the overgrowth of *G. amarae*. The results from the microbial kinetic studies also indicated a strong affinity between *G. amarae* and fatty acids, which are non-readily biodegradable organics. Based on this specific characteristic, the Feasting-Fasting Operation (FFO) was developed for foaming control as process operational strategy during the biological wastewater treatment process, in which the microorganisms grew in changing conditions with high and low F/M ratios. The Sludge Volume Index (SVI) was reduced to 80 ml/g and was then stabilized at approximately 70 ml/g. The resulting activated sludge system gradually dissociated from the stable foam, while the BOD removal rate was kept at a particular level to meet the discharge standard.

Excessive growth of filaments could be sufficiently avoided and the activated sludge settleability could be increased without any adverse effects on the treatment performance and process stability with this control technology. The Sequencing Batch Reactor (SBR) with FFO operation mode, which is the activated sludge process simulator used in this study, was modified to treat the paper mill wastewater. At optimal operation conditions, the SVI was successfully reduced without affecting the removal efficiency.

The first stage of this work involves the identification of the predominant filamentous microorganism that caused problems of foaming and bulking in the activated sludge from the paper mill wastewater treatment plant. Different diagnostic and examination methods were used to identify the particular microorganism. The predominant filamentous species was identified as *Actinomycetes spp.*, namely, *Gordonia amarae.* Based on the specifically developed novel FFO technique, the filamentous overgrowth, as well as the

related foaming and bulking problems, were effectively controlled. In the FFO process, the sludge microbes were subjected to repetitive switches between high and low F/M ratio conditions during the wastewater treatment process. Thus, the exposure time of the sludge microbes to a fixed F/M ratio was minimized. In the optimized implementation of FFO, in which the F/M ratio of the 'feast tank' was maintained at 0.89 d^{-1} compared with that in the 'fast tank' at 0.15 d^{-1}, of the filamentous growth was quickly suppressed as shown in the improved SVI and in preventing foaming in the system. The treatment system achieved not only an effective control of foaming but also a satisfactory BOD removal efficiency.

Based on previous kinetic findings and the novel FFO strategy, the second stage of this work involves the setting up of a lab-scale SBR system with the FFO to treat paper mill wastewater. The operating conditions, including Hydraulic Retention Time (HRT), aeration time in the SBR cycle, Volumetric Exchange Rate (VER), Mixed Liquor Suspended Solids (MLSS) concentrations and temperatures, were optimized to attain a stable and efficient treatment process.

The experimental results show that the optimal MLSS, aeration time, VER, temperature, and operation cycle for the SBR system were 4500 mg/l, 4 h, 0.50, 30℃, and 2 cycles per day. With the implementation of FFO, the SVI was reduced from as high as 260 ml/g in conventional activated sludge process to a desirable and stable level of 52.7 ±1.3 ml/g.

Key words**:** Activated Sludge; Feast-Fast Operation; Filament; Foaming; F/M

Publication Arising from the Thesis

1. Y. Yang, Y.F. Tsang, S.Y.M. Siu, N. Sin, Y.H. Liu, W.H. Lo, D. Zhong, H. Chua, Filamentous Foaming Control in Sequencing Batch Reactor for High Strength Wastewater Treatment, submitted to Journal of Environmental Management.

Acknowledgements

This research study has been financially supported by The Hong Kong Polytechnic University, which is hereby thankfully acknowledged. I am also grateful for the award of a research studentship offered by the University under the Teaching Company Scheme.

I sincerely acknowledge my supervisors, Dr. LO, Wai-Hung, Thomas and Professor CHUA, Hong, who have provided me with professional guidance, useful comments and valuable suggestions throughout the tour of my research study.

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List of Abbreviations

A/V	Surface-To-Volume
BOD	Biochemical Oxygen Demand
BOD_5	5-Day Biochemical Oxygen Demand
℃	Degree Celsius
$C_6H_{12}O_6$	Glucose
COD	Chemical Oxygen Demand
DAF	Dissolved Air Floatation
DO	Dissolved Oxygen
F/M	Food To Microorganism Ratio
FFO	Feast-Fast Operation
G.	*Gordonia*
HRT	Hydraulic Retention Time
KH_2PO_4	Potassium Hydrogen Phosphate
K_s	Half-Saturation Constant
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
N	Nitrogen
N.	*Nocardia*
NO	Nitric Oxide
P	Phosphorus
P.	*Pseduomonas*
PFR	Plug Flow Reactor
PHA	Polyhydroxyalkanoate
RAS	Recycle Activated Sludge

RBCOD	Readily Biodegradable Chemical Oxygen Demand
SBCOD	Slowly Biodegradable Chemical Oxygen Demand
SBR	Sequencing Batch Reactor
spp.	Species
SRT	Sludge Retention Time
SVI	Sludge Volume Index
TSS	Total Suspended Solid
UASB	Upflow Activated Sludge Blanket
UK	United Kingdom
VER	Volumetric Exchange Rate
μ_{max}	Maximum Specific Growth Rate

Chapter 1: Introduction

1.1 Background

Activated sludge process has been commonly applied in biological processes as the main and essential unit process for treating domestic sewage and industrial wastewaters for more than 100 years (Blackall *et al*., 2002; Frigon *et al*., 2006). Although the activated sludge process has been employed for a long time, it has been frequently reported to encounter bulking and foaming problems, which have been identified to have adverse effects on plant process operations and on treatment efficiency, as well as on the treated effluent quality (Eikelboom and Geurkink, 2002). The activated sludge process is popular as a microbial-driven process in which the carbonaceous organic matters of the wastewater provide energy sources for the production of new microbial cells for a mixed population of microorganisms in an aquatic aerobic environment (Francis *et al.*, 2002; Tsang *et al*., 2008). The typical biological processes include sequential or concurrent carbonaceous oxidation and nitrification process, in which the degradation of organic matter and ammonia in the wastewater occurs with flocculating biological growth, followed by the solid-liquid separation of the treated effluent via a physical sedimentation (Baxter *et al*., 1998; Zhu *et al*., 2004). This process has been proven to work effectively for over a hundred years involving full-scale operations with various design configurations.

However, despite being a fully developed technology, operating problems in activated sludge processes and compromised treatment efficiency caused by bulking sludge in the secondary sedimentation tank and biological foaming in the aeration unit have been cited as typical operational problems in sewage treatment plants in the United States, Japan, Hong Kong, South Africa, Singapore, and Australia. (Jenkins *et al*.,

2004 ; Thompson and Forster, 2003; Tsang *et al*., 2007; Yang *et al*., 2003). The overgrowth of filamentous microorganism results in bulking, worsening of the sludge settleability, and decreased sludge settling rate and incompact sludge blanket. These effects are usually indicated by an increased SVI (Jenkins *et al.*, 2004). Overall, these effects eventually result in the deteriorating quality of treated effluent. A number of filamentous species attach to and stabilize air bubbles to become thick, stable, and persistent foam. Foam is often observed to interfere with instruments such as level sensors, which hinders normal SBR operations. These operations heavily rely on instrumental measurements and feedback controls (Andreasen, 1995; Kanagawa *et al*., 2000). Furthermore, safety problems, such as foam spilled from aeration tanks onto walkways may occur in daily operation. In addition, dried foam results in the propagation of air-borne pathogens that cause public health problems. Thick foams often block scum removal systems and reduce oxygen transfer and supply between the gas-liquid interfaces (Kaptay, 2004). Bulking and foaming increase the BOD and SS levels of effluent because of non-settling sludge or escaping foam. (Bergeron and Pelletier, 2004; Elliott, 2002; Richard, 1991a; 1991b; Thompson and Forster, 2003; Tsang *et al.*, 2006).

The health of a microbial community in activate sludge depends on the profound balance among the floc formers in the sludge, such as *Zoogloea spp., Achrombacter spp*., and *Alcaligens spp.*, and in the filaments, such as *Nocardia spp., Rhodococcus spp*., and *Microthrix spp*. (Bergeron and Pelletier, 2004; Pagilla *et al.*, 2002; Thompson and Forster, 2003). These two pivotal organic degraders in sludge, namely, filaments and floc formers, comprise the skeletal matrix for the formation of compact microbial flocs that are critical in achieving a high level of settling properties. Filamentous organisms can provide strength and skeletal structures for floc formation. Theses also form a backbone within the floc particles and extend into the bulk

solution from the perimeter of the flocs, which results in a stable and prompt sludge settling (Blackall *et al*., 1988). Many factors can have significant effects on the sludge characteristics as well as on the performance of the activated sludge process. Filamentous *Microthrix parvicella*, *Nocardia spp*., *Haliscomenobacter hydrossis, Nostocoida limicola, Thiothrix spp*., *Sphaerotilus natans*, Type 0092, Type 0914, and Type 1701 are commonly found to be associated with bulking and foaming in wastewater treatment plants all over the world (Hwang and Tanaka, 1998; Jenkins, 1992; Pagilla *et al.*, 2002; Strom and Jenkins, 1984; Tay *et al.*, 2001). The overgrowth of filamentous species were found to be promoted by numerous conditions such as nutrient deficiency, low pH (Jenkins, 1992), oxygen deficiency (Palm *et al*., 1980), low F/M ratio and sludge return rate, long SRT (Pantano and Watts, 1984; Pitt and Jenkins, 1990). The presence of chemical substances such as, oil and grease, nonionic surfactants, volatile fatty acids, long chain fatty acids, and sulfide also favor the growth of filamentous microorganisms (Richard *et al*., 1985). Conventional reactor configurations namely, completely mixed and continuously fed aeration tank designs, inevitably promote filamentous overgrowth as well (Albertson, 1987).

Although many factors that affect the growth of filamentous bacteria have been studied over the years, in-depth investigations about the growth characteristics and physiological balances of the floc formers and filaments are insufficient. Their growth kinetics, existence of various quantities in an interspecies balance, and types of substrates are not yet clearly identified, which are vital in understanding the mechanisms of the causes of bulking and foaming, and the tailor-made control measures to maintain a balanced microbial community (Blackall *et al*., 2002; Lemmer *et al*., 2005).

A number of foaming and bulking control techniques have been developed over the

years, but each one has its shortcomings (Tsang *et al*., 2008). The use of toxic chemicals, such as hydrogen peroxide or chlorine, in the aeration tanks or the return sludge line between the aeration unit and the secondary sedimentation tank to kill or inhibit the filamentous microorganisms selectively can solve bulking and foaming problems (Jenkins *et al*., 1992). Metal ions, such as magnesium, calcium, and iron (Simpson *et al*., 1991; Thompson and Forster, 2003), as well as multi-component proprietary additives (Seka *et al*., 2001) and synthetic polymer (Juang, 2005), could effectively control bulking problems if the cost of chemical and secondary pollutions are not a main concern. In a paper mill effluent treatment plant, the use of mineral talc can effectively increase the settleability of sludge (Clauss *et al*., 1999). However, when the addition of chemicals was stopped, bulking and foaming problems would resume. In other words, the chemical method, despite its high cost, only provided a short-term effect. An alternative approach to control excessive filamentous growth is the use of biological selectors that are based on the kinetic selection theory (Chudoba *et al*., 1973). This theory states that a concentration gradient of the substrate that favors the growth of floc formers occurs to replace filamentous bacteria. These bacteria are among the microbe species in the activated sludge. After installing the selector upstream to the aeration tank, the applications were successfully accomplished (Patoczka and Eckenfelder, 1990), namely in intermittently fed operation (Houtmeyers *et al*., 1980; van Niekerk *et al*., 1987), or in the plug flow (Eikelboom, 2000). However, selectors can also fail to control filamentous overgrowth (Clayton *et al*., 1991; Lee *et al*., 1982; Linne and Chiesa, 1987; Van den Eynde *et al*., 1984) due to various reasons. The ultimate origin of filamentous growth is yet to be determined and relevant literatures contain contradictory views on this matter (Martins *et al*., 2004). Universal and reliable strategies of filamentous control are not available, for each specific and peculiar problem case, a specifically designed

analysis is necessary to determine the appropriate control method. However, these specific analyses are often far from effective.

In Hong Kong, communal municipal sewage treatment plants were surveyed and found to be frequently foaming, which has often led to process operation problems and deteriorated quality of treated effluent. Solutions and preventive measures such as chemical addition and the incorporation of biological selectors in the activated sludge process have been found to be less satisfactory (Tsang *et al*., 2006; 2008). Thompson and Forster 2003) also confirmed that the commonly used control measures all over the world, which includes chlorination, addition of proprietary anti-foam agents, dissolved oxygen control, and selector applications. Therefore, circumstances in ambient conditions and operating parameters that affect the causes of bulking and foaming in the activated sludge process should be further studied. In-depth investigation on the growth kinetics of foam-causing microbes, the process operational parameters that affect inter-species population balance and the relationship between organic contents in the mixed liquor and microbial growth are lacking in relevant literature. These factors are essential in creating a reliable basis for the design and execution of precautionary measures to prevent and restrain the problems of bulking and foaming effectively.

1.2 Objectives of the Study

1.2.1 General Objective

The main objective of this work is to develop and optimize an effective control method for filamentous overgrowth that, in turn, causes bulking and foaming problems in activated sludge processes. The investigation was conducted in a multi-staged systematic approach.

1.2.2 Specific Objectives

The specific objectives of this study are threefold as follows:

1. to identify the predominant species of microorganisms in the bulking and foaming sludge;
2. to quantify the effects of commonly found fatty acids in the activated sludge liquor on the growth of typical filamentous species, and the related causes of bulking and foaming problems; and,
3. to evaluate the effectiveness and reliability of a novel control method developed in this project in controlling common foaming problems in activated sludge processes.

1.3 Scope of the Study

This research work was designed as a two-stage project. The first stage aims to identify the predominant species of filamentous microorganism that is primarily responsible for foaming and bulking problems in the activated sludge process. Different diagnostic and examination methods were used to identify the particular predominant filamentous microorganism. The major causes of the overgrowth of the predominant filamentous species, which include process operating parameters and mixed liquor organic compositions, were then evaluated. The effects of various types and concentrations of fatty acids (chain length ranging from 9 carbons to 18 carbons) on filamentous bacteria and foaming in activated sludge were also investigated. Measurement techniques that characterize the severity of foam layer, such as foam stability and degree of foaming, were also introduced as a reliable and reproducible procedure. The characteristics of growth kinetics of both bacterial types, namely, foam-causing filamentous microbes and floc-forming non-filaments were used as a theoretical foundation for controlling foaming and bulking. The system performance, in terms of effectiveness in foaming and bulking control, and the effect of the control strategy on BOD removal efficiencies were also evaluated.

In the second stage of the project, the novel foam-prevention method was used in a lab-scale activated sludge simulator, namely, a sequencing batch reactor (SBR) system to treat paper-mill wastewater. The intended purpose was to check the feasibility and effectiveness of applying the foam prevention method. SBR is one of the popular technologies with high performance in treating wastewater. It is flexible and has an effective removal rate for industrial wastewater containing toxic compounds (Franta and Wilderer, 1997; Reid and Simon, 2000; Wilderer *et al.*, 2001). This technology has five sequencing stages, namely, feeding, reaction, settling,

decanting and idling. At optimized conditions, these five stages operate at different periods of time (Wilderer *et al.*, 2001). The optimal running time for each stage was determined based on the findings in pilot-scale studies from expert operators. The durations of the settle and draw phases were fixed. However, the duration of the react phase must be adequately controlled to improve the overall facility efficiency (Tsang *et al.*, 2007, 2008). Therefore, choosing the suitable technical parameters is essential and valuable at this stage of the work because of the peculiar characteristics of specific kinds of wastewater and its relevant foaming and bulking problems.

Chapter 2：Literature Review

This chapter reviews common municipal wastewater treatment processes, with special emphasis on the various configurations of activated sludge processes that commonly result in operating problems such as foaming and bulking. Furthermore, foaming and bulking sludge problems in activated sludge processes, and existing methods in controlling and alleviating these operational problems were also surveyed. Emphases were placed on comparing and contrasting the limitations of these methods under various operating conditions.

2.1 Wastewater Treatment Processes

This section reviews the studies that investigated the performances of current wastewater treatment technologies. Primary clarification followed by biological treatment is the commonly used process in communal municipal wastewater treatment plants.

As and when necessary, advanced treatment processes are also incorporated to enhance the quality of treated effluent, and to meet stringent discharge standards or requirements in cases of water recovery and reuse.

2.1.1 Pretreatment

Different suspended solids such as bark particles, fiber debris, wood fiber, filler and coating materials were observed in common municipal sewages and certain industrial effluents. These particles form common solid ingredients in wastewater (Moy *et al.*,

2002; Vesilind, 2003). Wastewater is commonly pretreated with coarse screen, fine screen, and degritting operations to remove grit, coarse fibers and debris. The pH is also neutralized during pretreatment. These processes are believed to remove a certain fraction of nitrogenous compounds in the sewage, which, in turn, affect the shift of inter-species population balance in subsequent biological processes (Moy *et al*, 2002; Philips *et al.,* 2003; Rothman, 1998; Theander and Pugh, 2003)

2.1.2 Primary Clarification

The term 'Primary Treatment' refers to the physical treatment or unit operation following pretreatment operations. Primary clarification is commonly used to remove the remaining suspended solids and colloidal particles left in the sewage after screening and degriting operations. Sedimentation is the most common clarification technique employed. In the primary clarification, more than 80% of the Total Suspended Solids (TSS) could be removed, particularly in the municipal sewage in Hong Kong (Tsang *et al.,* 2008) and the paper mill effluents in the UK (Thompson *et al*., 2001). Rajvaidya and Markandey (1998) stated that a typical sedimentation tank could have a TSS removal of 70% to 80% TSS. On the other hand, the TSS removal efficiencies of dissolved air floatation (DAF) units were also found to be satisfactory in particular applications (Gubelt *et al*. 2000; Horan and Chen, 1998; Wenta and Hartmen, 2002). DAF was used to treat "toxic and difficult" streams prior to biological treatment (Sandquist and Sandstrom, 2000). Inorganic coagulants and organic polymers are commonly added to improve clarification performance (Hamza, 1983; Li *et al.,* 2007; McCubbin, 1983). In such cases, the operation is a physic-chemical one. However, these operations can affect the potential for foaming and bulking in subsequent biological processes (Leppard *et al*., 2003; Lozada *et al*.,

2004), although insights on the definite mechanisms are not yet elucidated.

2.1.3 Biological Treatment

Biological treatment processes are commonly used after primary sedimentation. The most popular unit process is the activated sludge process, and is commonly used as the main process for the removal of water-soluble organic matter.

Two distinct unit operations usually constitute the activated sludge process and are performed in two separate and consecutive tanks, namely, the aeration tank and the secondary settling tank. When the sewage and oxygen are supplied by aeration, microorganisms are mixed and activated. The water-soluble organics in the incoming sewage are metabolized by the activated microbes to produce carbon dioxide, water, and new biomass in the treated wastewater. The activated sludge liquor must be adequate mixed to ensure a homogenous mixture of microbes, oxygen, wastewater, and nutrients, and to prevent the undesirable settling of active microorganisms in the aeration tank (Vesilind, 2003). The level of aeration, which in turn affects the dissolved oxygen (DO) level in the mixed liquor, is believed to have a profound effect on the growth of filamentous species amongst other species in the mixed culture of the activated sludge (Martins *et al*., 2004; Wagner and Loy, 2002). In more recent developments, a number of distinct design configurations of activated sludge processes, such as SBR, circulated activated sludge technology (CAST), and the Unitank, have been reported to have various capabilities to prevent foaming problems.

The flocculated biomass and other suspended solids must be separated from the treated wastewater. This secondary sedimentation operation is designed to ensure efficient pollution removal by settling the flocculated biomass. The clarified effluent is relatively

devoid of any suspended particles compared to the clarifier influent. This result indicates that activated sludge systems have effective sludge settling and low suspended solid levels in the final treated effluent. A portion of the sludge from the underflow is usually returned to the aeration basin to maintain the mixed liquor suspended solids (MLSS) levels, whereas the remaining portion of the sludge is discarded for further sludge treatments and possible reuse. Therefore, the efficiency of settling in the secondary sedimentation operation determines the health of the entire activated sludge process. Operational problems such as sludge bulking and rising sludge are commonly encountered. Optimizing the operating parameters to prevent or minimize bulking problems is a major concern.

In the aerobic biological treatment, MLSS level higher than 2000 mg/L and reaction time between 6 hours and 8 hours were reported to be the basic requirements to achieve a BOD removal efficiency between 83% and 88% (Raghuveer and Sastry, 1991). The food-to-microorganism ratio (F/M) is one of the most important factors that reflect the organic loading in the reaction system. The two-stage activated sludge process was proven to remove a significant amount of BOD in the wastewater (Knudsen *et al*., 1994). The removal efficiency of BOD was even increased from 51% to 91% by introducing a series f floating biological beds (Hansen *et al*., 1999). The dyes in the wastewater are slowly biodegradable BOD compounds. *Pseudomonas putida*, *Citrobacter spp*., and *Enterobacter spp*. have a higher removal rate of color-causing chemicals as well as phenolics and sulfide (Chandra, 2001).

Anaerobic biological processes are sometimes applied in specific designs of sewage treatment plants, particularly in treating sewage that contains a fraction of persistent or recalcitrant industrial components. In contrast to aerobic treatment, anaerobic treatment does not require air input and generates considerably smaller quantities of sludge. Anaerobic processes, namely, anaerobic trickling filter, upflow anaerobic sludge blanket

(UASB), anaerobic contactor, anaerobic lagoon, and fluidized bed technologies, are considered to be economical methods to degrade bigger organic compounds in the wastewater. However, anaerobic treatment can be used to treat more dilute wastewaters, such as domestic sewage, but process efficiency is also a main concern.

Anaerobic processes are commonly applied prior to the activated sludge process as a pretreatment to improve the biological treatability of the sewage, i.e., increasing the BOD/COD ratio. In order occasions, anaerobic processes are applied after the activated sludge process as a polishing stage to further improve the quality of the treated effluent (Grove *et al*., 2007). When the UASB was operated for 6 hours, the COD and sulfate removal rates were 66% and 73%, respectively (Chen and Horan, 1998). A technology developed by Sandquist and Sandstrom (2000) achieved a foul condensate removal rate of 99% at a pH of 4 and a methanol removal rate of 90% at low liquid/gas ratios. However, the removal efficiency of COD by an anaerobic process remained steady at around 80%, and the tailed COD concentration was approximately 800 mg/l. Therefore, further treatment is required to reduce the effluent COD level (Thompson *et al*., 2001). The UASB was not able to treat the pollutant because of the low biodegradability of the black liquor (Peerbhoi, 2000). The fluidized bed with porous packing has been reported to have an organic content removal rate of 81.5% compared with a removal rate of 50% in the anaerobic filter with corrugated plastic tubes (Perez *et al*., 1998).

2.1.4 Tertiary Treatment

Some wastewaters may still be unable to meet particular discharge standards for specific water quality parameters after treatment by biological processes. In addition, many plants have attempted to lower the cost of operation by reducing the amount of freshwater

consumption and effluent production by reusing the treated effluents. Therefore, further treatment of treated effluent for possible recovery and reuse of the water should be considered. In both cases, tertiary treatment processes are usually incorporated into the sewage treatment system to further reduce the concentrations of specific wastewater pollution constituents so that particular effluent discharge guideline or reuse requirements are met. Physicochemical processes are commonly used in tertiary treatment to remove the biological persistent or recalcitrant COD, residual suspended solids, color, and toxicity that are present in the effluent after conventional secondary treatments. Operating costs are usually the main issue for consideration in such cases.

2.2 Common Operating Problems in Biological Treatment Processes

The ability of a biological treatment process to produce an effluent with low suspended solid levels and acceptable treated effluent quality is typically limited by the sedimentation process in the settling tank. However, sludge separation problems have been frequently found since the development of the activated sludge process in the early 1900s (Hartley, 2008; Hug *et al*., 2005; Madoni *et al*., 2000). Table 2.1 lists the different types of problems associated with the separation of activated sludge solids, and are identified by their common names and causes (Jenkins, *et al*., 2004). Among these problems, the most common and widespread ones are associated with the overgrowth of filamentous microorganisms and the related bulking and foaming problems in activated sludge processes (Levantesi *et al.*, 2004).

Table 2.1 Causes and effects of separation problems in common activated sludge systems (Jenkins *et al.*, 2004)

Problem	Cause of Problem	Effect of Problem
Dispersed growth	Microorganisms are dispersed, and only small clumps or single cells are formed	Turbid effluent, no zone settling of activated sludge
Slime, viscous or zoogleal bulking; nonfilamentous bulking	Microorganisms are present in a number of extracellular material; in severe cases, extracellular material imparts a slimy or jelly-like consistency to activated sludge	Decreased settling and compaction rates; virtually no solids separation in severe cases, which results in overflow of sludge blanket from the secondary clarifier; viscous foam may appear in poor sludge dewatering
Pin floc	Small, compact, weak, roughly spherical flocs are formed; larger flocs settle rapidly; smaller flocs settle slowly	Low sludge volume index (SVI) and turbid, often high SS effluent
Filamentous bulking	Large amounts of filamentous microorganisms present in the bridge between the flocs or create diffuse flocs, which interfere with compaction, settling, and thickening	High SVI with very clear supernatant; low RAS and WAS solids concentration; in severe cases, the sludge blanket overflows the secondary clarifier; solid-handling processes become hydraulically overloaded
Blank rising	Denitrification in the secondary clarifier releases poorly soluble N_2 gas which attaches to the activated sludge flocs and floats them to the secondary clarifier surface	A scum of activated sludge forms on the surface of the secondary clarifier and on the aeration basin anoxic zones
Foam/scum formation	Caused by (i) under-graded surfactants and (ii) nocardiofoams, *M. parvicella*, or type 1863	Foams (scum) can float large amounts of SS to the surface of treatment units; nocardioforms and *M. parvicella* foams are persistent and difficult to break mechanically; foams accumulate and can putrefy; secondary effluent SS can be elevated; foams can overflow tank freeboards

2.3 Causes of Bulking and Foaming in Biological Treatment Processes

Activated sludge systems experience bulking problems because of the excessive growth of filamentous organisms, which numerous researchers have published and reported over the years.

Wastewater Composition

High-carbohydrate wastes appear to be favorable to and may even trigger excessive filamentous overgrowth. The growth of filaments was accelerated by carbohydrates such as, glucose, lactose, and maltose (Chudoba, 1985; Chudoba *et al*., 1985). RBCOD favored the growth of *S. natans*, *Thiothrix spp*. and Type 021N, whereas SBCOD could be consumed by *M. parvicella* and Type 0041 (Jenkins, 1992; Levantesi *et al.*, 2004). These cases usually resulted in a severe imbalance of inter-species population ratio in the activated sludge, and eventually resulted in operation problems (Theander and Pugh, 2003). In-depth investigations in these areas are urgently needed to provide more insights in the related problems.

Substrate Concentration

Another common cause of filamentous overgrowth is substrate concentration. Compared with floc-formers, filamentous bacteria have a low K_s (half-saturation constant) and μ_{max} (maximum specific growth yield). In other words, filamentous bacteria grow slower. However, filaments have a higher organic consumption rate than the floc-formers in low substrate concentration conditions (Chudoba *et al*., 1973,

Chudoba, 1985; Tsang *et al*., 2006). However, substrate concentration is related to organic loading rate, which is in turn related to MLSS in the process. Therefore, an important composite operational parameter (F/M ratio) often affects process efficiency (Tsang *et al*., 2006; 2007; 2008). However, definite F/M ranges that prevent excessive filamentous overgrowth, foaming and bulking are not yet available.

pH

Low pH value also favored the growth of filaments. The most suitable pH for aerobic process is approximately 7 to 7.5. Fungi growth of *Geotrichum*, *Candida*, and *Trichoderma* are favored at pH values lower then 6, which resulted in bulking (Pipes, 1967). Hu and Strom (1991) reported that pH values between 4.0 and 5.0 caused bulking because of the excessive growth of fungi and specific filamentous species. However, definite microbial genus or species were not identified, and related microbial growth kinetics was not determined.

Sulfide Concentration

The growth of filamentous microorganisms such as *Thiotrix*, Type 021N or *Beggiatoa*, is accelerated in high sulfide concentration conditions. The sulfide is taken up by the aforementioned bacteria to produce energy, becomes oxidized to sulfur molecule, and then stored as intracellular sulfur granules. Overgrowth in specific sulfur-oxidizing filaments, such as *Beggiatoa spp.*, commonly occurs in fixed-film bioreactors (Jenkins, 1992). However, these conditions are less common in conventional activated sludge processes.

Dissolved Oxygen Level

Dissolved oxygen levels in the mixed liquor of the activated sludge process are often accepted as an important parameter that is associated with foaming problems (Madoni and Davoli, 1996； Soddel, 1990; Tay *et al.*, 2001). A low dissolved level had accelerating effects for the overgrowth of filaments such as *Sphaerotilus natans*, *Haliscomenobacter hydrossis* and Type 1701 (Lau *et al*, 1984; Palm *et al*., 1980; Sezgin *et al*., 1978; Travers and Lovett, 1984). Common practice holds that aerobic systems should be operated at a minimum DO level of 2 mg/l, to avoid the dominance of specific filamentous microorganisms. However, studies have shown that no relation was observed between dissolved oxygen concentration and filamentous bacteria concentration if *M. parvicella* or Type 0041 were predominant in the activated sludge (Forster and Dallas-Newton, 1980). Thus far, no definite relationship has been observed between controlled DO and effectiveness in foaming and bulking control (Wilen and Balmer, 1999). More related research efforts in these areas are necessary.

Nutrient Deficiency

Deficiencies in nutrients, such as nitrogen and phosphorus, could cause the overgrowth of filamentous bacteria, including *Thiothrix spp.*, Type 021N, and *S. natans*. This could be explained by the kinetic selection theory (Chudoba *et al*., 1973). It was also reported that iron and trace elements deficiencies may induce filamentous foaming.

2.3.1 Studies of Mechanisms of Filaments Overgrowth

The mechanisms of filaments overgrowth have always been indeterminate problems, and have been the focus of considerable research in recent years. Recent published studies have tried to elucidate a general mechanism to explain the overgrowth of filaments in various environmental conditions. Four major mechanisms were determined from these published studies. However, all of these studies lacked experimental verification and concrete theoretical bases. Although no mechanisms are recognized and universally accepted, the studies and their preliminary results form the current basic theoretical framework to approach and understand filamentous overgrowth.

2.3.1.1 Mechanisms of Diffusion-based Selection

Filamentous overgrowth is believed to be the result of the improved competitiveness of filaments at specific conditions in the mixed liquor of the activated sludge process (Baxter *et al*, 1998; Madoni *et al.*, 2000). A number of studies were conducted to investigate the substrate uptake of filaments at low concentrations of oxygen or nutrients and the results confirm that the filaments were more competitive. The filamentous bacteria were stimulated with a higher surface-to-volume (A/V) ratio by competing with filamentous and floc-formers (Pipes, 1967). The mass transfer from the liquid phase to the interface on the bacterial cells was facilitated more easily at a high A/V ratio, which benefited the filamentous microorganism at low substrate concentrations. Thus, a high growth yield of filaments was observed (Rossetti *et al.*, 2005). Table 2.2 summarizes the conditions associated with filamentous overgrowth.

Table 2.2 Summary of conditions associated with filamentous overgrowth

Causative Condition	Filamentous Microorganism
Low F/M	*M. parvicella, Nocardia spp., H. hydrossis*, 0041, 0675, 0092, 1851, 0803, 0961
Low DO	*S. natans*, 1701, *H. hydrossis*, possibly 021N and *Thiothrix spp*.
Sulfides	*Thiothrix spp, Beggiatoa spp*., 021N, 0914
Low pH	Fungi
Nutrient deficiency (N/P)	*Thiothirx spp, N. limicola, H. hydrossis, S. natans*, 021N.

In other studies, the filamentous bacteria had high penetrability outside the microbial flocs in the activated sludge. In a low substrate concentration condition, that the filamentous bacteria had a higher substrate concentration compared with non-filamentous bacteria inside the flocs (Kappeler and Gujer, 1994; Lau *et al*., 1984). Schramm *et al*. (1999) predicted the micro-gradients of substrate concentration in the flocs through experimental observation. Comparing between biofilm growth and floc growth, the micro-gradients mechanisms had been further developed (Martins *et al*., 2003; 2004). The filamentous biofilm structures were predominant in cultures with a low substrate concentration, whereas compact and smooth biofilms were predominant in cultures with a high substrate concentration (Picioreanu *et al*., 1998; Van Loosdrecht *et al*., 1995). The bacterial colony and the microbial cell morphology of a pure culture were also dependent on the substrate micro-gradients. In other words, the low substrate concentrations, which results in the appearance of filamentous colony morphology, are often observed in the activated sludge (Ben-Jacob *et al*., 1994). Hence, the floc structure becomes more open and filamentous overgrowth often occurs in low substrate concentration conditions (Martins *et al*., 2003).

2.3.1.2 Mechanisms of Kinetic Selection

Based on the pilot-scale studies conducted using mixed cultures with defined substrates in activated sludge process simulators, the settling abilities were related to the mixing condition and the substrate concentration macro-gradients. In the aeration tank, if a low axial mixing rate and a high macro-gradient of substrate level could be maintained, filaments growth could be suppressed (Schwarzenbeck *et al.*, 2004). Therefore, the settling abilities of the sludge in the sedimentation were improved. The macro-gradient of substrate concentration at the inlet zone would have a selection mechanism for the floc-former for the subsequence zone. Thus, the growth of filamentous bacteria would be suppressed (Chudoba *et al*., 1973).

Based on the selecting mechanisms of filamentous and floc-forming bacteria in substrate-limiting conditions, the theory of kinetic selection can be used to explain the occurrence or suppression of filamentous microorganisms in activated sludge systems in previous studies. These studies found that, the filamentous bacteria (K_s-strategists) grew slowly compared with floc-forming bacteria (μ_m-strategist) due to the low maximum specific growth rate (μ_{max}) and affinity constant (K_s) (Nielsen *et al*., 2000; Tsang *et al*., 2008; Miana *et al*., 2002). However, the filamentous bacteria could be more competitive in substrate seizing because of the higher specific growth rate in the low substrate condition, such as continuously fed completely mixed system (especially $C_s<K_s$),. On the contrary, if the substrate concentration was sufficiently high in the system, such as in plug flow reactor (PFR) and sequencing batch reactor (SBR), the competition result would be reversed. The growth of filamentous bacteria would be suppressed due to the low growth rate compared with floc-formers (Chudoba *et al*., 1973). From the pure culture studies of the filamentous (Lau *et al*., 1984; Richard *et al*., 1985; Rossetti *et al*., 2002; Van den Eynde *et al*., Van Niekerk *et*

al., 1987; 1983; Van Veen *et al*., 1982) and floc-foaming bacteria (Van den Eynde *et al*., 1983; Van Niekerk *et al*., 1987) carried out in various studies, the kinetic selection theory were verified. However, few signs were available to verify if the floc-forming bacteria could represent the functional bacteria in the activated sludge process. Non-dominant microorganisms were commonly found to be abundant in activated sludge using molecular probes (Wagner *et al*., 1993). A pioneering technique was developed to measure in situ the kinetics of filamentous bacteria by utilizing quantitative MAR and FISH (Nielsen *et al*., 2002). However, this technique must be modified to accurately examine the filamentous and floc-foaming bacteria.

The mechanism of kinetic selection is promising, but requires further verification. Whether or not the μ_{max} of the filamentous bacteria was lower than those of other bacteria in the activated sludge remains uncertain, and if the reason for a lower growth rate is due to filamentous morphology needs further studies.

2.3.1.3 Mechanism of Storage Selection

In general, the non-filamentous bacteria have excellent abilities to store substrates in a high substrate condition such as in PFR, SBR and selector systems. The growth of non-filamentous bacteria would be stimulated (Chiesa *et al*., 1985; Majone *et al*., 1996; Van den Eynde *et al*., 1983). During famine, the stored material can be metabolized to produce proteins and generate energy. Therefore, in fierce competition, these microorganisms have a strong selective advantage compared with other filaments and floc-formers. However, compared with well-settling sludge, the bulking sludge, which contains high concentrations of filamentous bacteria, could have a similar or even a higher storage capacity (Beccari *et al*., 1998; Martins *et al*., 2003;

2004). Some filamentous bacteria were proven to have high storage abilities at various dissolved oxygen conditions, such as aerobic, anoxic and anaerobic, by using pure culture (Andreasen and Nielsen, 2000; Nielsen *et al*., 2002; Rossetti *et al*., 2002). The lower storage capacity of filamentous bacteria may not be the major selection parameters and could not be considered to satisfy completely the rules in the selection mechanism. However, the intrinsic processes, including regeneration and storage, appeared more important in the selection system. Hence, the use of these parameters is more suitable in describing the metabolism mechanisms in foaming and healthy systems. In addition, further study could be conducted on these parameters to determine control strategy for the foaming problems (Davenport *et al*., 2008; Pujol *et al*., 1991).

2.3.1.4 Hypothesis of Nitric Oxide

A hypothesis, which assumes that the filamentous and non-filamentous bacteria compete for organic substrate in different denitrification conditions, was proposed for the proliferation of filamentous bacteria in low F/M biological nutrient removal systems (Casey *et al*., 1992; 1994; 1999). This hypothesis is somewhat related to the growth kinetics between two prevalent species, namely, filaments and non-filaments, in the activated sludge, and may be useful in foam control (Kotlar *et al*., 1996). In this hypothesis, nitrite and NO, which were the intermediates in the denitrification process, could only be accumulated at the same time in the floc-forming bacteria compared with filamentous bacteria. The filamentous bacteria only performed denitrification when all the intermediates were nitrite. The filamentous bacteria could utilize the slowly biodegradable chemical oxygen demand (SBCOD) in the aerobic condition and will not cause accumulation of NO, which is the intermediate inhibiting

compound, in the competition with floc-formers. Thus, this process would have advantages that result in higher growth. In the aerobic part, the low rate of readily biodegradable chemical oxygen demand (RBCOD) and nitrite were persistently produced because the SBCOD is hydrolyzed. Therefore, the growth of floc-forming bacteria was continuously inhibited (Lakay *et al*., 1999; Musvoto *et al*., 1999). Although the hypothesis was promising, experimental verification was lacking.

2.4 Control of Filamentous Overgrowth

Over the years, numerous methods and procedures have been developed for the prevention and control of filamentous overgrowth. Several of these methods are more effective than others at specific conditions. However, no single method is universally applicable to all processes. Essentially, two approaches can be followed and adopted to suppress filamentous bacteria overgrowth in the activated sludge, namely, specific and non-specific methods.

2.4.1 Non-specific Methods

The non-specific method indicates control in general, and includes the addition of hydrogen peroxide, ozonation, chlorination, and so on. The filaments are usually located outside the flocs. Thus, they are more susceptible to oxidants for filaments compared with floc-formers (Klitzing and Muller, 2002).

Chlorination is a popular non-specific method to control the excessive growth of filaments in the activated sludge in the United States. The procedures for this method are well-documented (Jenkins *et al*., 1993). However, the application of chlorination

is limited in several European countries because potential undesirable by-product such as halogenated organic compound may be formed, which can result in environmental issues. In the others, where adverse effects can occur, such as in the slow-growing bacteria, nitrifiers require considerable time to recover when affected by the oxidants, which could deteriorate the quality of the effluent.

Synthetic organic polymers, talc, and divalent metal salts are also regularly used to increase sludge settleability (Clauss *et al.*, 1999; Juang, 2005; Seka *et al.*, 2001; Simpson *et al.*, 1991; Thompson and Forster, 2003). However, the solid load was increased by adding metal salts and talc, and the cost of organic polymers became high. In addition, these methods could not eliminate the causes for filament overgrowth, and solid separation problems resumed when chemical additions were stopped.

The drawbacks of transient remediation effect are also suitable for specific short-term control methods, such as manipulation of return activated sludge flow rates and aeration tank feed point. The redistribution of biomass from the clarifiers to the aeration units contribute to the rate of sludge wasting.

2.4.2 Specific Methods

Specific methods are precautionary techniques. The target objective favors the growth of floc-forming microorganisms at the expense of filamentous microorganisms. In using specific methods, identifying the causative filamentous microorganisms and the operational problems that favor the excessive growth of filaments is necessary. The successful application could maintain a stable control of filamentous microorganism overgrowth caused by solid separation problems; thus, they should be adopted

preferentially. These methods are potentially effective prevention and control strategies in solving foaming and bulking problems (Thompson and Forster, 2003), and can generally be classified into three categories shown below.

2.4.2.1 Nutrient Deficiency

Carbon: nitrogen: phosphorus ratios in the mixed liquor that deviate from normal levels are commonly believed to affect the microbial balances of various species (Jenn *et al*., 2002; 2007). When the microbial identification results suggest that filamentous overgrowth is caused by nutrient deficiency, the system is likely to be N- or P-deficient. $BOD_{5,}$ N, and P ratios in the wastewater influent should be determined. Sufficient macronutrients are present when the wastewater that is fed to the aeration tank contains BOD_5: N: P at a weight ratio of 100:5:1.

Nitrogen deficiency has long been recognized as a major cause of microbial population shift. Thus, prevention of nitrogen deficiency becomes a major concern in process operation and control (Andreasen, 1995). Richard *et al*. (1985) illustrated the importance of preventing nitrogen deficiency. Intermittent supply of NH_3-N allowed filamentous Type 021N to dominate, whereas continuous N supply that matched the carbonaceous load led to the predominance of floc-former microorganisms. Jenkins *et al.* (2004) introduced anaerobic zones to remove phosphorus prior to the aerobic process. Thus, the phosphorus could be absorbed in the subsequent processes as nutrients, thereby maintaining organic loading.

In treating specific industrial effluents, special consideration must be taken into account with regards to nutrient requirements (Zhu *et al.*, 2004). When treating wastewater with high organic loading but containing RBCOD, such as wastewater

from paper making industry, the normal N and P levels should range between 1 mg/L to 3 mg/L to meet the requirements for high substrate uptake rate and fast growth (Reid and Simon, 2000; Richard, 1991a; 1991b). Therefore, nutrient considerations are substantially different for the case of industrial effluent treatment, and filamentous growth characteristics are also context-dependent.

2.4.2.2 Low Dissolved Oxygen Concentration

The control of DO levels is an important method for foam control because of the different affinities towards DO in the mixed liquor of the activated sludge, which are in turn caused by cell shape and specific morphological features (Hug *et al.*, 2006; Sevior *et al*., 1994). In the morphology of filamentous bacteria, limited DO levels can cause the predominant growth of various filaments at different ranges of mean cell residence times (MCRTs) (Hao *et al.*, 1988; Richard *et al*., 1985).

The relationship between F/M ratio, DO uptake rate, and DO level in the mixed liquor of the activated sludge must be modeled to prevent foaming in a complete mixing process, which was conducted by Palm in 1980. Low DO bulking can be solved by decreasing the F/M ratio or increasing the DO concentration in the aeration tank. However, the implementation can be difficult, uneconomical, and fraught with undesirable outcomes. Raising the DO concentration to trigger nitrification or partial nitrification further increased the oxygen requirements. The DO concentration could be increased despite a low F/M ratio because of the high rate of nitrification and long MCRT conditions caused by increased endogenous respiration.

2.4.2.3 Selectors

A biological selector is placed in the initial zone of a biological reactor, which form an area with a low substrate dispersion space and a high macro-gradient level, to select the appropriate bacteria for the activated sludge treatment system (Chudoba *et al*., 1973). This method is extremely popular and is usually incorporated into the upstream of the aeration tank of the activated sludge process (Barbusinski and Koscielniak, 1995; Liao *et al*., 2006; Martins *et al.*, 2004). The activated sludge from the influent and the return path combine in this dedicated initial mixing zone and a high BOD uptake rate is achieved, thereby completely removing BOD (Jenkins *et al*., 1992). The name of the selector originates from the selection mechanisms for the desirable bacteria in the activated sludge with good settling properties.

In a typical biological selector, microorganisms can use the accumulated substrates as internal storage products in cells in high growth rate environments. The predominant microorganism must have a relatively high substrate uptake rate and a relatively large substrate storage capacity. The cell storage capacity should be replenished when the aeration time is long and when external substrates are unavailable (Chiesa and Irvine, 1985; Van den Eynde *et al*., 1983; Van Loosdrecht *et al*., 1997; Van Niekerk *et al*., 1987). Selectors were frequently installed in full-scale activated sludge systems. Selectors remain the most popular technique for the preventive control of filamentous-related solid separation problems. Selectors are generally categorized based on availability of DO and nitrate-N (NO_3-N). A sufficient DO level is important in an aerobic selector to ensure that metabolic requirements are met. The substrate would be stored and aerobically degraded in this condition. Compared with aerobic selectors, DO is insufficient in anoxic selectors. Another major requirement is sufficient nitrate-nitrogen level. The substrates are stored and denitrified in the anoxic

state. The last requirement is anaerobic selectors, in which DO and NO_3-N are both absent. The substrates are degraded via hydrolysis or fermentation. Table 2.3 illustrates specific general design criteria of aerobic, anoxic, and anaerobic selectors for consideration.

Aerobic selectors, which has an initial contact zone (with or without aeration), can effectively control major foaming problems, which are caused by the overgrowth of *S. natans, Thiothrix spp*., and Type 021N. However, the use of aerobic selectors has limited effect on the foaming sludge predominated by *M. parvicella* (Gabb *et al*., 1991; Houtmeyers *et al*., 1980; Jenkins *et al*., Martins *et al*., 2003; 2004; 1992; Rensink, 1973).

Table 2.3 Major design criteria for bioselector (Martins *et al.*, 2004)

Parameter	Value	Reference
Aerobic selector		
Number of compartments	≥ 3	Jenkins *et al.* (1993)
Contact time	10–15 min, But it depends on load, temperature, and wastewater composition (i.e. Fraction of RBCOD)	Eikelboom (1982), Daigger *et al.*(1985) Van Niekerk *et al.* (1987), and Martins *et al.* (2003a)
Sludge loading rate	12 (1st comp.), 6 (2nd comp.), and 3 (3rd comp.) Kg COD kg^{-1} MLSS d^{-1}	Jenkins *et al.* (1993)
Floc loading	50–150 g COD kg TSS^{-1} (1st comp)	Heide and Pasveer (1974), Eikelboom (1982), and Kruit *et al.* (1994)
DO concentration	≥ 2mg O_2 L^{-1}, but it depends on the sludge loading rate, floc loading rate, and/or substrate uptake rate. Sensor should be placed in the 1st comp	Casey *et al.* (1975), Sezgin *et al.* (1978), Palm *et al.* (1980), Albertson (1987), and Martins *et al.* (2003b)
Anoxic selector		
Number of compartments	≥ 3	Jenkins *et al.* (1993)
Sludge loading rate	6 (1st comp.), 3 (2nd comp.), and 1.5 (3rd comp.) kg COD kg^{-1} MLSS d^{-1}	Daigger and Nicholson (1990), Albertson (1991), and Jenkins *et al.* (1993)
Contact time	45–60 min	Kruit *et al.* (2002)
$(RBCOD/NO_3\text{-}N)_{consumed}$	Usually higher than –79 mg RBCOD mg $NO_3\text{-}N^{-1}$ due to storage	Randall *et al.* (1992), Jenkins *et al.* (1993), Wanner (1994), Van Loosdrecht *et al.* (1997), WEF (1998), and Beun *et al.* (2000)
Anaerobic selector		
Number of compartments	≥3, long channels (length-to-width ratio larger than 10:1)	Albertson (1987) and Kruit *et al.* (2002)
Contact time	1–2 h	WEF (1998) and Kruit *et al.* (2002)
$(COD_{VFA\,fermentable}/PO4\text{-}P)_{inf}$	9–20 g COD g P^{-1}	Wentzel *et al.* (1990) and Smolders *et al.* (1996)

Selective pressure is one of the important parameters. Many systems cannot reach the designed pressure to conduct optimal operation, even in a full loading situation (Wilderer *et al*., 2001). The contact time must also be considered for a strong typical design. The relation between contact time and sludge settleability was non-linear (Martins *et al*., 2003). This relationship is important in creating strategies to suppress excessive filamentous growth. If the contact time is excessively short, the un-adsorbed soluble substrate will flow into the main reaction tank. Therefore, the overgrowth of filamentous bacteria will occur. If the contact time is excessively long, a low substrate concentration will be formed in the contact zone, which is similar to the main reaction zone. In this case, no selection effects can be observed. Designated SVI could only be achieved with a precise design of the contact zone and control of operation parameters. In most practical cases, the temperature, throughput, and inflow concentration vary from time to time. Hence, the selectors will have no effect in controlling SVI. (WEF, 1998; Wilderer *et al*., 2001) In addition, sequencing running of the activated sludge system improves performance with kinetic limitations (Scuras *et al*., 2001).

Studies have shown that the DO level is an important factor to maintain the dissolved organic loading rate in the aerobic bioselector and in the main reaction zone (Palm *et al*., 1980; Martins *et al*., 2004; Sezgin *et al*, 1978; Gaval and Pernelle, 2003). The consumed COD could reach up to 15% to 30% in the selector in a short period of time (Jenkins *et al*., 1993; Martins *et al*., 2004). The removal rate in this selector depends on a sufficient DO concentration. An inadequate DO level negatively affects the SVI level (Martins *et al*., 2004). Therefore, DO level control is critical. The DO sensor should be placed in an area in which the oxygen requirement is greatest.

Studies have shown that inadequate F/M ratio, insufficient DO level, slowly

biodegradable COD (SBCOD) hydrolysis, non-aerobic reactor kinetics, and biomass oxygen deficiency lead to the overgrowth of filamentous microorganisms in the biological nitrogen or phosphorous removal process (Eikelboom *et al*., 1998; Gabb *et al*., 1991; 1996; Kristensen *et al*., 1994; Kunst and Reins, *et al*., 1994; Lakay *et al*., 1999). In the anoxic and anaerobic bioselector, the readily biodegradable COD (RBCOD) should be degraded prior to entering the main reaction zone. Otherwise, filament overgrowth easily occurs (Jenkins *et al*., 1993; Shao and Jenkins, 1989). In addition, the DO level in the selector should be precisely controlled in the anoxic and anaerobic zone. Otherwise, the SVI level is not met.

Different selectors capitalize on different operating parameters such as physical parameters from engineering designs, microbial kinetics from interspecies interactions, and biochemical mechanisms in microbial physiology (Eikelboom *et al*., 1998). These methods may be effective in foam control in specific conditions, but they often lack theoretical bases and are not universally reliable (Martins *et al*., 2003; Yang *et al*., 2003)

2.5 Filamentous Bacterial Genera in Activated Sludge

The activated sludge process is a widespread technology in treating dissolved organics in wastewaters such as domestic sewage and industrial wastewater (Martins *et al*., 2004).

Since the early development of the activated sludge process as early as 1914 by Ardern and Lockett, bulking and foaming problems have been frequently encountered, which have hampered treatment efficiency. The activated sludge is a mixed microbial culture system. At least 30 types of distinct identified filamentous bacteria cause

sludge foaming and bulking. These filamentous bacteria are important in maintaining the sludge floc structure, maintaining the efficiency of biological treatment, and facilitating the precipitation of suspended solids. Bulking and foaming are commonly associated with the overgrowth of a wide range of filamentous microorganisms such as *Gordonia amarae*, (Gram-positive, branched hyphens), *Nocardia pinesis*, (Gram-positive, pine-like), *Rhodococcus spp.*, and *Microthrix parvicella* (Gram-positive, filamentous, non-sheath non-branches) (Blackbeard et al., 1988; Hwang and Tanaka, 1998; Seviour et al., 1990; Wangh et al., 1994). Among these bacteria, the most common are *Gordonia amarae* and *Microthrix parvicella*.

Blackall *et al.*, (1988) indicated that either *Nocardia amarae* or *Nocardia pinensis* would be the predominant species in a great number of foaming problems. Their overgrowth causes the sludge-settling rate to decrease substantially, which results in a decrease in the settleability of the sludge and in incompact sludge blankets. These effects will eventually compromise the quality of the treated effluent (Pagilla *et al.*, 2002; Tsang *et al*., 2007).

2.6 Bacterial Physiological Characteristics in Activated Sludge

Excessive filament content in the activated sludge interferes with settlement because it physically prevents close packing of activated sludge flocs and entraps air bubbles within. Thus, the bulk density of the flocs decreases. The predominance of filaments keep the activated sludge flocs apart and result in the formation of thick, persistent, and scum-like foams. This predominance severely hinders settlement and causes filamentous bulking in secondary sedimentation tanks (Baxter *et al.*, 1998; Simona *et al*., 2005; Tay *et al*., 2001).

Bulking sludge usually has the distinctive characteristics of poor settling and poor compact ability. Filamentous microorganisms have been identified and reported as the major cause of thick, persistent, and scum-like foam. The health of an activated sludge microbial ecosystem relies on the profound balance between non-filamentous bacteria, such as *Pseudomonas spp.*, *Zoogloea spp.*, *Alcaligenes spp.*, and *Achrombacter spp.*, and the filaments, such as *Nocardia spp.* and *Microthrix spp*. Although floc formers are major organic degraders, filaments also degrade organics and, at the same time, provide the skeletal matrix to form compact flocs, which are essential in achieving good settling properties.

2.7 Limitations of Conventional Control Methods for Bulking and Foaming

The activated sludge process was frequently reported to encounter bulking and foaming problems, which are difficult to suppress and control by conventional methods, because of the overgrowth of filamentous bacteria (Rossetti *et al*., 2005; Stainsby *et al*., 2002). Many factors are known to cause this phenomenon. For instance, all biological reactions are affected by physical factors such as temperature. The temperature coefficient is 1.015 for the floc-forming bacteria in a municipal wastewater (Eckenfelder, 2000). Other causes such as the parameters of process design (e.g., configuration of feeding regime and aeration basin) and conditions of plant operation (e.g., low sludge return rate and high suspended solids amount in mixed liquor) also result in the growth of filaments (Metcalf and Eddy, 2003). These problems are extremely difficult to address and alleviate completely via conventional methods and procedures.

Chemical factors such as the composition of wastewater have also been reported to trigger foaming and bulking problems that are very difficult to control using conventional methods. For example, glucose and other readily biodegradable organics are known to favor the growth of filamentous organisms (Chudoba, 1985; Eckenfelder, 2000; Richard and Collins, 2003). Nutrient balance is also critical in governing and suppressing filament overgrowth (Liu and Liu, 2006; Tsang *et al*., 2006).

Bulking and foaming problems cause seriously adverse effects on plant operation and exert a significant influence on the quality of the treated effluent.

Severe foaming and bulking problems are nearly impossible to control using mechanical devices. These problems cannot be avoided by manipulating the recycling of sludge in treatment facilities. Foaming in the secondary sedimentation and aeration unit can be controlled by adjusting the operating conditions. However, the methods involved are often very complex and difficult to carry out by normal plant operators. The operating conditions should not only upset the formation of the foam and scum, but should also ensure the maintenance of normal and acceptable performance of the treatment process (Liu *et al*., 2005). Given that the occurrence and proliferation of the filamentous microorganisms were not correlated with any single treatment unit operation, foaming could not be controlled by installing conventional mechanical devices in a single activated sludge treatment unit.

Chapter 3: Methodology

This chapter establishes the experimental design and procedure into three parts, which are described in three sections. Section 3.1 discusses the preparation of the microbial culture to determine the predominant filamentous microorganism from activated sludge plants that encounter severe foaming and bulking problems. Experiments were performed to determine the change in the carbon chain length and concentrations relative to the growth rates of filaments. Section 3.2 discusses the design of the investigations to examine the pure cultures of *Gordonia amarae*, which is a recently discovered genus, and *Pseudomonas aeruginosa* and to monitor their growth in different fatty acids as the sole carbon source. The comparison and contrast of the growth kinetics of the filaments and the non filaments were used as basis for the next stage of the study. The results and data gathered in Sections 3.1 and 3.2 were used in Section 3.3 as a theoretical basis to establish a novel activated sludge operating procedure for the suppression of filamentous overgrowth. This procedure can solve the foaming and bulking problems. The synthetic wastewater was used to simulate a paper mill effluent, and a number of process operating parameters were selected to investigate the effectiveness of this novel procedure in foam control.

3.1 Identification of Foam Causative Microorganisms

3.1.1 Cell Cultures for Identification

The plated culture with Czapek's agar containing 0.4% yeast extract was used to isolate the microorganisms in the activated sludge process of a paper mill wastewater treatment plant. The plates were incubated at 28℃ for 14 to 21 days based on the

criteria described by Blackall *et al.*, (1988) and Jenkins *et al.* (2004). The microorganisms isolated from activated sludge were maintained in pure culture at 28℃ for 3 to 5 days. The mixed liquor was kept in 500 ml flasks with 100 ml liquid broth and shaken at 200 rpm. After obtaining the pure culture, the samples were divided into two groups in the shaking flasks with appropriate incubation. The first group of samples was incubated with fatty acid from the general wastewater as a carbon source and as a minimum salt medium (MSM). The second group was cultured with raw wastewater containing various fatty acids after autoclaving, which was commonly found in municipal sewage. The dry cell mass was measured to examine the growth of the microorganisms.

3.1.2 Foaming and Stability Analysis

A one-molar solution of NaOH was used to dissolve 500 mg/l fatty acid solutions (stearic acid, C_{18}) so that the degree of foaming and foam stability can be measured. 20 ml of the prepared solution was mixed with 980 ml activated sludge with 3400 mg/l mixed liquor volatile suspended solids **(**MLVSS). A 1-L graduated measuring cylinder was used to contain the 0.5 L mixture. The mixture was then aerated by an aquarium air pump with the air supply of 240 l/h. The mixture in the cylinder was initially aerated for 0.5 min and then stopped to observe the change in foam height. Foam height above the 500-ml mark was noted for every 20 s during aeration and post-aeration periods. Fatty acid solutions with concentrations of 1000 mg/l and 1500 mg/l were used to investigate the relationship between the fatty acid concentration and the foaming appearance. The foam layer height during the aeration period is a measure of the degree of foaming, and the subsiding of the foam during the non-aeration period is a measure of stability of the biological foam.

G. amarae that were cultured in the shaking flask were harvested by centrifugation. The samples in the tube were washed and re-suspended twice. The products were collected in the supernatant with a concentration of 20000 mg/l. Three different amounts of the resulting solution containing *G. amarae* were added into the activated sludge mixture with an MLVSS concentration of 3400 mg/l. 5, 10 and 15 ml of the *G. amarae* solution mixed with 495, 490 and 485 ml of the activated sludge mixture, respectively, were used for the foaming and stability analysis as previously described.

3.2 Growth of Filamentous and Floc-forming Bacteria on Various Carbon Sources

3.2.1 Cell Cultures for FFO Analysis

Pseudomonas aeruginosa (CRCC 10261, from Culture Collection and Research Center, Taiwan) and *Gordonia amarae* (ATCC 27809, from American Type Culture Collection, Rockville, MD) were obtained to simulate the floc-forming and filamentous microorganisms in the wastewater treatment processes. These cells were cultured in yeast-extract glucose agar slants and stored in a refrigerator at 2-4^{o}C. *P. aeruginosa* and *G. amarae* were cultured at 30℃ for 24 and 72 h in a Luria-Bertani media before inoculation (Chua and Le, 1994). Fatty acids with different carbon chain lengths, which were purchased from a local chemical supplier with chemical purification level, were dissolved in a sodium hydroxide solution as the carbon source. The solution was adjusted to pH 7.0 and filtered through a 0.45-μm membrane. MSM was prepared as previously described and its pH was adjusted to 6.8 by adding 0.5 M NaOH. Each inoculation solution with a volume of 5 ml was mixed with 10 ml carbon source solution and 90 ml MSM solution in a conical flask with cap. The

cultures were stored in a shaking flask at 200 rpm at 30℃.

3.2.2 Cell Culturing Analysis

Bacterial growth was monitored by optical density using spectrophotometer at 600 nm (Spectronic-601, Milton Roy). The residual COD concentration in the medium was measured based on Standard Methods (APHA, 1998) to quantify the substrate uptake rate.

3.3 Development of a Novel Control Strategy for Bulking and Foaming

3.3.1 Preparation of Synthetic Wastewater

The synthetic wastewater was composed of 63 mg/l ammonium chloride, 26 mg/l potassium hydrogen phosphate and 0.8 g/L glucose. After complete mixing, the BOD_5 and COD of the solution were set at 470 mg/l and 800 mg/l, respectively, to maintain the desirable BOD_5: COD ratio, which was around 0.6, to simulate the paper mill effluents. The pH of the MSM solution was adjusted using 0.5 M NaOH. This solution was added to maintain the proper amount of trace elements required for bacterial growth.

3.3.2 FFO System Simulation

A two-stage activated sludge system (Figure 3.1) consisting of two 3-l aeration tanks, which represent the feasting and the fasting units, and one sedimentation tank was used for the FFO system simulation. The influent was fed to the two aeration tanks at designed portions. The effluent of the feasting tank was also fed to the fasting tank before liquid-sludge separation to achieve a more precise control of the desired F/M ratio in each tank for practical application consideration. However, in this study, the flow rates of the two tanks were precisely controlled by the peristaltic pump, thus, no bifurcation was required. The return sludge from the sedimentation tank was returned to the two aeration tanks to maintain the desired MLSS concentrations.

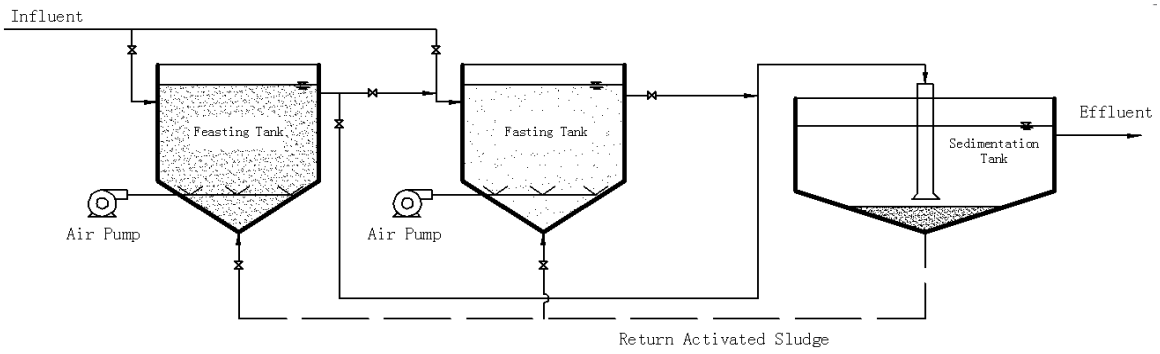

Figure 3.1 Activated sludge simulator

Prior to the start of the feast-fast operation (FFO), the system in Figure 3.1 was operated under conventional conditions for 90 days to reach a stable performance and a mature sludge culture. The two aeration tanks were operated at different operating conditions to achieve the FFO procedure. Therefore, the RAS ratio, HRT, SRT, and F/M, were different for each round of operation (Table 3.1). The feeding rates, sludge return rates, and HRT were calculated based on the desired F/M ratio. Other parameters, such as pH, SRT, and DO, were selected based on the general biological wastewater guideline. The system performance was determined by the water quality parameters, such as COD, BOD, MLSS, pH, DO and SVI according to Standard Methods (APHA, 1998).

Table 3.1 Major process operating conditions for conventional and FFO modes

Operation Conditions	Units	Conventional Mode	FFO Mode	
			Feasting unit	Fasting unit
pH		7±1	7±1	7±1
Effective volume	ml	6000	3000	3000
SRT	day	15	15	15
HRT	h	10	8.3	12.5
Sludge return rate	ml/h	250	60	210
Feeding rate	ml/h	600	360	240
Sludge disposal rate	ml/d	400	200	200
F/M ratio	d^{-1}	0.4	0.89	0.15
DO	mg/l	3±1	3±1	3±1

3.3.3 Laboratory Scale Reactors

The lab-scale system was used to study the combination of FFO and SBR. Figure 3.2 shows the system setup, which was constructed using four acrylic plastic tanks.

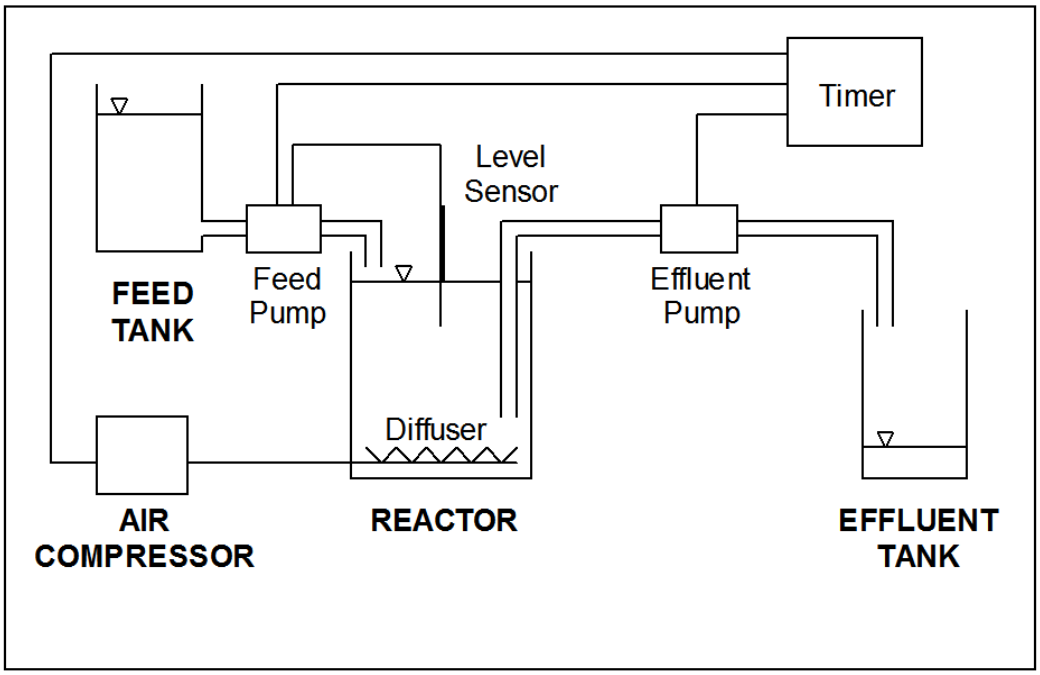

Figure 3.2 Control strategy of the lab-scale single-stage SBR system

In the SBR system, aquarium air pumps were used to supply oxygen through sintered-sand diffuser for aeration. MLSS concentration was controlled by peristaltic pumps at different sludge feeding and disposal rates. Wastewater was fed and drained using aquarium water pumps, and the water depths were controlled by level sensors. All of the facilities were controlled using pre-set timers in sequence.

The MLSS level was gradually reduced to 3000 mg/l at the start-up phase and then increased to 5000 mg/l to investigate filamentous foaming. The DO level was kept at approximately 3 mg/l during the experiment period. Sludge age was kept between 4 and 10 days by controlling the sludge wasting rates. Excess activated sludge was disposed at the end of the aeration phase to maintain the desired MLSS level and sludge age. The study was carried out at room temperature at approximately 25℃ and the water temperature was maintained by a circulation pump through a temperature controlling basin. Table 3.2 illustrates the major operating conditions for the single-stage SBR system.

Table 3.2 Major operating conditions for single-stage SBR system

Operation conditions	Units	Settings
Feeding	h	0.2
Reaction	h	3, 4, 5 and 6
Settling	h	0.5
Decanting	h	0.3
Idling	h	2, 6 and 18
Hydraulic retention time	h	16 to 64
Sludge age	d	4 to 10

The single-stage SBR was initially optimized by varying the settings of operating conditions. In optimized conditions, the system was switched to the FFO mode to investigate the performance in practical application. The FFO in the single-stage SBR

was fulfilled by sequential operation. The F/M ratios in the present and next stages had a high divergence but had a healthy average level for every two cycles, which was achieved by controlling the sludge disposal and wastewater feeding rates. Therefore, every two cycles is a pair operation. In this study, the F/M ratios for the pair cycles are 0.89 and 0.15 d^{-1}, respectively, which results in an average level of 0.5 d^{-1}. The effluent quality, removal efficiency, and sludge characteristics were used to assess the performance.

3.3.4 Inoculation and Influent

For the startup of the single-stage SBR system, activated sludge from Tai Po Sewage Treatment Works was used. The coarse matters were sieved (2000 micron pore size), and prior to inoculation, the sludge collected from the site was aerated for 48 h in the laboratory. During the startup phase, the SBR system was operated with diluted wastewater, and a high MLSS (>3000 mg/l) was maintained and gradually reduced until the system performance was stable.

For the practical performance study, wastewater from a paper making mill in Shenzhen City was used as the influent. The wastewater was collected from the equalization tank of the treatment system and then stored in a refrigerator at 4 ℃. $(NH_4)_2PO_4$ and the MSM mentioned earlier were dosed in the bacterial culture to adjust the BOD_5: nitrogen: phosphorus ratio to 100:5:1, which is the nutrient required for bacterial growth (Tsang *et al.*, 2006). Table 3.3 illustrates the properties of the collected wastewater.

Table 3.3 Influent wastewater qualities

Items	Units	Values
Chemical oxygen demand	mg/l	1300±100
Biochemical oxygen demand	mg/l	600±150
Total suspended solids	mg/l	350±150
Temperature	℃	23±3
pH	-	6.4±0.2

The effluent quality was estimated after every cycle of operation. The average level of the two cycles in each day was taken as the daily performance.

3.3.5 Analytical Methods

All water and sludge analyses carried out in this study were based on Standard Methods (APHA, 1998). COD, BOD_5, MLSS, and SVI measurements were strictly carried out based on standard codes 5220, 5210B, 2540D, and 2710D, respectively. DO was monitored using an electronic DO meter (YSI-5000). The pH value was measured using a pH meter (Fisher Scientific Accumet 900). Microorganisms were observed using a microscope (Meiji Techno ML5000) and photographed using a digital camera.

3.3.6 Statistical Analysis

The difference significance level and statistical reliability were evaluated to improve the analytical quality via t-test and ANOVA using $SPSS^R$ for Window Release 10.1. The COD removal rate defined in each section was determined by the data with a confidence level of 95%.

Chapter 4: Identification of Predominant Filamentous Filamentous Microorganism in a Papermaking Effluent Treatment Plant

Based on the three-stage work described in the previous chapter, the results and discussion are presented accordingly as follows:

4.1 The Identification of Foam-Causing Organisms

Through a series of microscopic observations on the morphological characteristics of the microbial species, a peculiar filamentous species was identified in the foaming sludge. The appearances of the different activated sludge foams were examined using a microscopic, as previously described (Richard, 2003). An overgrowth of branched-filamentous bacteria (Plate 4.1) could be observed in the stained samples of the foaming sludge. The peculiar morphological characteristics of this culture showed distinctive branching of the elongated cell structures.

Plate 4.1 *Gordonia spp*. in the foaming sludge (400X magnification)

This isolated culture from the foaming activated sludge was identified as *Gordonia spp*. because of the typical branched hyphae microscopic structure. A series of tests were carried out to confirm on this observation. The culture was further tested to be Gram-positive and Neisser-negative bacteria. The slender filaments had a right-angle branching at a magnification of 1,000X using an optical microscope. The branched filaments or hyphae were approximately 0.5-1.0 microns in diameter and 80-160 microns in length (Plate 4.2).

Plate 4.2 Right-angle branching in *G. amarae* cluster (1000X magnification)

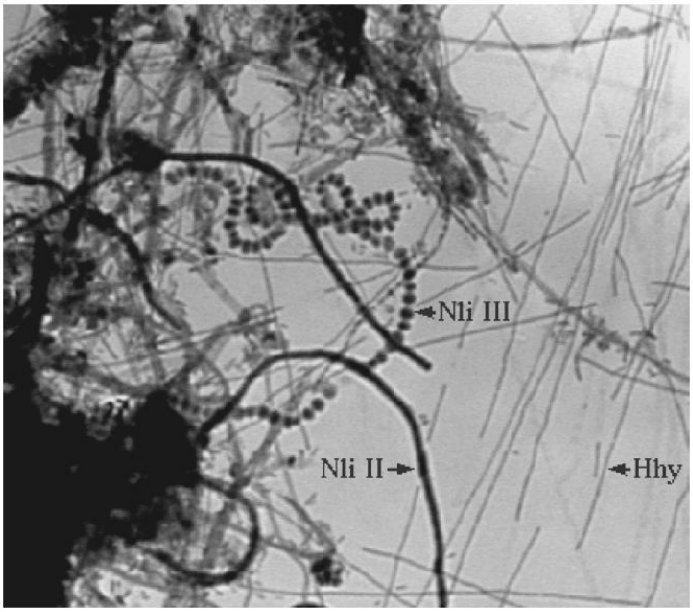

Figure 4.1 Hhy: *Haliscomenobacter hydrossis*, Nli II: *Nostocoida limicola II*, Nli III: *N. limicola* (Pernelle *et al.*, 2001)

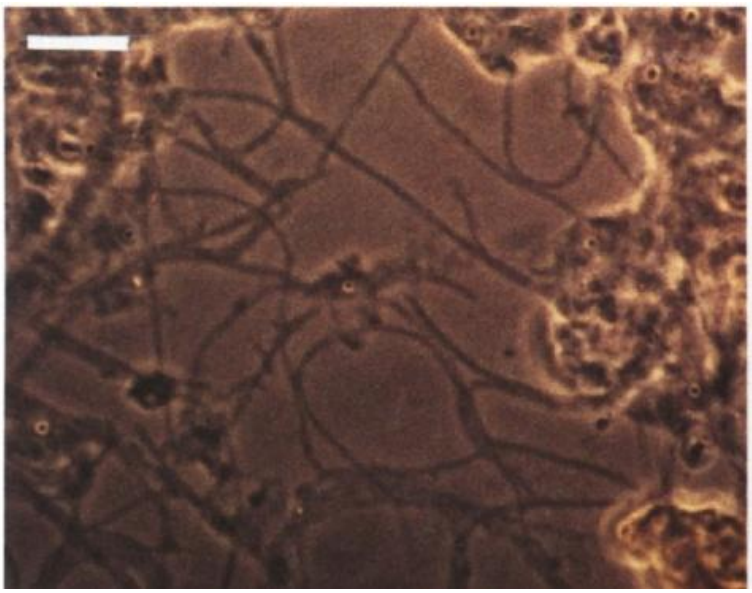

Figure 4.2 *Microthrix parvicella* (Erhart *et al.*, 1997)

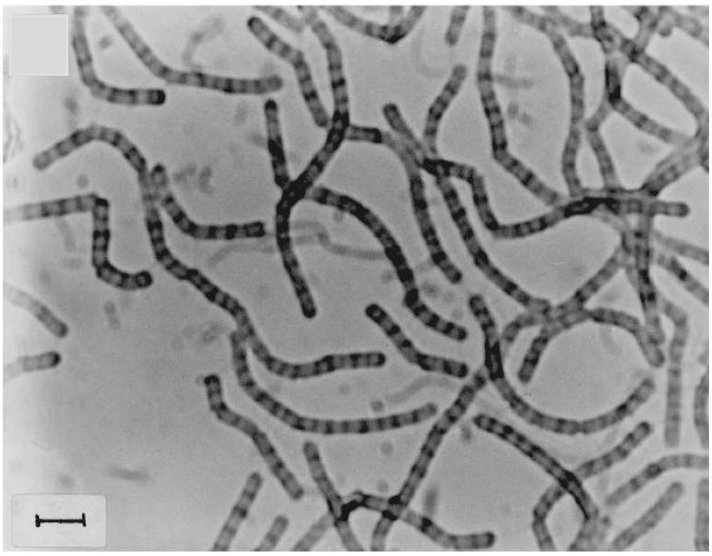

Figure 4.3 *Sphaerotilus natans* (1000x magnification) (Contreras *et al.*, 2000)

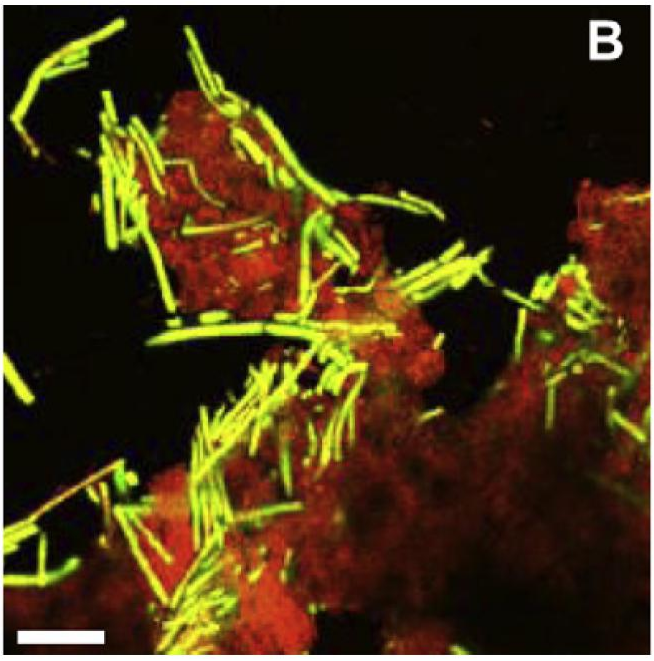

Figure 4.4 *Thiothrix spp.* (yellow) (Satoh *et al.*, 2009)

A series of physiological examinations were carried out to identify *G. amarae*. In the rigorous series of tests including a set of shake-flask cultures incubated at 28^{o}C, the

isolated bacteria generation time was 10.5 h. However, the bacteria can no longer survive at an incubation period of 8 h in a heat resistance test at 50^{o}C. The microorganisms shown in the plate cultures had a branching-net shape. The surface of the cell appeared slightly unpolished and chalky. Figures 4.1-4.4 show the common filamentous bacteria in various studies. Based on the morphological characteristics, the isolated microorganisms had unique differences, which differ from *Haliscomenobacter hydrossis*, *Nostocoida limicola*, *Microthrix parvicella*, *Sphaerotilus natans*, and *Thiothrix spp.* (Hwang and Tanaka, 1998). Therefore, based on the aforementioned unique physiological characteristics, filamentous bacteria, which were abundant in the foaming sludge, were identified to be associated with *G. amarae.*

The recently discovered genus *Gordonia*, as *Gordonia amarae*, in the foaming activated sludge in sewage treatment works in Hong Kong was generally similar to the *Nocardia amarae* species found worldwide. At the turn of the century and with the correct etymology, *G. amarae and N. amarae* were found to be closely related based on the phylogenetic analyses of nearly complete small-subunit ribosomal DNA sequences (Tsang *et al*., 2008); *Gordonia amarae* and *Nocardia amarae* were found to be closely related. Given that *G. amarae* is commonly and currently known as *N. amarae* in many relevant studies as well as engineering applications, they are sometimes considered to be genetically closely related (Juang and Hwu, 2003; Kaewpipat and Grady, 2002; Pagilla *et al.*, 2002). Thus, for the purpose of the present study and for general engineering applications in wastewater treatment, the genera and species terminologies of *G. amarae* were used in the experiments.

4.1.1 Growth of *G. amarae* in Sole Carbon Sources

The severe foaming sludge collected from Tai Po Sewage Treatment Plant was used to isolate *G. amarae*, which was then used to maintain a monoculture. The monoculture of the filaments was maintained by using different fatty acids as the sole carbon source on a 140-h growth cycle. Figure 4.5 shows the corresponding dry cell mass accumulation trends.

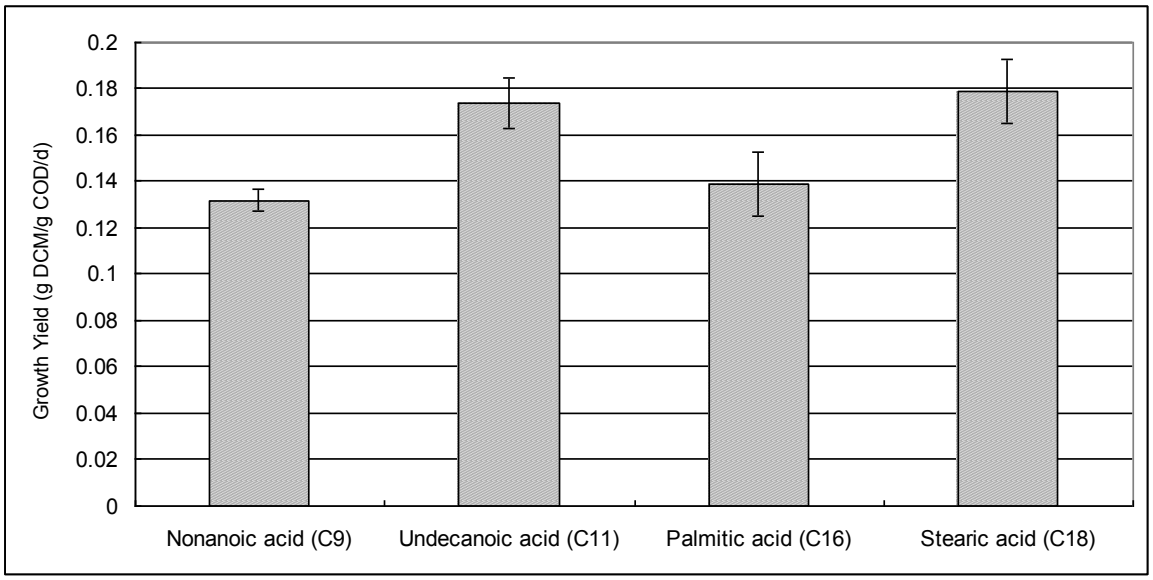

Figure 4.5 Growth yield in fatty acids as the sole carbon source

As shown in Figure 4.5, *G. amarae* had a higher growth yield when the chain length of the sole carbon source was increased. *G. amarae* is competitive among other organisms in sewage containing high concentrations of fatty acids, which could hardly be utilized by common microorganisms. A longer the carbon chain in the fatty acids indicates that the organic molecule becomes less biodegradable. These results show that the filamentous *G. amarae* has a high affinity toward persistent organics compared with the non-filamentous floc formers. Moreover, these findings on the

growth kinetics of the filaments also correspond with the normal observations which indicate that the filamentous foaming phenomenon always follows the sudden influx of grease-laden sewage into sewage treatment works.

4.1.2 Effects of Carbon Sources on *G. amarae* Growth

The chain length of the carbon sources is related to the degradability of organic matters. Figure 4.6 shows the different growth patterns of *G. amarae* based on the effects of different fatty acids in autoclaved raw sewage. Different trends of the growth cycle with various fatty acids were compared and contrasted to find out the differences of growth kinetics in grease-laden sewage.

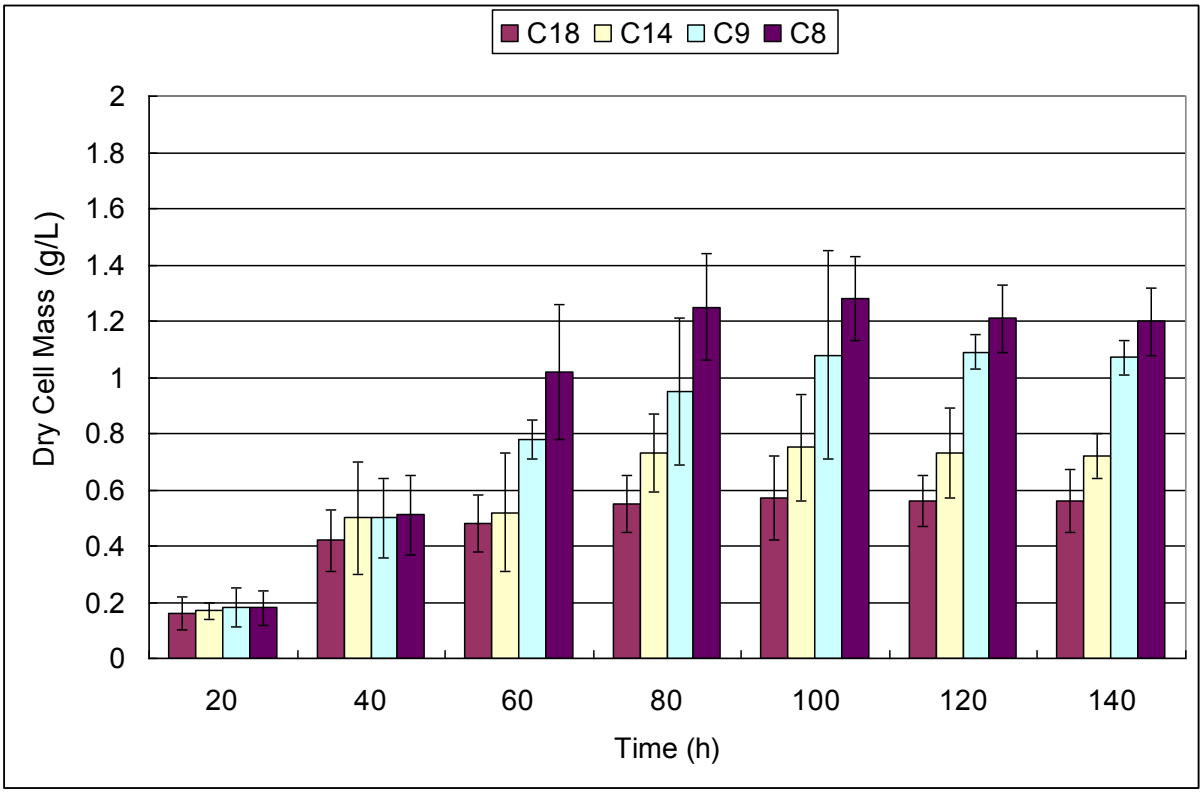

Figure 4.6 *G. amarae* growth with C_8, C_9, C_{14}, and C_{18} in sewage

Based on the results shown in Figure 4.6, *G. amarae* growth is more stimulated in short chain fatty acid cultures compared with long ones. This finding is an expected trend based on the absence of competition. The raw sewage was autoclaved to remove or inactivate other competing microbial species. The trends on the red and dark blue columns in Figure 4.6, which represents the growth in C_{14} and C_{18}, show that *G. amarae* had a high growth rate in the long chain fatty acid condition. A high fatty acid concentration in sewage could also cause an excessive growth of *G. amarae*, thereby causing foaming in the activated sludge. Based on this phenomenon, incoming sewage that is rich in persistent organics such as grease, protein, and other persistent macromolecules, results in filamentous overgrowth, foaming, and bulking in the plant.

4.1.3 Effects of Carbon Source and *G. amarae* Overgrowth on Foaming and Stability

Based on previous results, we deduced that fatty acids in incoming sewage can promote foaming in the activated sludge. At high filamentous concentrations, the foam could be stably maintained for a long period of time after the aeration was interrupted based on the measurement procedure described in Chapter 3 (Figure 4.7). The fatty acids have active properties on the surface and cause the formation of bio-surfactants and improve the stability of foam to avoid breaking. This phenomenon causes an increased degree of foaming with increasing fatty acid concentrations. However, fatty acids caused filamentous overgrowth, and the knitted network structure of branched filaments in the liquid film of air bubbles on the sludge surface also increased the surface tension. Thus, the stability of the air bubbles is increased, which forms stable foam.

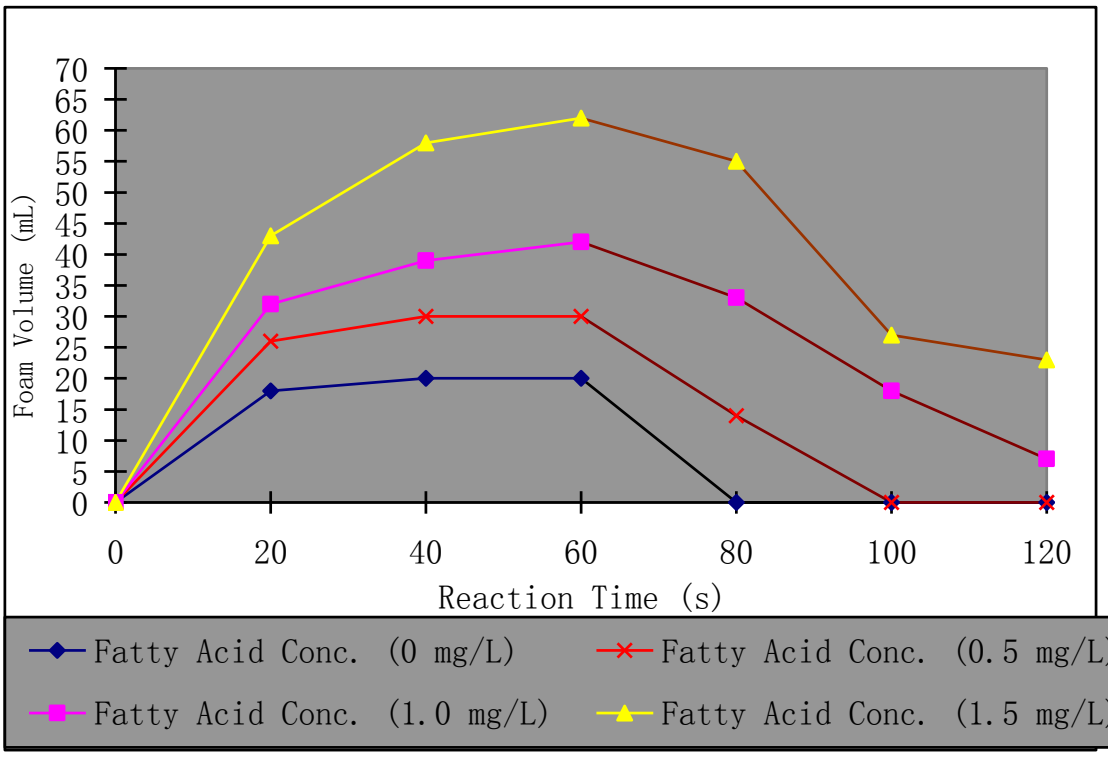

Figure 4.7 Effects of fatty acid (stearic acid, C_{18}) concentration on foaming and stability

The results in Figure 4.8 show that the foaming degree and stability also increased with increasing extent of overgrowth of *G. amarae* in the activated sludge. Figure 4.7 shows that in a 1.5-mg/l fatty acid culture, 50% of the foam remained stable even if aeration was cut off for 40 s. Based on these results, this method is reliable and reproducible and can therefore be applied in sewage treatment and can be used as a universal indicator to determine the severity of foam formation problem. The severity of foaming problems is a concerted effect of degree of foaming and foam stability, both of which can be accurately determined in the testing procedure.

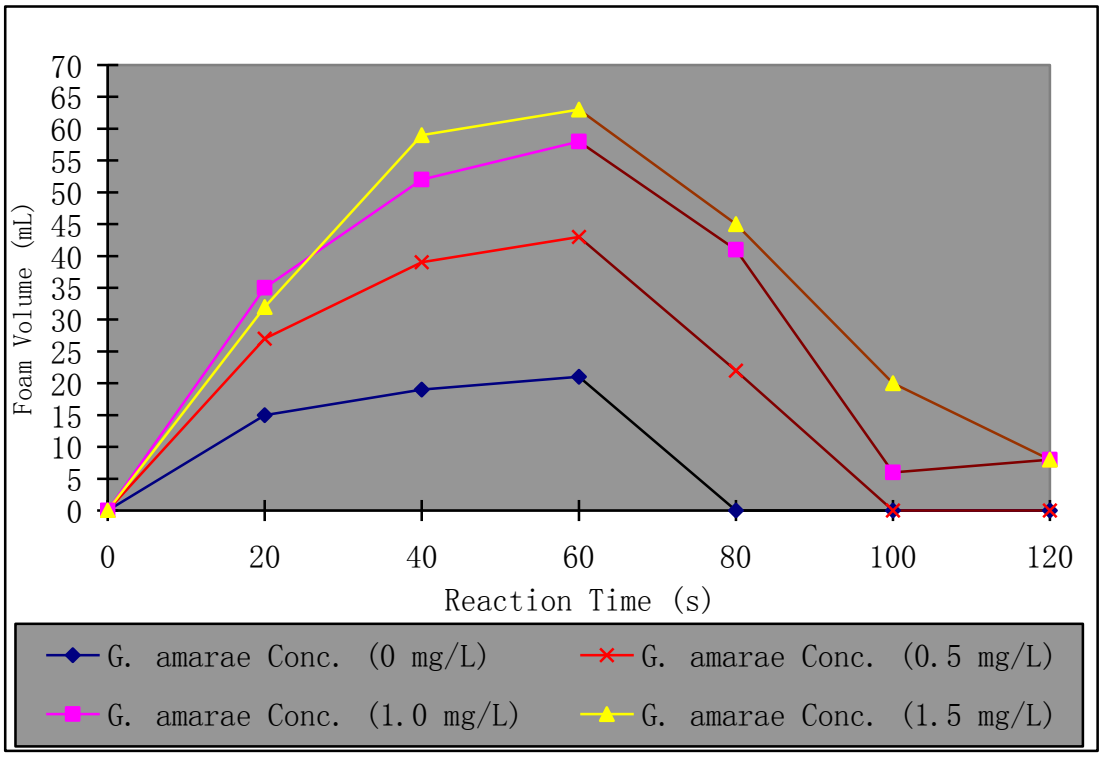

Figure 4.8 Effects of *G. amarae* concentration on foaming and stability

4.2 Growth of Filamentous and Floc-forming Microorganisms in Different Carbon Sources

Figure 4.11 shows the *G. amarae* growth, which was measured by the accumulation of dry cell mass, with different chain lengths of sole carbon sources in the form of fatty acids. Based on the culture in the acetic acid (C_2) condition, the logarithmic growth phase lasted for approximately 60 h as a part of the overall growth cycle. The growth then increasingly reached a plateau of 0.285 g/l (Figure 4.9), which indicates the onset of the stationary phase of the growth cycle. Other fatty acids, namely, C_{12}–C_{24}, also enhanced *G. amarae* growth but in a less pronounced behavior with a longer chain length. These results were not unexpected because the affinity of the filaments toward the fatty acids decreased with carbon chain length as observed in previous findings. Therefore, the culture of lignoceric acid (C_{24}) could only grow to a maximum of approximately 0.043 g/l in this investigation, which was substantially lower compared with shorter carbon chain acids as sole carbon sources.

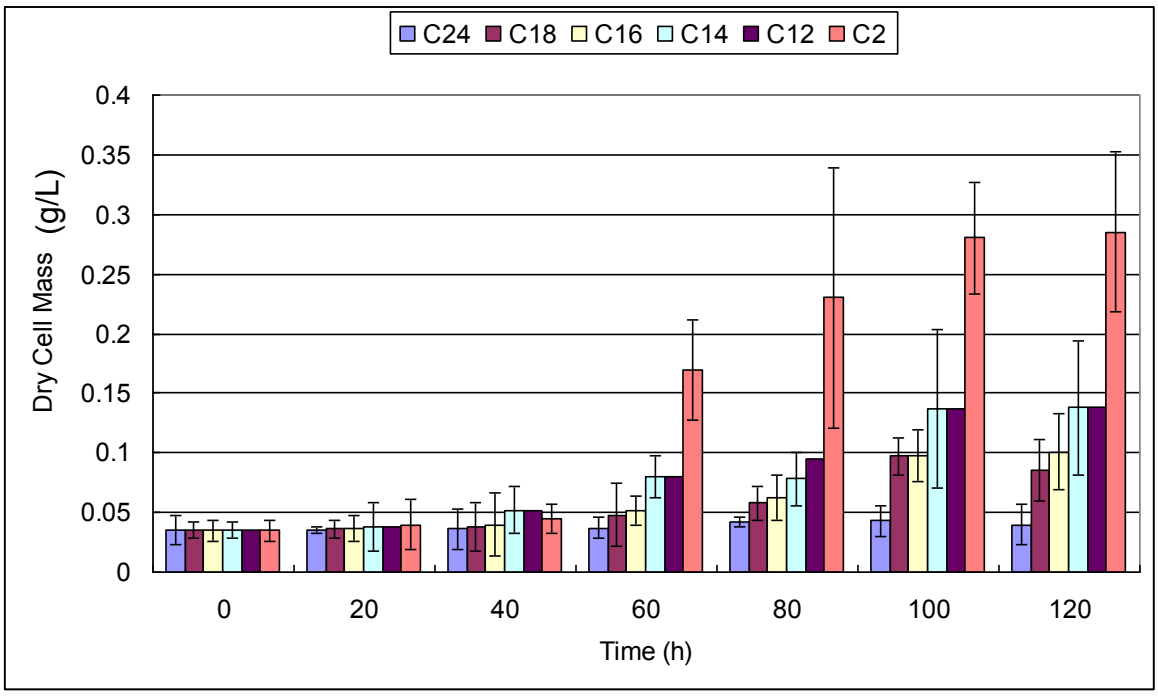

Figure 4.9 *G. amarae* growth in C_{24}, C_{18}, C_{16}, C_{14}, C_{12}, and C_2 conditions

As a comparative investigation of filaments and non-filaments for the growth kinetics on a competitive culture similar to that in an activated sludge, the growth of *P. aeruginosa* was studied in a growth cycle similar to previous investigations. Figure 4.10 shows the growth trends of *P. aeruginosa* in fatty acids of different chain lengths as sole carbon sources. Compared with *G. amarae*, *P. aeruginosa* in the acetic acid condition reached a value of 0.26 g /l which lasted for approximately 5 h. However, floc-formers could hardly utilize the long chain length fatty acids for their growth and a constant dry cell mass was retained over the culture period, particularly for lignoceric acid (C_{24}). This result indicates that an insignificant slight growth was detected. Based on the experimental results, both bacteria grew better in sewage with fatty acids with shorter chain lengths, which are obviously known to be more readily biodegradable.

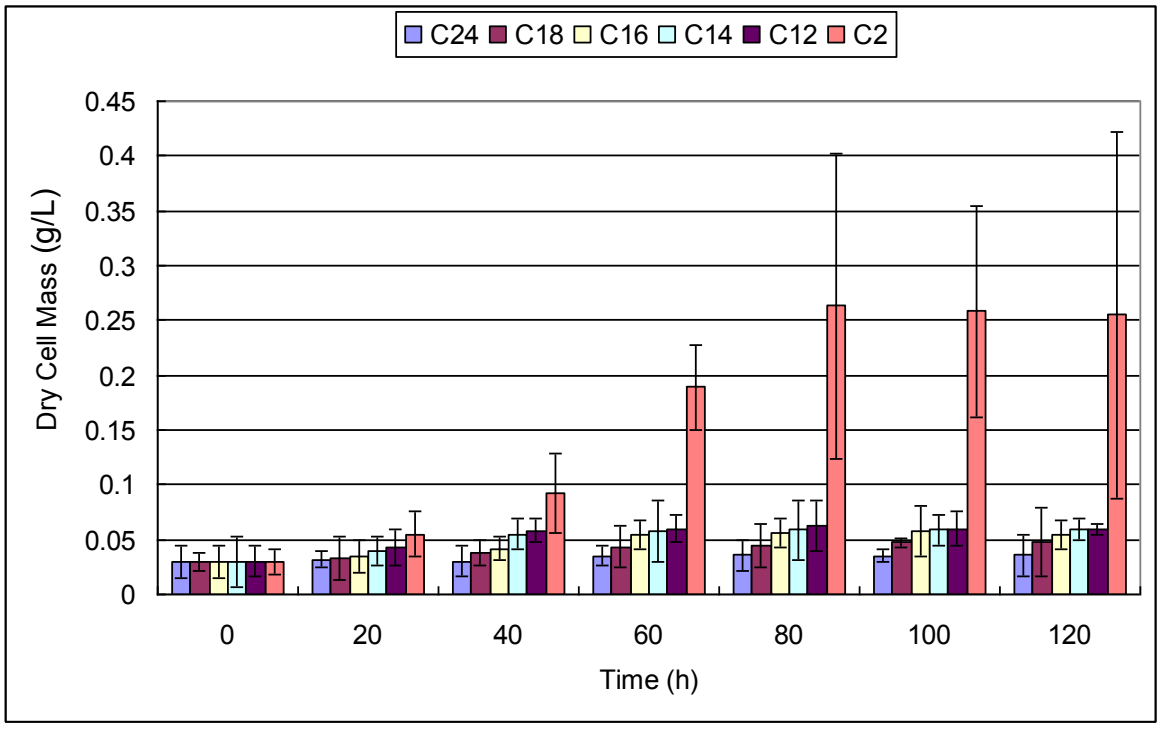

Figure 4.10 *P. aeruginosa* growth in C_{24}, C_{18}, C_{16}, C_{14}, C_{12}, and C_2 conditions

4.2.1 Kinetic Selection

The growth and substrate utilization data (Monod Growth Model) were analyzed by Line Weaver-Burk and linear regression data processing techniques to calculate the maximum specific growth rates (μ_m), saturation constants (K_s), and growth yields ($Y_{x/s}$) for both bacteria with various fatty acids as the sole carbon source (Tables 4.1 and 4.2). The specific growth rate indicates the condition of the bacterial species in the culture conditions. Therefore, their tendency to become the predominant species in the culture can also be determined. On the other hand, the saturation constant shows an inverse proportion of the affinity of the species toward the rate limiting carbon source. Thus, the competitiveness of the microbial species in the mixed culture can be delineated, similar to that in activated sludge. From the obtained results, the bacteria all possessed the same inclination and general trends toward the increasing chain length of fatty acids. As a general observable trend, μ_m and K_s were lesser and greater with increasing chain lengths, respectively. The growth characteristic figures were smaller for *G. amarae* compared with the typical floc formers based on the same carbon source, particularly with the availability of readily biodegradable C_2. These results were in line with that of Contreras' findings (2000), which indicates that different genera of filamentous bacteria (*Sphaerotilus natans*) have lower values of μ_m and higher values of K_s than floc formers (*Acinetobacter anitratus*) with more biodegradable organics as the sole carbon source. The credibility of the experiment was proven and the distinctive feature of growth for filamentous and non-filamentous bacteria was determined by conducting a number of experimental cycles. Moreover, similar growth cycles were carried out with various fatty acids, which resulted in gradually varying trends.

Table 4.1 Growth profile of *G. amarae*

Fatty Acid / Unit	Acetic Acid	Lauric Acid	Myristic Acid	Palmitic Acid	Stearic Acid	Lignoceric Acid
Specific Growth Rate μ_m (h^{-1})	0.092	0.071	0.070	0.069	0.068	0. 046
Saturation Constant K_s (g/g)	0.032	0.090	0.155	0.309	1.405	1.523
Growth Yield $Y_{x/s}$ (h^{-1})	0.483	0.420	0.426	0.430	0.415	0.446

Table 4.2 Growth profile of *P. aeruginosa*

Sole Carbon / Unit	Acetic Acid	Lauric Acid	Myristic Acid	Palmitic Acid	Stearic Acid	Lignoceric Acid
Specific Growth Rate μ_m (h^{-1})	3.560	1.012	0.995	0.720	0.351	0.120
Saturation Constant K_s (g/g)	2.009	7.426	8.730	10.449	11.146	16.631
Growth Yield $Y_{x/s}$ (h^{-1})	0.658	0.104	0.095	0.074	0.039	0.010

Figure 4.11 shows the comparison between *G. amarae* and *P. aeruginosa* in terms of their specific growth rates in different stearic acid concentrations, which resulted in different F/M. The growth cycles, which were measured as the specific growth rates calculated on dry cell mass, of the two cell lines were contrasted.

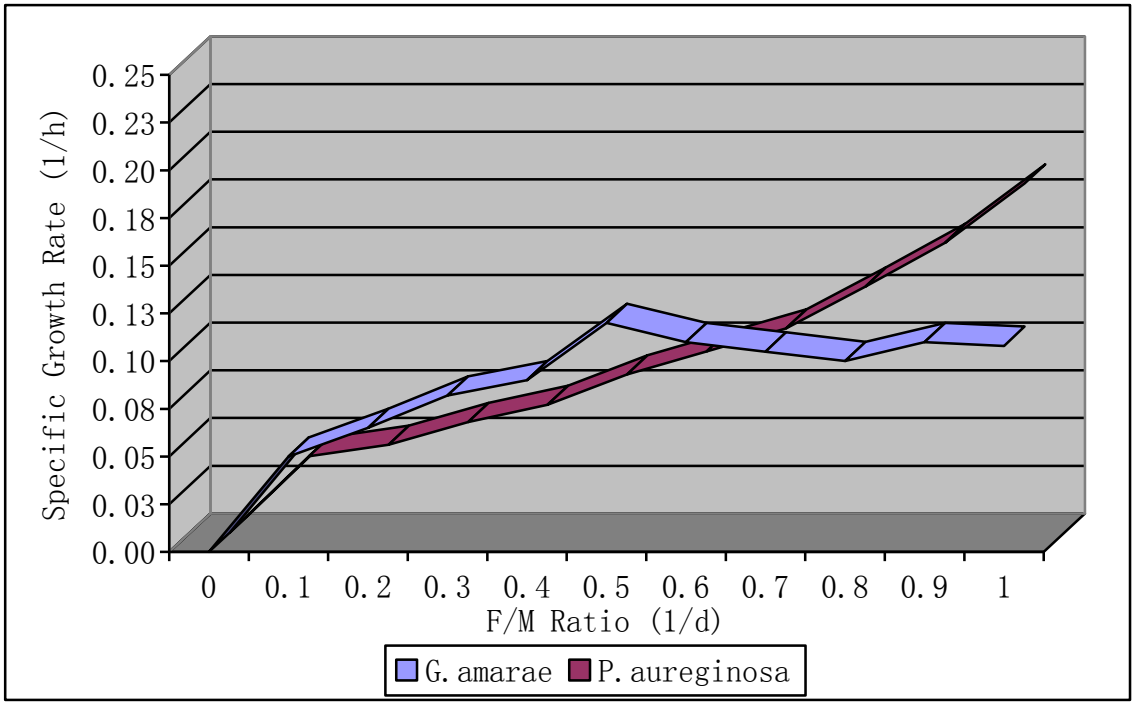

Figure 4.11 Specific growth rates at varying stearic acid concentrations

In this kinetic selection, *P. aeruginosa* growth was rapidly stimulated by increasing F/M ratio. However, in the low fatty acid concentration condition, the growth was slow. Compared with the *P. aeruginosa*, *G. amarae* growth was slow but had a better growth rate at low F/M condition. The specific growth rate of the non-filament surpassed that of the filament at an F/M range of 0.6-0.7. Therefore, *N amarae* was a K_s-strategist, whereas *P. aeruginosa* was a μ_m-strategist. These results indicate that the filament has low values of saturation constant and has a stronger affinity toward the fatty acid used as the sole carbon source. However, in better growth conditions, the non-filamentous bacteria have larger specific growth rate constants, thereby outgrowing the filaments when the culture conditions are favorable. The results shown in Figure 4.11 are in agreement with the previous findings, because an F/M ratio of 0.6 d^{-1} was the dividing line between the two bacteria. If the F/M ratio is lower than 0.6 d^{-1}, the growth of *G. amarae* will be greater than that of *P. aeruginosa*, and vice versa. Aside from the aforementioned data, filamentous bacteria were

reported to become μ_m-strategist when the RBCOD concentration is high and become K_s-strategist if only SBCOD is available (Chudoba *et al.*, 1973; Hartley *et al.*, 2008). However, in the present study, this phenomenon was not observed. The growth yield of *G. amarae* was fairly constant with decreasing amount of biodegradable acid (from 0.413 g/g to 0.487 g/g). By contrast, the growth yield of *P. aeruginosa* was higher in acetic acid but drastically decreased when exposed to long chain length fatty acid conditions (0.665 g/g to less than0.105 g/g).

The experimental data show that the substrate utilization rate of *P. aeruginosa* was not proportional to its growth in various fatty acid conditions. On the other hand, *G. amarae* shows a higher growth rate in longer chain length fatty acid conditions. Municipal wastewater contains approximately 30% long chain length fatty acids, which are produced via hydrolysis from oil and fats. In this case, the floc-forming bacteria, such as *Pseudomonas spp.*, *Flavobacterium spp.*, *Alcaligenes spp.*, *Achromobacter spp.*, and *Zoogloea spp.*, had minimal growth rates in long chain length fatty acid conditions. By contrast, a higher recalcitrant pollutant concentration favored the growth of filamentous bacteria, which resulted in filamentous foaming.

These results provide a strong proof that the growth kinetics of filaments and non-filaments in various culture conditions may be used to devise a foam control strategy. This strategy can solve the root cause of foaming problems because it is based on the underlying growth mechanisms and is expected to be more reliable and pragmatic compared with conventional foam control techniques.

4.3 Development of a Novel Control Strategy for Bulking and Foaming

Based on the growth analysis and the essential microbial kinetic data presented in Figure 4.11, the overgrowth of filaments, such as *G. amarae,* would be unavoidable if the operating condition is maintained at a continuous F/M ratio of 0.2 d^{-1} to 0.6 d^{-1} for a prolonged period of time. This overgrowth is inevitable because the aforementioned F/M range has long been proven to be the optimized operating parameters and would provide satisfactory treatment performance and process stability. Any alteration to this range to solve the foaming problems would upset the normal process operation. Based on this feature, the operation design that can alter the filament growth was examined. With the development and establishment of the novel FFO procedure, the feasting unit (the first aeration column) was used in trial experiments and operated at pre-selected SRT, influent rate, and the return sludge ratio, which resulted in a high F/M ratio of 0.89 d^{-1}. In the same manner, these key parameters in the fasting unit (the second aeration column) were maintained at a level to obtain a low F/M ratio of 0.15 d^{-1}. By applying the FFO, the F/M ratio of the unit tank varied, whereas the overall average F/M ratio of the whole treatment process was kept at a normal range, which was approximately 0.6 d^{-1}. In this manner, the overgrowth of filaments could be prevented and satisfactory results in treatment performance could be achieved at the same time. The alternating ‘Feasting’ and ‘Fasting’ causes a lack of exposure of the microbial culture to the normal F/M environment for a prolonged period, thus, filamentous overgrowth was not triggered. Meanwhile, the overall process was maintained in a normal F/M operational range to maintain a satisfactory treatment performance.

4.3.1 Performance of the Process

During conventional operation and FFO, the variations of the F/M ratio and MLSS of the activated sludge simulator are shown in Figures 4.12 and 4.13, respectively. In the FFO system start up phase, which was the normal running phase before eventually arriving at the stable phase, the F/M smoothly increased from 0.22 d^{-1} to 0.56 d^{-1} within 90 days. Given that the BOD of the influent was constant, the F/M was changed by adjusting the MLSS from 5800 mg/l to 2100 mg/l to achieve a stable status. Figure demonstrates that the two aeration columns show a similar tendency in the increase in F/M and the decrease in MLSS level in normal operational conditions. However, starting from Day 91, the two aeration columns were run into two different routes after switching the FFO. In the feasting and fasting tanks, the F/M ratios were controlled by appropriately adjusting the rates of inflow rate and RAS. In the feasting tank, the inflow rate and the RAS rate were 0.36 l/h and 0.062 l/h, respectively. Thus, MLSS of 2000 mg/l and F/M of 0.89 d^{-1} were achieved. On the other hand, the inflow rate and the RAS rate of the fasting tank were 0.24 l/h and 0.21 l/h, respectively, to maintain MLSS of 5800 mg/l and F/M of 0.15 d^{-1}, respectively.

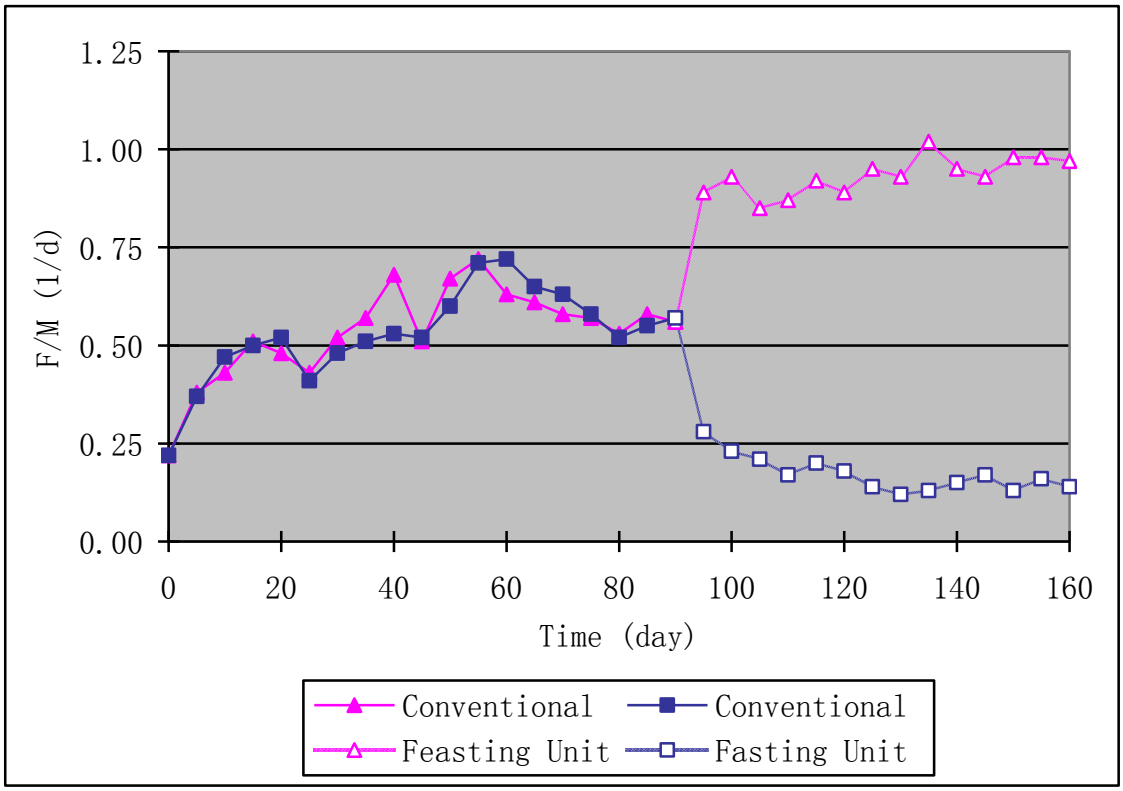

Figure 4.12 F/M ratio during conventional operation and FFO

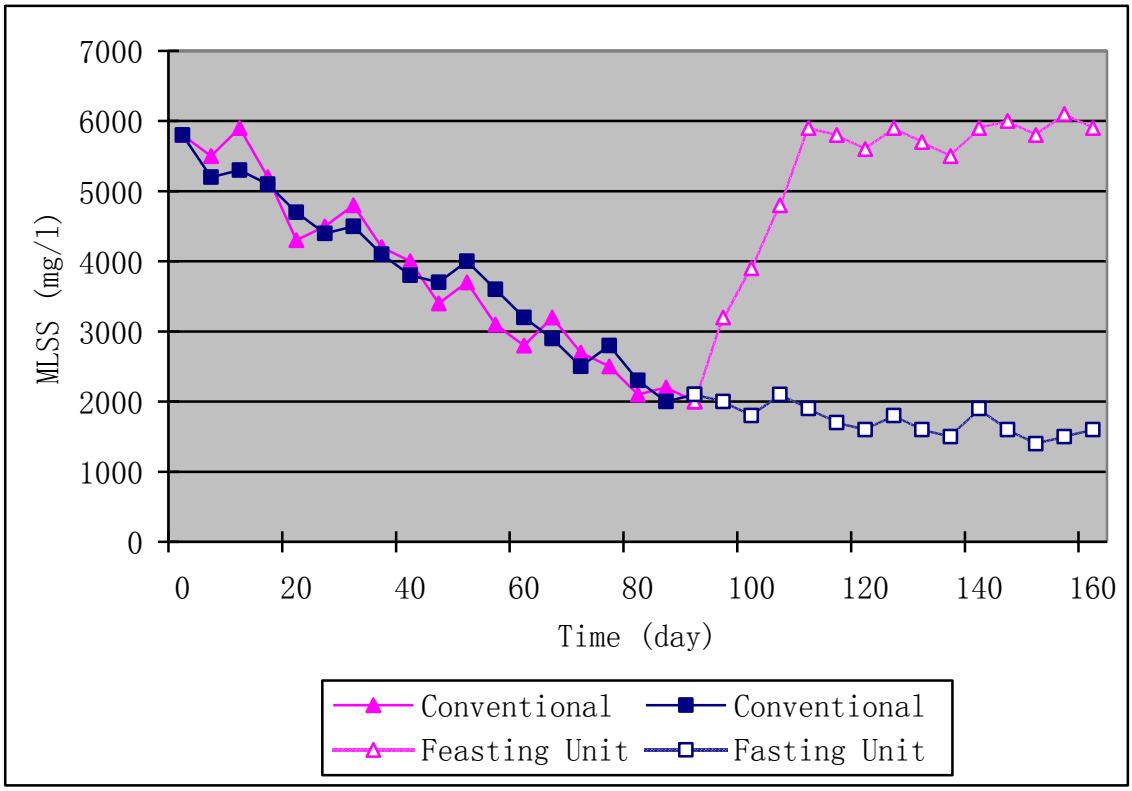

Figure 4.13 MLSS during conventional operation and FFO

The treatment performance, which is affected by the dominant filaments, could be observed by increasing the SVI (Tsang *et al.*, 2007; Jenkins *et al.*, 2004). As shown in Figures 4.14 and 4.15, the SVI increased from 65 ml/g to 310 ml/g in the start-up phase, which resulted in severe bulking and rising sludge, indicating that the dominant filaments affected the sludge settling performance. In the 91-day start-up phase, F/M was continuously set to 0.56 d^{-1}, which was the favorable condition for filament overgrowth. This phenomenon resulted in severe foaming and bulking in subsequent biological unit processes and physical unit operations, respectively.

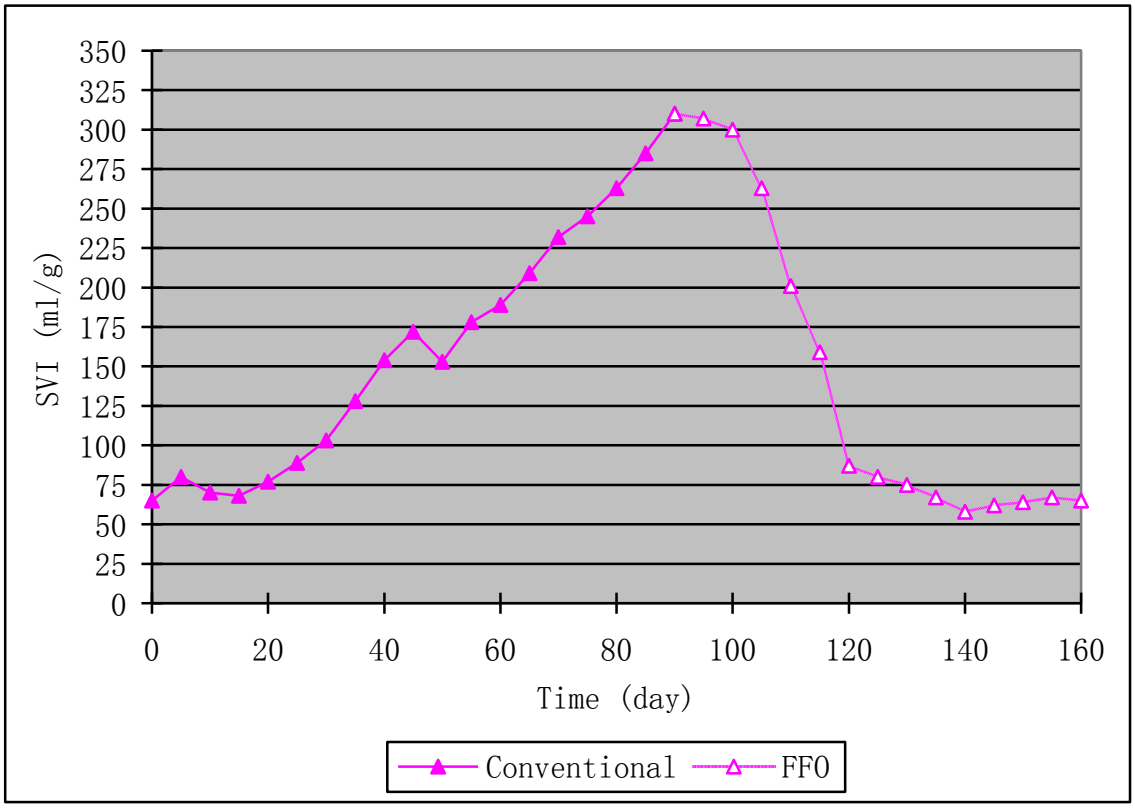

Figure 4.14 SVI during conventional operation and FFO

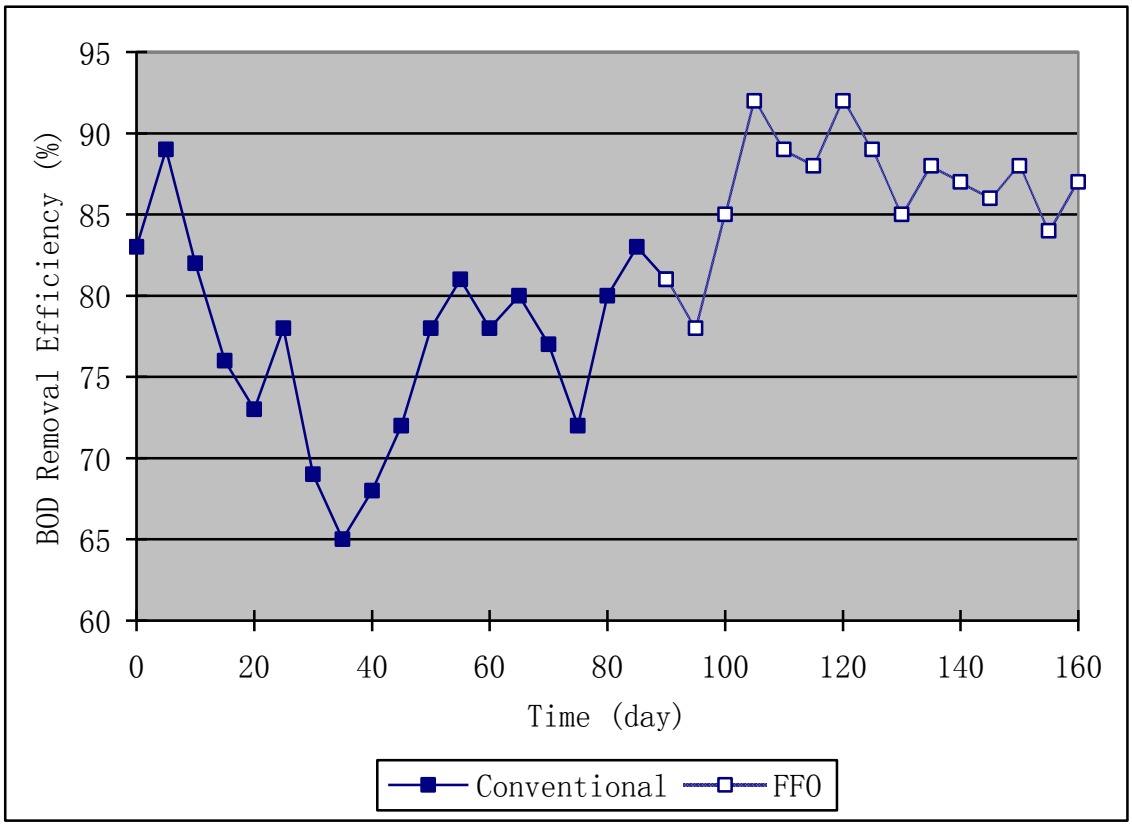

Figure 4.15 BOD removal efficiency during conventional operation and FFO

Plate 4.3 shows the excessive overgrowth of *G. amarae*, which is distinct in branched filamentous cellular structures, and among the foaming activated sludge in the conventional culture conditions in the FFO simulator. This result is in agreement with previous findings, and the overgrowth and dominance of filamentous *G. amarae* occurred at the F/M of 0.2 d^{-1} to 0.6 d^{-1}.

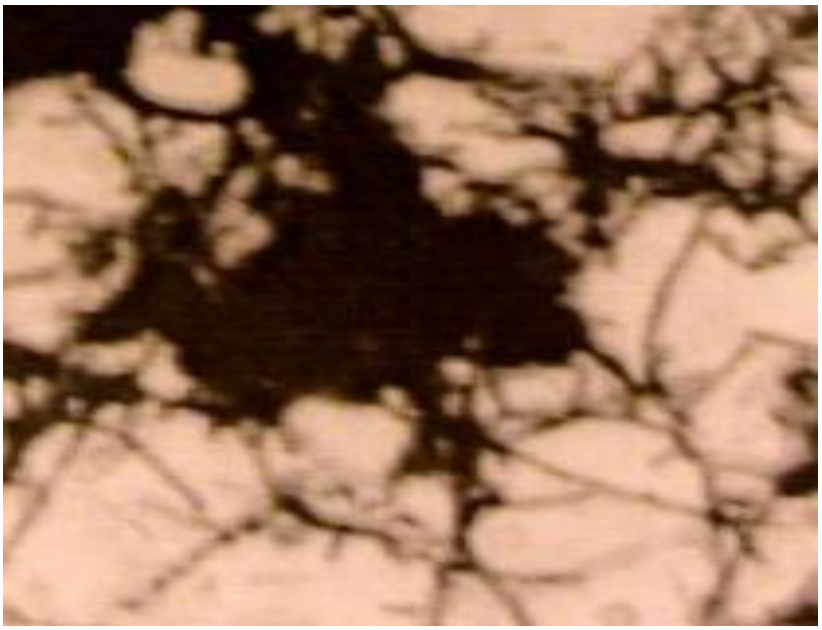

Plate 4.3 Filamentous dominance in the activated sludge (400X magnification)

These aforementioned results were obtained from the FFO simulator in conventional operation and were used as the basis for comparison. After the 91-day normal operation of the start-up phase, the system operating conditions were modified to achieve the feasting and fasting alternative feeding operation in the novel FFO. Figure 4.14 shows that the first 30-day FFO operation resulted in a quick increase of the sludge settleability to a satisfactory status. The SVI decreased to approximately 87 ml/g and was further stabilized at 60 ml/g. The results are in agreement with the findings from the study carried out by Chua *et al.* (1996) and Hartley *et al.* (2008). At a high F/M, the growth of the filamentous bacteria was weaker than that of floc formers, which is in line with the previous growth kinetic studies. The SVI pattern also confirmed that the FFO successfully suppressed filamentous overgrowth and improved sludge settleability resulting in high quality of the treated effluent.

The settled sludge from the sedimentation tank could be randomly recycled between the feasting and the fasting units using the FFO configuration. This process prevents

the prolonged exposure of the sludge to a consistently low F/M, which is between 0.2 d^{-1} and 0.4 d^{-1}. The filaments cannot adapt to a suitable growth condition, which results in foaming in the activated sludge. Conversely, the growth of the floc former is suitable in increasing its predominance in the ecological system and in functioning as the original support of the filamentous growth. A healthy distribution of bacterial species and an appropriate interspecies population ratio resulted in the effective establishment of good bacterial floc in the activated sludge, which would facilitate settling and avoid foaming.

The BOD removal efficiency was also not significantly altered during the whole operation of the novel FFO process when it was switched from the conventional operation to the specifically designed foaming-arresting FFO procedure. The residual BOD_5 of the treated effluent was maintained at approximately 26 mg/l to achieve a consistent BOD removal efficiency of 90%. The FFO rendered a relatively high F/M in the feasting unit and a relatively low F/M in the fasting unit, which suppressed the excessive growth of filamentous bacteria. Moreover, the FFO allowed the F/Ms remain within the normal working range, which does not cause any adverse effect on organic biodegradation and process stability. Therefore, we conclude that the novel FFO procedure is a superior method for the prevention and control of filamentous overgrowth, foaming, and bulking problems. In addition, the FFO does not possess the disadvantages of other conventional techniques, and requires costly chemical dosing, and hardware construction (e.g. selectors). This process control is relatively simple and normal sewage treatment efficiency is not substantially affected while controlling foaming problems.

Chapter 5: Combination of FFO and SBR system

The COD concentration in the effluent from the SBR system should be lower than 100 mg/l. However, in this study, the COD concentration in the effluent from the paper mill was higher than the discharge standard before the SBR process was optimized. Several operating problems that were encountered include the overgrowth of filamentous bacteria and poor sludge settling, which led to high SVI value of 260 ml/g.

5.1 System Performances on Different MLSS Concentrations

MLSS concentration was studied because it is one of the most important operating parameters. The tanks were set at 3000 mg/l at the first stage. After the system reached a stable stage, the MLSS was changed to 3500 mg/l. The MLSS was increased by 500 mg/l, for each stage, which resulted in an MLSS value of 5000 mg/l in the fifth stage. During the experiment, the average influent COD concentration was approximately 1264.8 ± 102.3 mg/l (n = 17). Figure 5.1 shows the COD removal efficiencies at different MLSS conditions.

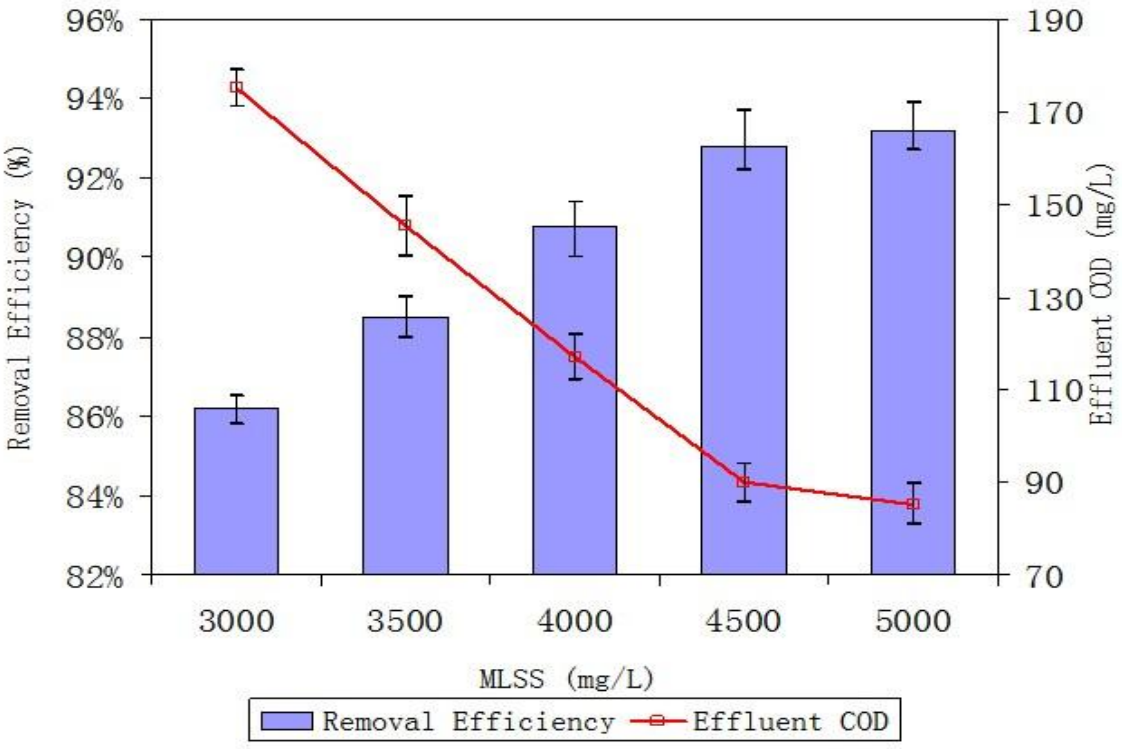

Figure 5.1 COD removal efficiencies and effluent COD concentrations under various MLSS

Figure 5.1 shows that the COD removal efficiencies in the SBR system were improved as the MLSS concentration was changed from 3000 mg/l to 5000mg/l with confidence level of 95%. Along with the COD removal efficiencies, the average effluent COD concentrations decreased from 175.2 mg/l (MLSS = 3000 mg/l) to 85.3 mg/L (MLSS = 5000 mg/l). The results show that the COD removal rates increased when the MLSS concentration was changed. These results are in agreement with the findings from other studies (Tsang *et al*., 2007; Wanner and Grau, 1998). However, the results of t-test show that there is no significant difference of removal rate at MLSS concentrations of 5000 mg/l and 4500 mg/l ($p>0.05$). This result is in agreement with Figure 5.1, which shows that no significantly improved effects could be achieved when the MLSS level was higher than 4500 mg/l. Furthermore, a higher suspended solids content in the effluent, a longer settling time and aeration time would be required if a higher MLSS concentration was introduced in the reaction.

The effluent COD level was reduced to 89.9 mg/l when the MLSS level reached 4500 mg/l (Figure 5.1 and Table 5.1). Compared with the unsatisfactory effluent quality at MLSS concentrations between 3000 mg/l and 4000 mg/l, testing at an MLSS concentration of 4500 mg/l was necessary. Compared with the high effluent quality at MLSS concentration of 5000 mg/l, the effluent quality at 4500 mg/l was satisfactory. According to the discharge standard requirement, the MLSS concentration at 4500 mg/l was optimum.

Table 5.1 Average effluent COD results at different operating conditions

Item	Condition	Effluent COD conc. (mg/l)	Satisfactory with discharge standard	Item	Condition	Effluent COD conc. (mg/l)	Satisfactory with discharge standard
MLSS	3000 mg/l	175.2	×	Cycle	3	94.7	√
	3500 mg/l	145.4	×		2	90.9	√
	4000 mg/l	117.0	×		1	89.1	√
	4500 mg/l	89.9	√	Aeration	6 hr	82.4	√
	5000 mg/l	85.3	√		5 hr	91.0	√
					4 hr	101.1	×
VER	37.5%	83.3	√		3 hr	123.0	×
	50%	90.3	√	Temp.	25℃	92.1	√
	62.5%	112.0	×		30℃	82.8	√
	75%	125.0	×		35℃	89.2	√

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5.2 System Performances on Different VERs

Wilderer *et al*. (2001) described the VER as the treatment volume of wastewater fed to the reactor. The VER value indicates the treatment capacity of the system. Martins *et al.* (2004) stated that a high VER value would be beneficial for filamentous foaming control because the organic concentration has a high disparity in the filling period. The SBR were operated at VERs of 37.5%, 50%, 62.5% and 75% to determine the optimal parameter based on the effluent COD concentration and removal efficiency. During the experimental period, the average COD influent concentration was 1196 ± 108.6 mg/l (n = 16).

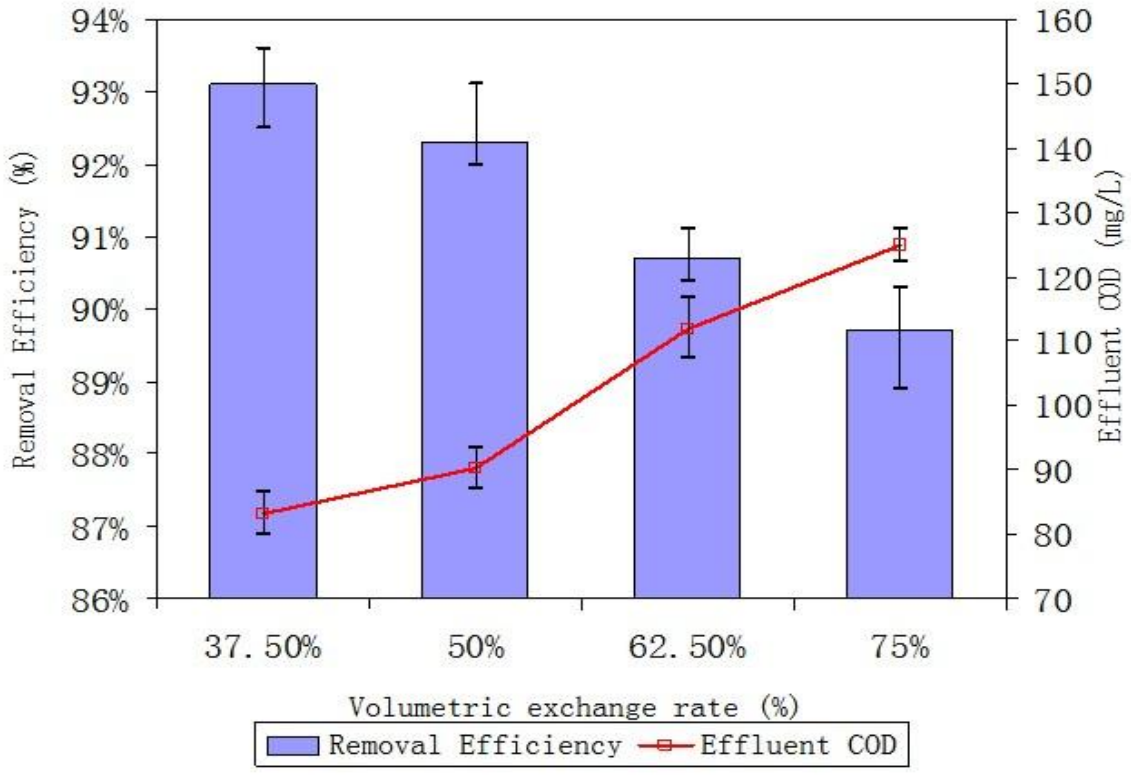

Figure 5.2 COD removal efficiencies and effluent COD concentrations at various VERs

Figure 5.2 shows that the increasing VER values obviously induced the decrease in COD removal rates and the increase in effluent COD level ($p<0.05$). The COD removal rate decreased from 93% to 89.5% because the VER was changed from 37.5% to 75%, which resulted in the increase in the effluent COD level to 125.01 mg/l from 83.25 mg/l. Therefore, the effluent COD concentration did not meet the standard if the VER reached up to 62.5% even if the removal rate was kept above 89%. At high VER values, the organic amount in the influent was high and exceeded the treatment capacity of the microorganisms in the mixed liquor. The F/M ratio reached up to 0.574 d^{-1} at a VER of 75% and 0.483 d^{-1} at 62.5%. Longer aeration time and higher MLSS concentration were required to treat the excess organic content so that the effluent quality would not deteriorate. Therefore, in the same comparison condition, a VER value higher than 62.5% would result in a poor effluent quality. Considering the stable performance and treatment capacity, a VER of 50% would be considered optimum.

5.3 System Performances on Different Aeration Periods

Aeration time is an important factor for aerobic wastewater treatment process and sequencing batch reactor (Qin *et al.*, 2004). Given that the aeration time is closely related to DO concentration, microbial growth, and operation cost, it should be neither too long nor too short. The SBR system was operated at aeration time between 3 and 4, and between 5 and 6 h to determine the optimal time based on the COD removal efficiency and effluent quality. The average influent COD concentration was approximately 1175.2 $\pm$ 116.4 mg/l (n = 19).

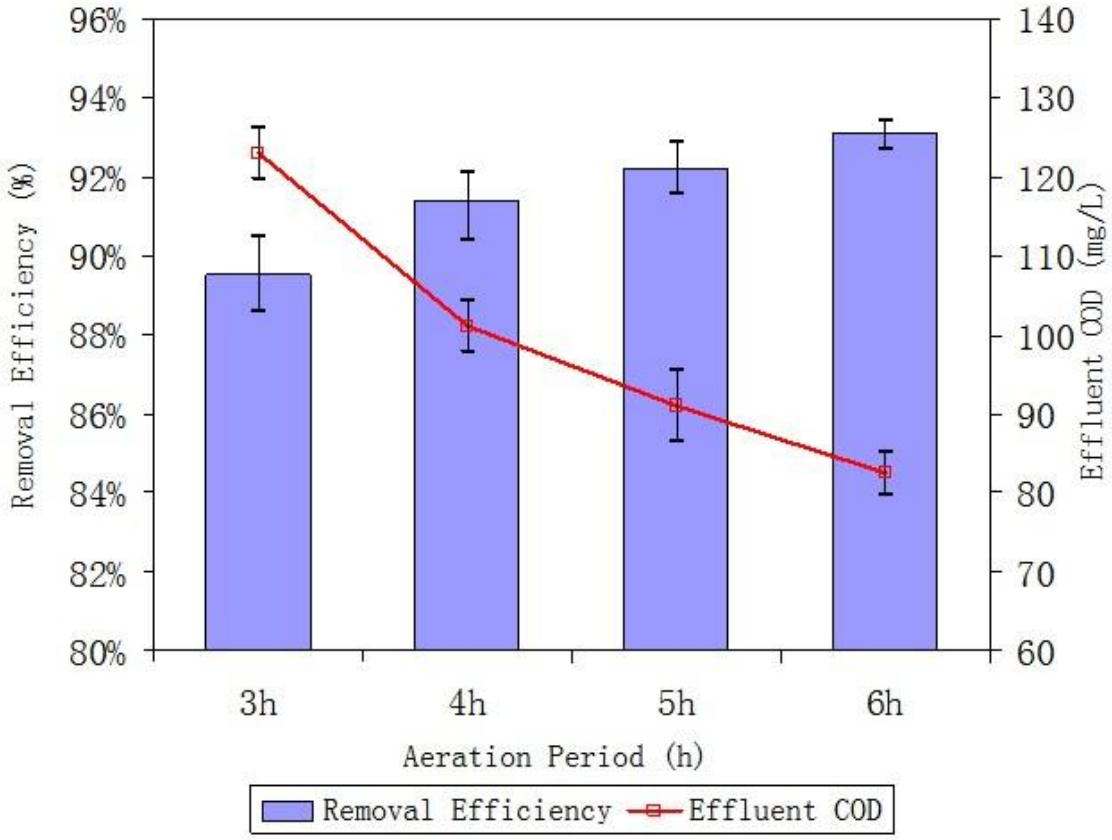

Figure 5.3 COD removal efficiencies and effluent COD concentrations at various aeration periods

Figure 5.3 shows that the COD removal rates increased with increasing aeration time, thereby improving treatment performance ($p<0.05$). The average effluent COD level of 123.0 mg/l at an aeration time of 3 h was obviously decreased to 82.4 mg/l at 6 hr. These results show that the COD removal rate was low and the effluent COD level exceeded the discharge standard at an aeration time of 3 h. When the aeration time reached 4 h, the results show the effluent quality was improved and met the discharge standard. A higher aeration time resulted in a more complete consumption of the organic matter and a higher removal rate. However, the high operation cost for the longer aeration time does not make the limitless increase possible. If the aeration time was too long, the activated sludge would undergo an endogenous respiration period and energy would be wasted for aeration. In addition, the COD removal efficiency

had insignificant differences at aeration times of 4 and 5 h, which indicates that the average effluent COD concentration decreased to 98 and 89 mg/l, respectively. Considering both effluent quality and operation cost, the optimum aeration time was 4 h.

5.4 System Performances on Different Temperatures

Temperature is one of important factors not only in microbial metabolic activities in reaction but also in the settleability of sludge and gas transfer rates. When the temperature was increased, rates of biochemical reactions and mass transfer processes also increased. Therefore, several influential factors were considered to achieve an optimal temperature for SBR. For example, in the mixed liquor, an increase in temperature results in a decrease in the solubility of oxygen, which induces a lower degradation rate for the aerobic microorganisms. Furthermore, during the settling stage of SBR, with higher temperature, the metabolic rate of microorganisms are higher. The sludge is difficult to settle and the SVI is higher. The average influent COD concentration was 1213.9 $\pm$ 107.7 mg/l (n = 19).

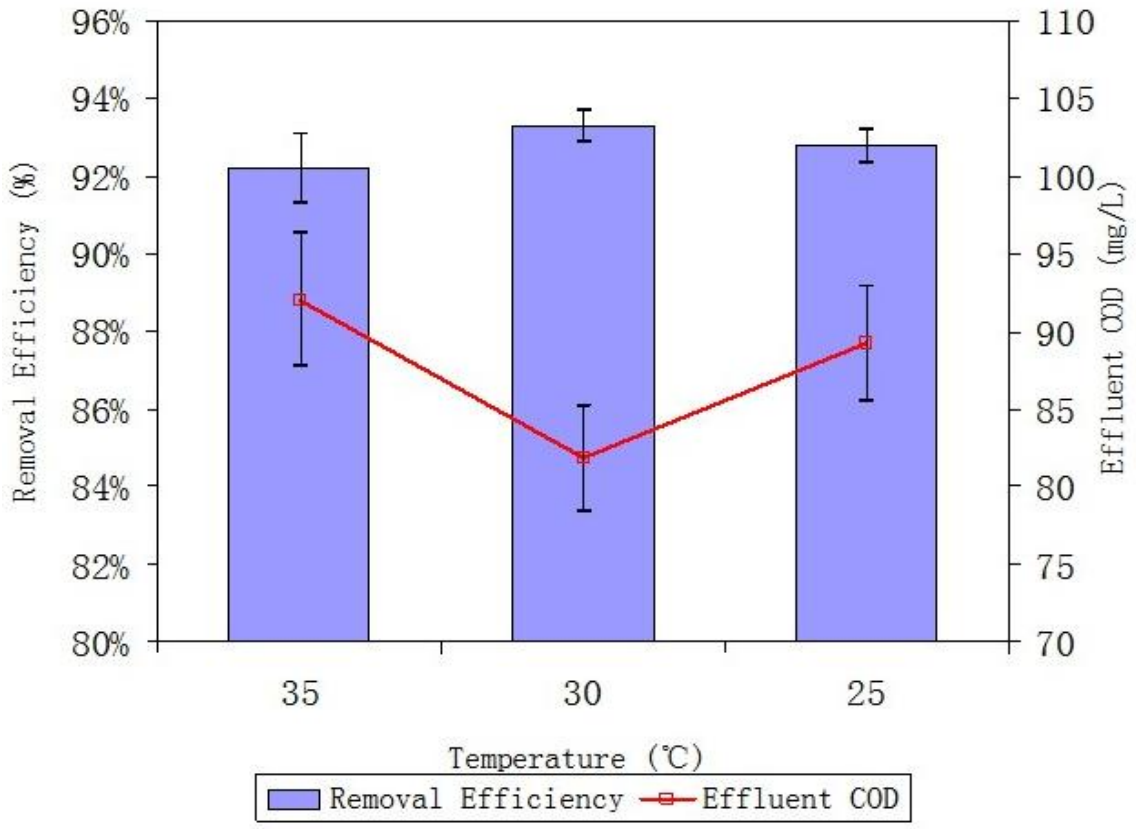

Figure 5.4 COD removal efficiencies and effluent COD concentrations at various temperatures

Figure 5.4 shows that no significant difference in the removal rates at temperatures of 25℃, 30℃ and 35℃ was observed ($p > 0.05$). The average effluent COD levels fluctuated within 82.8 and 92.1 mg/l and the lowest value obtained was 82.1 mg/l. Despite the small significance, these results all met the discharge standard. The temperature also affected the activated sludge settling characteristics and the gas transfer rates. Figure 5.5 shows the performance of SVI at temperatures of 25℃, 30℃ and 35℃.

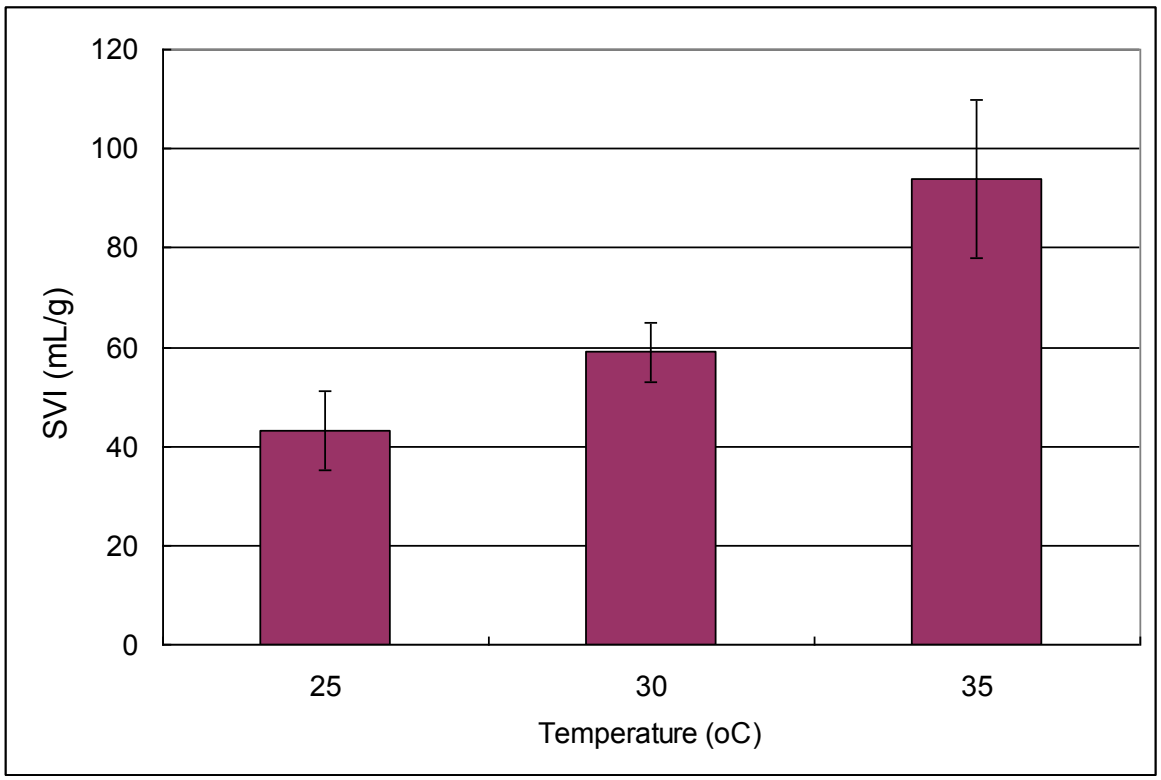

Figure 5.5 Results of SVI under different operating temperatures

Figure 5.5 shows that the SVI had obvious differences at different temperatures. Although both effluent quality and removal rates were satisfactory in the three temperatures, a high temperature led to a high SVI, resulting in the low sludge settleability. Furthermore, the growth of microorganisms at a high temperature was unstable leading to fluctuating SVI values. At higher temperatures, the flocculation of bacteria was looser, which resulted in higher SS and COD levels in the effluent (Hartley *et al.*, 2008). Considering the effluent quality and the sludge settleability, the optimum operating temperature was 30 ℃.

5.5 System Performances on Different Daily Operating Cycles

The system was operated at 1, 2, and 3 cycles per day to determine the optimum COD removal rate and effluent quality. Figure 5.6 shows the average COD removal

efficiencies and the effluent COD concentrations at different daily operating cycles.

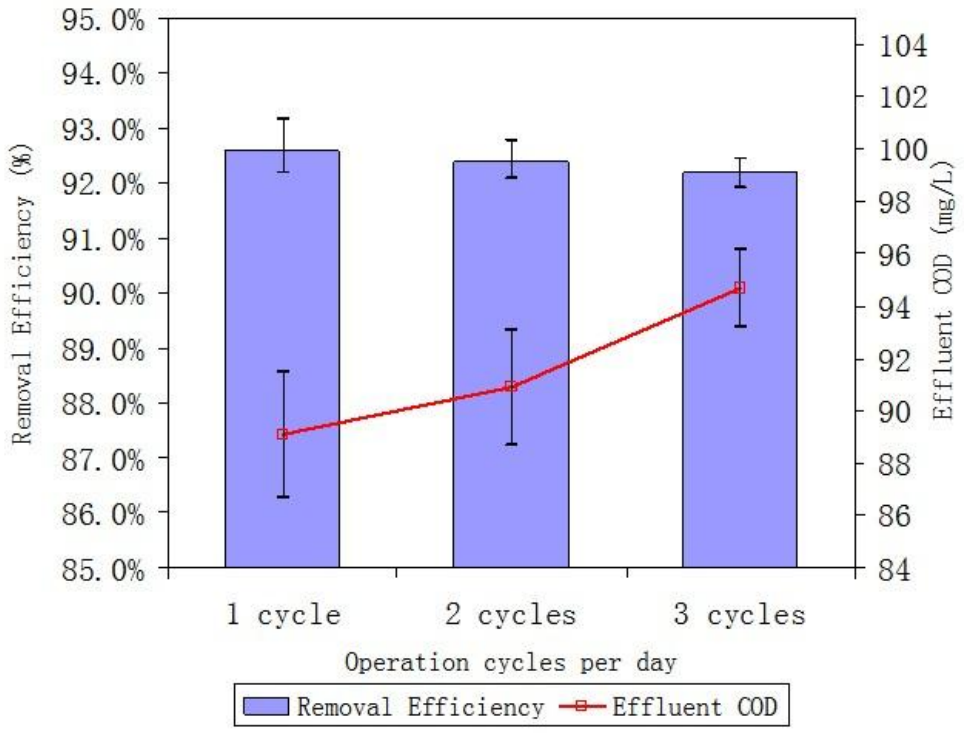

Figure 5.6 COD removal efficiencies at different daily operating cycles

During the experiment, the average influent COD concentration was 1233±111.6 mg/l (n = 19). There is no significant difference in the removal rates between 1 and 2 cycles ($p > 0.05$) (Figure 5.6). However, the treatment performance of 2 cycles per day operation was obviously better than that of 3 cycles per day ($p < 0.05$). The result also shows that different idle periods may affect the treatment performance in SBR. Idling time is related to the number of cycles per day. In SBR operation the idling period allows the microorganisms to digest the adsorbed organic matters. The daily operating cycles could also be calculated from the idling time in each cycle. Based on 0.2 h feeding, 5 h reaction, 0.5 h settling, 0.3 h decanting time, 1 daily operation cycle represented 18 h of idling time. Similarly, 2 and 3 daily cycle stood for 6 and 2 h of idling time, respectively. Consequently, judging on the average effluent COD concentrations, the 2 daily operation cycles was the optimum condition.

5.6 System Performance on Optimal Operating Conditions

Filamentous foaming is one of the most persistent problems in activated sludge process, especially in the treatment of paper mill wastewater (Gaval and Pernelle, 2003; Pipes, 1979). The problem of filamentous foaming was severe in the SBR under conventional operation in treating the paper mill effluent in the present study. Based on the finding from previous sections, the performance of SBR in the FFO mode was satisfactory in both the removal rate and the effluent quality. This finding shows that the optimal operation conditions including MLSS, aeration time, daily operating cycles, VER and temperature, were 4500 mg/l, 4 h, 2 cycles, 50%, and 30 ℃**,** respectively. Based on the results in this experiment, the effluent quality met the discharge standard and showed a stable performance with the optimal parameters. Given the good performance, these parameters should be considered as the optimal operating conditions in treating paper mill wastewater. Moreover, no bulking problem was observed and the SVI was kept within the normal range.

Chapter 6: Conclusions

The communal municipal sewage treatment plants in Hong Kong have been extensively surveyed and were found to have severe foaming problems. Foaming problems in the activated sludge and the associated sludge bulking problems in the secondary sedimentary tanks have continuously led to plant operating and control problems and have adversely affected the process efficiency. Furthermore, plant safety and public health concerns make effective foaming and bulking control an urgent issue.

A series of in-depth investigations were designed to overcome the limitations in conventional foam control techniques. The predominant foaming and bulking problems were identified to be mainly caused by the overgrowth of a specific filamentous bacterium, which is the predominant microbial species in the biological wastewater treatment system. The predominant filamentous bacterial genus (*G. amarae*), which is a recently discovered genus, was identified by morphological and physiological characteristics. A number of bacterial physiological and morphological evidences were used to identify the specific genus. This species is closely related to the previously identified *N. amarae* species.

The growth kinetics of *G. amarae* and *P. aeruginosa* were investigated and used as the theoretical basis for the subsequent study of designing an operational strategy for filamentous foaming preventive control. The kinetic study of bacterial growth indicates that F/M, one of the important operational parameters, was critical in *G. amarae* growth. The results from the microbial kinetic studies show that *G. amarae* has a high affinity for slowly

biodegradable COD (SBCOD), in long chain length fatty acid conditions. Based on the specific findings, Feast-Fast Operation (FFO), an operational strategy, was developed to control the filamentous foaming. The use of FFO enables the F/M to be controlled at two distinguished extreme values. However, at a normal average value, the growth of filamentous bacteria is suppressed. Thus, the foaming problem can be prevented.

After a series of studies, *G. amarae* were morphologically and physiologically identified as the predominant bacteria, which caused filamentous foaming and bulking. The mechanisms and the growing conditions were elucidated. FFO, a novel and laboratory-proven control method, was developed and its treatment performance was verified. Based on the FFO result, the SBR-activated sludge simulator system combined with the FFO was used to treat paper mill wastewater. In optimal operating conditions, the SVI was successfully reduced and the COD removal efficiency was maintained at a satisfactory level.

6.1 Predominant Bacteria Causing Filamentous Foaming

By analyzing the bulking activated sludge from a local wastewater treatment plant, the generation time of the isolated bacteria cultured in a shaking flask at 28 ℃ was 10.5 h. However, the bacteria were denatured after 8 h of incubation in a heat resistance experiment at 50 ℃. Based on the morphological and physiological characteristics, the isolated microorganism was *G. amarae*, which was the major foaming causative germ in municipal wastewater treatment plants in Hong Kong (Blackall *et al.*, 1988; 2002; Hartley *et al.*, 2008).

6.2 Different Growing Conditions and Behaviors

Based on the findings of kinetic experiments performed using pure cultures of *G. amarae* and *P. aeruginosa* (typical floc-forming bacteria), *G. amarae* had lower saturation constants and smaller maximum specific growth rates, and can utilize all fatty acids with different chain lengths for growth. *G. amarae* was competitive in the conditions that had low concentration of fatty acids. Compared with *G. amarae, P. aeruginosa* could hardly grow in pure culture containing fatty acids with 12 or more carbons. Therefore, *G. amarae* was one of the competitive bacteria commonly found in wastewater containing high recalcitrant pollutants.

6.3 FFO Control Strategy

Pure cultures of *G. amarae* and *P. aeruginosa* were studied at different F/M ratios. The results indicate that *G. amarae* had a strong affinity toward the long chain organic compounds and was able to survive in low organics conditions. By contrast, the growth of *P. aeruginosa* was faster but relied on a high concentration of readily biodegradable COD (RBCOD). *G. amarae* growth was predominant in F/M values lower than 0.7 d^{-1}, which resulted in filamentous foaming. Based on the findings of growth kinetics and inter-species interaction, a reliable control strategy, called FFO, was developed to minimize bulking and foaming sludge problems and to attain satisfactory treatment performance. By applying the feasting (F/M ratio of 0.89 d^{-1}) and fasting (F/M ratio of 0.15 d^{-1}) operation, the system achieved a satisfactory SVI of 60 ml/g without deteriorating the effluent quality.

6.4 Laboratory-scale study on SBR with FFO

The combination of FFO and a lab-scale SBR system was used to treat the paper industry wastewater and to investigate the feasibility of FFO application in practice. After optimizing the MLSS, aeration time, VER, temperature, and daily operating cycles during fourth month, the SBR in the FFO mode appeared stable and reliable. The effluent quality and removal rates were greatly improved. The optimal conditions, such as MLSS (4500 mg/l), aeration time (4 h), VER (50%), daily operating cycles (2 cycles) and temperature (30 ℃) resulted in an excellent performance in terms of effluent quality and removal rate as well as the healthy SVI. The effluent COD level met the local discharge standard. A healthy SVI indicates that no filamentous foaming occurred under the optimal conditions. This study showed that FFO could be applied in the SBR process and provided reference operating conditions for filamentous foaming control in treating wastewater from paper making industry.

In summary, this study confirmed that *G. amarae*, also known as *N. amarae*, was the major causal microorganism in the foaming sludge. In addition, *G. amarae* utilized commonly found fatty acids in sewage for growth. *G. amarae* was compared with non-filamentous *P. aeruginosa* by using fatty acids (C_2 - C_{24}) as the sole carbon source. This species had a much lower saturation constant (K_s=1.520 g-COD/l), indicating a stronger affinity toward grease-laden sewage, and a lower maximum specific growth rate (μ_m = 0.048 h^{-1}) than *P. aeruginosa* on C_{24}. Thus *G. amarae* had a relatively higher affinity for RBCOD and *G. amarae* was able to consume fatty acids with different chain lengths with a constant growth yield (0.413 - 0.487 g/g-COD). In treatment system with an F/M lower than 0.7 d^{-1}, the specific growth rate of *G. amarae* was found to be predominant compared with floc-foaming bacteria. Based on this finding, FFO, an operational strategy, was developed to prevent and control

foaming. The F/M ratios of feasting and fasting units were 0.89 and 0.15 d^{-1}, respectively, but the average F/M ratio was carefully maintained within a normal level in the overall process to avoid favorable growing conditions for *G. amarae*. The combination of SBR and FFO also shows satisfactory results. The results indicate that the FFO is a reliable and feasible operation method in the SBR system in treating wastewater from pulp and paper making industry for control of filamentous foaming.

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Appendices

Raw data of Figure 4.5: Growth yield in fatty acids as sole carbon source

Growth Yield (gDCM/gCOD)	Nonanoic acid (C9)	Undecanoic acid (C11)	Palmitic acid (C16)	Stearic acid (C18)
Trial 1	0.137	0.185	0.146	0.188
Trial 2	0.131	0.173	0.123	0.163
Trial 3	0.128	0.164	0.148	0.186
Mean	0.132	0.174	0.139	0.179
S.D.	0.005	0.011	0.014	0.014

Raw data of Figure 4.6: The growth of G.amarae with C8, C9, C14, C18 in sewage

		Time (h)						
Dry Cell Mass (g/L)		20	40	60	80	100	120	140
C18	Trial 1	0.15	0.45	0.56	0.44	0.55	0.48	0.68
	Trial 2	0.11	0.51	0.51	0.58	0.43	0.55	0.53
	Trial 3	0.22	0.3	0.37	0.63	0.73	0.65	0.47
	Mean	0.16	0.42	0.48	0.55	0.57	0.56	0.56
	S.D.	0.06	0.11	0.10	0.10	0.15	0.09	0.11
C14	Trial 1	0.19	0.45	0.65	0.66	0.93	0.86	0.66
	Trial 2	0.13	0.33	0.63	0.89	0.55	0.55	0.69
	Trial 3	0.19	0.72	0.28	0.64	0.77	0.78	0.81
	Mean	0.17	0.5	0.52	0.73	0.75	0.73	0.72
	S.D.	0.03	0.20	0.21	0.14	0.19	0.16	0.08
C9	Trial 1	0.23	0.43	0.72	1.15	0.78	1.05	1.13
	Trial 2	0.21	0.66	0.86	1.04	0.96	1.16	1.02
	Trial 3	0.1	0.41	0.76	0.66	1.5	1.06	1.06
	Mean	0.18	0.5	0.78	0.95	1.08	1.09	1.07
	S.D.	0.07	0.14	0.07	0.26	0.37	0.06	0.06
C8	Trial 1	0.11	0.66	1.21	1.43	1.13	1.25	1.34
	Trial 2	0.23	0.38	1.1	1.26	1.43	1.31	1.11
	Trial 3	0.2	0.49	0.75	1.06	1.28	1.07	1.15
	Mean	0.18	0.51	1.02	1.25	1.28	1.21	1.2
	S.D.	0.06	0.14	0.24	0.19	0.15	0.12	0.12

Raw data of Figure 4.7: Effect of fatty acid (Stearic acid, C18) concentration on foaming and stability

Fatty Acid Conc. (mg/L)	Foam Vol. (mL)	Reaction Time (s)						
		0	20	40	60	80	100	120
0	Trial 1	0	20	18	22	0	0	0
	Trial 2	0	21	21	21	0	0	0
	Trial 3	0	13	21	17	0	0	0
	Mean	0	18	20	20	0	0	0
	S.D.	0	4	2	3	0	0	0
0.5	Trial 1	0	21	35	36	19	0	0
	Trial 2	0	32	21	33	11	0	0
	Trial 3	0	25	34	21	12	0	0
	Mean	0	26	30	30	14	0	0
	S.D.	0	6	8	8	4	0	0
1.0	Trial 1	0	41	45	48	36	16	7
	Trial 2	0	31	36	41	34	21	5
	Trial 3	0	24	36	37	29	17	9
	Mean	0	32	39	42	33	18	7
	S.D.	0	9	5	6	4	3	2
1.5	Trial 1	0	45	63	46	69	36	19
	Trial 2	0	31	65	71	54	31	24
	Trial 3	0	53	46	69	42	14	26
	Mean	0	43	58	62	55	27	23
	S.D.	0	11	10	14	14	12	4

Raw data of Figure 4.8: Effects of concentration of G. amarae on foaming and stability

G. amarae Conc. (mg/L)	Foam Vol. (mL)	Reaction Time (s)						
		0	20	40	60	80	100	120
0	Trial 1	0	19	21	25	0	0	0
	Trial 2	0	13	16	17	0	0	0
	Trial 3	0	13	18	21	0	0	0
	Mean	0	15	19	21	0	0	0
	S.D.	0	3	3	4	0	0	0
0.5	Trial 1	0	33	31	46	16	0	0
	Trial 2	0	26	45	40	25	0	0
	Trial 3	0	22	41	43	25	0	0
	Mean	0	27	39	43	22	0	0
	S.D.	0	6	7	3	5	0	0
1.0	Trial 1	0	36	63	71	55	8	6
	Trial 2	0	43	47	43	37	5	9
	Trial 3	0	26	46	60	31	5	9
	Mean	0	35	52	58	41	6	8
	S.D.	0	9	10	14	12	2	2
1.5	Trial 1	0	35	67	69	56	21	9
	Trial 2	0	24	54	54	36	15	4
	Trial 3	0	37	56	66	43	24	11
	Mean	0	32	59	63	45	20	8
	S.D.	0	7	7	8	10	5	4

Raw data of Figure 4.9: Growth of G. amarae in C24, C18, C16, C14, C12, C2 condition

Dry cell mass (mg/L)		Reaction Time (h)						
		0	20	40	60	80	100	120
C24	Trial 1	0.043	0.031	0.051	0.046	0.045	0.053	0.05
	Trial 2	0.021	0.037	0.017	0.028	0.038	0.048	0.02
	Trial 3	0.041	0.037	0.04	0.037	0.043	0.028	0.05
	Mean	0.035	0.035	0.036	0.037	0.042	0.043	0.04
	S.D.	0.012	0.003	0.017	0.009	0.004	0.013	0.017
C18	Trial 1	0.038	0.028	0.016	0.049	0.043	0.115	0.096
	Trial 2	0.027	0.043	0.043	0.022	0.061	0.085	0.055
	Trial 3	0.04	0.037	0.055	0.073	0.07	0.091	0.104
	Mean	0.035	0.036	0.038	0.048	0.058	0.097	0.085
	S.D.	0.007	0.008	0.020	0.026	0.014	0.016	0.026
C16	Trial 1	0.043	0.046	0.06	0.066	0.069	0.123	0.086
	Trial 2	0.026	0.025	0.01	0.043	0.078	0.089	0.079
	Trial 3	0.036	0.04	0.05	0.047	0.042	0.082	0.138
	Mean	0.035	0.037	0.04	0.052	0.063	0.098	0.101
	S.D.	0.009	0.011	0.026	0.012	0.019	0.022	0.032
C14	Trial 1	0.022	0.031	0.066	0.11	0.067	0.211	0.12
	Trial 2	0.028	0.047	0.074	0.03	0.084	0.143	0.055
	Trial 3	0.055	0.036	0.016	0.1	0.083	0.057	0.239
	Mean	0.035	0.038	0.052	0.08	0.078	0.137	0.138
	S.D.	0.018	0.008	0.031	0.044	0.010	0.077	0.093
C12	Trial 1	0.043	0.024	0.066	0.06	0.085	0.213	0.114
	Trial 2	0.029	0.029	0.061	0.09	0.079	0.094	0.098
	Trial 3	0.033	0.061	0.029	0.09	0.121	0.104	0.202
	Mean	0.035	0.038	0.052	0.08	0.095	0.137	0.138
	S.D.	0.007	0.020	0.020	0.017	0.023	0.066	0.056
C2	Trial 1	0.045	0.06	0.031	0.215	0.335	0.226	0.342
	Trial 2	0.032	0.03	0.055	0.133	0.117	0.315	0.211
	Trial 3	0.028	0.02	0.049	0.162	0.238	0.299	0.302
	Mean	0.035	0.04	0.045	0.17	0.23	0.28	0.285
	S.D.	0.009	0.021	0.012	0.042	0.109	0.047	0.067

Raw data of Figure 4.10: Growth of P. aeruginosa in C24, C18, C16, C14, C12, C2 condition

Dry cell mass (mg/L)		Reaction Time (h)						
		0	2	4	6	8	10	12
C24	Trial 1	0.013	0.041	0.021	0.026	0.051	0.036	0.041
	Trial 2	0.043	0.028	0.023	0.035	0.034	0.029	0.052
	Trial 3	0.034	0.027	0.046	0.044	0.023	0.04	0.015
	Mean	0.03	0.032	0.03	0.035	0.036	0.035	0.036
	S.D.	0.015	0.008	0.014	0.009	0.014	0.006	0.019
C18	Trial 1	0.037	0.024	0.044	0.029	0.067	0.043	0.022
	Trial 2	0.022	0.055	0.046	0.036	0.041	0.051	0.039
	Trial 3	0.031	0.02	0.024	0.064	0.027	0.047	0.083
	Mean	0.03	0.033	0.038	0.043	0.045	0.047	0.048
	S.D.	0.008	0.019	0.012	0.019	0.020	0.004	0.031
C16	Trial 1	0.029	0.048	0.054	0.067	0.066	0.083	0.069
	Trial 2	0.016	0.019	0.037	0.041	0.061	0.041	0.044
	Trial 3	0.045	0.038	0.035	0.057	0.041	0.047	0.052
	Mean	0.03	0.035	0.042	0.055	0.056	0.057	0.055
	S.D.	0.015	0.015	0.010	0.013	0.013	0.023	0.013
C14	Trial 1	0.024	0.052	0.061	0.078	0.034	0.074	0.053
	Trial 2	0.055	0.027	0.039	0.026	0.055	0.046	0.055
	Trial 3	0.011	0.041	0.065	0.07	0.088	0.057	0.072
	Mean	0.03	0.04	0.055	0.058	0.059	0.059	0.06
	S.D.	0.023	0.013	0.014	0.028	0.027	0.014	0.010
C12	Trial 1	0.043	0.055	0.069	0.064	0.086	0.074	0.055
	Trial 2	0.016	0.049	0.048	0.047	0.059	0.042	0.061
	Trial 3	0.031	0.025	0.057	0.069	0.041	0.064	0.064
	Mean	0.03	0.043	0.058	0.06	0.062	0.06	0.06
	S.D.	0.014	0.016	0.011	0.012	0.023	0.016	0.005
C2	Trial 1	0.041	0.031	0.114	0.225	0.245	0.361	0.124
	Trial 2	0.031	0.062	0.112	0.147	0.411	0.243	0.198
	Trial 3	0.018	0.072	0.05	0.195	0.133	0.17	0.443
	Mean	0.03	0.055	0.092	0.189	0.263	0.258	0.255
	S.D.	0.012	0.021	0.036	0.039	0.140	0.096	0.167

Raw data of Figure 4.11: Specific growth rates at varying stearic acid concentration

SGR		F/M ratio										
		0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
G		0	0.063	0.044	0.114	0.105	0.152	0.146	0.13	0.096	0.122	0.084
		0	0.048	0.062	0.068	0.084	0.046	0.057	0.101	0.057	0.076	0.111
		0	0.039	0.089	0.064	0.081	0.162	0.127	0.084	0.147	0.132	0.129
	Mean	0	0.05	0.065	0.082	0.09	0.12	0.11	0.105	0.1	0.11	0.108
	SD	0	0.012	0.023	0.028	0.013	0.064	0.047	0.023	0.045	0.030	0.023
P		0	0.059	0.053	0.073	0.042	0.094	0.114	0.056	0.114	0.166	0.156
		0	0.042	0.042	0.056	0.075	0.074	0.074	0.088	0.142	0.134	0.243
		0	0.019	0.043	0.045	0.084	0.081	0.097	0.177	0.131	0.156	0.15
	Mean	0	0.04	0.046	0.058	0.067	0.083	0.095	0.107	0.129	0.152	0.183
	SD	0	0.020	0.006	0.014	0.022	0.010	0.020	0.063	0.014	0.016	0.052