ABSTRACT

LOU, IN CHIO. Analysis of the factors affecting the competition between filaments and floc-formers in activated sludge. (Under the direction of Dr. Francis L. de los Reyes III).

Filamentous bulking, the most common solids separation problem in activated sludge systems, is caused by the excessive growth of filamentous bacteria over floc-forming bacteria, resulting in decreased sludge settling ability. The competition between the two types of organisms has been historically described using kinetic selection. However, it has been suggested that other factors, such as the presence of a filamentous "backbone", differences in decay rates, bacterial storage abilities, and substrate diffusion limitation may also affect the microbial selection. In this research, various hypotheses that integrated several of those factors were tested using modeling, reactor and molecular studies, and a new conceptual qualitative model combining kinetics and diffusion explaining bulking was developed.

As a first step, a bacterial competition model integrating kinetic selection theory, filamentous backbone theory, decay rates and storage abilities of filaments and floc formers was set up to predict and explain coexistence in a completely mixed reactor. Sensitivity analysis showed that the kinetic parameters μ_{max} and Ks, storage rate constants and backbone coefficient had the greatest effect on the simulation results. Monte Carlo simulation showed the effect of storage, and the ranges of dilution rates wherein one group outcompetes the other were delineated. Since bacterial storage was an important factor in microbial selection, respirometry-based kinetic parameter measurement was reevaluated by considering cell storage. Substrate uptake tests combined with metabolic modeling were used to include bacterial storage in determining the kinetic parameters for

bulking and non-bulking sludge. It was found that non-bulking sludge had higher maximum substrate uptake rates than bulking sludge, consistent with results from respirometry. Quantitative fluorescence in situ hybridization (FISH) showed that the filaments Eikelboom Type 1851, Type 021N and *Thiothrix nivea* were dominant in bulking sludge, comprising 42.0 % of mixed liquor volatile suspended solids (MLVSS), with 61.6% of the total filament length extending from flocs into bulk solution. Only low levels of Type 1851 filament length (4.9% of MLVSS) occurred in non-bulking sludge, 83.0% of which grew inside the flocs. This result seemingly supported the kinetic selection theory, but contradicted our previous experimental data that showed that bulking and non-bulking sludge have similar levels of total filaments length, and thus supporting the diffusion limitation hypothesis. To resolve this contradiction, a new conceptual qualitative framework was developed in this study. We hypothesize that the growth rates of filaments and floc formers are affected by the combination of kinetic selection and substrate diffusion limitation. Three different regions (bulking, transitional and non-bulking region) based on substrate concentration are suggested. In the bulking and non-bulking regions, kinetic selection controls the growth rate process and favors filaments and floc formers, respectively. However, in the transitional region, substrate diffusion limitation, determined by the floc size, plays an important role in causing bulking. To test this framework, sequencing batch reactors (SBRs) were operated with various influent substrate concentrations, and sludge settleability was measured at various floc sizes induced by different mixing strength. A model integrating both mechanisms was developed to simulate the substrate concentrations at different floc sizes. The modeling results showed the occurrence of diffusion limitation inside the flocs at a certain range of activated sludge floc sizes, and the experimental data supported this framework in the bulking and transitional regions.

ANALYSIS OF THE FACTORS AFFECTING THE COMPETITION BETWEEN FILAMENTS AND FLOC-FORMERS IN ACTIVATED SLUDGE

by
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INTRODUCTION

In the activated sludge process, the most widely used wastewater treatment unit process around the world, microorganisms are used to convert organic wastes to carbon dioxide and biomass. Under normal operating conditions, the population balance between filamentous and floc-forming organisms leads to well-settling flocs that are efficiently separated from effluent flow, producing a clarified effluent. A moderate amount of filaments is needed to provide a "macrostructure" for well-formed flocs (the filamentous backbone theory) (Sezgin *et al.* 1978). However under certain conditions, the excessive growth of one or more "types" of filaments results in costly process upsets. The most common solids separation problem caused by filaments in activated sludge is filamentous bulking, wherein excessive levels of filaments interfere with solids settling and compaction (Grady and Daigger 1999), resulting in solids inventory problems - high suspended solids concentrations in the effluent, overburden of waste sludge dewatering, and process failure due to uncontrolled losses of biomass. Bulking occurs all over the world (Table 1), and according to one survey (Keith and de los Reyes, unpublished), 63% of wastewater treatment plants (WWTPs) in North Carolina have experienced bulking. It has been estimated that the cost associated with bulking problem is in the billion-dollar range (Martins 2004; Sayler 1996).

Table 1 Percentage of plants in different studies reporting bulking

	Reference	No. of plants surveyed	% of plants bulking
North Carolina	Keith and de los Reyes, unpublished	47	63
Australia	Seviour *et al.* 1994	65	82
Italy	Rossetti *et al.* 1994	39	49
Massachusetts	Switzenbaum *et al.* 1992	50	60
Netherlands	Eikelboom 1994	70	70
South Africa	Blackbeard *et al.* 1986	111	32

Over the last two decades, the number of full-scale plants where mechanisms for selection of floc-formers is explicitly or implicitly employed has increased, and yet the relationships of operation, design, and performance parameters to the microbial community dynamics are not fully understood. Unless these relationships are fully understood, control measurements will remain on a trial-and-error basis. It is thus important to: (1) understand the mechanisms of the competition between filaments and floc-formers, and (2) determine the impact of different factors on the selection of one type of microorganism over another in activated sludge systems.

The most widely cited microbial selection theories and factors with respect to the growth of filaments in activated sludge are (1) the kinetic selection theory (Chudoba *et al.* 1973a; Chudoba *et al.* 1973b; Chudoba *et al.* 1973c), (2) the filamentous backbone theory (Sezgin *et al.* 1978), (3) storage phenomena, and (4) decay rates of filaments and floc formers. In the kinetic selection theory, floc formers are generally thought to be favored at higher substrate concentrations (Chudoba 1985), while filaments are thought to have higher growth rates at lower substrate concentrations. In the filamentous backbone theory, a fraction of filaments can be incorporated in the floc to serve as backbone, which decreases the effect of filaments on bulking and thus increases the sludge settleability. Two other factors, storage phenomena (the formation of storage compounds such as polyphosphates, glycogen, and polyhydroxyalkanoates inside cells) and decay rates of floc formers and filaments have not been thoroughly studied, although it is obvious that the effect of a lower decay rate is to increase net growth rate. It is highly probable that a combination of kinetics, storage phenomena, decay rates and the filamentous backbone theory can be integrated to form a

unified hypothesis for explaining the competition between specific filaments and floc formers.

Respirometry based on Monod kinetics is currently used to measure the kinetic parameters of activated sludge and is a widely used technique in analyzing full-scale plant operation. However, as storage is an important factor that affects the growth rates of organisms, the traditional respirometric measurements of kinetic parameters in past studies may not be applicable. The values of these parameters need to be reevaluated by substrate uptake tests that reflect bacterial storage formation as well as microbial growth.

The "substrate diffusion limitation" (SDL) hypothesis has also been recently suggested for the competition between filaments and floc formers (Martins *et al.* 2003a). Similar values of maximum substrate uptake rates were obtained for bulking and non-bulking sludge developed using different feeding patterns. It was hypothesized that instead of the kinetic selection effect, substrate gradients in sludge flocs causes bulking. At low bulk substrate concentration, floc formers cannot access enough substrate for their growth because of SDL. On the other hand, filaments, because of their morphology, can more quickly grow outside the flocs to access substrate, even if the growth rates are similar to floc formers, and bulking thus occurs. However, at high bulk substrate concentration, filaments have no such advantage and are not found extending outside the floc, and bulking would not happen. Our previous results (Liao *et al.* 2004) seemingly supported the SDL hypothesis for bulking. Quantitative FISH showed that high levels of filamentous lengths were observed for both bulking and non-bulking sludge. However, our later study (Lou *et al.* 2005b) using substrate

uptake tests found that non-bulking sludge have higher maximum growth rate than filaments, seemingly confirming the kinetic selection theory. It still remains unclear whether filamentous bulking is due to SDL or KST, or some combination of both mechanisms.

The focus of this research is to combine modeling, reactor studies and molecular techniques to determine the main parameters that affect the competition between filaments and floc formers that cause bulking in activated sludge systems. Sequencing batch reactors (SBR) with different feeding patterns were used to produce bulking and non-bulking sludge, and different experimental methods were performed to measure the kinetic parameters of bulking and non-bulking sludge. A new conceptual qualitative framework was also suggested and tested in experiments where the floc size distribution was controlled. A substrate diffusion model was developed and simulated the feeding condition in sequencing batch reactors to determine the substrate diffusion limitation inside the flocs at different floc sizes.

The thesis is divided into four parts. The first part is a literature review of filamentous bulking. Traditional and molecular approaches in identifying specific filaments were compared, and different theories or factors in microbial selection and the integrated models for explaining the competition between filaments and floc formers are presented. The second part describes the experiments and their results, in four chapters. Chapter 1 describes the development and simulation of a model incorporating kinetics with the filamentous backbone concept, storage phenomena, and decay rates to predict the competition between filaments and floc formers. The effect of the various parameters on filamentous bulking was determined. Chapter 2 describes the application of respirometry to determine the kinetic

parameters of bulking and non-bulking sludge, and FISH to identify the dominant filament. Chapter 3 describes the application of substrate uptake tests and metabolic modeling to determine the kinetic parameters of bulking and non-bulking sludge, and quantitative FISH to identify the dominant filaments and correlate the filament lengths to the degree of bulking. Chapter 4 presents a new conceptual qualitative framework that integrates substrate diffusion limitation and kinetic selection for explaining filamentous bulking, and tests this hypothesis using floc size control experiments. A substrate diffusion model for different floc sizes under intermittent feeding condition was also developed to simulate the substrate concentration at different layers of various flocs. The third part of the thesis summarizes the modeling and experimental results discussed in Part II, and suggests further studies for filamentous bulking. The fourth part includes the appendices, providing the experimental data, modeling programs and results in Part II.

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Part I

Literature Review

I.1 Traditional approaches for identifying filaments

Early research on bulking focused on the identification of causative organisms. In the 1970s, workers (Eikelboom 1975; Eikelboom 1977; Farquhar and Boyle 1971) attempted to identify filaments in activated sludge using morphology, physiology, and staining characteristics. Today, Eikelboom's "Type" identification keys, later modified by Jenkins *et al.* (2003) are widely used to determine which filaments are present in bulking and foaming plants (Eikelboom and Geurkink 2002; Wanner 1994; Wanner 1997). Based on these identification keys, approximately 80 different morphotypes of filamentous organisms have been identified in domestic and industrial bulking plants (Eikelboom and Geurkink 2002; Jenkins *et al.* 2003; Martins 2004).

The large number of filamentous "types" observed point to the complexity of bulking phenomena. While these early identification keys have paved the way for a more thorough analysis of bulking, they suffer from severe limitations. Morphotype-based methods are tedious, and similarities in morphology often lead to misidentification (Amann *et al.* 1998; Wagner *et al.* 1994; Wanner 1997). Depending on environmental conditions, the morphology and staining characteristics of the same organism may change (Foot *et al.* 1993; Jenkins *et al.* 2003; Wagner *et al.* 1994). These limitations have led to conflicting reports on the causative organisms, and have resulted in contradicting control measures (Soddell *et al.* 1992; Soddell and Seviour 1990). In addition, the use of these methods for quantitative studies is problematic. This has limited their application to subjective studies that are not amenable to rigorous, quantitative modeling and theory testing.

I.2 Molecular approaches for identification and quantification of filaments

Compared with traditional identification methods, molecular methods, such as ribosomal RNA-targeted hybridization, are more accurate for identification and quantification of specific microbial populations. These methods have become standard in microbial ecology studies in the past ten years.

I.2.1 Principle of molecular approaches

The rRNA-based approach is based on the comparison of the differences in RNA nucleotide sequences, usually the 16S rRNA sequences. These differences are used to reconstruct the phylogenetic relationships between organisms, because closely related organisms have more sequence similarities than unrelated organisms. 16S rRNA sequences have highly conserved regions, yet contain sufficient sequence variability in other regions to serve as excellent phylogenetic chronometers (Brock *et al.* 1994). This allows us to design oligonucleotide probes targeting different phylogenetic levels based on the complementary bases, i.e., if the target sequence (e.g. in a specific species of organism) is known, a complementary sequence (oligonucleotide probe) can be synthesized that can hybridize with the targeted sequence under certain conditions.

Two approaches are currently used for probe hybridization: whole cell hybridization (fluorescent *in-situ* hybridization, FISH) and dot blot hybridization (membrane hybridization).

In FISH, fluorescent dyes are incorporated into the probe for targeting the whole cells in the samples directly, making the cells visible by epifluorescence microscopy. The probe, whose sequence is complementary to the target rRNA sequence, will only bind to specific rRNA in target cells, and the rest of the fluorescent probe is washed off. The abundance (cell number) of target populations can be estimated by manual or automated counting methods. In membrane hybridization, rRNA is extracted from the environmental samples and blotted onto a membrane. A radioactively-labeled probe complementary to targeted species is used to hybridize with the RNA of interest in the membrane. The levels of hybridized RNA in the sample can be determined through quantification of the bound signal, by comparing with a standard series. This method is quantitative and allows analysis of many samples with multiple probes simultaneously.

I.2.2 Application of molecular approach to identification *in-situ* growth rate quantification

Molecular methods have previously been used in the identification of foam-forming filaments (de los Reyes *et al.* 1998a; de los Reyes *et al.* 1998b) and bulking-causative filaments in activated sludge (Aruga *et al.* 2002; Beer *et al.* 2002; Bjornsson *et al.* 2002; Blackall *et al.* 2000; Bradford *et al.* 1996; Howarth *et al.* 1998; Hugenholtz *et al.* 2001; Kanagawa *et al.* 2000; Kohno *et al.* 2002; Liu *et al.* 2000; Liu *et al.* 2001; Rossetti *et al.* 1997; Schade *et al.* 2002; Snaidr *et al.* 2002; Thomsen *et al.* 2002; Wagner *et al.* 1994). A suite of probes for targeting about 20 specific filaments, such as *Haliscomenobacter spp., Sphaerotilus spp. Leptothrix spp., Thiothrix spp., Leucothrix mucor, Beggiatoa spp., Nostocoida limicola spp., Microthrix parvicella,* Eikelboom Type 021N, Type 1863, Type

0041, Type 0803 and Type 1851 are summarized by Jenkins *et al.* (2003). A quantitative approach for relating the levels of Type 1851 to bulking was developed (Liao *et al.* 2004), and the combination with fluorescence in situ hybridization and real-time PCR were used recently to detect and quantify Type 021N in activated sludge (Vervaeren *et al.* 2005).

A molecular technique for estimating the *in-situ* growth rate of particular species is by using rRNA- and rDNA-targeted probes to measure the rRNA/rDNA ratio and correlating this to growth rate. The amount of DNA per cell is relatively stable and can be exploited to measure the number of cells present, whereas the amount of cellular RNA will change with growth rate in continuous growth cultures. The ratio of RNA to DNA can therefore be used to measure metabolic activity (growth rate, μ) (Muttray and Mohn 2000). Previous researchers have shown that growth rate is directly proportional to ribosome content (DeLong *et al.* 1989), and *in-situ* growth rates of individual cells can be inferred from fluorescent signal intensities (Poulsen 1993). This phenomenon has also been observed for *Salmonella typhimurium* (Schaechter *et al.* 1958), *Aerobacter aerogenes* (Neidhardt and Magasanik 1960), *Escherichia coli* (Bremer and Dennis 1987), slowly-growing marine isolates (Kemp *et al.* 1993; Kerkhof and Ward 1993) and the resin acid-degrading bacteria (Muttray and Mohn 1998). The results showed that there was exponential (Bremer and Dennis 1987; Maaloe 1960) or linear relationships (Herbert 1961; Muttray and Mohn 1998) between growth rate and rRNA/rDNA ratio or (total RNA)/(total DNA) (Kerkhof and Ward 1993), respectively, for various species. Thus, the 16S rRNA/rDNA ratio is a useful parameter for measuring metabolic activity of specific organisms within a complex microbial community, and

oligonucleotide hybridization probes can be used to estimate species-specific growth rate (μ) for modeling specific populations in activated sludge.

I.3 Respirometry

Based on Monod kinetics, respirometric tests are widely used to determine the biodegration kinetics of specific organic compounds, by tracking the mineralization of a compound without having to measure the compound analytically. The change in dissolved oxygen (DO) concentration as a result of biodegradation is measured. Respirometry is often an attractive alternative in biodegradation studies since DO concentration can be monitored easily and continuously.

Theoretical analysis and experimental results reported by Chudoba *et al.* (1992) have shown that the initial substrate to biomass (S_0/X_0) ratio plays an important role in batch cultivations, and the biokinetic parameters evaluated at high and low S_0/X_0 ratios can differ significantly. From the classification proposed by Grady *et al.* (1996), batch kinetic tests can be classified as intrinsic (high S_0/X_0 ratio) or extant (low S_0/X_0 ratio, usually less than 0.02). Extant kinetics refers to the biodegradation kinetic parameters currently existing in the activated sludge system. An extant kinetic test is a test that attempts to measure those kinetic parameters as close to the original conditions as possible (Ellis *et al.* 2002).

I.4 Substrate uptake test

Recent studies (Goel *et al.* 1998; Kohno *et al.* 1991; Krishna and van Loosdrecht 1999; Majone *et al.* 1996; van Loosdrecht *et al.* 1997) have suggested that storage phenomena may

be more important than kinetics in microbial selection, since the dynamic nature of substrate flows into wastewater treatment plants elicit transient responses from microorganisms.

Respirometry results should thus be reevaluated by considering storage in the cells, and a more reliable method should be developed to include bacterial storage to determine growth and metabolism of filaments and floc formers.

In wastewater treatment processes volatile fatty acids are produced from the primary soluble substrate. It has been shown that activated sludge organisms respond to feast/famine regimes by the production of storage polymers (van Loosdrecht *et al.* 1997). PHAs (polyhydroxyalkanoates), glycogen and Poly-P (polyphosphate) are three major types of bacterial storage products that have been reported in activated sludge wastewater treatment systems.

PHAs are polyesters of various hydroxyalkanoates that are synthesized by many Gram-positive and Gram-negative bacteria from at least 75 different genera (Reddy *et al.* 2003). Existing in the cell cytoplasm as 0.2 to 0.5 μm granules surrounded by a membrane (Sudesh *et al.* 2000), these polymers are accumulated intracellular by at levels as high as 90% of the cell dry weight under conditions of nutrient stress, and act as a carbon and energy reserve (Madison and Huisman 1999). The most common PHA stored by bacteria, polyhydroxybutyrate (PHB) (Madigan *et al.* 2000), is probably the most dominant polymer as it is directly formed out of the central metabolite acetyl-CoA (van Loosdrecht *et al.* 1997).

Glycogen is a branched polymer made up of glucose monomers connected by α (1-4) and α (1-6) glycosidic bonds. Its granules are smaller and more dispersed than PHA granules (Prescott *et al.*, 1990). Glycogen is formed only when sugars are present in the influent, and it plays an essential role in bacterial metabolism, e.g., in growth of the "G" bacteria, recently found to belong to the genus *Amaricoccus*, in biological phosphorus removal systems (van Loosdrecht *et al.* 1997). In enhanced biological phosphorus removal (EBPR), it is consumed under anaerobic conditions to supply part of the reducing equivalents needed to convert fatty acids into PHAs. Thus, glycogen reserves are restored during the aerobic period (Satoh *et al.* 1992; Serafim *et al.* 2002).

Poly-P stored by polyphosphate-accumulating microorganisms (PAOs) is a linear polymer of ortho-P monomers joined by an ester bond (Prescott *et al.* 1990). The degree of polymerization varies greatly, and for activated sludge, the acid-soluble fraction contains between 3 and 20 monomer units while the acid-insoluble fraction contains a higher number of monomers (Kulaev 1979). In biological systems, poly-Ps are stabilized by metal cations or small basic proteins (Serafim *et al.* 2002).

van Aalst-van Leeuwen *et al.* (1997) formulated a metabolic model describing the stoichiometry of PHB metabolism by a pure culture of *Paracoccus pantotrophus* under dynamic substrate supply. A similar metabolic model for glycogen was developed by Dircks (2001a). These metabolic models are based on six or seven internal reactions (Figure I.4-1 and Figure I.4-2) and results in two linear equations describing the conversion processes (one for feast phase and one for famine phase). The advantage of the metabolic models is that the

yields and maintenance coefficients for both phases are only dependent on two metabolic parameters that can be solved by the two set-up equations.

I.4.1 Metabolic mechanism for PHB production and consumption

(van Loosdrecht et al. 1997)

When acetate is used as singular carbon source, PHB is formed inside the cell. The aerobic metabolism of a bacterium capable of storing PHB growing on ammonia as sole nitrogen source and either acetate or PHB as carbon and energy source can be described by the seven internal reactions described below. A schematic representation of the model structure is given in Figure I.4-1.

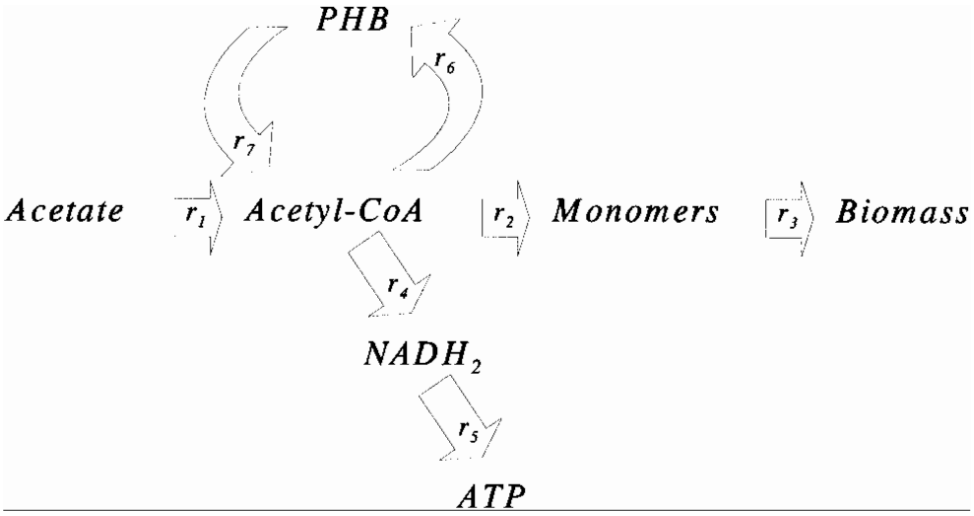

Figure 1. Schematic representation of the metabolism of an organism capable of producing and consuming PHB (van Loosdrecht *et al.* 1997)

I.4.2 Metabolic mechanism for glycogen production and consumption

(Dircks *et al.* 2001a)

When glucose instead of acetate is used as the singular energy source, glycogen is formed. The metabolic model (Figure I.4-2) describes the aerobic metabolism of a bacterium, where

growth occurs on the primary substrate, glucose in feast period, or the secondary substrate, stored glycogen in famine period. In this case ammonium is the N source.

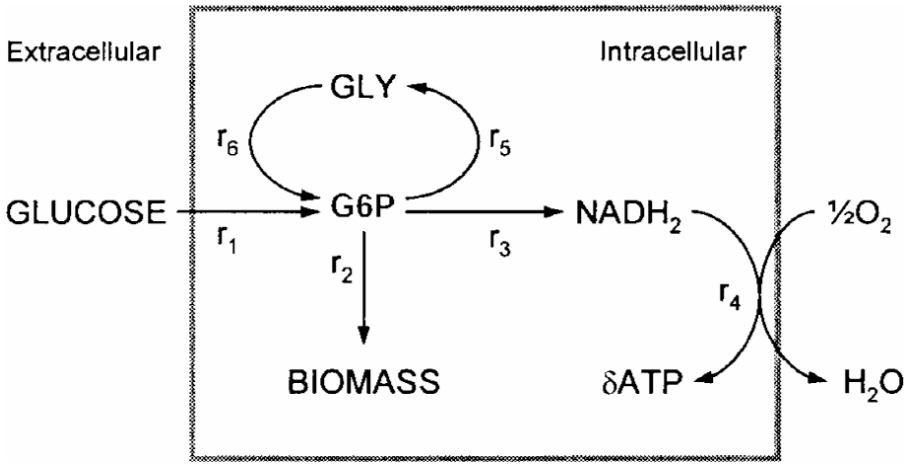

Figure 2. Schematic representation of the metabolism of an organism capable of producing and consuming glycogen (Dircks *et al.* 2001a)

I.5 Microbial selection in activated sludge

The current most widely favored microbial selection theories and factors with respect to the growth of filaments in activated sludge are (1) the kinetic selection theory (Chudoba *et al.* 1973c; Chudoba *et al.* 1973b; Chudoba *et al.* 1973a), (2) the filamentous backbone theory (Sezgin *et al.* 1978), (3) storage phenomena, and (4) difference of decay rates.

I.5.1. Kinetic selection theory

The kinetic selection theory is perhaps the most widely used theory for modeling selection and competition between filaments and floc formers. First formulated by Chudoba *et al.* in the 70's (1973c; 1973b; 1973a), kinetic selection theory uses Monod kinetics to model organism growth, using the assumption that filaments and floc-formers have different kinetic constants K_s (half-saturation constant, g/l) and μ_{max} (maximum growth rate, 1/h) for a soluble

substrate. Floc formers are generally thought to have high μ_{max} and K_s for soluble substrate, and thus favored at higher substrate concentrations (Chudoba *et al.* 1985). On the other hand, filaments are thought to have a lower K_s and thus have a higher growth rate at lower substrate concentrations.

A practical consequence of the theory is the use of smaller reactor before the main aeration basin. This smaller reactor will have a higher process loading factor (because of a lower hydraulic retention time), and therefore will serve to select for floc-forming bacteria. The design and operation of such selectors has recently been the subject of intensive research based on the kinetic selection of filaments and floc formers (Gaval and Pernelle 2003; Martins *et al.* 2003b; Pernelle *et al.* 2001; Scuras *et al.* 2001; Wilderer *et al.* 2001). However, some design and operational guidelines, such as initial F/M ratio and system performance number (SPN), are not generally accepted, and several contradictions have been reported (Chiesa and Irvine 1985; Salameh 1990; Salameh and Malina 1989; Wilderer *et al.* 2001). The selector failure in the control of bulking activated sludge was cited by Ekama *et al.* (1996).

I.5.2 Filamentous backbone theory

The filamentous backbone theory (Sezgin *et al.* 1978) assumes that the structure of the activated sludge floc is formed at two levels: microstructure and macrostructure. Microstructure depends on the bioflocculation of floc-forming bacteria. Microstructure flocs are small, spherical and compact, but mechanically weak. On the other hand, large activated sludge flocs exhibit a macrostructure provided by filamentous microorganisms. It is

postulated that filamentous microorganisms form a backbone within the floc, to which floc formers are firmly attached by their extracellular polymers.

I.5.3 Storage phenomena

The storage concept is based on the assumption that floc-formers have higher substrate uptake rates and capacities, and are therefore favored at high substrate concentrations. Thus, it is the storage capacity that is determining the outcome of microbial competition, as long as the capacity is adequately regenerated (all substrate taken up is subsequently oxidized). The storage concept forces us to question the assumption of direct coupling of substrate uptake to growth rate. Recent studies have suggested that storage phenomena may be another important factor in microbial selection (Goel *et al.* 1998; Kohno *et al.* 1991; Krishna and Loosdrecht 1999; Majone *et al.* 1996; van Loosdrecht *et al.* 1997). This realization has led the IWA Task Group on Mathematical Modeling to propose ASM3 (Gujer 2001), an activated sludge model that explicitly includes storage formation and use. However, the effect of storage on the competition between filaments and floc formers is not clear. There is evidence that floc-formers outcompete filaments under intermittent feeding or plug-flow conditions because of their ability to store substrates during imposed transients and subsequently reuse them for growth (Beccari *et al.* 2001; Beccari *et al.* 1998; Carta *et al.* 2001; Dircks *et al.* 2001b; Jacquart *et al.* 1973; Kohno *et al.* 1991; Majone *et al.* 1999; Majone *et al.* 1996; Martins *et al.* 2003a; Van den Eynde *et al.* 1983; Verachtert *et al.* 1980). However, storage products within filaments have also been found (Holt 1994; Jenkins *et al.* 2003; Seviour and Blackall 1999; Staley *et al.* 1989).

I.5.4 Differences in decay rates

The other factor that may affect competition is the difference in the decay rates of floc formers and filaments. Previous studies have hypothesized that filaments may have lower overall "decay" rates, since filaments are less likely to be preyed upon by higher organisms (Curds *et al.* 1968; Gude 1979; Hahn and Hofle 1998; Jurgens *et al.* 1999). This factor is not thoroughly studied, although it is obvious that the effect of a lower decay rate is to increase net growth rate.

I.6 Modeling

Earlier work has indicated that uptake of soluble substrate by activated sludge cultures can be attributed to 1) conversion of substrate to bacterial storage products or 2) growth. The two functions work together to properly predict total organism and substrate behavior. Blackwell (1971) first formulated this concept in a mathematical model. The total organism was considered to be composed of storage mass, i.e., storage products, and active mass without bacterial storage. A different approach was taken recently by Konopka (2000), who modeled the "starvation response" of *E. coli* by allowing the cells to switch to a lower death rate. The modeling exercise showed that the time interval of substrate addition would impact microbial competition.

However, previous models of filamentous bulking were still based on Monod kinetics, without incorporating the effect of storage or physiological state of cells. A dual substrate competition model was developed by Lau *et al.* (1984b) for a floc-former (*Citrobacter*) and

the filament *Sphaerotilus*. Van Niekerk *et al.* (1987; 1988) set up a competitive growth model for *Zoogloea ramigera* and Type 021N, again using measured kinetic parameters.

The role of storage processes has often been neglected when substrate uptake is mathematically described in activated sludge models: Activated Sludge Model No. 1 (Henze *et al.* 1987) and Activated Sludge Model No. 2 (Henze *et al.* 1995) do not deal with the storage process in heterotrophs. The possible conceptual approaches to modeling bacterial storage, structured and unstructured models were discussed by Majone (1999). In unstructured models, the storage is modeled only in an implicit way by adjustment of growth and decay parameters. When the purpose is only to describe carbon removal in a process where the storage is not very important, it is likely that this approach gives adequate results. However, if the population dynamics has to be taken into account, structured modeling appears to be necessary. The structured approaches increase the number of expressions and parameters to be optimized.

Recently the IWA Task Group proposed Activated Sludge Model No. 3 (Gujer *et al.* 1999), which includes the carbon storage process. In ASM3, it is hypothesized that all heterotrophic microorganisms can store substrate. Soluble substrates are removed only by storage and then growth occurs only on internally stored polymers. ASM3 has been used in activated sludge simulations (Koch *et al.* 2000) and respirometric methods have been proposed for calculation of storage yield (Karahan-Gui *et al.* 2002). However, it was recognized that this is not an accurate depiction of reality. In the case of simultaneous storage and growth, three yield factors have to be introduced; one for growth of the active biomass on the substrate, one for

the storage on the substrate and one for the growth on the stored substrate, and related kinetic expressions have to be defined accordingly. Based on experimental observations, a new model structure was proposed, in which simultaneous storage formation and bacterial growth are allowed (Krishna and van Loosdrecht 1999). The results showed that the behavior of the growth rate was adequately modeled. To test the data obtained on real plants, ASM3 and two derived models were compared in the interpretation of experimental data with reference to synthetic substrate (Beccari *et al.* 2001). ASM3 was found to describe the experimental data only by assuming a stored product formation much higher than the analytically measured value. On the other hand, the model that assumed a parallel growth and storage on the substrate can describe the observed stored product profile only by assuming the contribution to direct growth to be much higher than estimated from ammonia consumption. The model that assumed an accumulation / biosorption stage as first step of substrate removal was found to describe the whole experimentally observed behavior better.

I.7 Diffusion limitation on microorganisms' growth

I.7.1 Diffusion limitation on biofilm, activated sludge and anaerobic granules

Early researchers (Pasveer 1954; Wuhrmann 1963) have speculated that the diffusion phenomenon through the biofilms and flocs surrounding the microorganisms can be the most significant rate process in wastewater treatment reactors, which limits the overall degradation reaction. To test this hypothesis, a theory that incorporated the substrate diffusion concept for prediction of the performance of the biological film reactor was introduced and confirmed by experiment (Atkinson *et al.* 1968). The substrate diffusion limitations within the microbial mass, and the biofilm thickness on the substrate utilization and microbial growth,

were investigated (Atkinson and Daoud 1970; Atkinson and Davies 1974a; Atkinson and How 1974b). A mathematical model was developed based on the Monod maximum utilization rate and half-saturation coefficients, the biofilm stagnant liquid depths, the substrate diffusion coefficients through the biofilm and water, the biofilm density, and the bulk liquid substrate concentration (Williams and McCarty 1976). These biological rate equations formulated by the microbial kinetics and substrate diffusion have been widely used in the design of biofilm reactors.

In aerobic biological systems, substrate diffusion limitation (electron donor) and dissolved oxygen diffusion deficiency (electron acceptor) was also found inside the floc particles that limit the metabolic rates of cell (Knudson *et al.* 1982; Mueller *et al.* 1968). The biomass in activated sludge is composed of many bacterial floc particles (microbial suspension aggregate), and the aerobic granules were formed under certain appropriate conditions (Morgenroth *et al.* 1997; Peng *et al.* 1999; Tay *et al.* 2001). The metabolic reactions will occur simultaneously with mass transfer within the floc matrix, and a concentration gradient will be established. Thus the metabolic rates of the cells inside the flocs are limited by diffusion of oxygen and substrate. Such condition may lead to endogenous respiration and cell lysis near the center of flocs if the substrate is exhausted, or a change from aerobic respiration to anaerobic fermentation if oxygen is exhausted (Benefield and Molz 1983).

In addition to the aerobic system, the mass transfer resistance phenomenon was also used to explain the kinetics of the anaerobic processes of methane formation and substrate utilization in anaerobic granules (Dolfing 1985; Giraldo-Gomez *et al.* 1992; Gonzalez-Gil *et al.* 2001;

Wu *et al.* 1995), and bioavailability in soil (Harms and Bosma 1997). The experimental data indicated that mass transfer resistance in methanogenic biolayers became of significance only at low substrate concentrations and in thick biolayers with high methanogenic activities (Dolfing 1985). Girado-Gomez (1992) measured the half saturation constant for H_2 uptake by mixed-cultures without considering the internal biomass transfer limitation, and found that the value obtained was two orders of magnitude smaller than the other previously reported values that consider the internal limitation. Substrate utilization rate was observed to increase with decreasing the granule size. These results were also confirmed by Gonzalez-Gil *et al.*'s experiment (2001) showing that increase in Ks was found at increasing granule diameters.

I.7.2 Diffusion coefficient and diffusivity measurement

To test the importance of diffusion limitation inside the flocs, a number of researchers attempted to measure the diffusion coefficients of various reactants through biological material. Matson and Characklis (1976) developed a direct measure of mass flux to determine the variation in the diffusion coefficients of glucose and oxygen through microbial aggregates grown under various experimental conditions, and found that oxygen diffusivity varied from 20 to 100% and glucose diffusivity from 30 to 50% of their values in water. These values agreed with the research results of Siegrist and Gujer (1985) in biofilm experiments showing the biofilm matrix reduced molecular diffusion to about 50-80% of its values in pure water.

Maxham and Hickman (1974) defined the effectiveness factor, the ratio of the actual floc uptake rate to that which would be obtained in the absence of diffusive resistance. This was shown to be a universal function of a dimensionless floc radius and substrate concentration. However, Mikesell (1984) found that this factor was a simple function of sludge age and hydraulic residence time in his experiment and implied that the diffusion resistance probably explained why some of the kinetic parameters for the activated sludge process reported in the literature seem so unreasonable. Thus diffusion limitation is the crucial process in many aspects of the biology of bacteria (Koch 1990).

I.7.3 Diffusion limitation on filamentous bulking

Previous research has suggested that oxygen diffusion limitation (Sezgin *et al.* 1978) and/or substrate diffusion limitation (Martins *et al.* 2003a) inside the activated sludge floc may induce filamentous bulking. Filaments grow in only one or two dimension, while floc formers grow in three dimensions and form the floc matrix. Then the floc formers would grow inside the flocs, and are affected by substrate diffusion limitation. Assuming the same kinetics for both types of organisms, it is predicted that filaments have a higher outward growth velocity than floc formers and win the competition, because they can easily access the bulk liquid substrate. Recent research (Martins *et al.* 2003a) suggested that filamentous bulking was not due to the kinetic selection, but the substrate diffusion limitation, since from their substrate uptake experiment, they obtained similar maximum substrate uptake rates from bulking and non-bulking sludge. This result contradicts to the kinetic selection theory for explaining the competition between filaments and floc formers. It is still not clear that which mechanism, diffusion limitation or kinetic selection, affects filamentous bulking.

To our knowledge, the combination of diffusion limitation and microbial growth for explaining the competition of different bacterial morphotypes is new. Brandt and Kooijman (2000) presented a simple two-parameter (floc size at division and diffusion length) extension of growth models for cells suspensions to account for the reduction of the degradation rate due to the diffusion limitation. The model revealed that the effect of stirring on degradation rates occurs through a reduction of floc size at division. However this model was not related to the filamentous bulking. Martins, *et al.* (2004) simulated three-dimensional dual-morphototype species (e.g., filaments and floc formers) of activated sludge flocs using the adapted individual-based model originally developed for a biofilm system, allowing spatial development of the floc according to the bacterial morphology, diffusion, reaction ,and growth processes. The model indicated that filamentous bacterial morphology and substrate microgradients are important aspects in the formation of bacterial structures. In mass transport-limited regimes, filamentous bacterial structures prevail, whereas in growth-limited regimes irregular shaped flocs within figure-like structures are dominant. The model results support the hypothesis that floc-macrogradients can be the most important parameter for development of bulking sludge.

I.7.4 Floc size control and measurement

To study the mass transfer of substrate and oxygen inside the flocs, it is essential to estimate the floc size and size distribution. Floc size can be controlled under different hydrodynamics, and its distribution can be varied with different impeller ((Bouyer *et al.* 2004). Couette bioreactor was recently used to generate different floc size at various

rotational speeds (Liu and Evans 2003). They found that the floc size decrease linearly with increasing rotational speed that caused high shear rate. Numerous studies using various traditional techniques for measuring the floc size were summarized by Knudson *et al.* (1982). In recent studies, floc size measurement was determined using Malvern Mastersizer (Biggs and Lant 2000; Wilen *et al.* 2004), Coulter counter/multisizer (Andreadakis 1993; Barbusinski and Koscielniak 1995; Choi *et al.* 2004) or morphological analysis (Dougherty and Pelz 1991).

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Part II

Modeling and Experimental Results

Chapter 1.

Bacterial competition model development and simulation integrating decay, storage, kinetic selection and filamentous backbone

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INTEGRATING DECAY, STORAGE, KINETIC SELECTION, AND FILAMENTOUS BACKBONE FACTORS IN A BACTERIAL COMPETITION MODEL

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ABSTRACT: Filamentous bulking in activated sludge systems occurs when filamentous organisms outgrow floc-forming bacteria and interfere with sludge settling. The competition between filaments and floc formers has been described previously using the kinetic selection and filamentous backbone theories. We hypothesized that differences in decay rates and storage abilities also affect this competition. We tested this hypothesis by integrating these four factors into a model to predict and explain coexistence in a completely mixed reactor. Filamentous and non-filamentous sludges were developed in laboratory-scale reactors and analyzed to determine decay rates. The modeling results showed coexistence of the two organism types, and sensitivity analysis showed that the kinetic parameters, the storage rate constants, and the backbone coefficient had the greatest effect on the simulation results.

Monte Carlo simulation showed the effect of storage, and the ranges of dilution rates wherein one group outcompeted the other were delineated.

KEYWORDS: bulking, kinetic selection, filamentous backbone, decay rate, storage, modeling

Introduction

The production of a clear effluent in activated sludge wastewater treatment is dependent on the formation of highly settleable flocs, which in turn is dependent on the balance between floc-forming and filamentous bacteria. While some filamentous "backbone" is needed to form strong flocs, an overgrowth of filaments leads to solids separation problems (Sezgin *et al.*, 1978). The most common solids separation problem is bulking, wherein excessive levels of filaments interfere with solids settling and compaction (Grady, Daigger and Lim, 1999), resulting in solids inventory problems and high suspended solids concentrations in the effluent. It is thus important to understand the competition between floc-formers and filaments, and determine the impact of different factors on the selection of one type of microorganism over another in activated sludge systems.

The kinetic selection theory (KST) is perhaps the most widely used theory for modeling selection and competition between filaments and floc formers. First formulated by Chudoba *et al.* in the 70's (1973a; 1973b; 1973c), KST uses Monod kinetics to model organism growth, using the assumption that filaments and floc-formers have different kinetic constants K_s (half-saturation constant, g/l) and μ_{max} (maximum growth rate, 1/h) for a soluble substrate. Floc formers are generally thought to have high μ_{max} and K_s for soluble substrate, and thus favored at higher substrate concentrations (Chudoba, *et al.*, 1985). On the other

hand, filaments are thought to have a lower K_s and thus have a higher growth rate at lower substrate concentrations. KST has been used in a variety of models for predicting the outcome of the competition between the two organism types. For example, a dual substrate competition model was developed by Lau *et al.* (1984) for a floc-former (*Citrobacter*) and the filament *Sphaerotilus*. The model incorporated the effect of carbonaceous substrate and dissolved oxygen diffusion into the floc. Van Niekerk *et al.* (1987; 1988) developed a competitive growth model for *Zooglea ramigera* and the filament Eikelboom Type 021N, using experimentally derived kinetic parameters. The model allowed the analysis of the effects of selectors on the growth of filaments and floc formers. Similarly, the modeling approach developed by Sheintuch and Tartakov (1997) used kinetics based on the AEROFIL model (Kappeler and Gujer, 1994).

Despite its popularity, KST does not adequately provide a general framework for microbial selection. Notwithstanding experimental evidence for the high K_s and μ_{max} of several flocculating bacteria (Chudoba *et al.*, 1985; Van den Eynde *et al.*, 1983; Verachtert *et al.* 1980), it is becoming increasingly clear that other factors contribute to the selection process, and need to be integrated into mathematical models of competition. A key shortcoming of KST is a consequence of the competitive exclusion principle: the number of species coexisting cannot exceed the number of limiting resources (Hardin, 1960). In activated sludge systems, this implies that the experimentally observed generic coexistence of floc forming and filamentous species growing on a single limiting substrate cannot be predicted with simple models according to kinetic selection (Cenens *et al.* 2000b). Several different additions to KST were used to overcome this limitation. Chiesa and Irvine (1985) hypothesized that a time-varying growth environment could explain coexistence. Lau *et al.*

(1984) used double Monod (interactive) kinetics to account for the effect of dissolved oxygen in flocs. Cenens *et al.* (2000a) used a different approach; they combined the filamentous backbone theory (Sezgin *et al.*, 1978) with kinetic selection to account for the fraction of filaments incorporated in the floc serving as backbone.

The filamentous backbone theory assumes that the structure of the activated sludge floc is formed at two levels: microstructure and macrostructure. Microstructure depends on the bioflocculation of floc-forming bacteria. Microstructure flocs are small, spherical and compact, but mechanically weak. On the other hand, large activated sludge flocs exhibit a macrostructure provided by filamentous microorganisms. It is postulated that filamentous microorganisms form a backbone within the floc, to which floc formers are firmly attached by their extracellular polymers. This modeling effort led to the delineation of ranges of dilution rates that allow generic coexistence of filaments and floc formers.

Although many external factors such as microbial interactions, time variations in the growth environment, mass transport limitations, etc., may affect the competition, two intrinsic factors have not been thoroughly investigated. The first is storage (the formation of storage compounds such as polyphosphates, glycogen, and polyhydroxyalkanoates inside cells). Recent studies have suggested that storage phenomena may be more important than kinetics in microbial selection (Goel *et al.*, 1998; Kohno et. al., 1991; Krishna and van Loosdrecht, 1999; Majone *et al.*, 1996; van Loosdrecht et. al. 1997). This is particularly important since the dynamic nature of substrate flows into wastewater treatment plants elicit transient responses from microorganisms. This realization has led the IWA Task Group on Mathematical Modeling to propose ASM3 (Gujer *et al.* 2000), an activated sludge model that explicitly includes storage formation and use. However, the effect of storage on the

competition between filaments and floc formers is not clear. There is evidence that floc-formers outcompete filaments under intermittent feeding or plug-flow conditions because of their ability to store substrates during imposed transients and subsequently reuse them for growth (Verachtert, *et al.* 1980; Van den Eynde, *et al.* 1983). However, storage products within filaments have also been found (Holt, 1994).

The second factor that may affect competition is differences in the decay rates of floc formers and filaments. This factor is not thoroughly studied, although it is obvious that the effect of a lower decay rate is to increase net growth rate. We hypothesized that filaments have lower overall decay rates in activated sludge, and therefore gain a competitive advantage. To test this hypothesis, we experimentally measured the decay rates of filamentous and non-filamentous sludge using respirometry. Further, we hypothesized that a combination of kinetics, storage phenomena, and the filamentous backbone theory can be integrated to more fully explain the competition between specific filaments and floc-forming bacteria. We attempted to integrate these factors in this study, and explored the effects of the different factors. The primary objective of the modeling study was to determine the relative importance of the different factors on the competition between filaments and floc formers. For the purpose of the study, the ability of the model to predict filamentous or floc-forming biomass was not directly assessed.

Materials and methods

Reactor study. Two laboratory reactors, each with an active volume of 8 L, were operated to develop bulking and non-bulking sludges. The experiment was performed in 2 stages. In Stage 1 (Day 0 to Day 36), filamentous bulking in the sludge inoculum was suppressed by running both reactors as sequencing batch reactors (SBRs) with an anaerobic

phase. In Stage 2, one reactor was operated as an SBR and the other was operated as a completely mixed reactor (CMR). Both reactors were seeded with the combined sludge from Stage 1. The SBR simulated a plug flow reactor (PFR) by having a short fill time (12 min), while the CMR had a fill time of 24 hours. The mean cell retention time (MCRT) and the hydraulic residence time (HRT) for both reactors were 16 days and 12 hours, respectively. The reactors were seeded with activated sludge from the North Cary Water Reclamation Facility (Cary, NC). The reactors treated synthetic wastewater (250 mg /L glucose, 150 mg/L yeast extract, 50 mg/L $MgSO_4 \cdot 7H_2O$, 7 mg/L KCl, 150 mg/L NH_4Cl, 196.4 g/L KH_2PO_4, 55.5.6 mg/L $NaHCO_3$, 5 mg/L $MnSO_4 \cdot 7H_2O$, 3.8 mg/L $CaCl_2$, and 2.2 mg/L $FeSO_4 \cdot 7H_2O$). The MLSS (mixed liquor suspended solids concentration), MLVSS (volatile suspended solids concentration), SV (30 minute settled volume) and SVI (sludge volume index) were periodically measured using Standard Methods (Greenberg *et al.*, 1998).

Evaluation of decay rate k_d. To evaluate the value of k_d, a sample of biomass was taken from the reactors and aerated for 10 days. A sample of the biomass was taken to measure the endogenous oxygen uptake rate (OUR) once every day. The decay coefficient was estimated by plotting ln(OUR) versus time of biomass aeration (Grady, Daigger and Lim, 1999). The measured k_d value was temperature-adjusted using the temperature coefficient value of 1.029 as described by Dold *et al.* (1980).

Model development. The model was set up for a continuously mixed reactor (CMR) followed by a settler with sludge recycling. The kinetic equations for growth on soluble substrate Cs, and aerobic growth and aerobic respiration of storage products C_{STO}, are based on Monod kinetics. Filamentous biomass C_{xfil} and floc former biomass C_{xfloc} levels can be

determined in the model separately. The model and mass balance equations for the CMR are shown below, and the model schematic is shown in Figure 1.

CMR

$$\frac{dCs}{dt} = D(Cs,in - (1+r)Cs) - \frac{\mu_{floc}}{Y_{floc}} C_{Xfloc} - \frac{\mu_{fil}}{Y_{fil}} C_{Xfil} - \frac{\mu_{STOSfloc}}{Y_{STOSfloc}} C_{Xfloc} - \frac{\mu_{STOSfil}}{Y_{STOSfil}} C_{Xfil}$$

$$\frac{dC_{Xfloc}}{dt} = -\overline{D} C_{Xfloc} + \mu_{floc} C_{Xfloc} + \alpha \frac{Y_{floc}}{Y_{fil}} C_{Xfil} - k_{dfloc} C_{Xfloc} + \mu_{STOXfloc} C_{Xfloc}$$

$$\frac{dC_{Xfil}}{dt} = -\overline{D} C_{Xfil} + \mu_{fil} C_{Xfil} - \alpha C_{Xfil} - k_{dfil} C_{Xfil} + \mu_{STOXfil} C_{Xfil}$$

$$\frac{dC_{STOfloc}}{dt} = \mu_{STOSfloc} C_{Xfloc} - b_{STOfloc} C_{STOfloc} - \frac{\mu_{STOXfil}}{Y_{STOXfloc}} C_{Xfloc}$$

$$\frac{dC_{STOfil}}{dt} = \mu_{STOSfil} C_{Xfil} - b_{STOfil} C_{STOfil} - \frac{\mu_{STOXfil}}{Y_{STOXfil}} C_{Xfil}$$

$$\overline{D} = \frac{w(1+r)}{r+w} D$$

$$\mu_{floc} = \mu_{\max\ floc} \frac{C_s}{K_{sfloc} + C_s}; \mu_{fil} = \mu_{\max\ fil} \frac{C_s}{K_{sfil} + C_s}$$

$$\mu_{STOSfloc} = k_{STOfloc} \frac{C_s}{K_{sfloc} + C_s}; \mu_{STOSfil} = k_{STOfil} \frac{C_s}{K_{sfil} + C_s}$$

$$\mu_{STOXfloc} = \mu_{\max\ STOXfloc} \frac{C_{STOfloc}/C_{Xfloc}}{K_{STOfloc} + C_{STOfloc}/C_{Xfloc}}; \mu_{STOXfil} = \mu_{\max\ STOXfil} \frac{C_{STOfil}/C_{Xfil}}{K_{STOfil} + C_{STOfil}/C_{Xfil}}$$

Where: D = dilution rate; μ = growth rate; Ks = half saturation coefficient; k = storage rate; Y = yield: C_{STO} = concentration of storage products; r = recycle ratio; α = rate of incorporation of filaments to flocs; b = aerobic decay rate of storage products; k_d = biomass decay rate; w= fraction of sludge wasted; (subscripts: fil = filaments, floc = floc formers, STOS = formation of storage products, STOX = growth on storage products)

It is considered that the substrate is used for formation of storage polymers as well as for biomass growth. After depletion of the substrate, microorganisms depend solely on the accumulated storage polymers for growth. The growth rates were set up as Monod functions wherein the rates vary with the appropriate substrate concentration levels. With this model setup, the kinetic selection theory, the filamentous backbone theory (α values), and the effect

of storage phenomena (with simultaneous growth on exogenous substrate and storage products) are integrated. The model was implemented in MATLAB (The MathWorks, Inc., Natick, MA).

The following are the model assumptions and limitations:

1. The microbial community is composed of two populations: filaments and floc formers. Filaments, once incorporated into the floc, are considered part of the floc biomass and do not contribute to settling problems.
2. Growth of floc formers and filaments in activated sludge is limited by a single limiting carbon substrate present in the influent, and all other growth factors are present in excess.
3. The model can only be applied to the completely-mixed activated sludge process consisting of an aerated basin followed by a settler with sludge recycling and waste.
4. Substrate uptake, conversion to biomass, and conversion to storage compounds can be modeled using Monod kinetics.
5. Substrate transport effects into the floc (e.g. diffusion) are not considered in the model.

The model is conceptually similar to those proposed by van Aalst-van Leeuwen *et al.* (1997) and by Krishna and van Loosdrecht (1999). However, the model is unique in that it integrates four different factors in the competition between filaments and floc formers. While the model can be used to predict filamentous and floc former biomass levels in activated sludge, the value of the modeling study is in determining the relative importance of the different conversion processes. Since substrate conversions to biomass and storage are segregated, the importance of storage kinetics can be assessed.

Results and Discussion

Laboratory-scale reactors and decay rate estimation. In Stage 1 of reactor operation, the objective was to suppress filamentous growth and reduce SVI so that the filamentous growth can be readily observed during Stage 2. Incorporating an anaerobic period in the SBR operation was successful in decreasing SVI. The SVI decreased from about 160 ml/g at the beginning of Stage 1 to around 50 ml/g at the end of Stage 1 (Day 36, Fig. 2). Typically, sludge with an SVI greater than 150 ml/g is considered bulking sludge. In Stage 2, one week after the change in operation, the CMR experienced bulking, with an SVI increase from 125 ml/g to 190 ml/g in one day. On the other hand, the SBR sludge remained non-bulking, with an SVI of 50 ml/g. The sludge bulking in the CMR continued, with the SVI reaching the maximum of 523 ml/g at the end of Stage 2. During this entire stage, the SBR did not experience bulking, and the SVI stayed in the 46.3 –56.6 ml/g range. These results are in accordance with the kinetic selection theory (Chudoba *et al.*, 1973), which predicts bulking under CMR conditions, and non-bulking under PFR conditions.

Further microscopic observation showed that the CMR was dominated by filamentous bacteria extending outside the flocs. The filaments were identified as Eikelboom Type 1851 using Jenkins' modified Eikelboom classification method (Jenkins *et al.*, 1993) and by fluorescence in situ hybridization with a Type 1851 16S rRNA-targeted probe (Liao *et al.*, submitted). These filaments were estimated by microscopic examination to comprise up to 35% of the sludge in the CMR. Interestingly, while low levels of Type 1851 filaments were observed *outside* the flocs in the non-bulking reactor (SBR), high levels of filaments were observed *inside* the same flocs. These results may be taken as conflicting KST, since little or no growth of filaments is predicted by KST for SBRs. Recently, Martins *et al.* (2003)

formulated an alternative hypothesis wherein filaments gain competitive advantage because of substrate gradients created by diffusion limitation inside the floc. According to this hypothesis, at low substrate concentrations, filaments gain an advantage by extending outside the floc to obtain access to substrate. Conversely, at high substrate concentrations, the filaments can remain inside the floc without suffering a disadvantage because substrate is not limited inside the floc. Our data appears to support this "diffusion limitation" hypothesis, and contradicts the KST prediction that there should be no filamentous growth under high substrate concentrations. However, additional evidence is needed to determine whether kinetic selection or "substrate diffusion limitation" is the dominant mechanism for filamentous growth in bulking sludge.

The decay rate measurements closely matched the values from other studies (Table 1), and showed that filaments have a lower k_d than floc formers. This has the effect of increasing the net growth rate of filaments, as compared to the assumption of equal decay rates for both filaments and floc formers. Table 1 compares the kinetic parameters and decay rates of floc formers and filaments from the literature.

Parameter evaluation and modeling results. For the simulations, kinetic parameters from the literature and decay rates estimated from the OUR tests were used. For the kinetic parameters μ_{max} and Ks, the values were derived from pure culture studies in the literature. However, in activated sludge, floc structure affects substrate access and thus the 'apparent' Ks. For example, filaments extending beyond the floc would not suffer from substrate transport effects (diffusion through the floc), effectively reducing the Ks of filaments compared to the Ks of floc formers. One could thus argue that the low Ks of filaments for carbonaceous substrate is not inherent to the organisms, but is a function of floc structure. In

this modeling study, the low Ks attributed to filaments is based on pure culture studies (Table 2). However, even if this reflects the 'apparent' Ks due to diffusion effects, the net mathematical impact on the model is the same even though the mechanism is different. Thus, the model still examines the effect of Ks (inherent or apparent) on microbial competition.

There is insufficient experimental data on the backbone coefficient and storage kinetic parameters for filaments and floc formers. Nevertheless, values from the literature (Carta *et al.*, 2001; Cenens *et al.* 2000a; Filipe *et al.*, 2001; Krishna and van Loosdrecht, 1999, Magbanua, *et al.*, 1998) were obtained, and engineering judgment was used to estimate kinetic values not available in the literature (Table 2). These were parameters mostly related to storage uptake and utilization, and were estimated based on the values of analogous storage kinetic parameters determined by previous research (Filipe *et al.*, 2001; Krishna and van Loosdrecht, 1999). In all simulations, the influent concentration was 500 mg COD/L and the dilution rate was held constant with $D=0.6\ h^{-1}$. This value was chosen since initial simulation results showed that coexistence of filaments and floc formers was predicted at this dilution rate. Cenens *et al.* (2000b) showed, using a model that included the filamentous backbone theory, that floc formers dominated (i.e., no filaments were predicted) at specific, wide ranges of dilution rates. To determine the effects of the different factors on microbial selection, three scenarios were tested at this constant dilution rate.

Effect of the rate of incorporation of filaments to floc. Conceptually, filaments can either grow from outside the floc and later incorporated in the floc, or grow from inside the floc. From a modeling standpoint, growth inside the floc does not impact settling unless the growth extends beyond the confines of the floc and the filaments bridge other flocs. In the

model, the growth of filaments is considered independently from location (inside or outside floc), and the rate at which filaments are incorporated into floc, α, can be considered a parameter that takes into account the 'filamentous backbone' formation. This parameter determines how fast filaments become part of the floc backbone, and is also related to the optimal level of filaments needed to produce the ideal, well-settling floc. Since this concept is relatively new, there is little experimental data on the value of α.

The effect of α on the competition between filaments and floc-formers was tested by running the model assuming: (1) no decay, (2) storage phenomena were not occurring, and (3) two levels of the rate of filament incorporation to floc, α : 0.01 h^{-1} and 0.02 h^{-1}. The simulations reached steady-state after a reactor run of 500 h (simulation results over time not shown). By varying the initial conditions of C_{Xfil} and C_{Xfloc} and running all simulations to steady-state, phase diagrams showing the model convergence to steady-state can be plotted. The results for Case 1 are shown in Figure 3, and show that the steady state concentration of floc formers relative to filaments increases (from 2.0 g/l to 4.0 g/l) when the incorporation rate of filaments to floc was increased from 0.01 h^{-1} to 0.02 h^{-1}. Thus, as the rate of "filamentous backbone" formation is increased, the floc biomass increases relative to filaments that do not form a floc backbone. The modeling effort emphasizes the importance of the value of α. To our knowledge, an experimental value for α has not yet been reported. However, the use of molecular techniques such as quantitative FISH (fluorescence in situ hybridizations) (Liao *et al.*, submitted) coupled with image analysis of flocs, should provide estimates of α in the future.

Effect of difference in decay rates. Previous studies have hypothesized that filaments may have lower overall "decay" rates, since filaments are less likely to be preyed upon by higher organisms (Jurgens *et al.*, 1999; Gude, 1979; Curds, *et al.*, 1968). The experimental data provided in this study showed that the decay rate for Type 1851-dominated sludge is one-third of the decay rate for well-settling sludge. The mechanism for this decreased decay rate is not yet completely understood, although decreased predation is one possibility. To show the effect of decay rates, simulations incorporating storage phenomena (same values of parameters for flocs and filaments), but using the same (Fig. 4a) and different (Fig. 4b) decay rates (k_d) for the two types of organisms, were conducted. For the latter simulation, the experimentally determined values of k_d were used. The simulations reached steady-state in 100 h (data not shown). The results show that Type 1851 filaments gain a competitive advantage because of their lower decay rates. To our knowledge, this study is the first to combine experimental results and modeling to show the competitive advantage of filaments with respect to decay rates.

Effect of difference in storage kinetics. Storage as a selective pressure in activated sludge has been reviewed in the 1970s (Blackwell, 1971; Dennis, 1978), and has been the subject of more recent studies that suggest storage as a critical parameter in microbial selection (Goel *et al.*, 1998; Kohno et. al., 1991; Krishna and van Loosdrecht, 1999; Majone *et al.*, 1996; van Loosdrecht et. al. 1997). However, Daigger and Grady (1982) reported that while the occurrence of storage during transient response is consistent with the principles of physiological adaptation, the storage response is not a general phenomenon, but one which can occur under specialized circumstances. The first condition is a biosynthetic growth limitation in the presence of excess substrate. The second condition is when storage

functions as a mechanism for preventing the accumulation of traumatic levels of intracellular metabolites. The clear role of storage in enhanced biological phosphorus removal suggests that storage may occur more often in activated sludge. Regardless of the disagreement on the role of storage in microbial selection in activated sludge, it is clear that it can be an important phenomenon that should be further investigated.

One mechanism for substrate removal postulated that growth can also occur directly on external substrate, in parallel with storage (van Aalst-van Leeuwen *et al.* 1997). For example, after depletion of external substrate, stored poly-β-hydroxybutyrate (PHB) can be used as energy source for growth. Microorganisms able to store substrates during imposed transients and subsequently reuse them for growth could have a strong competitive advantage. The beneficial effects of aerobic selectors or intermittent feeding seem to indicate that floc formers have such an advantage over filaments (Verachtert, *et al.* 1980; Van den Eynde, *et al.* 1983). This assumes that in intermittently fed systems (plug flow systems) floc forming bacteria become dominant as a result of higher substrate uptake rates, possibly to survive a starvation phase, by thriving on accumulated intracellular compounds.

Of all the model parameters, the values for storage kinetics were the most problematic to determine. These parameters are new, and there are no experimental data available on these values. The storage parameters in ASM3, a model that explicitly considers storage, were used as a guide in selecting values. This simulation was performed at $\alpha = 0.01\ h^{-1}$, equal decay rates of $0.006\ h^{-1}$ for both filaments and floc formers, and different storage rate constant (k_{STO} [g C_s/ g C_x –h]). In the first simulation (Figure 5a), the floc formers had a higher storage rate constant, and in the second simulation (Figure 5b), the filaments had a higher storage rate constant. The phase diagrams show that filaments will only be present if

the storage rate constant is not too high. This is because in the model, a high storage rate constant means that most of the substrate is being used to form storage products before being used for growth. This reduces the competitiveness of filaments in a reactor where the substrate is not limiting, as is the case in these simulations, since conversion of substrate to storage products and then to biomass is slower than direct conversion of substrate to biomass. Thus, in this scenario ($D = 0.6\ h^{-1}$), a high storage rate constant does not confer a competitive advantage. However, substrate conversion to storage is most likely to occur during conditions of excess substrate, implying that in reality storage may not be a key factor in competition between filaments and floc formers.

Sensitivity analysis of the model parameters

Sensitivity analysis of each parameter. A literature search showed wide ranges of reported parameter values (Table 1). In some cases, particularly for storage parameters, there is a lack of experimental data. Sensitivity analysis of the model parameters (kinetic parameters, backbone coefficient, decay rates and storage parameters) was performed to identify the parameters that most significantly impact the simulation results (C_{xfloc}, C_{xfil} and C_{xfloc}/C_{xfil}). The procedure involved increasing or decreasing the value of each parameter by 10% from its baseline value (Table 2) and comparing the simulation results to the results of the baseline case. The dilution rate was kept at $0.6\ h^{-1}$ in the analysis.

The results (Figure 6) showed that at $D = 0.6\ h^{-1}$, the least sensitive parameters were $\mu_{maxSTOXfloc}$, $\mu_{maxSTOXfil}$, $K_{STOfloc}$, K_{STOfil}, $b_{STOfloc}$ and b_{STOfil}, with minimal effect on the simulation outputs. The kinetic growth parameters and backbone coefficient affected the results most significantly. The ratio of C_{xfloc}/C_{xfil} was increased by more than 40% and 20% respectively, when $\mu_{maxfloc}$ and backbone coefficient α were increased by 10%. The storage

rate constants, $k_{STOfloc}$ and k_{STOfil} were the next sensitive parameters. The effect of decay rate was very small; simulation results were within 10% of the baseline.

Monte-Carlo Simulation. A more detailed analysis can be achieved with a Monte-Carlo simulation in which the parameters are changed randomly according to a given statistical distribution. The statistical distribution of the predicted value is obtained after many simulation steps, thus reflecting the uncertainty of the model prediction. We assumed that the parameter values follow a normal distribution with the baseline value as the mean. The operational conditions were the same as the above with D=0.6 h^{-1}.

The cumulative distribution curve of the ratio C_{xfloc}/C_{xt} (where C_{xt} is the sum of C_{xfloc} and C_{xfil}) is plotted in Figure 7 for the case with storage and the case without storage. The difference between the curves is therefore due to storage phenomena. It is apparent that storage phenomena do not significantly affect the competition between floc-formers and filaments. The cumulative frequency was about 0.80 when C_{xfloc}/C_{xt} was 0.75, i.e., the probability that C_{xfloc}/C_{xt} was less than 0.75 was around 0.80. Assuming that a ratio C_{xfil}/C_{xt} = 0.25 causes bulking, the simulation shows that there is 80% probability of bulking under the assumptions and conditions stated above.

Delineation of dilution rate D on the decay and storage effect of floc formers and filaments.

Dilution rate determines which factors (kinetic selection, backbone coefficient, decay rate or storage) would control the competition between floc former and filaments. The model was run to a number of steady states under the baseline conditions (simulation based on parameters shown in Table 2), and the concentrations of floc formers and filaments are shown in Figure 8. This plot can be divided into three regions:

Region I: $D < 0.48\ h^{-1}$, $C_{xfloc} > C_{xfil}$ with the peak C_{xfloc} at $D = 0.16\ h^{-1}$;

Region II: $0.48 < D < 0.52\ h^{-1}$, with the peak C_{xfil} and lowest values of C_{xfloc} at $D = 0.5\ h^{-1}$;

Region III: $D > 0.52\ h^{-1}$, with the peak C_{xfloc} at $D = 1.32\ h^{-1}$, above which only floc formers will be present, until D was increased to greater than $5.72\ h^{-1}$, above which both species will be washed out.

We investigated the other two factors, decay rates and storage phenomena, which may affect this competition at different regions of dilution rates. The peak points on the curve were chosen, and additional simulations at these dilution rates were performed. Three cases (Case 1, Baseline results; Case 2, Same decay rates, and Case 3, Same storage rates) were performed and the results are listed in Table 3.

At $D = 0.16\ h^{-1}$, the ratio C_{xfloc}/C_{xt} was increased by 17.2% due to the storage effect (Case 2), and by 3.8% due to the decay rate (Case 3). This indicated that the storage phenomena impact the ratio more than the decay rates of the two populations. This is because a lower dilution rate leads to inadequate substrate levels for growth. Therefore, storage becomes a competitive advantage, and floc formers, which are modeled as having faster storage kinetics, are favored. At $D = 0.5\ h^{-1}$, the percentage of change due to different decay rates (96.5%) is significantly higher than that due to storage (83.1 %). This may be because the substrate level is high enough to allow a higher concentration of filaments, which increases the effect of the decay rate. The difference between the decay rates becomes the dominant factor affecting competition. At $D = 1.32\ h^{-1}$, the effect due to decay rate and storage is very small. At this high dilution rate, the washout effect controls the complete process. The retention time is too low to allow filaments to be retained in the reactor.

Assuming that a C_{xfil}/C_{xt} ratio of 0.25 (i.e., 25% of the biomass is composed of filaments) is the threshold for bulking, then the above analysis shows that bulking will occur if the dilution rate is between 0.1 and 0.84 h^{-1}. The C_{xfil}/C_{xt} ratio as a function of MCRT is also plotted in Figure 9, which shows that the sludge bulking occurs when MCRT is between 0.7 and 5.8 d. This is because within this range, filaments having lower decay rates become dominant that can compete with floc formers. However, at extremely low and very high MCRT the combination of kinetic selection effect and storage phenomena are more favored for the growth of floc formers.

Conclusions

A model integrating kinetic selection theory, filamentous backbone theory, storage phenomena, and decay was developed to predict the competition between filaments and floc formers in activated sludge. The model was shown to generically predict coexistence of the two types of microorganisms, in contrast to simple kinetic selection models. The values of decay rates of floc formers and filaments were measured using oxygen uptake rate measurements of bulking and non-bulking sludges. The measured decay rate for filament-dominated sludge was lower than that of floc-dominated sludge, revealing another competitive advantage for filaments. Sensitivity analysis and Monte Carlo simulation were used to determine bulking potential and identify the major parameters that significantly affect the modeling results. Analysis of dilution rate effects showed that the storage effect was high at low dilution rates, while the decay effect became significant at high dilution rates. The modeling results indicate that the rate of filament incorporation into floc, α, the storage rate parameters, and the growth kinetic parameters should be the focus of future experimental work, as these determine the outcome of competition, depending on the dilution rates. A

procedure for identifying operational conditions (mean cell residence times and hydraulic residence times) that are conducive to bulking was outlined for the special case of a completely-mixed reactor. The results show that over the typical range of MCRTs, and after integrating kinetic selection, filamentous backbone, storage phenomena, and decay, bulking conditions are common in a CMR. Thus, this study reinforces the concept that to control filamentous growth, CMR conditions should be avoided.

Acknowledgments

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Table 1. Values of kinetic parameters and decay rates of floc formers and filaments (literature values and experimental values determined in this study)

Reference	Ks	μ_{max}	Y	k_d
Filaments				
Lau (1984) *Sphaerotilus* (glucose-limited growth)	10.0 (mg carbon source/L)	$6.5d^{-1}$	0.47-0.53 (g solids/g glucose)	$0.05d^{-1}$
Richard (1985) *Sphaerotilus*	10 (mg glucose/L)	6.5^{-1}	0.53 (mg cells/mg glucose)	$0.065d^{-1}$
Richard (1985) Type 1701	2 (mg glucose/L)	$2.6d^{-1}$	0.44 (mg cells/mg glucose)	$0.06d^{-1}$
Richard (1985) Type 021N	<1 (mg glucose/L)	$1.4\text{-}3.8d^{-1}$	0.52-0.67 (mg VSS/mg COD)	0.16-0.55 d^{-1}
Van Niekerk (1987) Type 021N	0.07 (mg HAc/L)	$3.9d^{-1}$	0.38 (g SS/g HAc)	$0.03d^{-1}$
Van Veen (1982) *Haliscomenobacter hydrossis*	5.3 (mg COD/L)	$1.2\text{-}2.14d^{-1}$	0.39-0.55 (mg VSS/mg COD)	---
This study				$0.002h^{-1}$ ($0.048\ d^{-1}$)
Floc formers				
Lau (1984) *Citrobacter* sp. (glucose limited growth)	5.0 (mg carbon source/L)	$9.2d^{-1}$	0.43-0.55 (g solids/g glucose)	$0.15d^{-1}$
Richard (1985) FF35	4.3 (mg glucose/L)	---	---	---
Van Niekerk (1987) *Zoogloea ramigera* Aeration basin	0.3 (mg HAc/L)	$5.5d^{-1}$	0.51 (g SS/g HAc)	$0.08d^{-1}$
Chiu (1972) (Mixed population)	26.5 (mg/L)	$16.6d^{-1}$	0.577 (mg VSS/mg COD)	$0.45d^{-1}$
Muck and Grady (1974)	130-172 (mg/L)	$4.9\text{-}20.8d^{-1}$	0.44-0.47 (mg VSS/mg COD)	$0.09\text{-}0.89d^{-1}$
This study				$0.006h^{-1}$ ($0.144d^{-1}$)

Table 2. Parameter values used in the model

	Kinetic selection (from literature)	Backbone coeff.	Decay rate (from experiment)	Storage
Floc former	$\mu_{maxfloc}=0.6(h^{-1})$	$\alpha=0.01h^{-1}$	$k_{dfloc}=0.006$ (h^{-1})	$k_{STOfloc}=0.3(h^{-1})$
				$K_{STOfloc}=1(g/L)$
	$K_{sfloc}=0.065(g/L)$			$Y_{STOSfloc}=0.5(g\ MLSS/g)$
				$Y_{STOXfloc}=0.5(g\ MLSS/g)$
	$Y_{floc}=0.88(g\ MLSS/g)$			$b_{STOfloc}=0.004\ (h^{-1})$
				$umax_{STOSXfloc}=1(h^{-1})$
Filament	$\mu_{maxfil}=0.2(h^{-1})$		$k_{dfil}=0.002$ (h^{-1})	$k_{STOfil}=0.15(h^{-1})$
				$K_{STOfil}=1(g/L)$
	$K_{sfil}=0.008(g/L)$			$Y_{STOSfil}=0.5(g\ MLSS/g)$
				$Y_{STOSXfil}=0.5(g\ MLSS/g)$
	$Y_{fil}=0.88(g\ MLSS/g)$			$b_{STOfil}=0.004(h^{-1})$
				$umax_{STOSXfil}=0.6(h^{-1})$

Table 3. Effect of storage and decay rates at different dilution rates

Case	1. Baseline result	2. Same k_d $k_{dfloc}=k_{dfil}=0.002\ h^{-1}$		3. Same storage rate $k_{STOfloc}=k_{STOfil}=0.15\ h^{-1}$	
Dilution rate (h^{-1})	C_{xfloc}/C_{xt}	C_{xfloc}/C_{xt}	% (C_{xfloc}/C_{xt}) increase	C_{xfloc}/C_{xt}	% (C_{xfloc}/C_{xt}) increase
0.16	0.66	0.84	17.2	0.75	3.8
0.5	0.50	0.62	83.1	0.66	96.5
1.32	1.00	1.00	0.04	1.00	0.04

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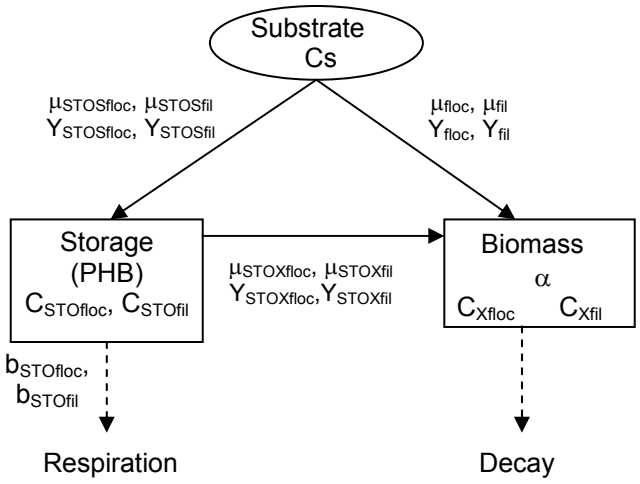


Figure 1. Schematic representation of model.

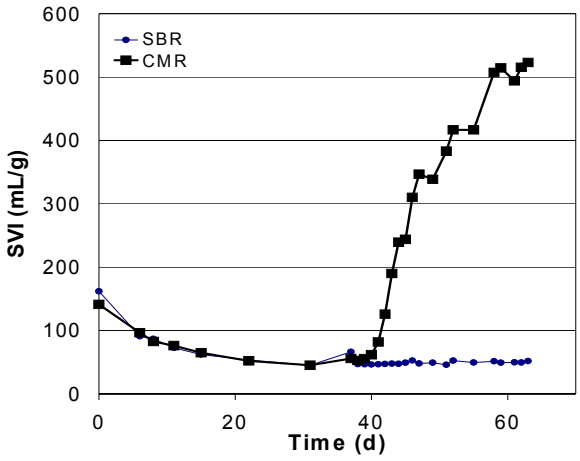


Figure 2. SVI change over time in laboratory-scale reactors.

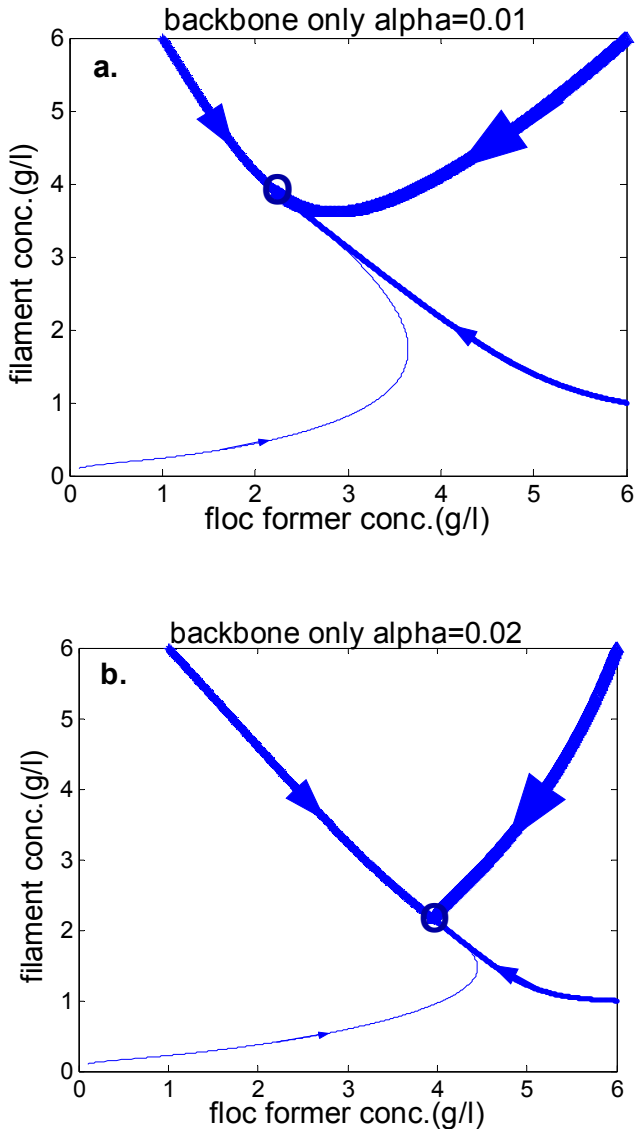


Figure 3. Phase diagrams showing effect of rate of filament incorporation into floc. (a) $\alpha = 0.01\ h^{-1}$, (b) $\alpha = 0.02\ h^{-1}$.

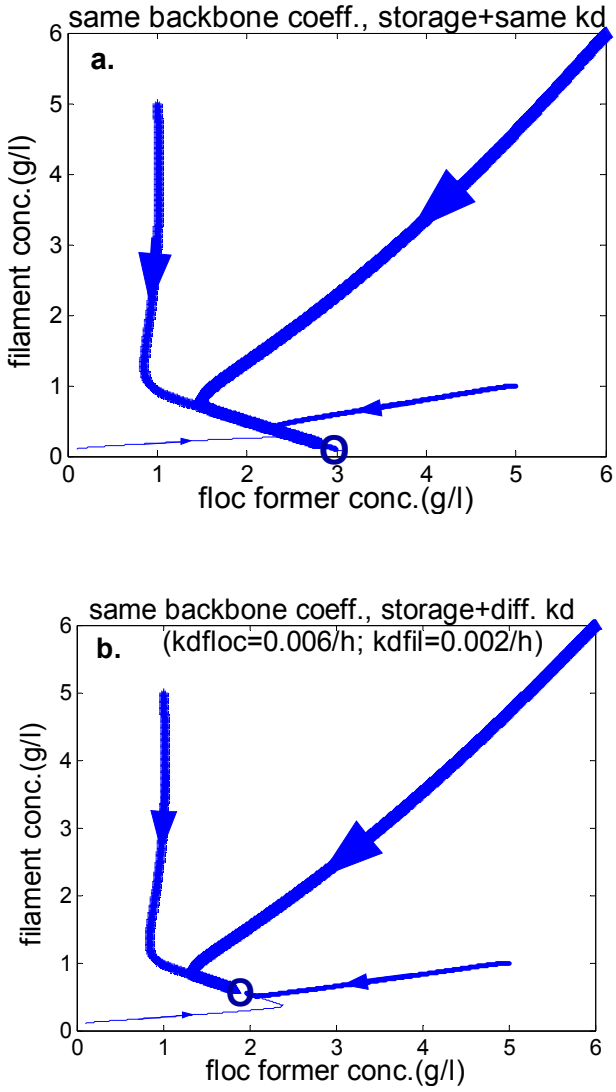


Figure 4. Phase diagrams showing effect of decay rates. α=0.01 h^{-1}, and (a) $k_{dfloc} = k_{dfil}$ = 0.006 h^{-1}, (b) k_{dfloc} = 0.006 h^{-1} and k_{dfil} = 0.002 h^{-1}.

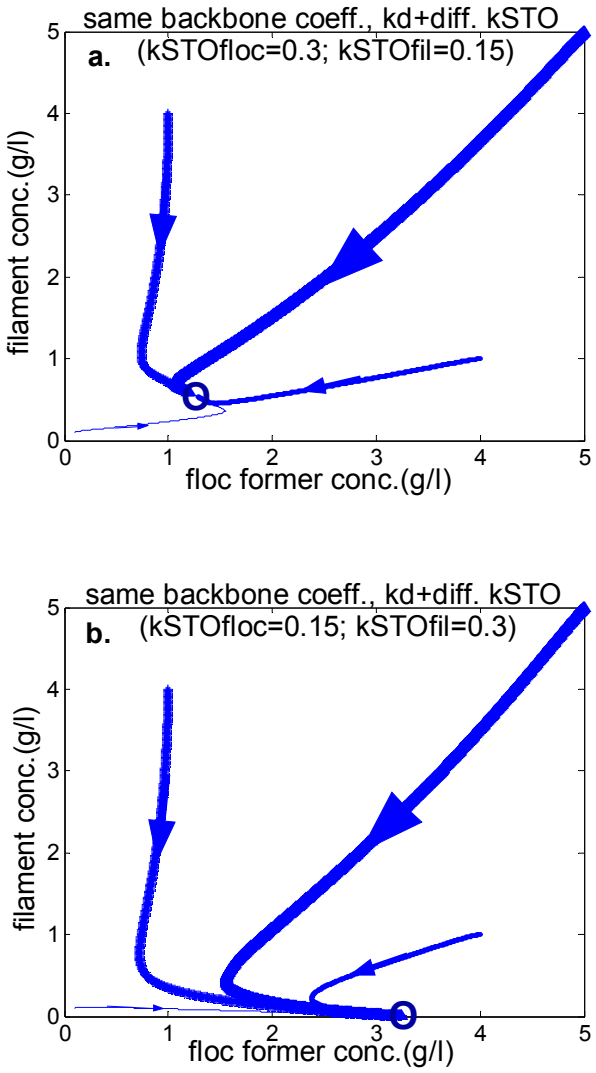


Figure 5. Phase diagrams showing effect of storage kinetics. (a) $k_{STOfloc}$ = 0.3 h h^{-1}, k_{STOfil} = 0.15 h^{-1}; (b) $k_{STOfloc}$ = 0.15 h^{-1}, k_{STOfil} = 0.3 h^{-1}.

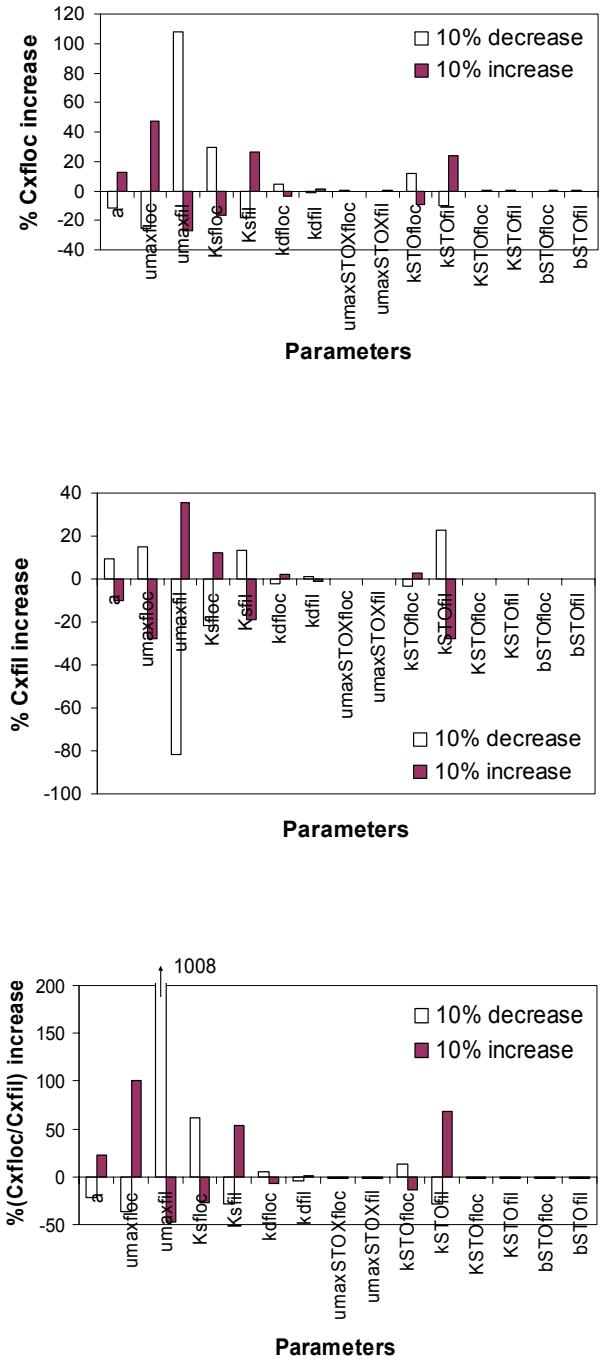


Figure 6. Sensitivity analysis. Each parameter was decreased or increased 10% from its baseline values.

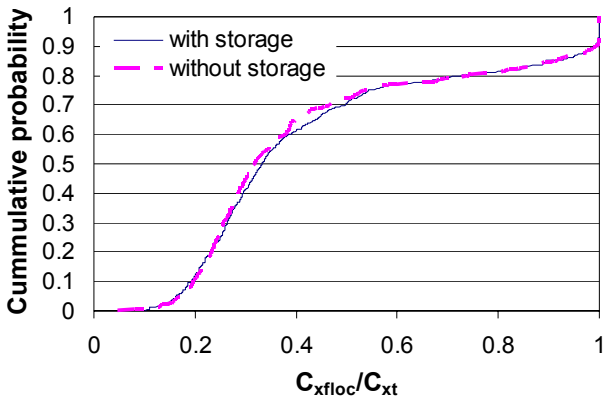


Figure 7. Monte-Carlo simulation to predict the bulking potential at D = 0.6 h^{-1}.

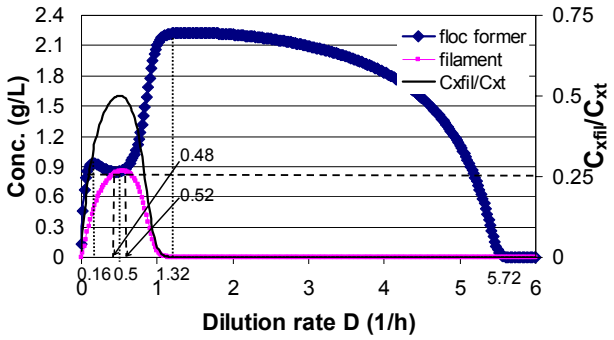


Figure 8. Effect of dilution rate on the concentrations of floc formers and filaments.

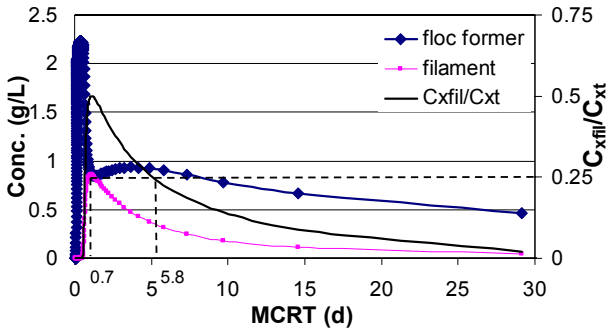


Figure 9. Effect of mean cell residence time (MCRT) on the concentrations of floc formers and filaments.

Chapter 2.

Kinetic parameter measurement using respirometry

Objective: Evaluate the concept of K_s and μ_{max} strategies, and measure the decay rates for bulking and non-bulking activated sludge, respectively, using traditional respirometric testing. The fluorescent *in-situ* hybridization (FISH) technique was used to identify the dominant filaments in bulking activated sludge developed in lab-scale reactor.

2.1 Materials and methods

2.1.1 Reactor study

Two laboratory reactors, each with an active volume of 8 L, were operated to develop bulking and non-bulking sludges. The experiment was performed in 3 stages. In Stage 1 (Day 0 to Day 36), filamentous bulking in the sludge inoculum was suppressed by running both reactors as sequencing batch reactors (SBRs) with an anaerobic phase. In Stage 2, one reactor was operated as an SBR and the other was operated as a completely mixed reactor (CMR) (Figure 1). Both reactors were seeded with the combined sludge from Stage 1. The SBR simulated a plug flow reactor (PFR) by having a short fill time (12 min), while the CMR had a fill time of 24 hours. In Stage III, the CMR was switched to SBR operation as in Stage I. The mean cell retention time (MCRT) and the hydraulic residence time (HRT) for both reactors were 16 days and 12 hours, respectively. The reactors were seeded with activated sludge from the North Cary Water Reclamation Facility (Cary, NC). The reactors treated synthetic wastewater (all mg/L: 250 glucose, 150 yeast extract, 50 $MgSO_4 \cdot 7H_2O$, 7

KCl, 150 NH_4Cl, 196.4 KH_2PO_4, 55.5.6 $NaHCO_3$, 5 $MnSO_4 \cdot 7H_2O$, 3.8 $CaCl_2$, and 2.2 $FeSO_4 \cdot 7H_2O$). The MLSS (mixed liquor suspended solids concentration), MLVSS (volatile suspended solids concentration), SV (30 minute settled volume) and SVI (sludge volume index) were periodically measured using Standard Methods (Greenberg *et al.* 1998).

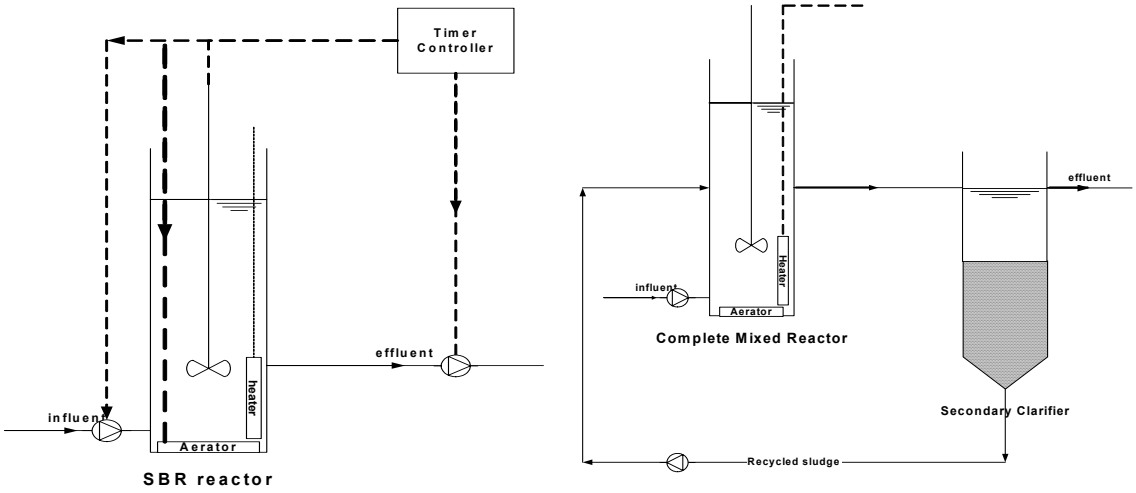


Figure 1. SBR and CMR configuration

2.1.2 Respirometric test

A respirometer (Tudor Scientific, Belvedere, SC) with an internal volume of 230 – 250 mL was used (Figure 2). The respirometer was water-jacketed, and the temperature during the tests was maintained at approximately 25 ˚C by a circulating constant temperature water bath. Mixing during the tests was provided by magnetic stir bars. The dissolved oxygen (DO) concentration was measured by a DO meter (YSI 5100 Dissolved Oxygen Meter, Yellow Spring Instruments, OH) that was interfaced with a personal computer for data acquisition. DO was automatically recorded every second.

Prior to the test, the DO meter was calibrated, and the biomass was aerated for approximately 4 hours to consume any residual substrate. The biomass was transferred to the respirometer,

and the DO concentration was adjusted about 8.3 – 8.5 mg/L. Endogenous respiration was collected 5 min prior to injection of the biomass. DO data were collected until the oxygen uptake rate (OUR) returned to the background endogenous rate. Two extant kinetic tests were performed with each biomass sample.

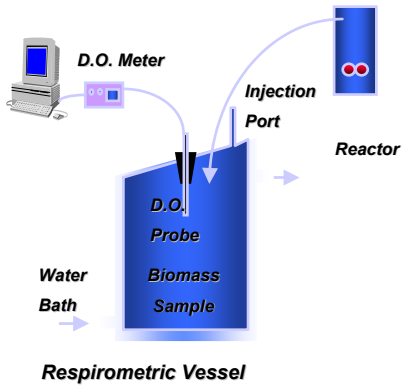


Figure 2. Configuration of respirometric vessel

The oxygen uptake ($DO_0 - DO_{(t)}$) data were subsequently used to estimate the kinetic parameters μ_{max}, K_s, and Y for the biodegradation of glucose by minimizing the residual sum-of-squares error between the measured and theoretical DO concentrations. The following equations expressing the variation of substrate (S) and biomass (X) concentrations as a function of time during a batch experiment were used.

Y=1-(DO consumption)/S_0

$$\frac{dX}{dt} = (\frac{\mu_{\max} \cdot S}{K_s + S}) \cdot X - K_d \cdot X \quad \text{(i)}$$

$$\frac{dS}{dt} = -\frac{1}{Y}(\frac{\mu_{\max} \cdot S}{K_s + S}) \cdot X \qquad \text{(ii)}$$

$$DO_0 - DO(t) = (S_0 - S(t)) - (X(t) - X_0) \qquad \text{(iii)}$$

Minimize RSSE= $\sum_{i=1}^{n} [(DO_{obs})_i - (DO_{th})_i]^2$

Where S_0 and X_0 are the concentrations (mg COD/L) of substrate and biomass, respectively, at the start of biodegradation.

The data analysis was performed as previously described by Grady *et al.* (1998).

The simultaneous estimation of μ_{max}, K_s, and Y involved assuming an initial set of parameter values and calculating a theoretical oxygen uptake curve by solving equations (i) to (iii). A minimal residual sum-of-squares error, associated with the difference between the theoretical curve and the oxygen uptake data obtained in the respirometer, had to be reached. The values of μ_{max}, K_s, and Y associated with the minimum constitute the best estimation of the parameters (Ellis *et al.* 1996).

2.1.3 Evaluation of decay rates k_d

To evaluate the value of k_d, a sample of biomass was taken from the reactors and aerated for 10 days. A subsample of the biomass was taken to measure the endogenous oxygen uptake rate (OUR) once every day. The decay coefficient was estimated by plotting ln(OUR) versus time of biomass aeration (Grady and Daigger 1999). According to the equation (iv), the decay coefficient should be the slope of this line. The measured k_d value was adjusted to temperature using the temperature coefficient value of 1.029 as described by Dold *et al.* (1980).

$$\ln(OUR) = \ln(X_0 k_d) - k_d t \quad \text{(iv)}$$

2.1.4 Fluorescent in-situ hybridization (FISH)

Oligonucleotide probes

The oligonucleotide probes used for FISH and membrane hybridizations are listed in Table 1. For FISH, Cy3 labeled probes were obtained from Sigma-Genosys (The Woodlands, Texas) and Integrated DNA Technologies, Inc. (Coralville, IA.)

Table 1. Probe names, target groups and hybridization conditions used in this study

Probe name[a]	Target group	Probe use	Tw (°C)	*FA %*	Reference
S-S-S.nat-0656-a-A-18	*Sphaerotilus natans*	FISH	46	45	(Wagner 1994a)
S-*-1851-0592-a-A-21	Eikelboom Type 1851	FISH	N/A	35	(Beer *et al.* 2002)

[a] Probe names have been standardized according to the Oligonucleotide Probe Database (Alm *et al.* 1996)

2.2 Results

2.2.1 Lab-scale reactors

In Stage I, the objective was to reduce the SVI to less than 150 ml/g, which is generally regarded as the threshold SVI for bulking (Jenkins *et al.* 2003). By transferring the sludge from NCWRF to the lab scale reactors, and running the reactors as SBRs with an anaerobic stage followed by an aerobic stage, conditions that favored floc forming bacteria were created. In Stage I, mixed liquor suspended solids concentrations in both SBRs were maintained at 3.5 to 4.0 g l^{-1}. The SVI of both reactors decreased from 270 ml g^{-1} at the beginning of this stage to around 50 ml g^{-1} (Figure 3). Microscopic observation (data not shown) showed that most filamentous bacteria were washed out or decayed. One month after start-up, the SVIs of both the reactors decreased to around 40 ml g^{-1}. This stage lasted for more than 4 months (Day -130 to Day 0) until the sludge stabilized to a yellowish, granular appearance with good settleability.

In Stage II, one reactor was operated as an SBR and the other as a CMR, and *S. natans* cells were added to stimulate bulking. One week after the change of the operational condition, the CMR began bulking suddenly, and the SVI increased from 92.9 ml g^{-1} to 198.2 ml g^{-1} (Day 10). During the same period, the SVI for the SBR remained constant at about 40.3 ml g^{-1}. The sludge bulking in the CMR continued, with the SVI reaching the maximum of 1283 ml g^{-1} (Figure 3). In Stage III, the CMR was switched to SBR operation as in Stage I. The SVI of the CMR decreased sharply and within one MCRT ((Day 36 to Day 45) returned to non-bulking status with a final concentration of 72.5 ml g^{-1}. During the entire run, the SBR sludge remained non-bulking, and the SVI remained in the 40.3 to 83.8 ml g^{-1} range.

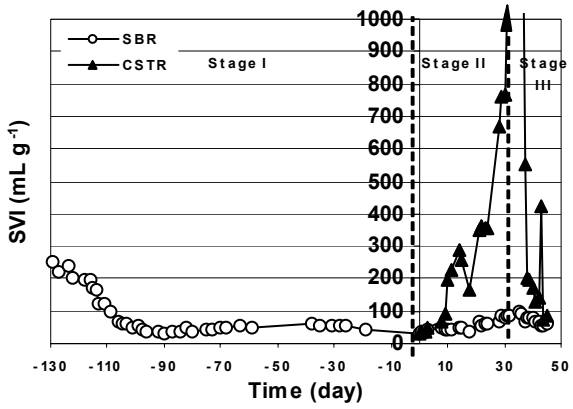


Figure 3. SVI change over time in laboratory-scale reactors

2.2.2 Respirometric test and decay rate measurement

The respirometric DO curve and Monod curve fitting after normalization were shown in Figure 4. The test results can be found in Figure 5 and 6. The respirometric test results showed that the non-bulking sludge (SBR) had a average value of μ_{max} that was about three times that of the bulking sludge (CMR). Similarly, the average apparent K_s for the non-bulking sludge was eight times that of the apparent K_s for the bulking sludge. These values

confirm the basic assumption of the kinetic selection theory for the filament Type 1851. Yield values for glucose degradation for both sludges were similar, and relatively high (0.88 g/g), because glucose, a readily degradable substrate, was used. The decay rate measurements (figure 2.2.2-3) closely matched the values from other studies (Table 1, Chapter 1), and showed that bulking sludge have a lower k_d than non-bulking sludge. This has the effect of increasing the net growth rate of filaments, as compared to the assumption of equal decay rates for both filaments and floc formers. Table 1 (Chapter 1.) compares the experimental kinetic parameters and decay rates of floc formers and filaments with values from the literature. Although different floc forming and filamentous species were used in other studies, all the experimental values of the parameters in this study, except for Y, are within the range of values found in the literature. However, the high Y value we measured (0.88 g VSS/g COD) is similar to that (0.89 gVSS/g COD) reported by Barbeau (1992), who also used glucose as substrate for determination of extant kinetic parameters.

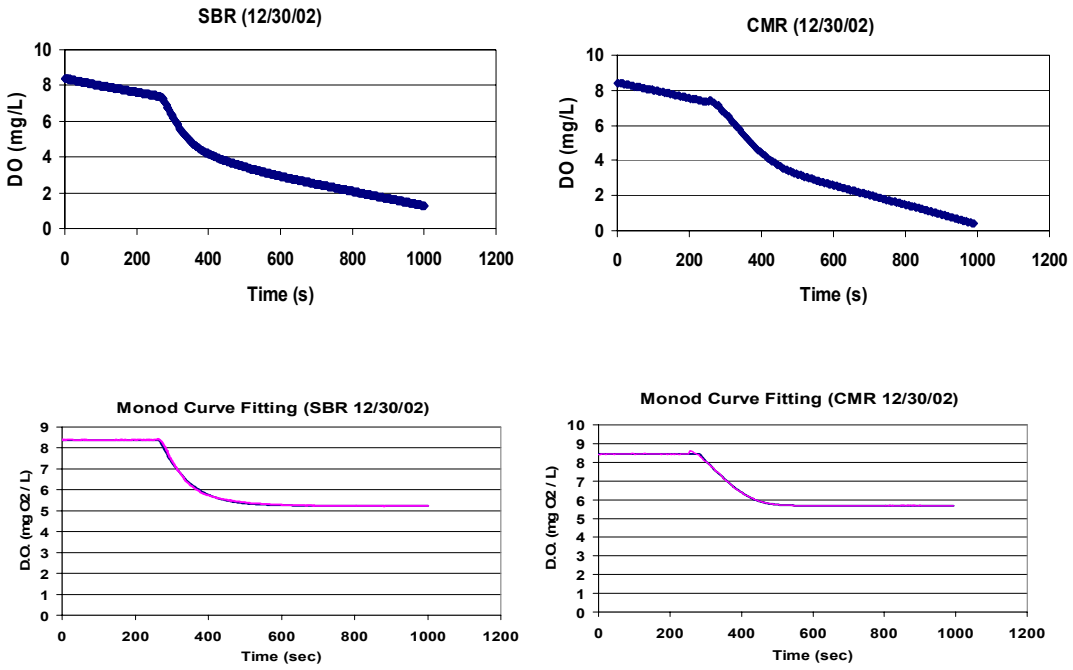


Figure 4. DO curve and Monod curve fitting after normalization

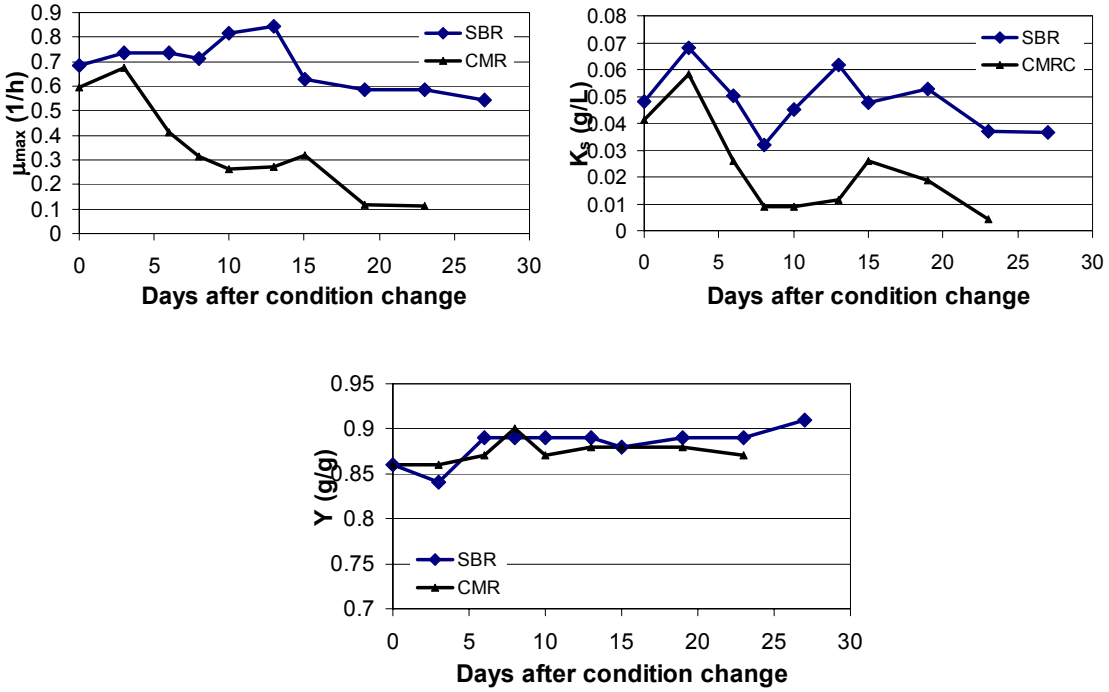


Figure 5. Kinetic parameters, μ, K_s, and Y change over time

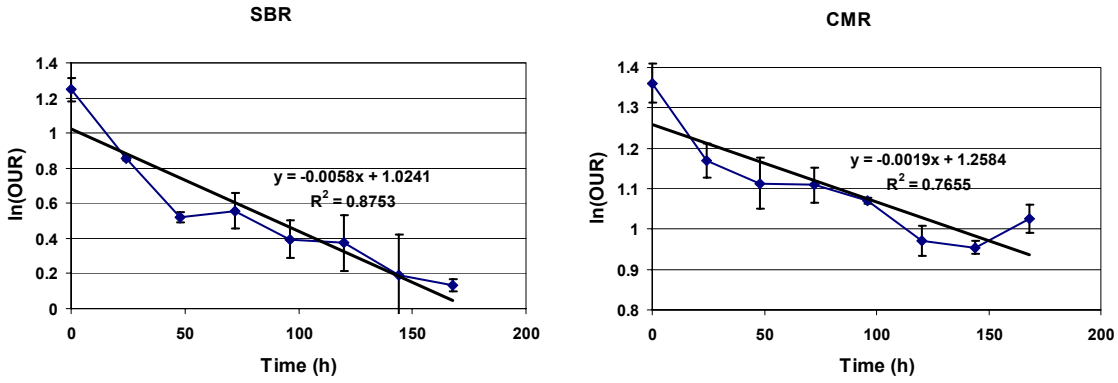
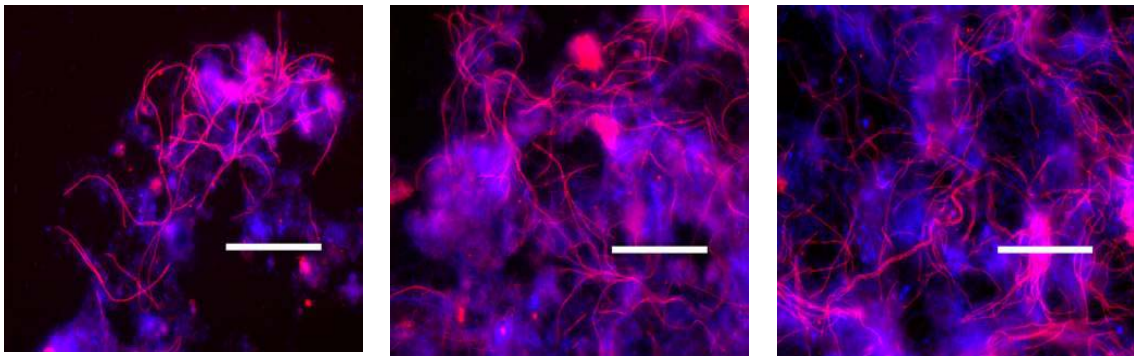


Figure 6. Relationship of ln(OUR) vs. time in SBR A and CMR C (determination of k_d)

2.2.3 FISH for identifying the dominant filament

A Gram-negative filament was found to be dominant during the whole course of bulking. Top-down FISH with available oligonucleotide probes targeting increasingly smaller phylogenetic groups showed that this filament was Eikelboom Type 1851 (Figure 7).



10 days sludge 15 days sludge 24 days sludge

Figure 7. Micrographs of Type 1851 filaments in the CMR over time

2.3 Discussion

The marked difference in SVIs among the two reactors is in accordance with the prevailing views on selection of filaments. The substrate gradient in a quick fill SBR allowed the floc formers to outcompete the filaments. On the other hand, the continuous low substrate concentration inside the CMR and the slow fill SBR allowed filaments to dominate. Traditionally, this has been explained by kinetic differences (Chudoba *et al.* 1985; Jenkins *et al.* 2003). High μ_{max} floc-formers outcompete low μ_{max} filaments under high substrate conditions, while low K_s filaments outcompete the floc formers under low substrate conditions. The kinetic selection theory, while popular, is incomplete in explaining the competition between floc-formers and filaments, since complete washout of one type never

occurs in activated sludge. Various modifications to the theory have been proposed, including the role of incorporated filaments into floc (Cenens *et al.* 2000), differences in substrate storage kinetics (Jenkins *et al.* 2003; Lou and Reyes 2002), and differences in decay rates (Lou and Reyes 2002). Recently, Martins *et al.* (2003a) showed that substrate uptake and potential for substrate storage were similar for floc formers and filaments. Accordingly, they formulated an alternative hypothesis wherein filaments gain competitive advantage because of substrate gradients created by diffusion limitation inside the floc. At low substrate concentrations, filaments gain an advantage by extending outside the floc to obtain access to substrate. Conversely, at high substrate concentrations, the filaments can remain inside the floc without suffering a disadvantage because substrate is not limited inside the floc. Our data appears to support this "diffusion limitation" hypothesis, and directly contradicts the prediction of the kinetic selection theory. Even under high substrate conditions in the SBR, high levels of filaments were observed, albeit inside the flocs. Additional research is needed to determine if there are no intrinsic differences in the K_s of floc formers and filaments. Regardless of the mechanism for development of filamentous structures, it is clear that low substrate concentrations favor the growth of Type 1851.

Type 1851 has been shown to belong to the *Chloroflexi* group (Kohno *et al.* 2002), is associated with high MCRTs (> 10 days) and low food-to-microorganism ratios (< 0.2 as kg BOD_5 / kg MLVSS), and is often seen when simple sugars are present in the wastewater (Jenkins *et al.* 2003). These conditions were present in the laboratory-scale reactors. Previous studies have shown that Eikelboom Type 1851 is an extremely slow grower in pure culture (Beer *et al.* 2002; Kohno *et al.* 2002). Type 1851 has been found in both industrial

wastewater (including cattle slaughtering, fruit juice processing, and chicken slaughtering industries) and domestic sewage (Kohno *et al.* 2002); in Australia (Beer *et al.* 2002) and in Japan (Kohno *et al.* 2002). Bjornsson *et al.* (2002) concluded that filamentous *Chloroflexi* (green non-sulfur bacteria) are abundant in biological nutrient removal WWTPs. This is consistent with the fact that the North Cary facility is a biological nutrient removal plant, as are many WWTPs in North Carolina. It is likely that Type 1851 bulking is not confined to this one plant. In a US survey, Type 1851 was the thirteenth most common filament found in WWTPs (Jenkins *et al.* 2003). In a survey conducted in 1989 to 1991 on Danish BNR plants, Type 1851 was the sixth dominant filamentous microorganism (Kristensen *et al.* 1994). Another survey of nutrient removal activated sludge systems in South Africa revealed that Type 1851 was the fourth in frequency (Lacko *et al.* 1999). Since more and more WWTPs are required to upgrade to perform BNR, the extent of Type 1851-caused bulking may increase.

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Chapter 3.

Kinetic parameter measurement using substrate uptake tests and quantitative FISH

(This chapter was published in Biotechnology and Bioengineering 92(6): 729-739)

SUBSTRATE UPTAKE TESTS AND QUANTITATIVE FISH SHOW DIFFERENCES IN KINETIC GROWTH OF BULKING AND NON-BULKING ACTIVATED SLUDGE

Competition between filaments and floc formers

INCHIO LOU and FRANCIS L. DE LOS REYES III*

Abstract

The competition between filaments and floc formers in activated sludge has been historically described using kinetic selection. However, recent studies have suggested that bacterial storage may also be an important factor in microbial selection, since the dynamic nature of substrate flows into wastewater treatment plants elicit transient responses from microorganisms. Respirometry-based kinetic selection should thus be reevaluated by considering cell storage, and a more reliable method should be developed to include bacterial storage in the analysis of growth of filaments and floc formers in activated sludge. In this study, we applied substrate uptake tests combined with metabolic modeling to determine the growth rates, yields and maintenance coefficients of bulking and non-bulking activated sludge developed in lab scale reactors under feast and famine conditions. The results of quantitative fluorescence in situ hybridization (FISH) showed that the filaments Eikelboom Type 1851, Type 021N and *Thiothrix nivea* were dominant in bulking sludge, comprising

42.0 % of mixed liquor volatile suspended solids (MLVSS), with 61.6% of the total filament length extending from flocs into bulk solution. Only low levels of Type 1851 filament length (4.9% of MLVSS) occurred in non-bulking sludge, 83.0% of which grew inside the flocs. The kinetic parameters determined from the substrate uptake tests were consistent with those from respirometry and showed that filamentous bulking sludge had lower growth rates and maintenance coefficients than non-bulking sludge. These results provide support for growth kinetic differences in explaining the competitive strategy of filamentous bacteria.

KEYWORDS

Growth rate, yield, maintenance coefficient, substrate uptake, metabolic modeling, quantitative FISH

INTRODUCTION

Understanding how filamentous organisms are selected over floc-forming bacteria in activated sludge systems is crucial, since the overgrowth of filaments lead to sludge bulking, the inability of sludge to settle and compact well in secondary clarifiers. The kinetic selection theory (KST) is the most widely used theory for explaining the competition between filaments and floc formers (Chudoba et al. 1973a; Chudoba et al. 1973b; Chudoba et al. 1973c). KST suggests that differences in Monod kinetic coefficients K_s (half-saturation constant) and μ_{max} (maximum growth rate) for a limiting soluble substrate can explain the selection of filaments over floc formers under certain substrate concentrations.

However, recent studies (Goel et al. 1998; Kohno et al. 1991; Krishna and van Loosdrecht 1999; Majone et al. 1996; van Loosdrecht et al. 1997) suggest that formation of storage

compounds inside cells may be an important factor that affects this competition. Activated sludge plants are hardly steady-state environments. Under unbalanced growth conditions, biomass composition changes. Rather than simple steady-state kinetics, storage of substrates as internal polymers becomes an important factor in determining the outcome of the microbial selection between filaments and floc-formers.

The storage effect has been considered and incorporated into the ASM3 model proposed by the IWA Task Group on Mathematical Modeling (Gujer et al. 1999). In addition, respirometric methods have been modified for calculation of storage yield (Karahan-Gui et al. 2002). In ASM3, it is hypothesized that heterotrophic microorganisms remove soluble substrates by first forming storage polymers, followed by growth occurring only on internally stored polymers. However, this is not realistic since simultaneous storage and growth processes are probably occurring. In the current study, we considered simultaneous storage and growth. Three yield factors are introduced: one for growth of the biomass on the substrate, one for storage formation on the substrate and one for biomass growth on the stored substrate, and related kinetic expressions were defined accordingly.

Respirometry, which is based on the relationships between substrate biodegradation, biomass growth, and oxygen consumption, has typically been used to determine kinetic parameters (Ellis et al. 1996; Grady et al. 1996). Though useful in the control of activated sludge process, traditional respirometry does not reflect the actual metabolic mechanism in cells, and does not consider bacterial storage. This aspect of respirometric measurements has not been explicitly stated in past studies, and may undermine the validity of experimental data

supporting the kinetic selection theory. Respirometric measurements should thus be reevaluated by considering the effect of storage. Previous researchers (van Aalst-van Leeuwen et al. 1997) have formulated a metabolic model for describing the stoichiometry of poly-β-hydroxybutyrate (PHB) metabolism using a pure culture of *Paracoccus pantotrophus* under dynamic substrate supply. Substrate uptake tests combined with the model was used to analyze the metabolism of PHB (Beun et al. 1999; Beun et al. 2000), the metabolism of glycogen (Dircks et al. 2001a) and their combination (Carta et al. 2001) in activated sludge.

Instead of KST, the "substrate diffusion limitation" (SDL) hypothesis has also been recently suggested for explaining filamentous bulking (Martins et al. 2003). Similar values of maximum substrate uptake rates were obtained for bulking and non-bulking sludge developed using different feeding patterns. It was hypothesized that bulking sludge occurs because of substrate gradients in sludge flocs. At low bulk substrate concentration, floc formers cannot access enough substrate for their growth because of SDL. On the other hand, filaments, because of their morphology, can more quickly grow outside the flocs to access substrate, even if the growth rates are similar to floc formers. However, at high bulk substrate concentration, filaments have no such advantage and are not found extending outside the floc. Our previous results (Liao et al. 2004) seemingly supported the SDL hypothesis for bulking. Quantitative FISH showed that high levels of filamentous lengths were observed for both bulking and non-bulking sludge. However, since the kinetic parameters for both types of sludge were not measured, and the filamentous biomass levels were not quantified, it still remains unclear whether filamentous bulking is due to SDL or KST, or some combination of both mechanisms.

In the present study, we hypothesized that filamentous bulking sludge and non-bulking sludge have different kinetic, storage and maintenance parameters. These parameters were measured by combining substrate uptake tests and metabolic modeling, and thus bacterial storage was explicitly considered. The total and extended filament length levels were determined using quantitative FISH, and the converted biomass concentrations in bulking and non-bulking sludge were compared.

MATERIALS AND METHODS

a. Substrate Uptake Test

Sequencing batch reactors (SBRs), each with active volumes of 8 L, were operated to simulate dynamic feast/famine conditions. The reactors were seeded with sludge from the North Cary Wastewater Reclamation Facility (Cary, NC), and treated synthetic wastewater [200-300 mg/L glucose, 50 mg/L $MgSO_4 \cdot 7H_2O$, 7 mg/L KCl, 150 mg/L NH_4Cl, 141.8 mg/L, KH_2PO_4, 54.91 mg/L K_2HPO_4, 555.6 mg/L $NaHCO_3$, and 1 mL/L trace element solution (Vishniac and Santer 1975)]. The pH and temperature of the reactors were maintained at 7.0 ± 0.1 and 22 ± 1 ^oC respectively. Three SBRs (A, B, and C) with mean cell residence times (MCRTs) of 4, 8 and 16 days respectively, were operated with a 5 min fill time to obtain non-bulking sludge (Liao et al. 2004; Martins et al. 2003), while two SBRs (D and E) with MCRTs of 8 and 16 days respectively, were operated with a fill time of 1.5 hours to produce bulking sludge. Filter units were installed in SBRs D and E to prevent sludge loss during effluent discharge. The operation of one cycle (period of 8 hrs) is shown in Table I.

Nitrification was prevented by adding 200 mg of allythioureum (ATU) to the reactor before the sampling cycle.

Mixed liquor suspended solids concentration (MLSS), mixed liquor volatile suspended solids (MLVSS), and 30 minute sludge volume index (SVI) were periodically measured using Standard Methods (Greenberg et al. 1998). Total organic carbon (TOC) was measured using a TOC analyzer (Model DC-190, Rosemount Analytical Inc., Chanhassen, MN). Dissolved oxygen (DO) was measured using a YSI5300 DO probe meter (YSI, Yellow Springs, OH). The oxygen uptake rate (OUR, in mg/L/h) of activated sludge during the famine phase was performed in batch respirometric experiments with small initial glucose concentrations as described by Ellis *et al.* (1996). DO depletion due to glucose degradation in a 250 ml glass respirometer (Tudor Scientific Inc., Belvedere, S.C.) was continuously recorded during a respirometric test, from which the DO uptake (in mmol/cycle) during the famine phase was estimated using the OUR, the reactor active volume, and the duration of the famine period. Glucose and glycogen were measured using a kit (Boehringer Mannheim, Cat. No.716251). The elemental composition (C, H, N, S) of the biomass was measured with an elemental analyzer (Soil Science Laboratory, North Carolina State University), and the oxygen content of biomass was calculated by subtracting the percentage of C, H, N, S, and ash from 100%. The biomass concentration was calculated as $TOC_{biomass}=TOC_{total} - TOC_{filter}$, where $TOC_{biomass}$ is the biomass concentration measured as TOC; TOC_{total} is the concentration of mixed liquor measured as TOC; TOC_{filter} is the concentration of supernatant measured as TOC after passing through 0.45 μm filters. The start and end of feast and famine phases during one cycle were estimated using the DO curve. Macrobal software (Hellinga 1992)

was used for balancing all the converted amounts. The obtained data were used as inputs to a metabolic model to determine the stoichiometric parameters and consequently, growth rates of bulking and non-bulking sludge. The maximum specific substrate uptake rates during feast phases were measured using two different methods. For bulking sludge, the maximum specific substrate uptake rates were obtained by measuring the differences in glucose concentration and dividing by biomass concentration and the time (measured during the first 15 minutes of the feast phase). For non-bulking sludge, because the feast period was so short that the glucose concentration cannot be measured accurately, the maximum specific substrate uptake rates were assumed to be equal to the specific substrate uptake rates during the feast phase, as estimated from the Macrobal software.

b. FISH and filament length quantification

Quantitative FISH as previously described (Liao et al. 2004) was used for identifying filaments and measuring the filament length in bulking and non-bulking sludge. FISH probes (Table II) targeting Type 1851, Type 021N, *Sphaerotilus natans*, *Haliscomenobacter hydrossis*, *Thiothrix nivea* (TNI), *Leucothrix mucor* and *Microthrix parvicella* were used. Previous statistical analysis (Liao et al. 2004) was adopted to determine the appropriate number of microscope wells and fields of view within a well for filament length quantification. Twelve wells and five random pictures per well were measured to give a power of 1.0, i.e. the probability that the F test would find a statistically significant difference in mean log counts across samples is 1.0 if the true difference is 0.3 at a significance level of 0.05. The filament biomass concentrations for all types of filaments were estimated based on the correlation between filament length and filament biomass for *L. mucor* (Liao et al. 2004).

c. Metabolic model

The metabolic model previously described by Dircks *et al.* (2001) was used. The model consists of a linear equation for the feast period (1), describing glucose uptake, biomass growth, glycogen production and maintenance, and another linear equation for the famine period (2), describing glycogen consumption, biomass growth and maintenance.

$$-r_s = \frac{1}{Y_{sx}^{\max}} r_X + \frac{1}{Y_{sg}^{\max}} r_g^{feast} + m_s C_X \qquad (1)$$

$$\text{where } Y_{sx}^{\max} = \frac{2\delta + 2/3}{1/2\,\gamma_B \delta + 3.14};\ Y_{sg}^{\max} = \frac{\delta + 1/3}{\delta + 1/2};\ m_s = \frac{m_{ATP}}{2\delta + 2/3}$$

$$-r_g^{famine} = \frac{1}{Y_{gx}^{\max}} r_X + m_g C_X \qquad (2)$$

$$\text{where } Y_{gx}^{\max} = \frac{2\delta + 5/6}{1/2\,\gamma_B \delta + 3.14};\ m_g = \frac{m_{ATP}}{2\delta + 5/6}$$

$-r_s$: glucose uptake rate;

r_X : biomass growth rate;

r_g^{feast} : glycogen production rate in feast phase;

r_g^{famine} : glycogen consumption rate in famine phase;

C_X : biomass concentration;

γ_B : degree of reduction of the biomass;

$Y_{sx}^{\max}$: maximum yield of biomass on glucose;

$Y_{sg}^{\max}$: maximum yield of glycogen on glucose;

$Y_{gx}^{\max}$: maximum yield of biomass on glycogen;

m_s : maintenance coefficient for growth on glucose;

m_g : maintenance coefficient for growth on glycogen;

δ: ATP produced per $NADH_2$ oxidized;

m_{ATP}: maintenance coefficient based on ATP

The parameters $r_s, r_X, r_g^{feast}, C_X$, and r_g^{famine} were determined from the substrate uptake tests, and γ_B was calculated from the biomass composition. Overall, the model consists of

two unknowns δ and m_{ATP}, and two equations. These values are independent of the MCRT, because the metabolic pathways are the same at different MCRTs. After solving these two equations, Y_{sx}^{max}, Y_{sg}^{max}, Y_{gx}^{max}, m_s and m_g were obtained.

RESULTS

a. Substrate uptake tests

Sludges that settled well were obtained in SBRs A, B and C with SVI decreasing from 450 mL/g to around 50 mL/g (Figure 1a). Bulking sludges were produced in SBRs D and E, with SVIs above 400 mL/g (Figure 1b). The SVI decrease between days 20 and 36 was due to pumping problems in the reactors which affected MLSS levels, and did not reflect the settling characteristics. The substrate uptake tests were performed on days 52, 71 and 83 for reactors A, B and C (non-bulking sludge), and days 53 and 66 for reactors D and E (bulking sludge).

The results confirm the finding that filaments are favored in SBRs with long fill times, and can be suppressed in SBRs that simulate plug flow condition by having short fill times. The feast and famine period for both types of sludge were determined from the DO curves during the substrate uptake tests (Figure 2). Table III shows the durations of the feast and famine phases. The DO levels increased in the first 10 min because of mixing and aeration of the mixed liquor in the reactors. The DO concentration then decreased until the glucose was completely degraded, after which the DO again increased and stabilized until the rapid decrease during the settling period. The change in DO concentration clearly defined the feast

phase where DO levels from the injection point decrease to the minimum, and the famine phase was calculated by subtracting the feast phase from the cycle period.

The levels of TOC, biomass growth, and DO uptake in the famine phase were measured or calculated and shown in Table III. The feast period was too short for the glucose concentrations in the reactors to be accurately measured; thus the reactor glucose concentrations were estimated from the influent concentrations. The original glucose concentrations in the reactors were calculated as 2/3 of the glucose concentrations in the influent container, since for each cycle, wastewater corresponding to 2/3 of the active volume was replaced.

For both types of sludge, higher MCRTs resulted in higher C_x, higher DO uptake, and less biomass growth. When glucose was present, a decrease in glucose and an increase in glycogen concentration were measured during the feast period (Figure 3). The glucose uptake rate during the first 5 min can be considered the maximum glucose uptake rate because during the feast period glucose was in excess. After glucose depletion, the glycogen concentration slowly decreased. This result verified that glycogen is an important energy source that affects the growth of microorganisms during the whole cycle. Under the same amount of substrate, the glycogen conversion rate was 0.030 (C-mmol)/(C-mmol biomass) for non-bulking sludge, which is 20% higher than that for bulking sludge, 0.025 (C-mmol)/(C-mmol biomass). This indicates that non-bulking sludge has a higher storage ability than bulking sludge to convert glucose into glycogen during the feast phase.

b. FISH and filament length quantification

Microscopic observation and FISH analysis (Figure 4) showed that flocs and filaments coexisted in both types of sludge in all the reactors. However the floc structure and the level of filaments in the two types of sludges were different. In bulking sludge (Fig. 4b1 to 4b3) most of the filaments were extending from flocs into the bulk solution, while in non-bulking sludge (Fig. 4a) the filaments grew mostly inside the flocs. Type 1851, Type 021N and *T. nivea* were identified as the dominant filaments in bulking sludge, with the extended filament lengths of 3.03×10^9, 0.75×10^9 and 1.54×10^9 μm ml^{-1} respectively (Figure 5). Thus the respective ratios of extended (outside the flocs) filament to total filament length were calculated as 61.1%, 47.3 and 74.3%. On the other hand, only Type 1851 was observed to be present in non-bulking sludge, with levels of 0.30×10^9 μm ml^{-1} extended filament length and 1.78×10^9 μm ml^{-1} total filament length, i.e., only 17.0% of the total length grew outside the flocs. These quantitative FISH results are consistent with the filamentous backbone theory (Sezgin et al. 1978) and our previous results showing the correlation between bulking and the extended filament length outside the flocs (Liao et al. 2004). We also compared the MLVSS fraction of filaments (MLVSS of 1443.2 mg/L for bulking sludge and 2588.8 mg/L for non-bulking sludge) on the days of sampling for quantitative FISH (Figure 6). Since the ratios between the filament lengths of Type 1851, Type 021N and *T. nivea* to their biomass are not available, the value of 14.133×10^8 μm filament length per MLVSS (mg ml^{-1}) determined for *L. mucor* (Liao et al. 2004) was used. This approach assumes that the length-to-biomass ratios of different filament species are approximately the same. Using this assumption, the percentages of the filament biomass to the MLVSS of Type 1851, Type 021N and *T. nivea* were approximately 24.5%, 7.8% and 9.6% respectively for bulking

sludge. For non-bulking sludge, Type 1851 was only 4.9% of the MLVSS (Figure 5). Thus, a filament biomass fraction of 41.6% of MLVSS led to filamentous bulking, while 4.9% filamentous MLVSS did not lead to bulking in the lab-scale reactors.

c. Metabolic model

The biomass compositions were calculated as $CH_{1.33}O_{0.81}N_{0.18}$ (γ_B =3.17) for non-bulking sludge and $CH_{1.06}O_{0.87}N_{0.13}$ (γ_B =2.93) for bulking sludge. The Macrobal elemental composition matrices for each reactor were constructed (Table IV for SBR E). The converted amount of all compounds in non-bulking activated sludge and bulking sludge during one cycle (Table V) were balanced with Macrobal software (Hellinga 1992), as previously described (Beun et al. 1999; 2000).

After input of the balanced conversion rates for the different components, we obtained a δ of 1.94 ± 0.33 mol ATP/mol $NADH_2$ and a m_{ATP} of 0.012 ± 0.004 mol/(Cmol h) (average ± st. dev.) for non-bulking sludge; a δ of 0.59 ± 0.24 mol ATP/mol $NADH_2$ and a m_{ATP} of 0.0002 ± 0.0002 mol/(Cmol h) for bulking sludge. From these two values the maximum yields, maintenance coefficients and the growth rates for filaments and floc formers were calculated according to equations (1) and (2). The results (Table VI and Table VII) showed that in either feast or famine period, the floc formers have higher growth rate (μ) and lower maintenance coefficients (m_s or m_g) than filaments, which agree with our previous results (Lou and de los Reyes, in press) showing that filaments have a competitive advantage by having lower decay rates to compensate for low growth rates. These values are similar to the results of Dirck's study (2001) for well-settling sludge. However, the coefficients of

variation (COV=standard deviation/mean) were higher for the bulking sludge tests. This might be due to the changing filament populations in the reactors. The dynamics (Huisman and Weissing 1999) of the different filament populations may have caused the high standard deviation of the parameter values. Despite this disadvantage, the combination of substrate uptake testing and metabolic modeling provided an effective way for estimating the parameter values for bulking and non-bulking sludge.

DISCUSSION

a. Quantitative FISH and converted filament biomass

The bulking threshold concept was used previously to determine the relationship between SVI and the extended filament length of Type 1851 (Liao et al. 2004). The concept states that above a certain level of extended filament length, bulking starts to occur. In this study, a total of 5.23×10^9 μm ml^{-1} extended length for the three types of filaments led to bulking, while an extended length of 0.3×10^9 μm ml^{-1} for Type 1851 was found in non-bulking sludge. These results are consistent with the bulking threshold value of 6×10^8 μm ml^{-1} extended filament length for Type 1851 (Liao et al. 2004).

The correlation factors between filament length and filament biomass for *S. natans* and *L. mucor* were previously determined to be 20.3×10^8 and 14.1×10^8 μm ml^{-1}, respectively (Liao et al. 2004). We applied this method to estimate the biomass concentration of uncultured filaments. In this study, filaments comprised 42.0% of VSS in bulking sludge, while the non-bulking sludge consisted of only 4.9% of filaments by VSS. This difference in filament biomass may account for the difference in kinetic parameters measured in this study.

b. Substrate uptake tests and metabolic modeling

From Table VI, we can compare the direct biomass formation on glucose ($Y_{sx}^{\max}$) with indirect biomass formation via glycogen ($Y_{sg}^{\max} * Y_{gx}^{\max}$) for the two types of activated sludges by calculating the ratio of ($Y_{sg}^{\max} * Y_{gx}^{\max}$)/($Y_{sx}^{\max}$) (Beun et al. 2000). The ratio was 0.965 for non-bulking sludge, and 0.919 for bulking sludge. This means that stimulating the glycogen pathway may increase the biomass yield for non-bulking sludge compared to bulking sludge. Our experimental data also suggest that non-bulking sludge has higher values of $Y_{sg}^{\max}$, $Y_{gx}^{\max}$, and $Y_{sx}^{\max}$ than bulking sludge. Thus, high substrate gradients will favor the growth of non-bulking sludge. Non-bulking sludge can easily convert substrate into storage products, allowing the organisms to survive during the famine period. On the other hand, bulking sludge has maintenance coefficients (m_s) that are 20 times lower than non-bulking sludge. The maintenance coefficients can be considered analogous to the decay rates of bacteria. The lower maintenance coefficients of filaments allow them to compete with floc formers at lower substrate concentrations.

At each MCRT, non-bulking sludge had average specific growth rates during the feast period that are about 3 to 4 times higher than that of bulking sludge (Table VII). The lower the MCRTs, the higher the μ_{feast} values. This is consistent with previous results showing that during the feast phase, both types of sludge will temporarily increase growth rate and storage (Beun et al. 2000; Dircks et al. 2001a).

During the feast period, the specific substrate uptake rates q_{sfeast} (Table VII) for non-bulking sludge was in the range of 0.5 to 0.7 Cmmol/(Cmmol.h), which is lower than previously reported (Dircks et al. 2001a). However, the ratio of q_{gfeast}/q_{sfeast} was nearly the same, 0.82-0.88 Cmmol/Cmmol, implying that about 45% of the amount of glucose consumed was used for synthesis of glycogen and the remainder (about 55%) was used for growth and maintenance processes. This ratio was higher than that obtained using acetate as substrate and resulting in PHB production (Beccari et al. 1998; Beun et al. 2000; van Aalst-van Leeuwen et al. 1997). This suggests that storage as glycogen is more favorable than PHB storage (Goel et al. 1998). For either bulking or non-bulking sludges, the ratios of q_{sfeast}/q_{gfeast} were constant at different MCRTs. When applying the storage concept, we should note that substrate uptake during the feast period has two different pathways: biomass formation and glycogen production. The amount of substrate used for biomass growth in the feast period represented only 12-18% of q_{feast} for non-bulking sludge, but was 93-94% of q_{feast} for bulking sludge (Table VII).

The measurement of the maximum specific substrate uptake rates shows that bulking sludge has about 60% lower $q_{maxsfeast}$ value than non-bulking sludge, which seems to confirm the kinetic selection theory that states that floc formers have higher $q_{maxsfeast}$ than filaments. These results contradict previous research showing both types of sludges to have similar values of the maximum specific substrate (acetate) uptake rates (Martins et al. 2003). However, it is still possible that the differences in the measurement methods, the dominant species of filaments in the reactors, and the kinds of substrates (acetate versus glucose) resulting in different formation of storage products (PHB versus glycogen), may account for

the discrepancies between these results and that of Martins et al. (2003). Our data support the kinetic selection theory, but cannot exclude the effect of diffusion limitation on the competition between filaments and floc formers. Further studies need to investigate which of the mechanisms (kinetic selection, diffusion limitation, or a combination of both) can explain the growth of filaments over floc formers.

Table VIII shows the comparison of kinetic parameters, μ_{max} and Y, estimated from respirometry and substrate uptake tests for bulking and non-bulking sludge. In kinetic parameter measurement using respirometry, the substrate is assumed to be used only for biomass growth, and the oxygen uptake is assumed to be directly coupled to substrate uptake for growth. Thus only one Y and one μ_{max} are obtained. Table VIII shows that the values of measured Y from respirometry are higher than those estimated from the substrate uptake tests. This may be because of the underestimation of oxygen consumption in the respirometric measurement, as Y is defined as (1-oxygen consumed/substrate utilized). The process of indirect growth, via the storage formation pathway, should be slow. However, because the respirometric measurement test is rapid, normally less than 15 mins, the complete biodegradation of storage products in the biomass is not guaranteed. Thus during the test, it often happens that the DO consumption at the end is higher than the endogenous DO consumption at the beginning, resulting in a higher slope at the end of the DO curve. Since the values of $Y_{sg}^{\max}$ are high for bulking (0.84) and non-bulking sludge (0.93), the overall yield will increase if the process of biomass growth on glycogen is incomplete. However, it is not clear why the respirometry-derived values of Y for both types of sludge are similar, while the values from substrate uptake tests are different.

The results of this study are in agreement with previous respirometric results (Lou and de los Reyes, 2005), that showed that non-bulking sludge had a higher μ_{max} than bulking sludge. Compared with μ_{max} (0.6 h^{-1}) obtained from respirometry, the lower μ_{max} (0.39 h^{-1}) for non-bulking sludge obtained from the substrate uptake test may be due to the estimation of the corresponding q_{max} during the whole feast phase. Since the substrate uptake should be higher at the beginning of the feast phase, the estimated value may not represent the true maximum substrate uptake rate,

In the substrate uptake tests, the half saturation coefficients (K_s), which represent the affinity for the substrate, are not part of the metabolic model. It remains to be clarified whether the proliferation of filaments in bulking sludge is due to substrate affinity or due to the combination of diffusion limitation inside the flocs and filament morphology, which allows filaments to extend outside the flocs faster than floc formers can grow. Additional studies should attempt to determine K_s, so that a more comprehensive model of the competition between filaments and floc formers in activated sludge systems can be tested. Such models will eventually lead to better understanding of bulking phenomena.

CONCLUSIONS

FISH analysis showed that filaments were present in both bulking and non-bulking sludges. However, in non-bulking sludge, a low level of filaments grew inside the floc, while in bulking sludge, the high levels of filaments extended from the flocs into the bulk solution. The substrate uptake tests coupled with quantitative FISH technique showed that lower

values of growth rates were associated with higher percentages of filament biomass as MLVSS.

Respirometry is an attractive technique that is widely used, because the biodegration kinetics is determined solely from a single oxygen consumption curve that can be monitored easily and continuously. However, respirometry does not reflect the actual mechanism of substrate degradation wherein some portion of substrate is used for biomass growth, and some portion is used to form storage products. In this study, respirometric measurements led to high yields and maximum growth rates. In contrast, the combination of substrate uptake tests and metabolic modeling was used to determine the growth rates, yields and maintenance coefficients during feast and famine phases of bulking and non-bulking activated sludge. The results show that there are differences in the kinetic growth parameters of bulking and non-bulking sludge. However it is still not clear if diffusion limitation affects the competition between filaments and floc formers.

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LIST OF TABLES

Table I. Cycle operation

Phase	Time (min)	
	SBR A - C	SBR D - E
Start	0 - 10	0 - 10
Influent (16/3 L)	10 - 15	10 - 100
Reaction	10 - 310	10 - 310
Settling	310 - 355	310 - 400
Effluent (16/3 L)	355 - 370	400 - 415
Idle	370 - 480	415 - 480

Table II. FISH probes used, their target groups and hybridization conditions used in this study

Probe name[a]	Target group	***FA %***	Reference
S-S-S.nat-0656-a-A-18	*Sphaerotilus natans*	45	Wagner et al. 1994
S-S-L.muc-0652-a-A-18	*Leucothrix mucor*	35	Wagner et al. 1994
S-S-M.par-0223-a-A-18	*Microthrix parvicella*	20	Erhart et al. 1997
S-*-1851-0592-a-A-21	Eikelboom Type 1851	35	Beer et al. 2002
S-*-021N-0652-a-A-18	Eikelboom Type 021N	35	Wagner et al. 1994
S-*-H.hyd-0655-a-A-18	*Haliscomenobacter hydrosis*	20	Wagner et al. 1994
S-*-T. niv-0652-a-A-18	*Thiothrix nivea*	45	Wagner et al. 1994

Table III. Duration of feast and famine phases for bulking and non-bulking activated sludges and the corresponding concentrations of biomass and biomass growth for one cycle

	SRT (d)	Cx (Cmmol/l)	Feast phase (min)	Famine phase (min)	Biomass growth (Cmmol/cycle)	DO uptake (mmol/cycle)
Non-bulking AS	16 (SBR C)	131.27	4.5	475.5	10.66	34.14
	8 (SBR B)	94.43	6.5	463.5	15.66	26.89
	4 (SBR A)	46.25	9	491	21.19	21.25
Bulking AS	16 (SBR E)	89.73	45	435	11.24	20.69
	8 (SBR D)	61.43	48	432	16.86	20.35

Table IV. Macrobal elemental composition matrix for SBR E

	C1	N1	red1	C2	N2	red2	CO_2t	O_2t
Glucose[1*]	1	0	4	0	0	0	0	0
Biomass[1]	1	0.13	2.93	0	0	0	0	0
Glycogen[1]	1	0	4	0	0	0	0	0
CO_2^1	1	0	0	0	0	0	1	0
O_2^1	0	0	-4	0	0	0	0	1
NH_4^1	0	1	0	0	0	0	0	0
Biomass[2]	0	0	0	1	0.13	2.93	0	0
Glycogen[2]	0	0	0	1	0	4	0	0
CO_2^2	0	0	0	1	0	0	1	0
O_2^2	0	0	0	0	0	-4	0	1
NH_4^2	0	0	0	0	1	0	0	0
CO_2^t	0	0	0	0	0	0	-1	0
Biomass[t]	0	0	0	0	0	0	0	0
O_2^t	0	0	0	0	0	0	0	-1

* 1 represents the feast phase; 2 represents famine phase and t represents total

Table V. Converted amount in (C)mmole/cycle for all compound in bulking activated sludge and non-bulking activated sludge during one cycle

	Compound	Conversion rate (C)mmol/cycle				
		Non-bulking activated sludge			Bulking activated sludge	
		SRT 16 d	SRT 8 d	SRT 4 d	SRT 16 d	SRT 8 d
Feast	Glucose	-42.64	-39.42	-36.82	-28.92	-32.70
	Biomass	1.192	2.522	3.027	2.926	4.027
	Glycogen	38.43	33.83	30.26	18.29	22.30
	O_2	-3.256	-3.572	-4.138	-8.487	-7.450
Famine	Biomass	9.437	13.14	18.16	8.314	12.83
	Glycogen	-38.43	-33.83	-30.27	-18.29	-22.30
	O_2	-30.88	-23.32	-15.73	-12.20	-12.90
Total	Biomass	10.63	15.66	21.19	11.24	16.86
	O_2	-34.14	-26.89	-21.25	-20.69	-20.35

Table VI (a). Yield and maintenance coefficients of non-bulking activated sludge

SRT (d)	7/21/03			8/9/03			8/21/03			Average	COV	Dircks, 2000
	4	8	16	4	8	16	4	8	16			
δ	2.7484	1.4524	1.5966	1.6798	1.8439	1.5313	1.9035	2.3506	2.3226	1.9386	0.173	1.8
m_{ATP}	0.0792	0.0185	0.0121	0.0070	0.0123	0.0116	0.0097	0.0162	0.0175	0.0124	0.318	0.017
Y_{sx}^{max}	0.82	0.65	0.68	0.69	0.71	0.67	0.72	0.78	0.77	0.7248	0.061	0.60
Y_{sg}^{max}	0.95	0.91	0.92	0.92	0.93	0.92	0.93	0.94	0.94	0.9306	0.010	0.91
Y_{gx}^{max}	0.84	0.68	0.71	0.72	0.74	0.70	0.75	0.80	0.80	0.7517	0.056	0.63
m_s	0.0129	0.0052	0.0031	0.0017	0.0028	0.0031	0.0022	0.0030	0.0033	0.0027	0.225	0.0030
m_g	0.0125	0.0049	0.0030	0.0017	0.0027	0.0030	0.0021	0.0029	0.0032	0.0026	0.226	0.0029
$(-q_s)$ Cmmol/cycle	65.34	57.52	43.71	37.12	40.34	43.72	36.82	39.42	42.64			
$(-q_g)$ Cmmol/cycle	54.53	48.78	38.66	30.73	34.06	38.56	30.26	33.83	38.43			

Table VI (b). Yield and maintenance coefficients of bulking activated sludge

	10/25/03		11/7/03		Average	COV
SRT (d)	8	16	8	16		
δ	0.9188	0.4992	0.6095	0.3512	0.594687	0.405
m_{ATP}	0.0001	0.0001	0.0001	0.0006	0.000242	0.923
Y_{sx}^{max}	0.54	0.42	0.46	0.37	0.448505	0.162
Y_{sg}^{max}	0.88	0.83	0.85	0.80	0.842429	0.039
Y_{gx}^{max}	0.58	0.47	0.50	0.41	0.489502	0.141
m_s	0.000047	0.000087	0.000068	0.000420	0.000156	1.139
m_g	0.000044	0.000079	0.000063	0.000375	0.000140	1.121
$(-q_s)$ Cmmol/cycle	32.70	28.92	39.42	35.44		
$(-q_g)$ Cmmol/cycle	22.30	18.29	26.69	21.63		

Table VII. Specific growth rates, specific glucose uptake rate in feast period (q_{sfeast}) and specific glycogen production rate (q_{gfeast}) for non-bulking and bulking activated sludges

	SRT (d)	μ_{feast} (h^{-1})	μ_{famine} (h^{-1})	q_{sfeast} Cmol/(Cmol.h)	q_{gfeast} Cmol/(Cmol.h)	q_{gfeast}/q_{sfeast} Cmol/Cmol	$q_{maxsfeast}$ Cmol/(Cmol.h)
Non-bulking AS	16 (SBR C)	0.0124	0.0012	0.5551	0.4896	0.8820	0.5551
	8 (SBR B)	0.0301	0.0022	0.4921	0.4162	0.8458	0.4921
	4 (SBR A)	0.0467	0.0064	0.6674	0.5537	0.8296	0.6674
Bulking AS	16 (SBR E)	0.0054	0.0016	0.0537	0.0035	0.0654	0.3488
	8 (SBR D)	0.0102	0.0036	0.0832	0.0063	0.0758	0.3203

Table VIII. shows the comparison of kinetic parameters estimated from respirometry and substrate uptake test for bulking and non-bulking activated sludge

	MCRT (d)	This study		Respirometry	
		non-bulking sludge	bulking sludge	non-bulking sludge	bulking sludge
μ_{max}^{*} (h^{-1})	16	0.38	0.16	0.60	0.20
	8	0.33	0.14		
	4	0.45	---		
	Avg.	0.39	0.15		
Y	Direct growth (Y_{sx}^{max})	0.72	0.45	0.88	0.88
	Indirect growth ($Y_{sg}^{max} * Y_{gx}^{max}$)	0.67	0.41		
	Overall**	0.68	0.45		

* $\mu_{max} = q_{feast}$ x $Y_{overall}$

**$Y_{overall}$ is estimate as ($Y_{direct\ growth}$ x 16% + $Y_{indirect\ growth}$ x 84%) for non-bulking sludge, and ($Y_{direct\ growth}$ x 93.5% + $Y_{indirect\ growth}$ x 6.5%). This is because the amount of substrate used for biomass growth in the feast period represented only 12-18% of q_{feast} for non-bulking activated sludge, but was 93-94% of q_{feast} for bulking activated sludge.

LIST OF FIGURES

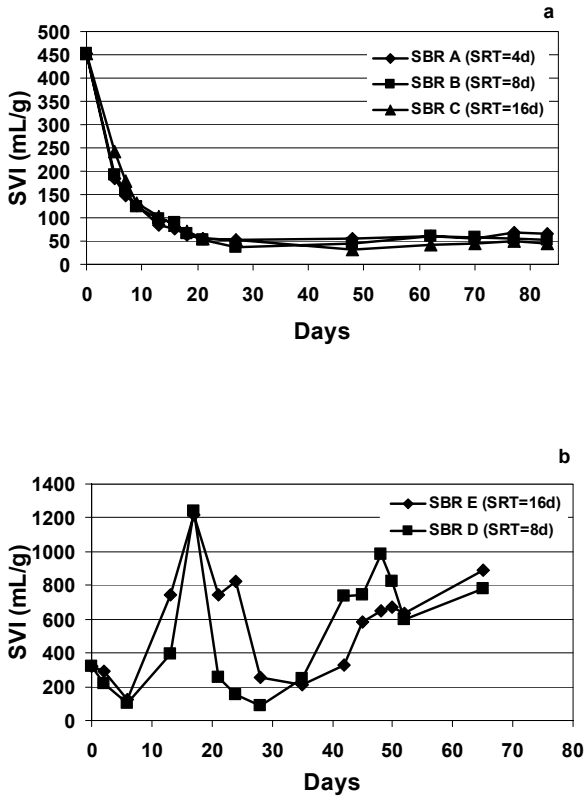

Figure 1. Change in SVI over time (a) non-bulking settling activated sludge; (b) bulking activated sludge

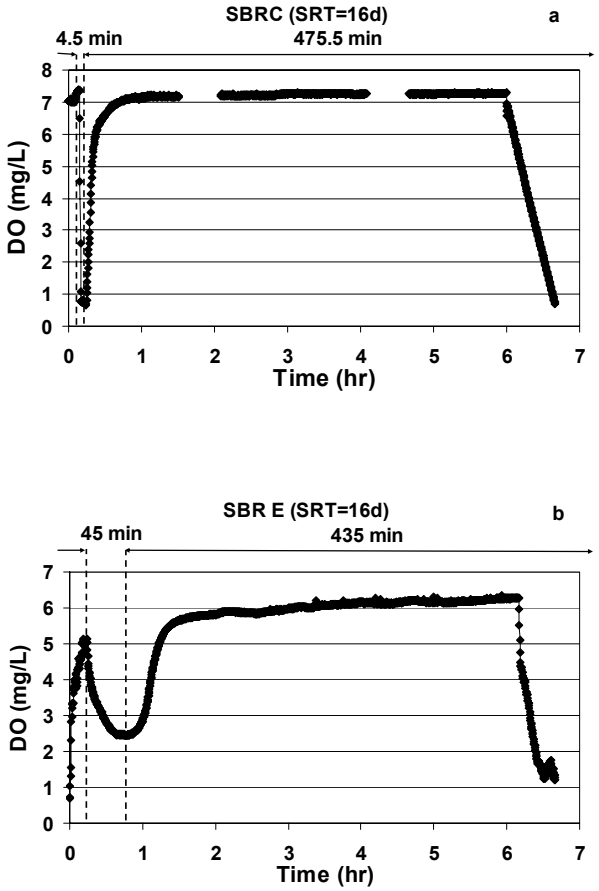

Figure 2. DO change over one cycle (a) SBR C (MCRT=16d);
(b) SBR E (MCRT=16d)

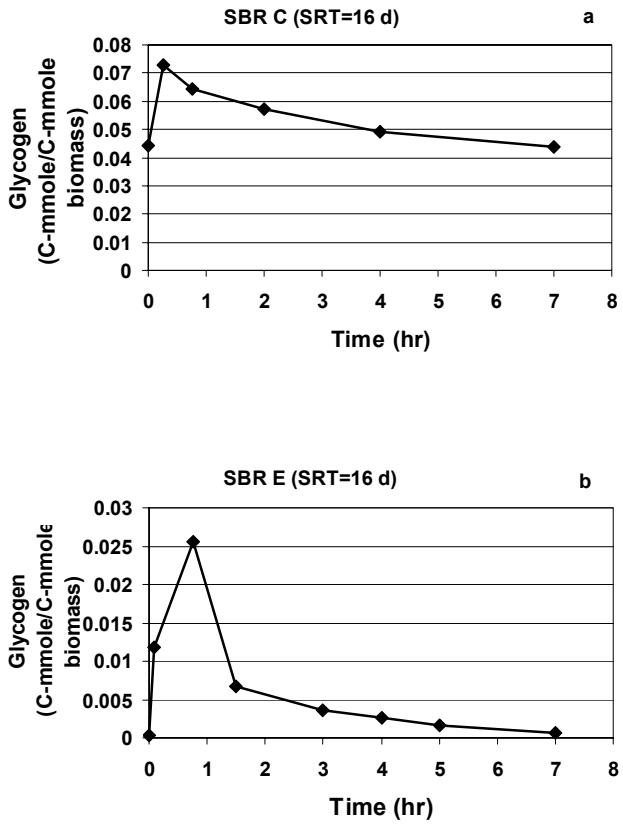

Figure 3. Change of glycogen concentration over one cycle in (a) SBR C (non-bulking activated sludge) and (b) SBR E (bulking activated sludge)

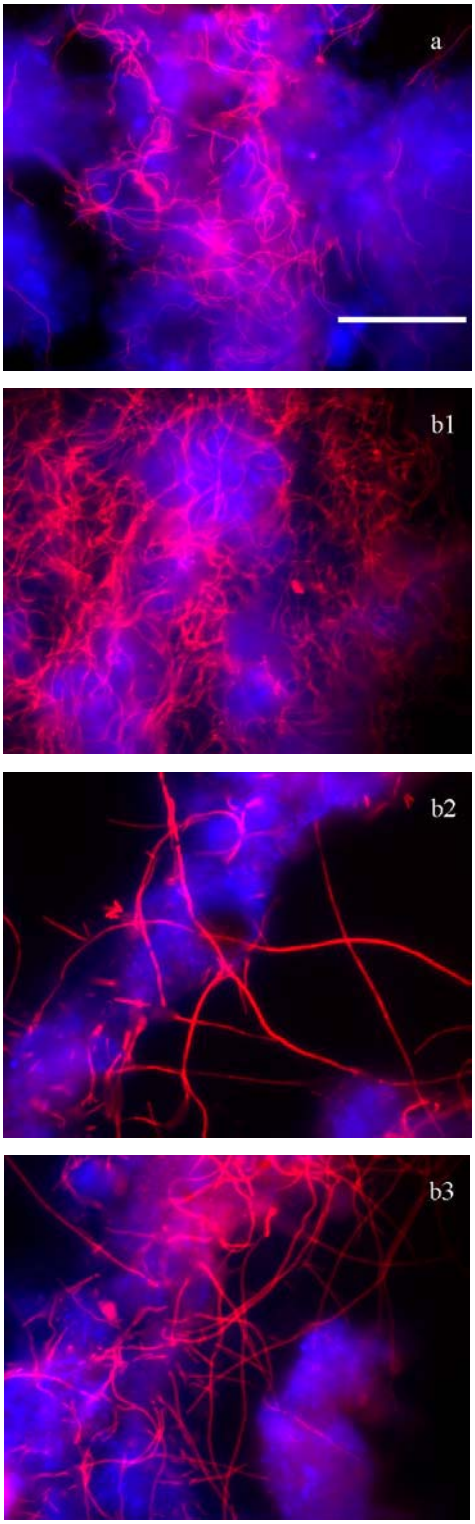

Figure 4. FISH micrographs showing (a) Type 1851 (red) in SBR C (non-bulking sludge) on Day 23; and (b1) Type 1851, (b2) Type 021N and (b3) TNI (red) in SBR E (bulking sludge) on Day 23. This experiment used probe S-*-1851-0592-a-A-21, S-*-021N-0652-a-A-18 and S-*-T. niv-0652-a-A-18 with Cy3, counterstained with DAPI (blue)

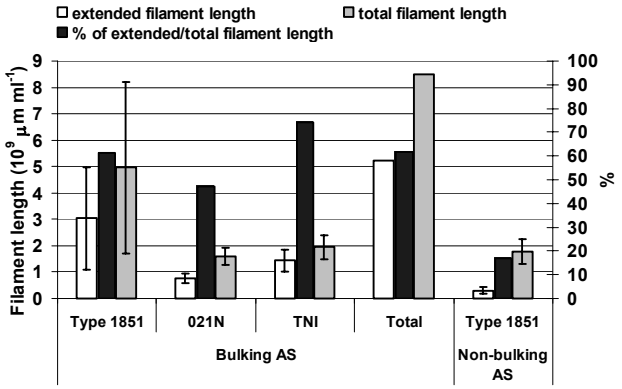

Figure 5. Comparison of filament length and ratio of extended filament length to total filament length for bulking and non-bulking activated sludge. Error bars represent standard deviation of filament length means for each well.

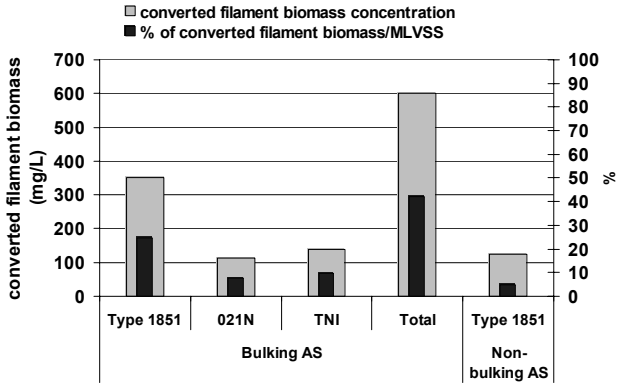

Figure 6. Filament biomass concentrations, and their respective MLVSS percentages for bulking and non-bulking activated sludge

Chapter 4.

A new hypothesis for analyzing filamentous bulking in activated sludge: roles for kinetics and diffusion

(This chapter is prepared for publication in Water Research)

ABSTRACT

Filamentous bulking is caused by the excessive growth of filaments over floc formers, and their competition was previously described using kinetic selection. However recent studies reported that instead of kinetic selection, diffusion limitation inside the flocs may be the crucial factor in microbial selection. To clarify roles of these factors in explaining filamentous bulking, a new conceptual qualitative framework was developed in this study. We hypothesize that the growth rates of filaments and floc formers are affected by the combination of kinetic selection and substrate diffusion limitation. Three different regions (bulking, transitional, and non-bulking region) based on substrate concentration are suggested. In the bulking and non-bulking regions, kinetic selection controls the growth rate process and favors either filaments and floc formers respectively. However, in the transitional region, substrate diffusion limitation, determined by the floc size, plays an important role in causing bulking. To test this framework, sequencing batch reactors (SBRs) were operated with influent COD of 100, 300, 600 and 1000 mg/L, and the sludge settleability was measured at various floc size distributions developed using different mixing strengths. A model integrating the two mechanisms was developed to simulate the substrate concentrations in different layers of floc sizes under intermittently feeding conditions. The

modeling results confirmed that substrate diffusion limitation occurs inside the flocs at a certain range of activated sludge floc sizes over the operation cycle, and the experimental data in the bulking and transitional regions supported the proposed framework.

KEYWORDS

Activated sludge, filamentous bulking, diffusion limitation, floc size

INTRODUCTION

Filamentous bulking is caused by the excessive growth of filamentous organisms outside the activated sludge flocs (Jenkins et al. 2003). The difference in growth kinetics between filaments and floc formers was widely used for explaining their competition (Chudoba et al. 1973c; Chudoba et al. 1973b; Chudoba et al. 1973a). Floc formers are thought to have high values of μ_{max} (maximum growth rate) and are favored at high substrate concentration, while filaments are thought to have low K_s and thus have high values of growth rate at low substrate concentration (Figure 1). Based on the substrate concentration, the bulking and non-bulking regions can be determined. In the non-bulking region, the growth rate of filaments is higher than that of formers.

However, a recent study (Martins et al. 2003a) suggests that instead of kinetic selection, substrate diffusion limitation inside the flocs may be the important factor in microbial selection. It was hypothesized that filaments and floc formers have the same kinetics. At low substrate concentration in bulk solution, because the diffusion of substrate inside the floc is very low, floc formers cannot access substrate for their growth, while filaments, due to

their morphology, can grow faster than floc formers and easily reach substrate outside the flocs. Extended filaments then cause bulking. On the other hand, at high substrate concentration, filaments have no such diffusion limitation advantage to be predominant, and most grow inside floc, and bulking does not occur. If this mechanism is correct, there is no need to invoke differences in growth kinetics, μ_{max} and K_s of the two types of organisms for explaining bulking. The implication of this hypothesis is that under the same substrate concentration, bulking tends to occur at large floc size due to higher substrate diffusion resistance.

To clarify the roles and to what extent diffusion and kinetics influence filamentous bulking, we used quantitative fluorescence *in situ* hybridization (FISH) to measure the filament length inside and outside the activated sludge flocs in bulking and non-bulking sludge. It was found that both types of sludge have high levels of total filament length. The difference is that in non-bulking sludge, most of filaments grew inside the flocs, which was consistent with the diffusion limitation hypothesis (Liao, et al., 2004). However, our later study (Lou and de los Reyes III, 2005b) using substrate uptake tests and metabolic modeling showed that non-bulking sludge has a higher maximum growth rate than bulking sludge. This result agreed with the kinetic selection theory. Considering our previous experiments, we hypothesized in this study that the growth rates of filaments and floc formers are affected by the combination of kinetic selection and substrate diffusion limitation, and the dominant effect depends on the substrate concentrations and the floc sizes in the activated sludge system.

The concept of combining substrate diffusion limitation and microbial kinetics has been previously reported for predicting the performance of biological film reactors (Atkinson et al. 1968), and a series of mathematical models based on the Monod maximum utilization rate and the substrate diffusion coefficients were developed (Williams and McCarty 1976). Since the biomass in activated sludge is composed of many bacterial floc particles (microbial suspension aggregate), and under the appropriate conditions, aerobic granules are formed (Morgenroth et al. 1997; Peng et al. 1999; Tay et al. 2001), it is also reasonable to speculate that substrate diffusion limitation as well as kinetic selection affect the organisms' growth. Within the floc matrix, the metabolic reactions occur simultaneously with mass transfer and a concentration gradient would be established, which limits the metabolic rates of the cells inside the flocs. Such conditions may lead to endogenous respiration and cell lysis near the center of flocs if the substrate is exhausted or a change from aerobic respiration to anaerobic fermentation if oxygen is exhausted (Benefield and Molz 1983). Applying this diffusion-limitation concept, Pochana *et al.* (1999) developed a dynamic microbial model of the activated sludge flocs to simulate the behavior of nitrogen and carbon compounds in SBR, and Brandt and Kooijman (2000) presented a simple two-parameter (floc size at division and diffusion length) extension of growth models for cell suspensions to account for the reduction of the degradation rate due to the diffusion limitation.

Oxygen diffusion limitation inside the activated sludge floc has been mentioned to induce filamentous bulking (Sezgin et al. 1978). However, research integrating substrate-diffusion-limitation degradation and microbial growth for explaining filamentous bulking is very limited. In addition, most research has been conceptual, and not accompanied by

experimental data. Lau *et al.* (1984) set up a competition model for explaining the competition between filaments and floc formers by considering glucose and DO diffusion limitations in a CMR (completely mixed reactor). Martins, *et al.* (2004), taking into account the morphological differences between filaments and floc formers, simulated three-dimensional growth of both species in activated sludge flocs using the adapted individual-based model originally developed for a biofilm system. This model allowed spatial development of the floc according to the bacterial morphology, diffusion, reaction, and growth processes. The model results supported the hypothesis that floc-macrogradients can be the most important parameter for development of bulking sludge. However these models still have not been verified by experimental data.

In the present study, a new conceptual qualitative framework integrating kinetics and diffusion inside the activated sludge flocs for explaining filamentous bulking was developed. Three different regions (bulking, transitional and non-bulking region) based on substrate concentration are suggested. In the bulking and non-bulking regions, kinetic selection controls the growth rate process and favors filaments and floc formers, respectively. However, in the transitional region, substrate diffusion limitation, determined by the floc size, plays an important role in causing bulking. To test this framework, sequencing batch reactors (SBRs) were operated at various substrate concentrations and sludge settleability was measured at different floc size distributions. A model combining both mechanisms was developed to simulate the substrate concentrations inside the flocs with respect to different floc sizes, and the critical size that causes bulking was determined.

MATERIALS AND METHODS

I. Sequencing batch reactor for floc size control

The SBRs, each with active volumes of 8 L, were operated to produce bulking and non-bulking activated sludge. The reactors were seeded with sludge from the North Cary Wastewater Reclamation Facility (Cary, NC), and treated synthetic wastewater [50 mg/L $MgSO_4 \cdot 7H_2O$, 7 mg/L KCl, 150 mg/L NH_4Cl, 141.8 mg/L, KH_2PO_4, 54.91 mg/L K_2HPO_4, 555.6 mg/L $NaHCO_3$, glucose and yeast extract depending on the COD concentrations]. The pH and temperature of the reactors were maintained at 7.0 ± 0.1 and 22 ± 1 ^oC respectively. Four SBRs with glucose concentrations expressed as COD of 100, 300, 600 and 1000 mg/L respectively, were operated with a 2-hour fill time and 1-hour settling time. The cycle period of operation was 8 hours. Hydraulic retention time (HRT) and sludge retention time (SRT) were 12 hours and 16 hours, respectively. Two-thirds of active volume (5.33 L) of effluent was replaced by the new feed at the end of the period. The mixing strength or the shear rate, expressed as G [$=(\rho N_p N^3 D^5/\mu V)^{1/2}$ (ρ: density of fluid; N_p: dimensionless quantities power number; N: impeller speed, D: impeller diameter, μ: fluid viscosity; V: active volume of reactor], was controlled by the impeller speed, keeping other parameters constant. Two impellers speeds N, 50 rpm (revolutions per minute) and 126 rpm were used in the 100 mg/L and 300 mg/L influent COD reactors, and 50 rpm and 200 rpm were applied to 600 mg/L and 1000 mg/L influent COD reactors. A higher rpm (200 rpm), instead of 126 rpm was used in the high influent COD reactor to keep a similar range of floc size distribution, since at the higher COD concentrations, larger flocs were formed. Based on the equation for G, after inputting the referenced parameters (James, 1983), we obtain G_{200}=110.4 s^{-1}; G_{126}=55.2 s^{-1}; and G_{50}=13.8 s^{-1}, i.e., $G_{200}=2G_{126}=8G_{50}$ (subscripts represent the values of the rpm) for the

reactor operation. Thus the substrate diffusion can be compared, as the larger the floc size, the more difficult the substrate can diffuse into the flocs. The dissolved oxygen (DO) concentration was maintained at 6 to 7 mg/L to avoid oxygen limitation inside the activated sludge flocs (Lau et al. 1984).

MLSS (mixed liquor suspended solids concentration), MLVSS (mixed liquor volatile suspended solids) and SVI (30 minute sludge volume index) were periodically measured using Standard Methods (Greenberg et al. 1998). DO was measured using a YSI5300 DO probe meter (YSI, Yellow Springs, OH).

Floc size measurement

Depending on the biomass concentration in activated sludge, floc particles were stained with an appropriate amount of methylene blue solution [methylene blue: 0.5 g; KOH (1% solution): 1 mL; ethanol (95%): 30 mL; distilled water: 100 mL], and were immobilized in agarose gel for imaging. The images were captured with a Photometrics Sensys CCD camera mounted on a Nikon Optiphot II fluorescence microscope for size analysis using Metamorph^{TM} (Universal Imaging Corp., Silver Spring, MD). More than 500 floc particles (10 shots) were imaged and analyzed to obtain mean particle size values for each test. The average radius of floc size (R) was determined using $R=(3V/4\pi)^{1/3}$, where V is the average volume of particles was defined as the total volume of flocs divided by number of flocs.

The procedure of floc size analysis using Metamorph was performed in three stages. The measured distance was first calibrated, the floc sizes were then thresholded, and the size

distribution was measured using the internal program of integrated morphometry. Floc size thresholding was performed manually and based on personal judgment. Three types of thresholding (normal, overestimated and underestimated) were used to test the effect of personal judgment on the sensitivity of floc size measurement. The average floc size radii for the overestimated case (137.4 μm) and the underestimated case (110.0 μm) were within about 10% of the value of normal thresholding (123.4 μm).

II. ***Model development***

The model setup was conceptually similar to those developed by Pochana *et al.* (1999) and Lau *et al.* (1984) in integrating kinetic selection and substrate diffusion, and was extended to model intermittent feeding conditions. Instead of showing the competition between filaments and floc formers directly in terms of their respective biomass concentrations, our model was only used to simulate the substrate concentration inside the flocs to test the effect of the diffusion limitation inside the flocs. Thus one value of u_{max} and one value of K_s were used to represent the kinetics of floc growth. The substrate diffusion hypothesis implies that the differences in bacterial morphologies cause bulking. This model is unique in trying to show how diffusion limitation occurs over the range of activated sludge floc sizes and operational conditions in the lab-scale reactors. The model schematic is shown in Figure 2 and includes three steps. We assume

- the reactor is operated as SBR
- the floc has a spherical shape
- the transport of chemical species within the flocs is by molecular diffusion
- the floc density is taken as a constant

- the reactions follow Monod kinetics
- the diffusivity of substrate is constant
- oxygen is not limiting

Step 1. Model for a single floc

At steady state, for a spherical shell of thickness dr, the substrate uptake is equal to the net rate of substrate transport through the shell ((Lau et al. 1984).

$$4\pi \cdot r^2 N|_{(r+dr)} - 4\pi \cdot r^2 N|_r = 4\pi \cdot r^2 R dr$$

where N is mass flux of substrate passing through a surface; and other parameters are defined in Step 3.

Simplifying the equation and applying Fick's Law: $N = D \cdot \frac{dS}{dr}$, then

$$D\frac{d}{dr}(r^2 \frac{dS}{dr}) = r^2 R$$

i.e., the mass balance for substrate within a floc can be written as

$$\frac{\partial S}{\partial t} = D\,(\frac{\partial^2 S}{\partial r^2} + \frac{2}{r}\frac{\partial S}{\partial r}) \pm \sum_{k=1}^{n} r_k$$

where $r_k = \mu_{\max}(\frac{S}{S+K_s})(\frac{M}{Y})$

Step 2. Overall reaction rate (OR)

To simplify the model at this stage, we assumed that the internal floc concentration reaches steady state at each time period. Then the reaction rate for a particular particle is equal to the net mass flow of substrate though the surface of the floc. The overall rate of

reaction within a single floc can be obtained by multiplying the flux at the outer surface by the external surface area of the floc.

$$OR = \frac{V_F}{V_0 + Qt}\left(\frac{3}{R} D \left.\frac{\partial S}{\partial r}\right|_{r=R}\right)$$

where $\left.\frac{3}{R} D \frac{\partial S}{\partial r}\right|_{r=R}$ represents the reaction rate for a single floc

Step 3. Macro model for the whole reactor considering the changes of SBR operation

During the fill period, the total volume in the reactor and the bulk substrate solution can be expressed as

$$\frac{\partial S_B}{\partial t} = \frac{(S_f - S_B)Q}{(V_0 + Qt)}$$

The initial conditions are

- $S=S_0$ at t=0 and r>=R
- S=0 at t=0 and r<R

and the boundary conditions are

- $\frac{\partial S}{\partial r} = 0$ at r=0
- $\frac{\partial S_B}{\partial t} = \frac{(S_f - S_B)Q}{V_0 + Qt} - \frac{3V_F}{R(V_t + Qt)} D \frac{\partial S}{\partial r}$ (during feeding) **or**
- $\frac{\partial S_B}{\partial t} = -\frac{3V_F}{R(V_0 + Qt)} D \frac{\partial S}{\partial r}$ (reaction only)

where S: substrate concentration; S_B: substrate concentration in bulk liquid; S_0: initial substrate concentration; S_f: feeding substrate concentration; V_F: total volume of floc in mixed liquor; V_T: total liquid volume of the SBR; V_0: liquid volume in the reactor after effluent (=V_T/3); *R*: rate of substrate uptake; r: radial distance from the center of a spherical floc; R: radius of the floc; t: time.

The parameter inputs below are based on the literature and operational conditions of the experiment.

- S_0=20 mg/L; S_f=1000 mg/L (S_f: feed concentration);
- D=250 $\mu m^2/s$ (D: diffusivity) (Lau et al. 1984);
- μ_{max}=0.38 hr^{-1} (μ_{max}: maximum growth rate of flocs) (Lau et al. 1984);
- M=25000 mg/L (M: mass density within flocs) (Lau et al. 1984);
- Y=0.4 (Y; Yield) (Lau et al. 1984);
- K_s=5 mg/L (K_s: half saturation coefficient) (Lau et al. 1984);
- V_F=0.1 L; V_T=8 L; V_0=8/3 L
- Q=7.41x10^{-4} L/s (Q: flow rate);
- T=8 hr (T: period);
- R=50 – 300 μm;
- Fill time t=2 hr;

Diffusivity for glucose was chosen as the effective substrate diffusivity within flocs in this study, since the glucose was used as the carbon source in the synthetic wastewater. The model was developed for SBR operation, programmed using Matlab and the partial differential equations were solved using the finite difference method. The substrate concentration S versus r/R ratio was tested at different floc sizes.

RESULTS

a. Qualitative framework (hypothesis)

The hypothesis integrating kinetic selection and diffusion limitation effects on filamentous bulking is summarized in Figure 1, which describes the growth rates of filaments and floc formers versus the substrate concentrations with or without substrate diffusion limitation. When the organisms are free-living, and no floc effects exist, the growth rates for filaments and floc formers follow Monod kinetics, i.e., only kinetic selection affects the competition. If the substrate concentration is lower than C_{SA} (bulking region), filaments win the

competition and bulking occurs. However, when the activated sludge flocs composed of filaments and floc formers are formed, diffusion resistance is introduced to the system which results in differences in concentration in the bulk solution and in the floc interior. The larger and denser the flocs, the higher the diffusion resistance, and the measured apparent K_s value would be larger. The apparent K_s (in the dash growth rates of Figure 1) is the K_s combining the true value (in the solid growth rates of Figure 1) and the diffusion limitation effect. This causes the growth rate curves to shift to the right, while keeping the μ_{max} the same. If the substrate concentration is higher than C_{SB} (non-bulking region), bulking does not occur. Thus, in both the bulking and non-bulking region, diffusion limitation is insignificant and has no effect on sludge bulking. In the transitional region, bulking may or may not occur, depending on the degree of substrate diffusion limitation. Figure 1 illustrates that due to the effect of substrate diffusion limitation, the bulking region will be increased by the shaded area (C_{SA}-C_{SB}) that previously was in the non-bulking region in the absence of diffusion. The shaded region is reduced when the diffusion resistance is decreased, e.g., by decreasing floc sizes using higher shear. This framework may be used to explain the contradiction in our previous experimental results (Liao, et al., 2004; Lou and de los Reyes III, 2005b) that showed different percentages of filament levels inside and outside the flocs using the same substrate concentration and operational conditions. The hypothetical framework also predicts that decreasing floc size in the transition region would shift bulking sludge to non-bulking sludge. In addition, it should be noticed that in the framework, the values of the critical region-determined substrate concentrations, C_{SA} and C_{SB}, are not fixed, and can be varied depending on many factors, e.g., the definition of bulking threshold (SVI=150 mL/g); operational conditions, such as types of reactors, substrate concentration and fill time; and

floc properties, such as diffusion coefficient, and mass density. The significance of this framework is that it may explain the contradictory experimental data in the literature by showing how kinetic selection alone does not determine bulking occurrence, and how substrate diffusion may be a crucial factor under certain operational conditions.

b. SBR floc size- controlled experiment

Using different mixing strength in SBRs was successful for controlling the floc sizes in this experiment, and the measurements were repeatable (Table 1). The large particles, around 230 μm average radius, were formed when the impeller speed 50 rpm was applied, while the small particles, 130-140 μm average radius were obtained when the impeller speed was 126 or 200 rpm. Lower value of average radius of 192 μm, measured using 50 rpm in the 100 mg/L COD reactor, were possibly because of the low influent substrate concentration that decreased the organisms' growth. Much smaller particles determined in the 600 mg/L and 1000 mg/L influent COD reactors were due to the high mixing strength (200 rpm), for which the growth of flocs at higher substrate concentrations could not compensate. The volume distribution of the floc size is shown in Figure 4-7. In the 100 mg/L influent COD reactor, a high volume percentage (74.5%) of flocs was in the range of 500-800 μm at the low impeller speed, while only 16.9% of flocs in this range at the high speed. Approximately 66 to 74% of flocs were found in the 300 influent COD mg/L reactor ranging 900-1300 μm at the low speed, and 64% of flocs was from 500 to 800 μm at the high speed. For 600 mg/L and 1000 mg/L influent COD reactors, a high volume percentage (68%-76%) of flocs was found greater than 1000 μm at 50 rpm. However using the high mixing strength at 200 rpm, the floc sizes distributed homogenously in the range of 25 to 450 μm in the 600 mg/L COD

reactor, and about 70% of flocs were 375 to 700 μm in 1000 mg/L COD reactor. Thus the various impeller speeds produced different shear rate to control the floc sizes.

The experiment was performed in two stages (for the 100 mg/L influent COD reactor) or three stages (for the 300 mg/L influent COD, the 600 mg/L influent COD, and the1000 mg/L influent COD reactors). In the 100 mg/L and 300 mg/L influent COD reactors, the mixing impeller speed of 50 rpm was applied in stage 1 to form large floc particles. After inoculating with activated sludge, both reactors started to bulk with SVI increasing from 122 mL/g to 1278 mL/g in the 100 mg/L influent COD reactor, and from 101 mL/g to 353 mL/g in the 300 mg/L influent COD reactor. In stage 2, the impeller speed was changed from 50 rpm to 126 rpm. The reactors showed different behaviors. The SVI in the 100 mg/L COD reactor decreased from 1278 mL/g to 612 mL/g, and bulking still occurred. However, in the 300 mg/L COD reactor, the sludge became non-bulking with SVI decreasing from 353 mL/g to 97 mL/g, and corresponding small floc sizes (Figure 5) were measured. In stage 3, the impeller speed was changed back to 50 rpm, and the 300 mg/L COD reactor started to bulk again with SVI increasing from 94 ml/g to 205 mL/g at the end of the stage.

In the 600 mg/L and 1000 mg/L influent COD reactors, 200 rpm was used in stage 1 to reduce the floc sizes. A higher rpm was used in the first stage to maintain similar floc sizes as in the 300 mg/L influent COD reactor. Approximately one month after seeding activated sludge, non-bulking sludge was obtained for both reactors with SVI stabilizing at about 100 mL/g. In stage 2, the SVI for both reactors increased to more than 150 mL/g in about 4 days after the mixing strength was decreased from 200 rpm to 50 rpm. SVI was 200 mL/g at the

end of the stage. In stage 3, the high mixing strength (200 rpm) was applied again in both reactors, and the SVI decreased to 120 mL/g in the 600 mg/L reactor and 150 mL/g in the 1000 mg/L reactor in one week.

c. Modeling results

The substrate concentrations at different layers of various floc were simulated in the 1000 mg/L COD influent reactor at time 5 min, 15 min, 30 min, 45 min, 1 hr and 2 hr during the fill phase (2 hours) of the SBR operation. This phase was simulated to test the possibility of substrate diffusion limitation at a high bulk substrate concentration, and to determine the critical floc size at which bulking starts. It is believed that higher diffusion limitation may occur at lower substrate influent concentration and the remaining reaction phase in 1000 mg/L influent COD reactor because of low bulk substrate concentrations, if the particle size was kept the same. The modeling results (Figure 8) showed that the larger the floc sizes, the higher the degree of the substrate diffusion limitation inside the flocs and the higher the bulk substrate concentration. This is because, compared with small particles, large particles increased the difficulty of the substrate entering the particles, which resulted in less substrate degradation within the flocs, and thus increased the substrate concentration in the bulk liquid. The bulk substrate concentrations from this model were comparable to those obtained from our experiment (Figure 9). These figures also illustrate that during the fill phase, the bulk substrate concentration increased over time, and at a certain specific time, the substrate concentration decreases as the r/R is reduced. Diffusion limitation occurred at the critical floc size radius greater than or equal to 150 μm, for which the substrate concentrations were very low at the centers of flocs. This substrate diffusion limitation would be expected to be

more obvious in the 100mg/L, 300 mg/L and 600 mg/L influent COD reactors. The modeling results supported the presence of diffusion limitation in floc particles greater than 150 μm in radius. Since there is limited bulking and related floc size data in the literature, this critical value of 150 μm cannot be verified.

DISCUSSION

An important point of the proposed framework is that the values of substrate concentrations in Figure 1 do not need to be fixed for each time period, e.g., the constant substrate concentrations in CMR at steady state, but that these can represent the initial substrate concentrations in reactors, allowing different shapes of the Monod growth rate curves of filaments and floc formers at different conditions. Thus the operational conditions should be stated clearly when describing the region-determined substrate concentrations. In addition, this framework is a general framework that can be applied in all the operational conditions for explaining filamentous bulking that was subjected to kinetic selection and substrate diffusion limitation.

Our experimental data supported the hypothesis that filamentous bulking is caused by the combination of kinetic selection and substrate diffusion limitation. The kinetic selection is a dominant factor in controlling the bulking process at low substrate concentrations, where diffusion limitation is insignificant to the competition between filaments and floc formers. The filaments, because of their low K_s, have high growth rates at low substrate concentration, and thus win the competition. At the same substrate concentration, the decrease in SVI values with decreasing floc sizes indicated the substrate diffusion effect. However it cannot

compensate for the effect of kinetic selection and bulking continued. As the influent COD was increased from 100 mg/L to the higher substrate concentration, kinetic selection favored the floc formers and counteracted the substrate diffusion limitation effect on the excessive growth of filaments. The SVI profiles (Figure 3) show that the bulking occurred when particle sizes were large and non-bulking occurred when particle sizes were small. These phenomena were repeated in stage 3 for the 300, 600 and 1000 mg/L COD influent reactors. The results indicate that both kinetic selection and the substrate diffusion may occur in municipal wastewater treatment plants since the influent substrate concentration is around 250-300 mg/L, and thus diffusion limitation may be a critical factor in filamentous bulking in full-scale WWTPs.

To test the validity of the conceptual qualitative framework, we initially used the 100 mg/L influent COD reactor to test the bulking region, the 300 mg/L COD reactor to test the transitional region, and the 600 mg/L & 1000 mg/L reactors to test the non-bulking region in our floc size-controlled experiment. The experimental data supported the hypothesis in the bulking and transitional region, using SVI greater than or equal to 150 mL/g as bulking threshold. However, the non-bulking region has not been verified by our results. An influent substrate concentration of 1000 mg/L using 2-hour feeding seems be too low to test the non-bulking region, and apparently was still in the transitional region (Figure 3d). The SVI data indicated that bulking occurred at 240.9 μm average floc size radius and non-bulking occurred at 127.0 μm. This agreed with our modeling results that showed high substrate diffusion limitation was inside the flocs when the particle sizes are greater than 150 μm.

Future experiments should continue to test the non-bulking region using a higher influent COD at the same floc sizes.

Our study used different shear rates, expressed as G values, to control the floc size and consequently to test the effect of diffusion on bulking. The data seem to agree with our hypothesis that the occurrence of bulking is related to a combination of kinetics and substrate diffusion limitation. However, it is also possible that instead of diffusion limitation as the main factor that causes bulking, another mechanism may be present. One mechanism is physical shear that mechanically suppresses filamentous growth outside the floc directly, e.g., by cutting the filament length. Zahradka (1966) has previously reported that filaments were suppressed in the recycled activated sludge with high energy pump. This phenomenon was also observed by other researchers (Galil, et al., 1991; Shin, et al., 1992). However, small floc sizes were also obtained in their experiments, implying that the effect of diffusion limitation was low. Thus it is not clear which mechanism led to the suppression of filamentous growth in their studies. Our framework only considers the effect of substrate diffusion limitation on filamentous growth, and did not include the mechanical shear effect directly on the filaments.

Activated sludge is a complicated system and many factors may also be involved in the competition between filaments and floc formers. Bacterial storage may be one of the important factors (Lou and de los Reyes III 2005a; Lou and de los Reyes III, 2005b), since the dynamic nature of substrate flows into wastewater treatment plants elicit transient responses from microorganism that affect the growth rates of filaments and floc formers.

Floc formers have higher storage capacities and thus outcompete the filaments in famine condition. We do not include the storage phenomenon in our framework. In the SBR floc size-controlled experiments, we did not need to consider the storage effect on the bulking, since our previous experimental data (Lou and de los Reyes III, 2005b) have shown that at a 2 hr fill time and influent COD lower than 1000 mg/L, the concentration of the storage product (glycogen when glucose was used as carbon source) is very low. However, for testing the non-bulking region, it would be an important factor if a higher influent substrate concentration is used.

Our modeling results showed that the effect of substrate diffusion limitation would occur inside the flocs at a certain range of floc sizes. However, the input parameters in our model, e.g., kinetic parameters, diffusion coefficients, and mass density were taken from the literature, and some of them, especially the diffusion coefficients, may be sensitive to the modeling results. To simulate and predict the substrate concentration inside the flocs more accurately in the model, these parameters should be measured and calibrated from the experiments using the methods mentioned in the literature review.

CONCLUSIONS

Our experimental and modeling results are consistent with the predictions of the suggested conceptual framework. At low substrate concentration (the bulking region), bulking occurred and was independent of floc size (kinetic selection control). At a higher substrate concentration corresponding to the transitional region, the occurrence of the filamentous bulking is determined by the effect of substrate diffusion limitation. Bulking occurs at large

floc sizes and non-bulking occurs at smaller floc sizes. A mathematical model integrating both mechanisms was developed to simulate the substrate concentrate at different layers of flocs, and the critical floc sizes can be determined. The modeling results were consistent with our experimental data, and the framework explains the paradox of our experimental observations. The results demonstrate that using either kinetics or diffusion exclusively to explain microbial competition is incorrect. We realize that the substrate concentration boundaries between bulking, transition, and non-bulking regions may be dependent on substrate type, bioreactor configuration, bulking threshold SVI and other factors. Nevertheless, we are confident that the underlying concepts in the proposed framework are intrinsically correct. Further studies demonstrating the non-bulking region at higher substrate concentrations should consider bacterial storage. The new framework provides a more integrated understanding of the competition between filaments and floc formers under different operational conditions. Based on this understanding, conditions promoting filamentous bulking in activated sludge may be avoided by controlling floc sizes using different mixing strengths.

ACKNOWLEGEMENTS

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LIST OF TABLES

Table 1. Floc sizes measurement (μm in radius) at different operational conditions

Floc size radius (μm)	COD 100mg/L		COD 300mg/L			COD 600mg/L			COD 100mg/L		
	50 rpm	126 rpm	50 rpm	126 rpm	50 rpm again	200 rpm	50 rpm	200 rpm again	200 rpm	50 rpm	200 rpm again
0-25	408	277	354	1064	507	747	635	1867	437	456	287
25-50	124	117	125	339	206	345	204	1490	207	161	256
50-75	47	69	79	81	95	151	67	596	95	74	159
75-100	19	33	51	35	35	79	50	253	55	39	69
100-125	11	17	38	27	23	41	57	145	28	22	43
125-150	9	18	19	21	18	34	25	63	28	10	31
150-175	9	14	7	11	12	22	18	34	17	10	11
175-200	4	10	15	9	9	13	11	24	12	3	12
200-225	4	6	9	12	3	13	9	16	7	2	11
225-250	4	3	0	7	3	3	6	5	5	4	4
250-275	1	1	4	5	5	5	4	8	2	0	4
275-300	2	3	2	5	3	8	8	8	3	1	3
300-325	2	2	1	4	3	4	2	2	0	1	4
325-350	2	1	1	4	3	6	2	9	0	3	2
350-375	0	4	1	4	3	5	1	6	0	0	3
375-400	1	2	2	0	2	1	4	0	3	2	1
400-425	2	3	2	2	4	1	0	1	2	0	0
425-450	0	1	1	3	1	2	0	1	2	1	0
450-475	1	1	1	1	1	0	1	2	0	0	1
475-500	2	1	1	0	1	0	2	0	2	0	1
500-600	3	0	2	4	5	0	2	0	2	0	2
600-700	4	1	2	4	3	0	1	0	1	2	3
700-800	5	0	0	3	1	0	4	0	1	0	0
800-900	0	0	0	0	1	0	1	0	0	1	0
900-1000	0	0	2	0	1	0	0	0	0	1	0
1000-1100	0	0	0	0	1	0	1	0	0	0	0
1100-1200	0	0	2	0	1	0	2	0	0	1	0
1200-1300	0	0	1	0	2	0	3	0	0	2	0
1300-1400	0	0	0	0	0	0	0	0	0	0	0
1400-1500	0	0	0	0	0	0	0	0	0	1	0
Total #	664	584	722	1645	952	1480	1120	4530	908	797	907
Average radius (μm)	195.7	140.5	232.7	142.5	228.3	105.5	236.2	83.5	127.0	240.9	135.5

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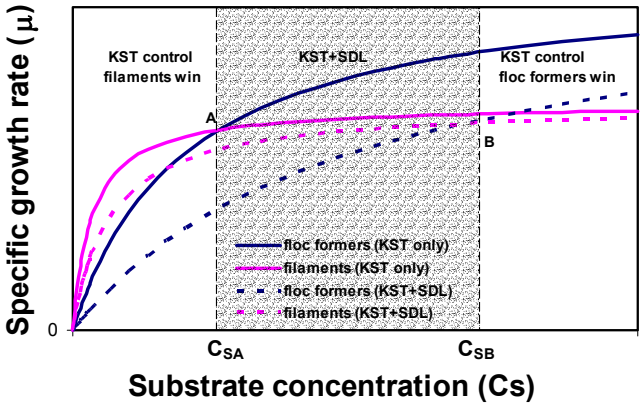


Figure 1. Specific growth rates of filaments and floc formers with/without diffusion limitation

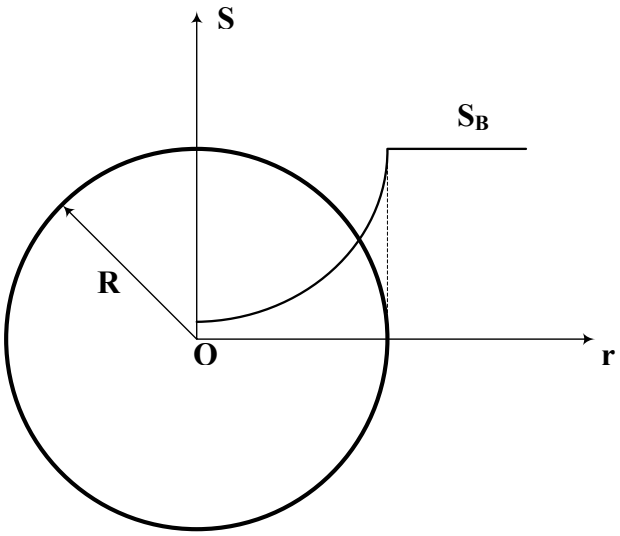
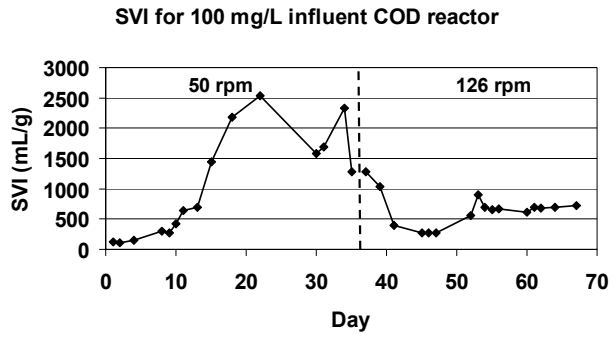
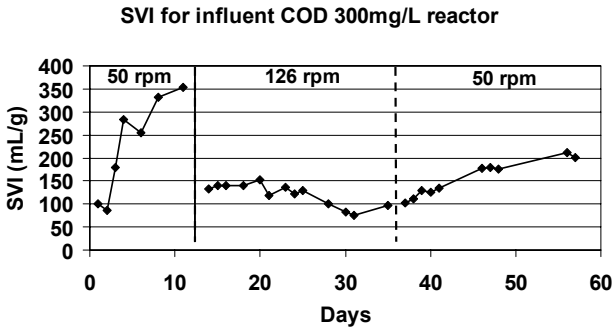
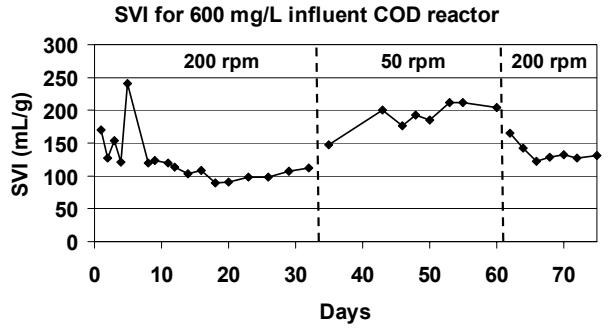


Figure 2. Schematic representation of model







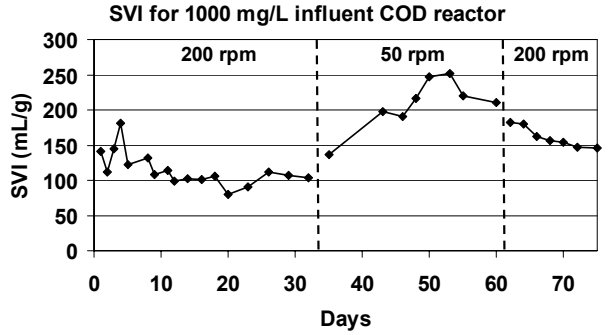


Figure 3. Change of SVI in the SBRs with different substrate concentration
(a) COD 100 mg/L; (b) COD 300 mg/L; (c) COD 600 mg/L; and (d) COD 1000 mg/L

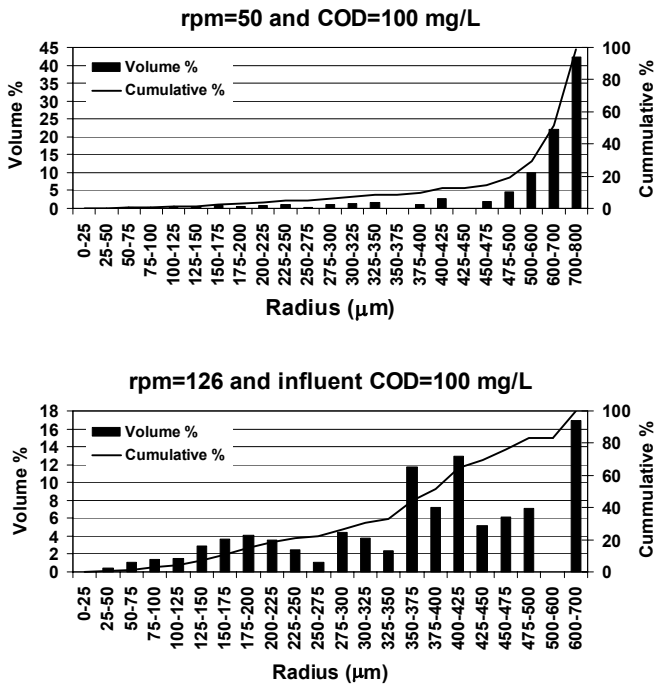


Figure 4. Floc size distribution in COD 100 mg/L SBRs with (a) 50 rpm and (b) 126 rpm

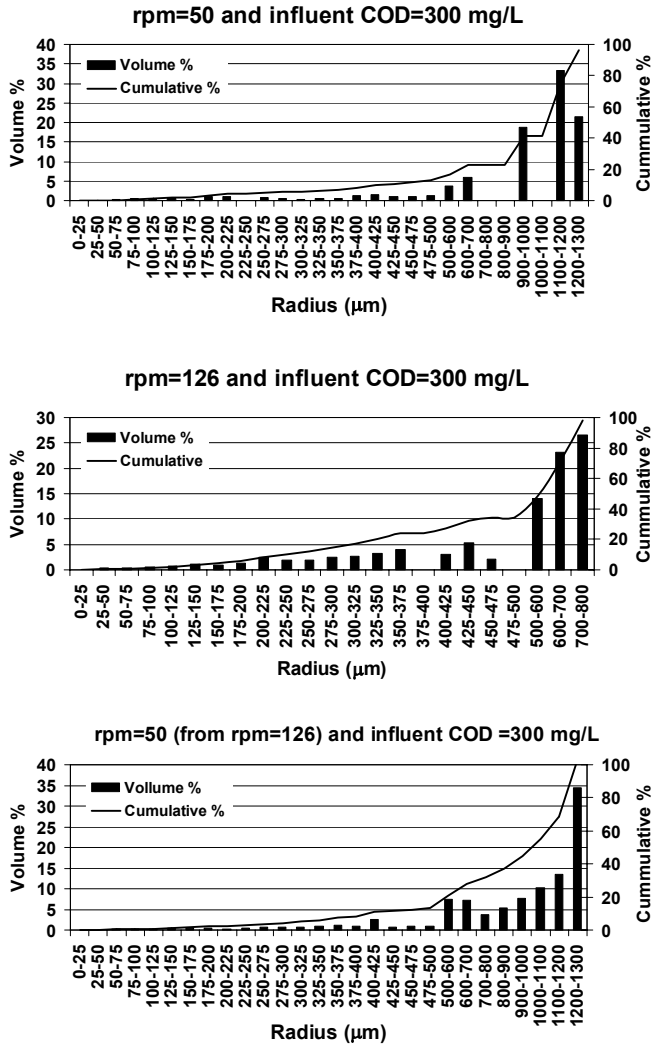


Figure 5. Floc size distribution in COD 300 mg/L SBRs with (a) 50 rpm; (b) 126 rpm and (c) 50 rpm (return from 126 rpm)

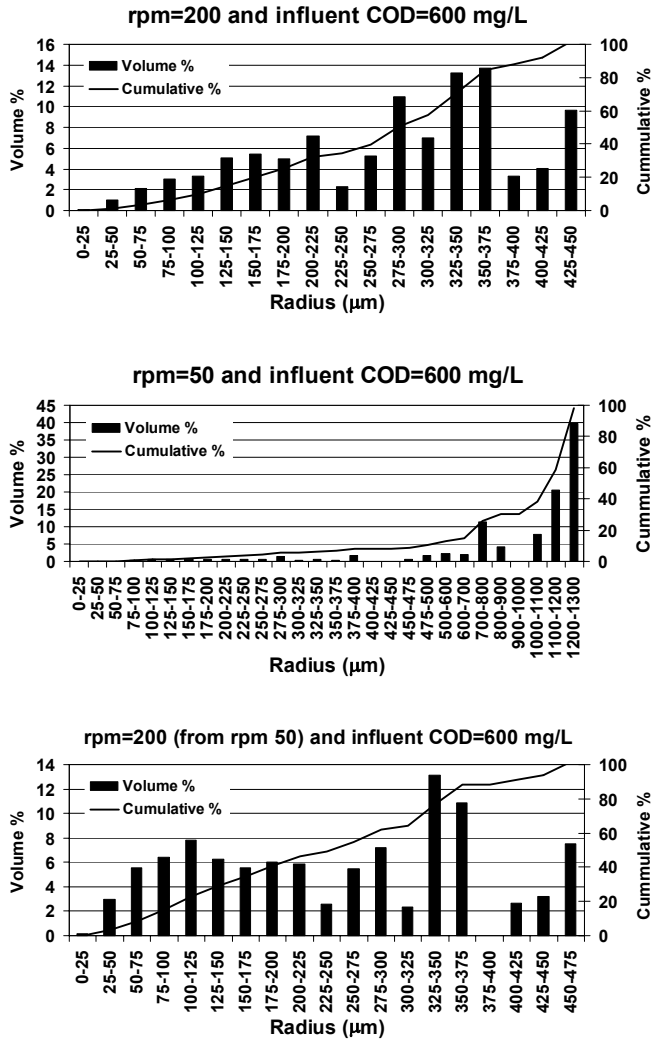


Figure 6. Floc size distribution in COD 600 mg/L SBRs with (a) 200 rpm, (b) 50 rpm and (c) 200 rpm (return from 50 rpm)

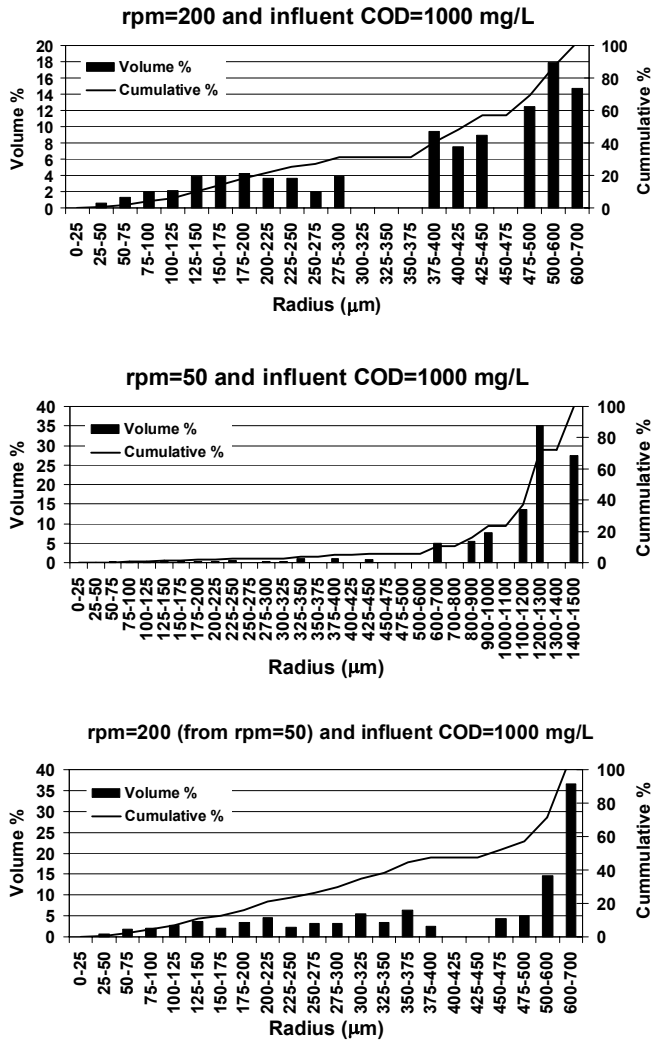


Figure 7. Floc size distribution in COD 1000 mg/L SBRs with (a) 200 rpm, (b) 50 rpmand (c) 200 rpm (return from 50 rpm)

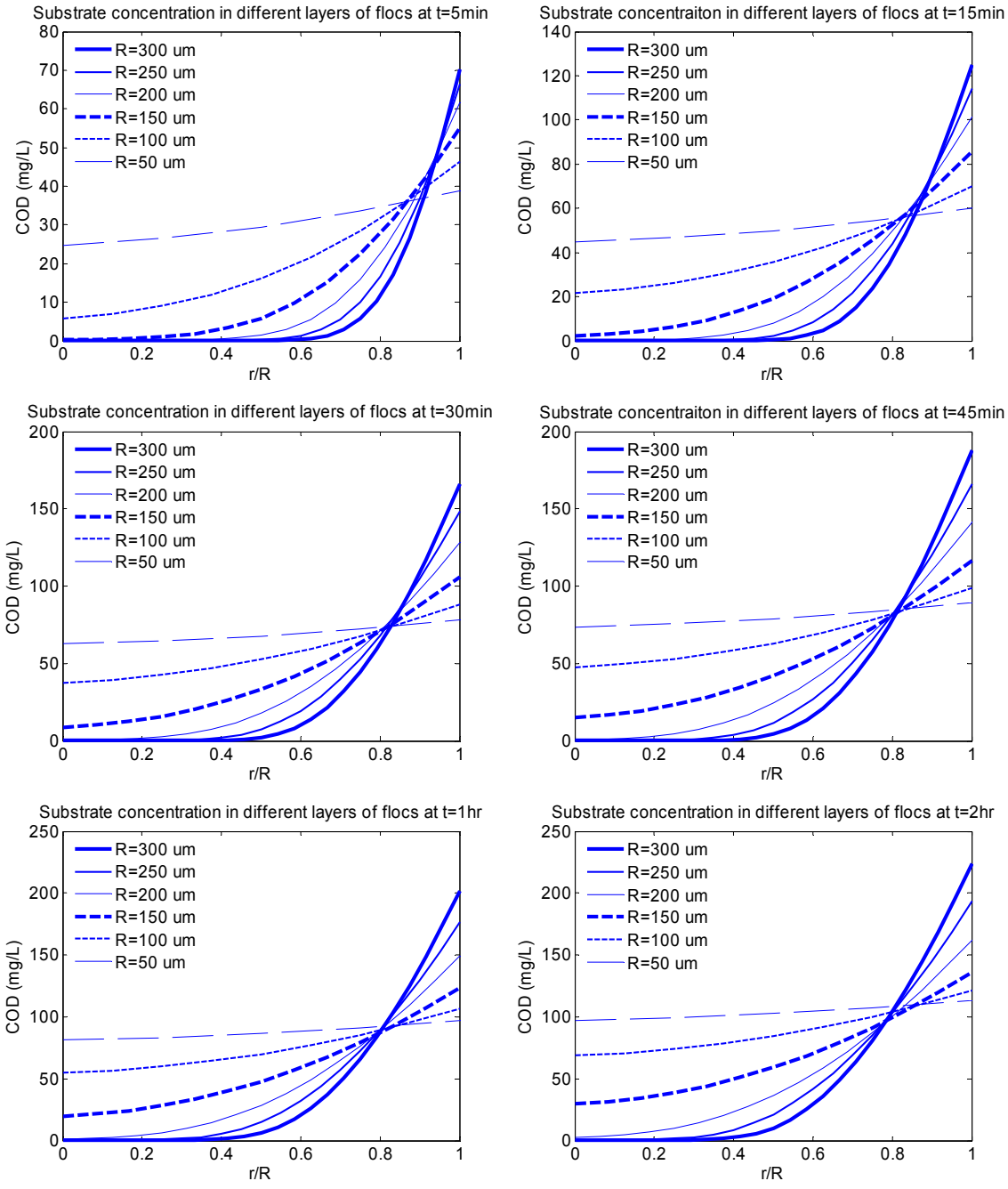


Figure 8. Substrate concentrations at different layers of various flocs at time 5 min, 15 min, 30 min, 45 min, 1 hr and 2 hrs

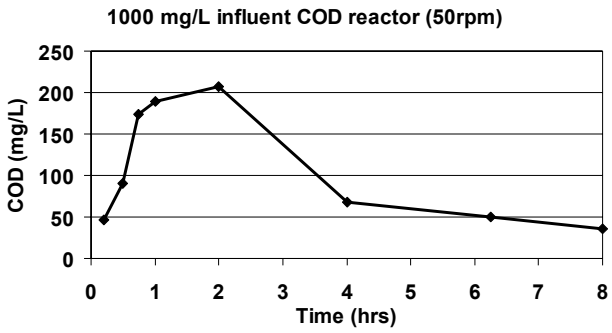
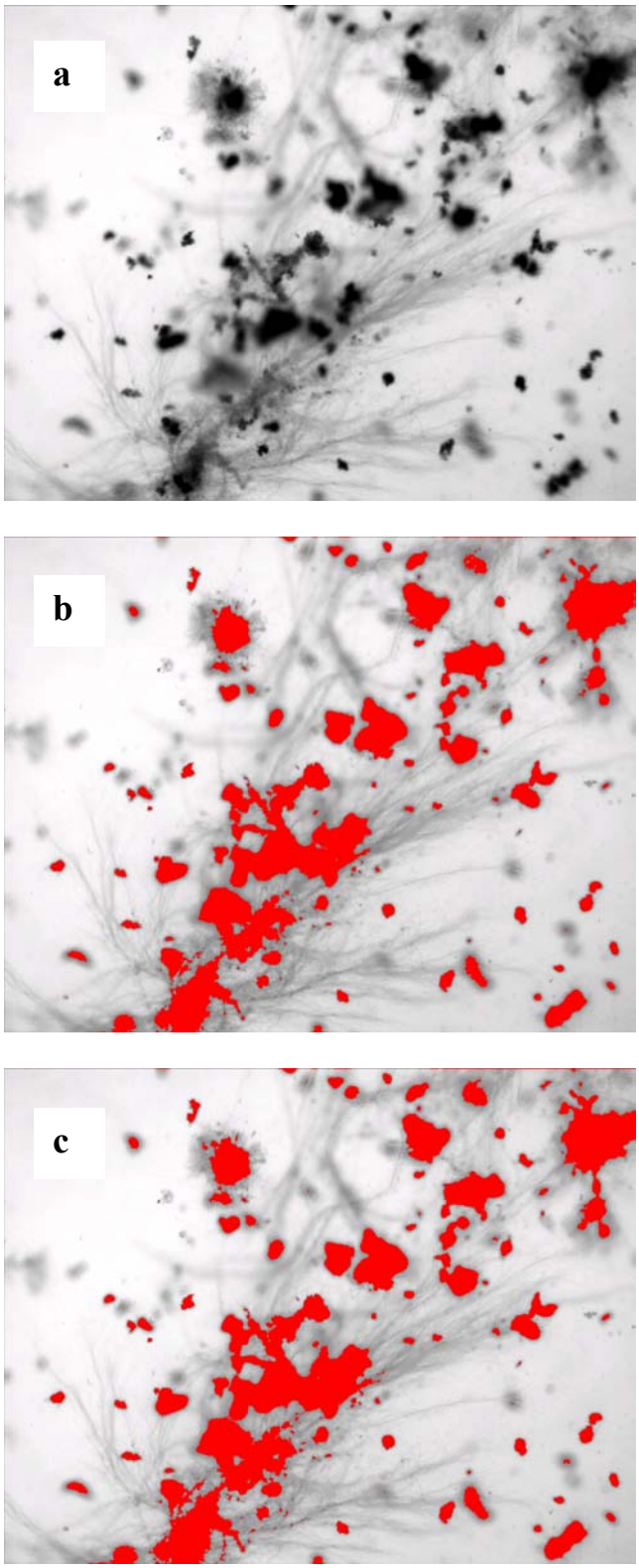


Figure 9.Substrate concentration in the 1000 mg/L influent COD reactor over one cycle


a
b
c

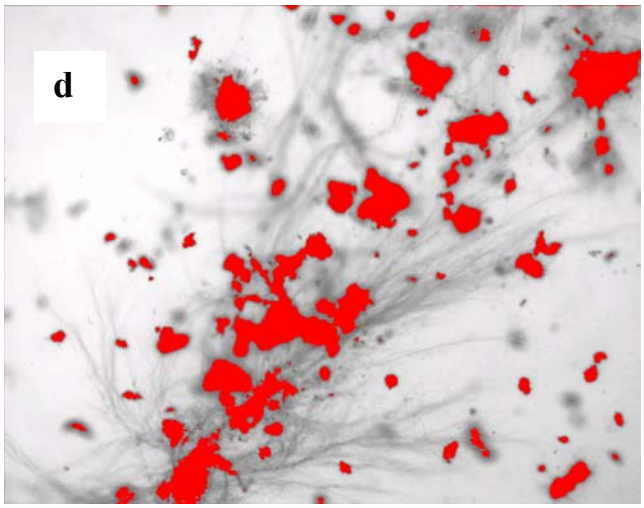


Figure 10. Threshold effect on floc size measurement (a) Typical image of floc size measurement; (b) Normal thresholding, average radius=123.4 μm;(c) Overestimated thresholding, average radius=137.4 μm; and (d) Underestimated thresholding, average floc radius=110.0 μm

Part III

Conclusions and Recommendations

In this part, conclusions and implications regarding various factors (kinetic selection, "filamentous backbone", decay rates, storage phenomena and diffusion limitation) affecting the competition between filaments and floc formers are drawn, different methods (respirometry and substrate uptake tests) used for kinetic parameters measurement are evaluated and compared, and recommendations are suggested for future research on filamentous bulking.

1. Conclusions

The purpose of this study was to analyze different factors affecting filamentous bulking. Thus producing bulking and non-bulking was the first important step, which allowed subsequent studies. Based on the experimental results in our reactors, it was believed that the occurrence of bulking was closely related to influent substrate concentration and the fill time. Non-bulking sludge was favored in SBRs with higher substrate concentration and short fill time, while bulking sludge was preferred in SBRs with low substrate concentration and long fill time (CMR in extreme case).

Considering the complicated nature of the activated sludge systems and different factors involved in microbial competition, we first applied a modeling approach integrating different factors (kinetic selection, "filamentous backbone", decay rates and storage phenomena) for explaining bulking. Sensitivity analysis showed that kinetic parameters such as the storage rate and backbone coefficient had the greatest effect on the simulation results. The ranges of dilution rates wherein one group outcompeted the other were delineated. (See Chapter 1)

Since the storage phenomenon inside the cells was an important factor in microbial selection, the substrate uptake test was developed to include bacterial storage and compared respirometry-based kinetic parameter measurement. Respirometry (without considering storage) and substrate uptake tests (considering bacterial storage) were used to measure the kinetic parameters, and the dominant filament causing bulking was identified. Both methods showed that non-bulking sludge had higher maximum growth rates than bulking sludge. The difference was that the values obtained from substrate uptake tests were lower than those determined using respirometry. Respirometric measurement is a simple and rapid method that is convenient to perform, while the substrate uptake test is a complicated technique to perform, but reflects the physiological state of microorganisms affecting the growth kinetics. Using FISH, it was found that Type 1851 was the dominant filament for both types of sludge. Bulking sludge comprised 42% of MLVSS, with 62% of the total filament length outside the flocs, while only low levels (5%) of Type 1851 filament length occurred in non-bulking sludge, about 83% of which grew inside the flocs. These provided support for growth kinetic differences in explaining the competitive strategy of filamentous bacteria (See Chapter 2 and Chapter 3), but did not exclude the diffusion limitation hypothesis suggested by Martins (2003).

To solve the paradox in our and others' (e.g., Martins et al., 2003) experimental results, we suggested a new framework combining kinetics and diffusion. The framework included three regions showing the growth rates of filaments and floc formers versus substrate concentration. In the bulking and non-bulking regions, kinetic selection controls the growth rates, while in the transitional region, diffusion limitation plays an important role in causing

bulking. We tested our hypothesis by controlling floc size distributions and changing the influent substrate concentration. The floc sizes were controlled using different mixing strengths. The experimental data supported our framework in the bulking and transitional regions. A model integrating both mechanisms was developed to simulate the substrate concentration at different floc sizes. The results showed that the substrate diffusion limitation occurred inside the flocs, and that a higher the degree of diffusion limitation was induced at larger the floc sizes. The critical size for bulking was determined to be about 150 μm in radius. These modeling results were consistent with our experimental data (See Chapter 4).

2. Recommendations

Based on the detailed studies of the filamentous bulking in this dissertation, the some recommendations for future work are summarized below.

In the short term:

(1) The framework has been tested and shown to be valid in the bulking and transitional regions, if we take SVI 150 mL/g as the bulking threshold. The research can be continued for testing the non-bulking region with higher influent substrate concentrations, while keeping other parameters the same. However, bacterial storage products may need to be measured during the operation cycle to guarantee that storage phenomena will not be involved in causing bulking. In future studies, the storage phenomena can be also incorporated into the framework.

(2) Floc size control is important in our experiment for testing the effect of diffusion limitation. Different mixing strengths in the SBR were used, but because of the heterogeneity of the flow pattern, ideal flocs with more uniform distribution were not attained. This problem can be solved using Couette reactors, a reactor specially designed for floc size control by shear. However the shear may function to mechanically suppress the filaments directly. This mechanism has the same effect as the diffusion limitation on bulking, and has not been clarified in our experiments. Further studies can be continued to resolve which mechanisms affect bulking. For example, quantitative FISH may be used to measure the extended filament length of different species before and after changing the mixing strengths. FISH may also allow direct observation of shear effects.

(3) The input parameters in our model, e.g., kinetic parameters, diffusion coefficients, and mass density, are important for model simulation, and should be measured in experiments using appropriate methods. Sensitivity analysis can also be performed to determine the greatest parameters that affect the modeling results. This will increase the robustness and accuracy of our model for application.

(4) The growth rates measured using respirometry or substrate uptake test are the average values for all the microorganisms in activated sludge system, which only reflect the growth rates of the dominant organisms, instead of the specific species. Different types of filaments coexisting in an activated sludge system may induce different levels of bulking. Thus it is important to measure the metabolic activities of various causative specific species using

molecular techniques. The 16S rRNA: rDNA ratio method may be used in future studies for determining *in-situ* growth rates of filaments and floc formers.

(5) Only the filamentous form of bacteria can cause bulking. Some filaments, such as *Sphaerotilus natans*, have been reported to have different morphologies. The morphological change of "filaments" from filamentous forms to single cells may become a new approach for preventing bulking. The bacterial morphology is controlled by different types of genes, and gene control techniques may be useful in future studies.

In the long term:

Activated sludge is a complicated system, and many factors have been reported in the last 20 years to be involved in bulking. It seems that current research methods that change one factor while keeping other factors constant may not work to solve the bulking problem. The concept is similar to our sensitivity analysis discussed in the Chapter 1, which showed that the storage effect is an important factor when the storage rates is increased or decreased independently of other factors. However, storage rate became insignificant when Monte Carlo simulation was used, in which all the parameters were changed. The other problem comes from the interaction of the factors, and the difficulty of changing one factor when blocking others, especially in microbial systems. These complications may mislead our data interpretation, and prevent us from determining the key factors that causing bulking. The solution, in the author's opinion, is to view the bulking problem using a systems approach. Systems biology is becoming an innovative approach and a major area in medical research (e.g., cellular networks and signaling pathway in solving cancer problems). To solve the

bulking problem, all the related parameters should be measured simultaneously, and principal component analysis or mechanism model should be applied to determine the key parameters that affect bulking.

In addition, the author speculates that the phenomenon of bulking may be a chaotic system, caused by the intrinsic properties of microorganisms. The coexistence of species of filaments and floc formers in activated sludge may generate oscillations and chaos when competing for limited substrates. The noisy fluctuating environment may perform as "triggers" to magnify the microbial dynamics of filamentous growth. The "triggers" can be such parameters as weather conditions, spatial heterogeneity, and temporal variability, among others. If so, these "triggers" need to be determined and controlled to avoid filamentous bulking.

Part IV

Appendices

Chapter 1

Table 1. Experimental data for Figure 2

Day	SV		MLSS		SVI	
	SBR	CMR	SBR	CMR	SBR	CMR
0	0.24	0.15	1.484	1.064	161.73	140.98
6	0.17	0.20	1.872	2.076	90.81	96.34
8	0.16	0.20	1.844	2.424	86.77	82.51
11	0.15	0.22	2.128	2.892	72.84	76.07
15	0.13	0.24	2.096	3.7	62.02	64.86
22	0.16	0.24	2.988	4.612	53.55	52.04
31	0.22	0.21	4.864	4.664	45.23	45.03
37	0.33	0.26	4.96	4.668	66.53	55.70
38	0.23	0.22	4.9	4.168	46.94	52.78
39	0.23	0.24	4.932	4.328	46.63	55.45
40	0.23	0.27	4.964	4.38	46.33	61.64
41	0.23	0.35	4.936	4.28	46.60	81.78
42	0.24	0.50	5.076	3.98	47.28	125.63
43	0.23	0.28	4.832	4.216	47.60	189.75
44	0.23	0.89	4.84	3.72	47.52	239.25
45	0.23	0.94	4.66	3.856	49.36	243.78
46	0.23	0.94	4.376	3.032	52.56	310.03
47	0.22	0.99	4.584	2.856	47.99	346.64
49	0.20	0.99	4.064	2.924	49.21	338.58
51	0.20	1.00	4.348	2.612	46.00	382.85
52	0.21	1.00	4.008	2.4	52.40	416.67
55	0.20	0.99	4.04	2.376	49.50	416.67
58	0.21	0.99	4.092	1.952	51.32	507.17
59	0.20	1.00	4.072	1.944	49.12	514.40
61	0.21	0.99	4.228	2.004	49.67	494.01
62	0.20	1.00	4.044	1.94	49.46	515.46
63	0.21	0.99	4.072	1.912	51.57	523.01

Program 1. Matlab program for Figure 3

```
%Matlab main program
[t,C]=ode45('conc1',[0 500],[0 0.1 0.1]);
plot(C(:,2),C(:,3),'-');
hold on;
[t,C]=ode45('conc1',[0 500],[0 1 6]);
plot(C(:,2),C(:,3),'*');
hold on;
[t,C]=ode45('conc1',[0 500],[0 6 1]);
plot(C(:,2),C(:,3),'.');
hold on;
```

```
[t,C]=ode45('conc1',[0 500],[0 6 6]);
plot(C(:,2),C(:,3),'^');
xlabel('floc former conc.(g/l)')
ylabel('filament conc.(g/l)')
title('backbone only alpha=0.02')

%Function
function dC=conc(t,C)
umaxfloc=0.6;Ksfloc=0.065;Yfloc=0.88;
umaxfil=0.2;Ksfil=0.008;Yfil=0.88;
a=0.02;
Bfloc=2.0;Bfil=1.1;
r=0.5;w=0.025;
Cs0=0.5;
V=10;Vsed=3;
D0=(umaxfil*Ksfloc-umaxfloc*Ksfil)/(Ksfloc-Ksfil);
D1=(D0-a-((-D0+a)^2-4*a*Ksfil*umaxfloc/(Ksfloc-Ksfil))^0.5)/2;
D2=(D0-a+((-D0+a)^2-4*a*Ksfil*umaxfloc/(Ksfloc-Ksfil))^0.5)/2;
D=0.6;
Dg=w*(1+r)/(r+w)*D;
ufloc=umaxfloc*C(1)/(Ksfloc+C(1));
ufil=umaxfil*C(1)/(Ksfil+C(1));

Cssed=(1+r)/(1-w)*C(1);
Cxflocsed=(1+r)/Bfloc/(w+r)*C(2);
Cxfilsed=(1+r)/Bfil/(w+r)*C(3);
dC=zeros(3,1);
dC(1)=D*(Cs0-C(1))-ufloc/Yfloc*C(2)-ufil/Yfil*C(3);
dC(2)=-Dg*C(2)+ufloc*C(2)+a*Yfloc/Yfil*C(3);
dC(3)=-Dg*C(3)+ufil*C(3)-a*C(3);
```

Program 2. Matlab program for Figure 4

```
%Matlab main program
[t,C]=ode45('conc2',[0 700],[0 0.1 0.1 0 0]);
plot(C(:,2),C(:,3),'-');
hold on;
[t,C]=ode45('conc2',[0 700],[0 1 5 0 0]);
plot(C(:,2),C(:,3),'*');
hold on;
[t,C]=ode45('conc2',[0 700],[0 5 1 0 0]);
plot(C(:,2),C(:,3),'.');
hold on;
[t,C]=ode45('conc2',[0 700],[0 6 6 0 0]);
plot(C(:,2),C(:,3),'^');
```

```
xlabel('floc former conc.(g/l)')
ylabel('filament conc.(g/l)')
title('same backbone coeff., storage+diff. kd (kdfloc=0.006/h; kdfil=0.002/h)')

%Function
function dC=conc(t,C)
umaxfloc=0.6;Ksfloc=0.065;Yfloc=0.88;kdfloc=0.006;
umaxfil=0.2;Ksfil=0.008;Yfil=0.88;kdfil=0.002;
kSTOfloc=0.15;KSTOfloc=1;YSTOSfloc=0.5;YSTOXfloc=0.5;bSTOfloc=0.004;umaxSTO
Xfloc=1.0;
kSTOfil=0.15;KSTOfil=1;YSTOSfil=0.5;YSTOXfil=0.5;bSTOfil=0.004;umaxSTOXfil=0.6;
a=0.01;
Bfloc=2.0;Bfil=1.1;
r=0.5;w=0.02;
Cs0=0.5;
V=10;Vsed=3;
D=0.6;
Dg=w*(1+r)/(r+w)*D;
ufloc=umaxfloc*C(1)/(Ksfloc+C(1));
ufil=umaxfil*C(1)/(Ksfil+C(1));
uSTOSfloc=kSTOfloc*C(1)/(Ksfloc+C(1));
uSTOSfil=kSTOfil*C(1)/(Ksfil+C(1));
uSTOXfloc=umaxSTOXfloc*(C(4)/C(2))/(KSTOfloc+C(4)/C(2));
uSTOXfil=umaxSTOXfil*(C(5)/C(3))/(KSTOfil+C(5)/C(3));
Cssed=(1+r)/(1-w)*C(1);
Cxflocsed=(1+r)/Bfloc/(w+r)*C(2);
Cxfilsed=(1+r)/Bfil/(w+r)*C(3);
dC=zeros(5,1);
dC(1)=D*(Cs0-C(1))-ufloc/Yfloc*C(2)-ufil/Yfil*C(3)-uSTOSfloc/YSTOSfloc*C(2)-
uSTOSfil/YSTOSfil*C(3);
dC(2)=-Dg*C(2)+ufloc*C(2)+a*Yfloc/Yfil*C(3)-kdfloc*C(2)-uSTOXfloc*C(2);
dC(3)=-Dg*C(3)+ufil*C(3)-a*C(3)-kdfil*C(3)-uSTOXfil*C(3);
dC(4)=uSTOSfloc*C(2)-bSTOfloc*C(4)-uSTOXfloc/YSTOXfloc*C(2);
dC(5)=uSTOSfil*C(3)-bSTOfil*C(5)-uSTOXfil/YSTOXfil*C(3);
```

Program 3. Matlab program for Figure 5

```
%Main Program
[t,C]=ode45('conc3',[0 500],[0 0.1 0.1 0 0]);
plot(C(:,2),C(:,3),'-');
hold on;
[t,C]=ode45('conc3',[0 500],[0 1 4 0 0]);
plot(C(:,2),C(:,3),'*');
hold on;
[t,C]=ode45('conc3',[0 500],[0 4 1 0 0]);
```

```
plot(C(:,2),C(:,3),'.');
hold on;
[t,C]=ode45('conc3',[0 500],[0 5 5 0 0]);
plot(C(:,2),C(:,3),'^');
xlabel('floc former conc.(g/l)')
ylabel('filament conc.(g/l)')
title('same backbone coeff., kd+diff. kSTO (kSTOfloc=0.3; kSTOfil=0.15)');

%Function
function dC=conc(t,C)
umaxfloc=0.6;Ksfloc=0.065;Yfloc=0.88;kdfloc=0.006;
umaxfil=0.2;Ksfil=0.008;Yfil=0.88;kdfil=0.006;
kSTOfloc=0.3;KSTOfloc=1;YSTOSfloc=0.5;YSTOXfloc=0.5;bSTOfloc=0.004;umaxSTOX
floc=1.0;
kSTOfil=0.15;KSTOfil=1;YSTOSfil=0.5;YSTOXfil=0.5;bSTOfil=0.004;umaxSTOXfil=0.6;
a=0.01;
Bfloc=2.0;Bfil=1.1;
r=0.5;w=0.025;
Cs0=0.5;
V=10;Vsed=3;
D=0.6;
Dg=w*(1+r)/(r+w)*D;
ufloc=umaxfloc*C(1)/(Ksfloc+C(1));
ufil=umaxfil*C(1)/(Ksfil+C(1));
uSTOSfloc=kSTOfloc*C(1)/(Ksfloc+C(1));
uSTOSfil=kSTOfil*C(1)/(Ksfil+C(1));
uSTOXfloc=umaxSTOXfloc*(C(4)/C(2))/(KSTOfloc+C(4)/C(2));
uSTOXfil=umaxSTOXfil*(C(5)/C(3))/(KSTOfil+C(5)/C(3));
Cssed=(1+r)/(1-w)*C(1);
Cxflocsed=(1+r)/Bfloc/(w+r)*C(2);
Cxfilsed=(1+r)/Bfil/(w+r)*C(3);
dC=zeros(5,1);
dC(1)=D*(Cs0-C(1))-ufloc/Yfloc*C(2)-ufil/Yfil*C(3)-uSTOSfloc/YSTOSfloc*C(2)-
uSTOSfil/YSTOSfil*C(3);
dC(2)=-Dg*C(2)+ufloc*C(2)+a*Yfloc/Yfil*C(3)-kdfloc*C(2)-uSTOXfloc*C(2);
dC(3)=-Dg*C(3)+ufil*C(3)-a*C(3)-kdfil*C(3)-uSTOXfil*C(3);
dC(4)=uSTOSfloc*C(2)-bSTOfloc*C(4)-uSTOXfloc/YSTOXfloc*C(2);
dC(5)=uSTOSfil*C(3)-bSTOfil*C(5)-uSTOXfil/YSTOXfil*C(3);
```

Program 4. Matlab program for Figure 6

```
%Matlab main program
fid=fopen('resultn.doc','a');
for i=1:1;
   umaxfloc=0.6;
   Ksfloc=0.065;
```

```
    Yfloc=0.88;
    kdfloc=0.006;
    umaxfil=0.2;
    Ksfil=0.008;
    Yfil=0.88;
    kdfil=0.002;
    kSTOfloc=0.3;
    KSTOfloc=1;
    YSTOSfloc=0.5;
    YSTOXfloc=0.5;
    bSTOfloc=0.004;
    umaxSTOXfloc=1;

    kSTOfil=0.15;
    KSTOfil=1;
    YSTOSfil=0.5;
    YSTOXfil=0.5;
    bSTOfil=0.004;
    umaxSTOXfil=0.6;
    a=0.01;
    Bfloc=2;
    Bfil=1.1;
    r=0.5;
    w=0.025;
    Cs0=0.5;
    D=0.6;
[t,C]=ode45(@conc,[0 500],[0 1 1 0
0],[],umaxfloc,Ksfloc,Yfloc,kdfloc,umaxfil,Ksfil,Yfil,kdfil,...
kSTOfloc,KSTOfloc,YSTOSfloc,YSTOXfloc,bSTOfloc,umaxSTOXfloc,kSTOfil,KSTOfil,
YSTOSfil,YSTOXfil,bSTOfil,umaxSTOXfil,...
    a,Bfloc,Bfil,r,w,Cs0,D);
udC=flipud(C);
fprintf(fid,'%10.4f %10.4f %10.4f %10.4f %10.4f\n',udC(1,:,:,:,:));
end;
fclose(fid);

%Function
function dC=conc(t,C,umaxfloc,Ksfloc,Yfloc,kdfloc,umaxfil,Ksfil,Yfil,kdfil,...
kSTOfloc,KSTOfloc,YSTOSfloc,YSTOXfloc,bSTOfloc,umaxSTOXfloc,kSTOfil,KSTOfil,
YSTOSfil,YSTOXfil,bSTOfil,umaxSTOXfil,...
    a,Bfloc,Bfil,r,w,Cs0,D)
V=10;Vsed=3;
Dg=w*(1+r)/(r+w)*D;
ufloc=umaxfloc*C(1)/(Ksfloc+C(1));
ufil=umaxfil*C(1)/(Ksfil+C(1));
uSTOSfloc=kSTOfloc*C(1)/(Ksfloc+C(1));
```

```
uSTOSfil=kSTOfil*C(1)/(Ksfil+C(1));
uSTOXfloc=umaxSTOXfloc*(C(4)/C(2))/(KSTOfloc+C(4)/C(2));
uSTOXfil=umaxSTOXfil*(C(5)/C(3))/(KSTOfil+C(5)/C(3));
Cssed=(1+r)/(1-w)*C(1);
Cxflocsed=(1+r)/Bfloc/(w+r)*C(2);
Cxfilsed=(1+r)/Bfil/(w+r)*C(3);

dC=zeros(5,1);
dC(1)=D*(Cs0-C(1))-ufloc/Yfloc*C(2)-ufil/Yfil*C(3)-uSTOSfloc/YSTOSfloc*C(2)-
uSTOSfil/YSTOSfil*C(3);
dC(2)=-Dg*C(2)+ufloc*C(2)+a*Yfloc/Yfil*C(3)-kdfloc*C(2)-uSTOXfloc*C(2);
dC(3)=-Dg*C(3)+ufil*C(3)-a*C(3)-kdfil*C(3)-uSTOXfil*C(3);
dC(4)=uSTOSfloc*C(2)-bSTOfloc*C(4)-uSTOXfloc/YSTOXfloc*C(2);
dC(5)=uSTOSfil*C(3)-bSTOfil*C(5)-uSTOXfil/YSTOXfil*C(3);
```

Table 2. Modeling results for Figure 6

Kinetic Parameters		%increase	Cxfloc	Cxfil	CSTOfloc	CSTOfil	% Cxfloc increase	% Cxfil increase	Cxfloc/Cxfil
α	α=0.009	-10	0.7885	0.9285	0.0102	0.0525	-11.9978	9.5058	0.8492
umaxfloc	umaxfloc=0.54	-10	0.6648	0.9772	0.0088	0.0564	-25.8036	15.2494	0.6803
umaxfil	umaxfil=0.18	-10	1.8601	0.1559	0.0304	0.0103	107.6004	-81.6134	11.9314
Ksfloc	Ksfloc=0.0585	-10	1.1616	0.6655	0.0169	0.0383	29.6429	-21.5120	1.7455
Ksfil	Ksfil=0.0072	-10	0.7346	0.9606	0.0088	0.0554	-18.0134	13.2917	0.7647
kdfloc	kdfloc=0.0054	-10	0.9345	0.8293	0.0124	0.0478	4.2969	-2.1937	1.1269
kdfil	kdfil=0.0018	-10	0.8858	0.8586	0.0117	0.0493	-1.1384	1.2619	1.0317
umaxSTOXfloc	umaxSTOXfloc=0.9	-10	0.8961	0.8478	0.0132	0.0489	0.0112	-0.0118	1.0570
umaxSTOXfil	umaxSTOXfil=0.54	-10	0.8952	0.8486	0.0119	0.0547	-0.0893	0.0826	1.0549
kSTOfloc	kSTOfloc=0.27	-10	1.0016	0.8197	0.0119	0.0473	11.7857	-3.3259	1.2219
kSTOfil	kSTOfil=0.135	-10	0.8061	1.0421	0.0097	0.0506	-10.0335	22.9036	0.7735
KSTOfloc	KSTOfloc=0.9	-10	0.8958	0.848	0.0107	0.0489	-0.0223	0.0118	1.0564
KSTOfil	KSTOfil=0.9	-10	0.8966	0.8473	0.0119	0.044	0.0670	-0.0708	1.0582
bSTOfloc	bSTOfloc=0.0036	-10	0.8958	0.848	0.0119	0.0489	-0.0223	0.0118	1.0564
bSTOfil	bSTOfil=0.0036	-10	0.8966	0.8473	0.0119	0.0489	0.0670	-0.0708	1.0582
Yfloc	Yfloc=0.3	-25	0.6348	0.7861	0.006	0.0254	-22.9892	2.6776	0.8075
Yfil	Yfil=0.3	-25	0.8663	0.6034	0.0082	0.0195	5.0952	-21.1860	1.4357
YSTOSfloc	YSTOSfloc=0.375	-25	0.7966	0.7398	0.0076	0.0239	-3.3604	-3.3699	1.0768
YSTOSfil	YSTOSfil=0.375	-25	0.7743	0.7191	0.0074	0.0232	-6.0658	-6.0737	1.0768
YSTOXfloc	YSTOXfloc=0.375	-25	0.9017	0.7314	0.0064	0.0236	9.3898	-4.4671	1.2328
YSTOXfil	YSTOXfil=0.375	-25	0.8351	0.862	0.0072	0.0191	1.3102	12.5914	0.9688
α	α=0.011	10	1.0097	0.764	0.0138	0.0449	12.6897	-9.8950	1.3216
umaxfloc	umaxfloc=0.66	10	1.3213	0.6138	0.0174	0.0353	47.4665	-27.6094	2.1527
umaxfil	umaxfil=0.22	10	0.6546	1.1496	0.0069	0.0567	-26.9420	35.5820	0.5694
Ksfloc	Ksfloc=0.0715	10	0.7462	0.9508	0.0091	0.0549	-16.7188	12.1359	0.7848
Ksfil	Ksfil=0.0088	10	1.129	0.686	0.0163	0.0395	26.0045	-19.0942	1.6458

kdfloc	kdfloc=0.066	10	0.8603	0.8652	0.0114	0.0499	-3.9844	2.0403	0.9943
kdfil	kdfil=0.0022	10	0.9065	0.8371	0.0121	0.0485	1.1719	-1.2737	1.0829
umaxSTOXfloc	umaxSTOXfloc=1.1	10	0.8958	0.848	0.0108	0.0489	-0.0223	0.0118	1.0564
umaxSTOXfil	umaxSTOXfil=0.66	10	0.8965	0.8473	0.0119	0.0442	0.0558	-0.0708	1.0581
kSTOfloc	kSTOfloc=0.33	10	0.8096	0.8713	0.0118	0.0503	-9.6429	2.7598	0.9292
kSTOfil	kSTOfil=0.165	10	1.1099	0.6123	0.0163	0.0416	23.8728	-27.7863	1.8127
KSTOfloc	KSTOfloc=1.1	10	0.8961	0.8478	0.0131	0.0489	0.0112	-0.0118	1.0570
KSTOfil	KSTOfil=1.1	10	0.8954	0.8485	0.0119	0.0538	-0.0670	0.0708	1.0553
bSTOfloc	bSTOfloc=0.0044	10	0.8961	0.8478	0.0119	0.0489	0.0112	-0.0118	1.0570
bSTOfil	bSTOfil=0.0044	10	0.896	0.8479	0.0119	0.0489	0.0000	0.0000	1.0567
Yfloc	Yfloc=0.5	25	1.0042	0.7461	0.0095	0.0241	21.8246	-2.5470	1.3459
Yfil	Yfil=0.5	25	0.7862	0.9128	0.0075	0.0295	-4.6221	19.2268	0.8613
YSTOSfloc	YSTOSfloc=0.625	25	0.8419	0.7819	0.008	0.0253	2.1351	2.1290	1.0767
YSTOSfil	YSTOSfil=0.625	25	0.8575	0.7964	0.0082	0.0257	4.0277	4.0230	1.0767
YSTOXfloc	YSTOXfloc=0.625	25	0.7592	0.7943	0.009	0.0257	-7.8976	3.7487	0.9558
YSTOXfil	YSTOXfil=0.625	25	0.826	0.6623	0.0088	0.0295	0.2062	-13.4927	1.2472

Program 5. Matlab program for Figure 7

```
%Matlab main program
id=fopen('resultn.doc','a');
for i=1:500;
   umaxfloc=normrnd(0.6,0.6/3.92);
   Ksfloc=normrnd(0.065,0.065/3.92);
   Yfloc=0.88;
   kdfloc=normrnd(0.006,0.006/3.92);
   umaxfil=normrnd(0.2,0.2/3.92);
   Ksfil=normrnd(0.008,0.008/3.92);
   Yfil=0.88;
   kdfil=normrnd(0.002,0.002/3.92);
   kSTOfloc=normrnd(0.3,0.3/3.92);
   KSTOfloc=normrnd(1,1/3.92);
   YSTOSfloc=0.5;
   YSTOXfloc=0.5;
   bSTOfloc=normrnd(0.004,0.004/3.92);
   umaxSTOXfloc=normrnd(1,1/3.92);
   kSTOfil=normrnd(0.15,0.15/3.92);
   KSTOfil=normrnd(1,1/3.92);
   YSTOSfil=0.5;
   YSTOXfil=0.5;
   bSTOfil=normrnd(0.004,0.004/3.92);
   umaxSTOXfil=normrnd(0.6,0.6/3.92);
      a=normrnd(0.01,0.01/3.92);
   Bfloc=2;
   Bfil=1.1;
   r=0.5;
   w=0.025;
   Cs0=0.5;
   D=0.6;
[t,C]=ode45(@conc,[0 500],[0 1
1],[],umaxfloc,Ksfloc,Yfloc,kdfloc,umaxfil,Ksfil,Yfil,kdfil,...
   a,Bfloc,Bfil,r,w,Cs0,D);
udC=flipud(C);
fprintf(fid,'%10.4f %10.4f %10.4f\n',udC(1,:,:));
end;
fclose(fid);

%Function
function dC=conc(t,C,umaxfloc,Ksfloc,Yfloc,kdfloc,umaxfil,Ksfil,Yfil,kdfil,...
   a,Bfloc,Bfil,r,w,Cs0,D)
V=10;Vsed=3;
Dg=w*(1+r)/(r+w)*D;
ufloc=umaxfloc*C(1)/(Ksfloc+C(1));
```

```
ufil=umaxfil*C(1)/(Ksfil+C(1));
Cssed=(1+r)/(1-w)*C(1);
Cxflocsed=(1+r)/Bfloc/(w+r)*C(2);
Cxfilsed=(1+r)/Bfil/(w+r)*C(3);
dC=zeros(3,1);
dC(1)=D*(Cs0-C(1))-ufloc/Yfloc*C(2)-ufil/Yfil*C(3);
dC(2)=-Dg*C(2)+ufloc*C(2)+a*Yfloc/Yfil*C(3)-kdfloc*C(2);
dC(3)=-Dg*C(3)+ufil*C(3)-a*C(3)-kdfil*C(3);
```

Program 6. Matlab program for Figure 8 and 9

```
%Matlab main program
fid=fopen('resultn.doc','a');
   %for i=1:301;
   for i=1:1;
   umaxfloc=0.6;
   Ksfloc=0.065;
   Yfloc=0.88;
   %kdfloc=0.006;
   kdfloc=0.006;
   umaxfil=0.2;
   Ksfil=0.008;
   Yfil=0.88;
   kdfil=0.002;
   kSTOfloc=0.3;
   KSTOfloc=1;
   YSTOSfloc=0.5;
   YSTOXfloc=0.5;
   bSTOfloc=0.004;
   umaxSTOXfloc=1;
   kSTOfil=0.15;
   KSTOfil=1;
   YSTOSfil=0.5;
   YSTOXfil=0.5;
   bSTOfil=0.004;
   umaxSTOXfil=0.6;
   a=0.01;
   Bfloc=2;
   Bfil=1.1;
   r=0.5;
   w=0.025;
   Cs0=0.5;
   %D=0.02*(i-1);
   D=0.5;
```

```
[t,C]=ode45(@conc,[0 500],[0 1 1 0
0],[],umaxfloc,Ksfloc,Yfloc,kdfloc,umaxfil,Ksfil,Yfil,kdfil,...
kSTOfloc,KSTOfloc,YSTOSfloc,YSTOXfloc,bSTOfloc,umaxSTOXfloc,kSTOfil,KSTO
fil,YSTOSfil,YSTOXfil,bSTOfil,umaxSTOXfil,...
   a,Bfloc,Bfil,r,w,Cs0,D);
udC=flipud(C);
%wk1write('testa',udC(1,:,:,:,:));
fprintf(fid,'%10.4f %10.4f %10.4f %10.4f %10.4f\n',udC(1,:,:,:,:));
end;
fclose(fid);

%Function
function dC=conc(t,C,umaxfloc,Ksfloc,Yfloc,kdfloc,umaxfil,Ksfil,Yfil,kdfil,...
kSTOfloc,KSTOfloc,YSTOSfloc,YSTOXfloc,bSTOfloc,umaxSTOXfloc,kSTOfil,KSTO
fil,YSTOSfil,YSTOXfil,bSTOfil,umaxSTOXfil,...
   a,Bfloc,Bfil,r,w,Cs0,D)
V=10;Vsed=3;
kdfloc)*Ksfil*umaxfloc/(Ksfloc-Ksfil))^0.5)/2;
Dg=w*(1+r)/(r+w)*D;
ufloc=umaxfloc*C(1)/(Ksfloc+C(1));
ufil=umaxfil*C(1)/(Ksfil+C(1));
uSTOSfloc=kSTOfloc*C(1)/(Ksfloc+C(1));
uSTOSfil=kSTOfil*C(1)/(Ksfil+C(1));
uSTOXfloc=umaxSTOXfloc*(C(4)/C(2))/(KSTOfloc+C(4)/C(2));
uSTOXfil=umaxSTOXfil*(C(5)/C(3))/(KSTOfil+C(5)/C(3));
Cssed=(1+r)/(1-w)*C(1);
Cxflocsed=(1+r)/Bfloc/(w+r)*C(2);
Cxfilsed=(1+r)/Bfil/(w+r)*C(3);
dC=zeros(5,1);
dC(1)=D*(Cs0-C(1))-ufloc/Yfloc*C(2)-ufil/Yfil*C(3)-uSTOSfloc/YSTOSfloc*C(2)-
uSTOSfil/YSTOSfil*C(3);
dC(2)=-Dg*C(2)+ufloc*C(2)+a*Yfloc/Yfil*C(3)-kdfloc*C(2)-uSTOXfloc*C(2);
dC(4)=uSTOSfloc*C(2)-bSTOfloc*C(4)-uSTOXfloc/YSTOXfloc*C(2);
dC(5)=uSTOSfil*C(3)-bSTOfil*C(5)-uSTOXfil/YSTOXfil*C(3);
```

Table 3. Modeling results for Figure 8 and 9

Dilution rate (1/h)	SRT (h)	SRT (d)	Floc former	Filament conc.
0.02	700	29.16667	0.4655	0.046
0.04	350	14.58333	0.6648	0.1111
0.06	233.3333	9.722222	0.7853	0.1806
0.08	175	7.291667	0.8578	0.2483
0.1	140	5.833333	0.9	0.3121
0.12	116.6667	4.861111	0.9228	0.3713
0.14	100	4.166667	0.9329	0.4255
0.16	87.5	3.645833	0.935	0.4751

0.18	77.77778	3.240741	0.932	0.5202
0.2	70	2.916667	0.9257	0.5613
0.22	63.63636	2.651515	0.9175	0.5985
0.24	58.33333	2.430556	0.9084	0.6323
0.26	53.84615	2.24359	0.899	0.663
0.28	50	2.083333	0.8897	0.6907
0.3	46.66667	1.944444	0.8809	0.7158
0.32	43.75	1.822917	0.8727	0.7384
0.34	41.17647	1.715686	0.8654	0.7587
0.36	38.88889	1.62037	0.859	0.7768
0.38	36.84211	1.535088	0.8537	0.7929
0.4	35	1.458333	0.8496	0.8071
0.42	33.33333	1.388889	0.8467	0.8194
0.44	31.81818	1.325758	0.8452	0.8299
0.46	30.43478	1.268116	0.845	0.8386
0.48	29.16667	1.215278	0.8465	0.8456
0.5	28	1.166667	0.8495	0.8508
0.52	26.92308	1.121795	0.8544	0.8542
0.54	25.92593	1.080247	0.8613	0.8557
0.56	25	1.041667	0.8703	0.8553
0.58	24.13793	1.005747	0.8818	0.8527
0.6	23.33333	0.972222	0.896	0.8479
0.62	22.58065	0.94086	0.9132	0.8406
0.64	21.875	0.911458	0.9341	0.8305
0.66	21.21212	0.883838	0.959	0.8174
0.68	20.58824	0.857843	0.9886	0.8006
0.7	20	0.833333	1.0237	0.7799
0.72	19.44444	0.810185	1.0653	0.7545
0.74	18.91892	0.788288	1.1141	0.7238
0.76	18.42105	0.767544	1.1712	0.6873
0.78	17.94872	0.747863	1.2375	0.6443
0.8	17.5	0.729167	1.3131	0.5948
0.82	17.07317	0.711382	1.3979	0.5388
0.84	16.66667	0.694444	1.4902	0.4775
0.86	16.27907	0.678295	1.5874	0.4128
0.88	15.90909	0.662879	1.6857	0.3473
0.9	15.55556	0.648148	1.7808	0.2839
0.92	15.21739	0.634058	1.8685	0.2256
0.94	14.89362	0.620567	1.9458	0.1743
0.96	14.58333	0.607639	2.0111	0.1312
0.98	14.28571	0.595238	2.0642	0.0964
1	14	0.583333	2.1059	0.0693
1.02	13.72549	0.571895	2.1377	0.0488
1.04	13.46154	0.560897	2.1615	0.0339
1.06	13.20755	0.550314	2.179	0.0231
1.08	12.96296	0.540123	2.1916	0.0156
1.1	12.72727	0.530303	2.2008	0.0104
1.12	12.5	0.520833	2.2073	0.0068
1.14	12.2807	0.511696	2.2121	0.0044
1.16	12.06897	0.502874	2.2156	0.0029

1.18	11.86441	0.49435	2.2182	0.0018
1.2	11.66667	0.486111	2.2201	0.0012
1.22	11.47541	0.478142	2.2216	0.0007
1.24	11.29032	0.47043	2.2228	0.0005
1.26	11.11111	0.462963	2.2238	0.0003
1.28	10.9375	0.455729	2.2246	0.0002
1.3	10.76923	0.448718	2.2253	0.0001
1.32	10.60606	0.441919	2.2258	0.0001
1.34	10.44776	0.435323	2.2262	0
1.36	10.29412	0.428922	2.2266	0
1.38	10.14493	0.422705	2.2269	0
1.4	10	0.416667	2.2271	0
1.42	9.859155	0.410798	2.2272	0
1.44	9.722222	0.405093	2.2273	0
1.46	9.589041	0.399543	2.2273	0
1.48	9.459459	0.394144	2.2273	0
1.5	9.333333	0.388889	2.2271	0
1.52	9.210526	0.383772	2.227	0
1.54	9.090909	0.378788	2.2268	0
1.56	8.974359	0.373932	2.2265	0
1.58	8.860759	0.369198	2.2262	0
1.6	8.75	0.364583	2.2258	0
1.62	8.641975	0.360082	2.2253	0
1.64	8.536585	0.355691	2.2249	0
1.66	8.433735	0.351406	2.2243	0
1.68	8.333333	0.347222	2.2238	0
1.7	8.235294	0.343137	2.2231	0
1.72	8.139535	0.339147	2.2225	0
1.74	8.045977	0.335249	2.2218	0
1.76	7.954545	0.331439	2.221	0
1.78	7.865169	0.327715	2.2202	0
1.8	7.777778	0.324074	2.2193	0
1.82	7.692308	0.320513	2.2184	0
1.84	7.608696	0.317029	2.2175	0
1.86	7.526882	0.31362	2.2165	0
1.88	7.446809	0.310284	2.2155	0
1.9	7.368421	0.307018	2.2145	0
1.92	7.291667	0.303819	2.2134	0
1.94	7.216495	0.300687	2.2122	0
1.96	7.142857	0.297619	2.211	0
1.98	7.070707	0.294613	2.2098	0
2	7	0.291667	2.2085	0
2.02	6.930693	0.288779	2.2073	0
2.04	6.862745	0.285948	2.2059	0
2.06	6.796117	0.283172	2.2045	0
2.08	6.730769	0.280449	2.2031	0
2.1	6.666667	0.277778	2.2017	0
2.12	6.603774	0.275157	2.2002	0
2.14	6.542056	0.272586	2.1986	0
2.16	6.481481	0.270062	2.1971	0

2.18	6.422018	0.267584	2.1954	0
2.2	6.363636	0.265152	2.1938	0
2.22	6.306306	0.262763	2.1921	0
2.24	6.25	0.260417	2.1904	0
2.26	6.19469	0.258112	2.1886	0
2.28	6.140351	0.255848	2.1868	0
2.3	6.086957	0.253623	2.185	0
2.32	6.034483	0.251437	2.1831	0
2.34	5.982906	0.249288	2.1812	0
2.36	5.932203	0.247175	2.1792	0
2.38	5.882353	0.245098	2.1772	0
2.4	5.833333	0.243056	2.1752	0
2.42	5.785124	0.241047	2.1731	0
2.44	5.737705	0.239071	2.171	0
2.46	5.691057	0.237127	2.1689	0
2.48	5.645161	0.235215	2.1667	0
2.5	5.6	0.233333	2.1644	0
2.52	5.555556	0.231481	2.1622	0
2.54	5.511811	0.229659	2.1598	0
2.56	5.46875	0.227865	2.1575	0
2.58	5.426357	0.226098	2.1551	0
2.6	5.384615	0.224359	2.1527	0
2.62	5.343511	0.222646	2.1502	0
2.64	5.30303	0.22096	2.1476	0
2.66	5.263158	0.219298	2.1451	0
2.68	5.223881	0.217662	2.1425	0
2.7	5.185185	0.216049	2.1398	0
2.72	5.147059	0.214461	2.1371	0
2.74	5.109489	0.212895	2.1344	0
2.76	5.072464	0.211353	2.1316	0
2.78	5.035971	0.209832	2.1288	0
2.8	5	0.208333	2.1259	0
2.82	4.964539	0.206856	2.1229	0
2.84	4.929577	0.205399	2.12	0
2.86	4.895105	0.203963	2.117	0
2.88	4.861111	0.202546	2.1139	0
2.9	4.827586	0.201149	2.1108	0
2.92	4.794521	0.199772	2.1076	0
2.94	4.761905	0.198413	2.1044	0
2.96	4.72973	0.197072	2.1011	0
2.98	4.697987	0.195749	2.0978	0
3	4.666667	0.194444	2.0944	0
3.02	4.635762	0.193157	2.091	0
3.04	4.605263	0.191886	2.0875	0
3.06	4.575163	0.190632	2.084	0
3.08	4.545455	0.189394	2.0804	0
3.1	4.516129	0.188172	2.0767	0
3.12	4.487179	0.186966	2.0731	0
3.14	4.458599	0.185775	2.0693	0
3.16	4.43038	0.184599	2.0655	0

3.18	4.402516	0.183438	2.0616	0
3.2	4.375	0.182292	2.0576	0
3.22	4.347826	0.181159	2.0536	0
3.24	4.320988	0.180041	2.0495	0
3.26	4.294479	0.178937	2.0454	0
3.28	4.268293	0.177846	2.0412	0
3.3	4.242424	0.176768	2.0369	0
3.32	4.216867	0.175703	2.0326	0
3.34	4.191617	0.174651	2.0282	0
3.36	4.166667	0.173611	2.0237	0
3.38	4.142012	0.172584	2.0192	0
3.4	4.117647	0.171569	2.0146	0
3.42	4.093567	0.170565	2.0099	0
3.44	4.069767	0.169574	2.0051	0
3.46	4.046243	0.168593	2.0003	0
3.48	4.022989	0.167625	1.9953	0
3.5	4	0.166667	1.9903	0
3.52	3.977273	0.16572	1.9852	0
3.54	3.954802	0.164783	1.98	0
3.56	3.932584	0.163858	1.9748	0
3.58	3.910615	0.162942	1.9694	0
3.6	3.888889	0.162037	1.964	0
3.62	3.867403	0.161142	1.9585	0
3.64	3.846154	0.160256	1.9528	0
3.66	3.825137	0.159381	1.9471	0
3.68	3.804348	0.158514	1.9413	0
3.7	3.783784	0.157658	1.9354	0
3.72	3.763441	0.15681	1.9293	0
3.74	3.743316	0.155971	1.9232	0
3.76	3.723404	0.155142	1.917	0
3.78	3.703704	0.154321	1.9106	0
3.8	3.684211	0.153509	1.9042	0
3.82	3.664921	0.152705	1.8976	0
3.84	3.645833	0.15191	1.8909	0
3.86	3.626943	0.151123	1.8841	0
3.88	3.608247	0.150344	1.8772	0
3.9	3.589744	0.149573	1.8701	0
3.92	3.571429	0.14881	1.8629	0
3.94	3.553299	0.148054	1.8556	0
3.96	3.535354	0.147306	1.8481	0
3.98	3.517588	0.146566	1.8405	0
4	3.5	0.145833	1.8328	0
4.02	3.482587	0.145108	1.8249	0
4.04	3.465347	0.144389	1.8169	0
4.06	3.448276	0.143678	1.8087	0
4.08	3.431373	0.142974	1.8003	0
4.1	3.414634	0.142276	1.7918	0
4.12	3.398058	0.141586	1.7831	0
4.14	3.381643	0.140902	1.7742	0
4.16	3.365385	0.140224	1.7652	0

4.18	3.349282	0.139553	1.756	0
4.2	3.333333	0.138889	1.7465	0
4.22	3.317536	0.138231	1.7369	0
4.24	3.301887	0.137579	1.7271	0
4.26	3.286385	0.136933	1.7171	0
4.28	3.271028	0.136293	1.7069	0
4.3	3.255814	0.135659	1.6964	0
4.32	3.240741	0.135031	1.6857	0
4.34	3.225806	0.134409	1.6748	0
4.36	3.211009	0.133792	1.6637	0
4.38	3.196347	0.133181	1.6523	0
4.4	3.181818	0.132576	1.6406	0
4.42	3.167421	0.131976	1.6287	0
4.44	3.153153	0.131381	1.6165	0
4.46	3.139013	0.130792	1.604	0
4.48	3.125	0.130208	1.5912	0
4.5	3.111111	0.12963	1.5781	0
4.52	3.097345	0.129056	1.5647	0
4.54	3.0837	0.128488	1.551	0
4.56	3.070175	0.127924	1.537	0
4.58	3.056769	0.127365	1.5225	0
4.6	3.043478	0.126812	1.5078	0
4.62	3.030303	0.126263	1.4926	0
4.64	3.017241	0.125718	1.4771	0
4.66	3.004292	0.125179	1.4612	0
4.68	2.991453	0.124644	1.4448	0
4.7	2.978723	0.124113	1.428	0
4.72	2.966102	0.123588	1.4108	0
4.74	2.953586	0.123066	1.393	0
4.76	2.941176	0.122549	1.3749	0
4.78	2.92887	0.122036	1.3561	0
4.8	2.916667	0.121528	1.3369	0
4.82	2.904564	0.121024	1.3171	0
4.84	2.892562	0.120523	1.2967	0
4.86	2.880658	0.120027	1.2757	0
4.88	2.868852	0.119536	1.254	0
4.9	2.857143	0.119048	1.2317	0
4.92	2.845528	0.118564	1.2088	0
4.94	2.834008	0.118084	1.185	0
4.96	2.822581	0.117608	1.1606	0
4.98	2.811245	0.117135	1.1353	0
5	2.8	0.116667	1.1092	0
5.02	2.788845	0.116202	1.0822	0
5.04	2.777778	0.115741	1.0544	0
5.06	2.766798	0.115283	1.0255	0
5.08	2.755906	0.114829	0.9956	0
5.1	2.745098	0.114379	0.9646	0
5.12	2.734375	0.113932	0.9326	0
5.14	2.723735	0.113489	0.8993	0
5.16	2.713178	0.113049	0.8647	0

5.18	2.702703	0.112613	0.8289	0
5.2	2.692308	0.112179	0.7916	0
5.22	2.681992	0.11175	0.7528	0
5.24	2.671756	0.111323	0.7124	0
5.26	2.661597	0.1109	0.6704	0
5.28	2.651515	0.11048	0.6265	0
5.3	2.641509	0.110063	0.5808	0
5.32	2.631579	0.109649	0.533	0
5.34	2.621723	0.109238	0.4831	0
5.36	2.61194	0.108831	0.4312	0
5.38	2.60223	0.108426	0.3772	0
5.4	2.592593	0.108025	0.3216	0
5.42	2.583026	0.107626	0.2654	0
5.44	2.573529	0.10723	0.2102	0
5.46	2.564103	0.106838	0.1585	0
5.48	2.554745	0.106448	0.113	0
5.5	2.545455	0.106061	0.0758	0
5.52	2.536232	0.105676	0.048	0
5.54	2.527076	0.105295	0.0287	0
5.56	2.517986	0.104916	0.0164	0
5.58	2.508961	0.10454	0.009	0
5.6	2.5	0.104167	0.0048	0
5.62	2.491103	0.103796	0.0025	0
5.64	2.48227	0.103428	0.0013	0
5.66	2.473498	0.103062	0.0006	0
5.68	2.464789	0.1027	0.0003	0
5.7	2.45614	0.102339	0.0002	0
5.72	2.447552	0.101981	0.0001	0
5.74	2.439024	0.101626	0	0
5.76	2.430556	0.101273	0	0
5.78	2.422145	0.100923	0	0
5.8	2.413793	0.100575	0	0
5.82	2.405498	0.100229	0	0
5.84	2.39726	0.099886	0	0
5.86	2.389078	0.099545	0	0
5.88	2.380952	0.099206	0	0
5.9	2.372881	0.09887	0	0
5.92	2.364865	0.098536	0	0
5.94	2.356902	0.098204	0	0
5.96	2.348993	0.097875	0	0
5.98	2.341137	0.097547	0	0
6	2.333333	0.097222	0	0

Chapter 2

Table 1. Experimental data for Figure 2

Day	SV		MLSS		SVI / DSVI	
	SBR	CMR	SBR	CMR	SBR	CMR
-129	0.52		2.053		253.29	
-127	0.50		2.284		218.91	
-124	0.70		2.905		240.96	
-122	0.78		3.812		204.62	
-118	0.65		3.349		194.09	
-116	0.67		3.38		198.22	
-115	0.62		3.613		171.60	
-114	0.57		3.446		165.41	
-113	0.50		3.994		125.19	
-111	0.48		3.956		121.33	
-109	0.36		3.604		99.89	
-106	0.22		3.224		68.24	
-105	0.20		3.266		61.24	
-103	0.19		3.004		63.25	
-101	0.15		2.968		50.54	
-99	0.15		2.745		54.64	
-98	0.13		2.878		45.17	
-96	0.10		2.848		35.11	
-92	0.13		3.488		37.27	
-90	0.14		4.896		28.59	
-87	0.16		4.088		39.14	
-84	0.16		4.432		36.10	
-82	0.17		3.292		51.64	
-80	0.15		3.812		39.35	
-75	0.15		3.536		42.42	
-73	0.16		3.648		43.86	
-70	0.18		3.628		49.61	
-68	0.19		3.904		48.67	
-63	0.22		3.972		55.39	
-59	0.20		3.96		50.51	
-38	0.22		3.688		59.65	
-35	0.21		3.648		57.57	
-31	0.22		3.868		56.88	
-28	0.21		3.868		54.29	
-26	0.19		3.608		52.66	
-19	0.16		3.516		45.51	
0	0.10	0.18	3.112	5.348	32.13	33.66
1	0.14	0.20	3.824	5.508	36.61	36.31
2	0.14	0.21	4.076	5.316	34.35	39.50
3	0.14	0.24	3.272	4.832	42.79	49.67
8	0.17	0.27	3.62	3.84	46.96	70.31
9	0.19	0.29	4.06	3.068	45.57	92.89
10	0.18	0.78	4.468	3.936	40.29	198.17

11	0.20	0.78	4.356	3.456	45.91	225.69
14	0.20	0.78	3.884	2.692	51.49	289.75
15	0.22	0.93	4.468	3.584	49.24	259.49
16	0.14	0.51	4.032	3.056	35.94	167.34
18	0.29	1.00	4.452	2.988	65.34	346.82
21	0.15	1.00	2.908	2.768	52.94	361.94
22	0.26	0.95	4.156	2.68	62.32	355.57
23	0.25	0.98	4.172	2.728	61.09	358.71
24	0.28	1.00	4.092	2.732	68.21	666.67
28	0.26	1.00	2.932	1.5	87.32	763.24
29	0.17	0.98	2.176	1.284	80.37	765.63
30	0.20	1.00	2.364	1.28	83.77	1066.81
31	0.19	1.00	2.268	0.928	83.06	1283.07
32	0.23	0.92	2.408	0.756	95.96	1219.21
35	0.18	1.00	1.98	0.812	91.88	1856.06
36	0.14	0.29	2.068	0.528	69.33	550.60
37	0.20	0.14	2.452	0.672	80.52	201.01
38	0.20	0.16	2.484	0.796	81.17	198.41
39	0.10	0.13	1.232	0.756	77.16	171.23
40	0.09	0.11	1.296	0.876	67.37	129.76
41	0.09	0.16	1.336	1.156	67.20	142.28
42	0.08	0.21	1.488	0.492	52.45	424.53
43	0.09	0.02	1.716	0.212	55.19	72.46
44	0.11	0.02	1.812	0.276	61.58	86.21

Table 2. Sample excel spreadsheet for kinetic parameters estimation

SPREADSHEET FOR MONOD-NO-GROWTH
Copyright 1990-1995 E.S.E. Clemson University
Modified March 2001 @ Iowa State University
Subject:
DATE :
Input Variables:

So =	27.376	mg SCOD / L
X =	5482	mg X / L
del T =	2	sec
del T =	0.000555556	hr

Calculated Parmeters

DOo =	8.380596895	mg O2 / L
DOf =	5.230376122	mg O2 / L
Y =	0.884927646	mg COD / mg COD
D.O.@10%=	8.065574817	mg O2 / L
TR(h) =	0.074444444	sec

Fit Parameters

TR =	266	sec
qm =	0.825687452	mg SCOD / mg bug*hr
Ks =	82.09838267	mg SCOD / L
um =	0.730673653	hr-1
SSE =	0.590978347	

Results
Summary

Yield	Ks	um	qm	X	So
0.884927646	82.09838267	0.73067365	0.82568745	5482	27.376

t(sec)	t(hr)	ko	k1	k2	k3	S	sec	DO Curve	Data Set	RSE
0.000	0.000	0.000	0.000	0.000	0.000	27.376	0.000	8.381	8.400	0.000
2.000	0.000	-0.629	-0.623	-0.623	-0.618	27.376	2.000	8.381	8.393	0.000
4.000	0.000	-0.629	-0.623	-0.623	-0.618	27.376	4.000	8.381	8.385	0.000

Chapter 3

Table 3. Experimental data for Table 3

TC, IC and TOC		Non-bulking sludge								
		end of cycle			beginning of cycle			Biomass growth		
		SBR A (4d)	SBR B (8d)	SBR C (16d)	SBR A (4d)	SBR B (8d)	SBR C (16d)	SBR A (4d)	SBR B (8d)	SBR C (16d)
Liquid (ppm)	TC	47.7	41.2	34.3	48.9	43.2	39.8			
		36.81	36.31	25.12						
	IC	9.239	5.5	2.451	10.19	5.93	8.74			
		8.923	5.058	2.027						
	TOC	38.46	35.7	31.85	38.71	37.27	31.06			
		27.89	31.25	23.1						
	Avg.	33.175	33.475	27.475						
ML (mg/L)	TC	582.4	1103.9	1523.1	561.9	1146.9	1590.3			
		593.9	1229.3	1682.3						
	Avg.	588.15	1166.6	1602.7						
Biomass	(Cmg/L)	554.975	1133.125	1575.225	523.19	1109.63	1559.24	31.785	23.495	15.985
	(Cmmol/L)	46.24792	94.42708	131.2688	43.59917	92.46917	129.9367	2.64875	1.9579	1.3321
							Cmmol/cycle	21.19	15.663	10.627

TC, IC and TOC		Bulking sludge					
		end of cycle		beginning of cycle		Biomass growth	
		SBR D (8d)	SBR E (16d)	SBR D (8d)	SBR E (16d)	SBR D (8d)	SBR E (16d)
Liquid (ppm)	TC	38.3	33.8	41.2	37.9		
		43.5	34.2	38.8	29.8		
	IC	6.712	5.702	6.874	8.089		
		8.424	4.877	5.977	7.449		

	TOC	31.588	32.1	34.326	29.811			
		35.076	29.3	32.823	22.351			
	Avg.	33.332	30.7	33.5745	26.081			
ML	TC	754.3	1132.6	721.5	1089.7			
(mg/L)		786.6	1082.3	769.3	1189.8			
	Avg.	770.45	1107.45	745.4	1139.75			
Biomass	(Cmg/L)						25.2925	16.861
	(Cmmol/L)	737.118	1076.75	711.826	1059.89		2.10771	1.40508
						Cmmol/cycle	16.8617	11.2407

Table 4. Results of elemental analysis for Table 4

Non-bulking sludge							
Research ID	ASL ID	% C	% H	% N	% S	% ash	% O
SBRA 1	Z 180	41.19	4.53	8.62	0.53		
SBRB 1	Z 181	40.96	3.79	8.79	0.51		
SBRC 1	Z 182	38.90	4.35	8.19	0.46		
SBRA 2	Z 183	41.54	4.91	8.55	0.53	0.401747	44.07
SBRB 2	Z 184	41.72	4.40	8.77	0.53	0.452997	44.13
SBRC 2	Z185	40.50	4.38	8.48	0.48	0.263352	45.90
Avg. (%weight)	(total samples)	40.80	4.39	8.57			
Avg.(%mole)		3.40	4.39	0.611905			
Ratio		1.000	1.292	0.180			
Avg. (%weight)	(sample 2)	41.25	4.56	8.60			44.70
Avg.(%mole)		3.438	4.563	0.614			2.794
Ratio		1.000	1.327	0.179			0.813
	Crucible (g)	Cru+sludge	Cru+ash	ash	%		
SBRA	18.5191	18.654	18.594	0.074	0.401747		
SBRB	18.5873	19.205	18.672	0.084	0.452997		
SBRC	18.948	19.348	18.998	0.050	0.263352		
Summary							
$CH_{1.33}O_{0.8}1N_{0.18}$							

Bulking sludge							
Research ID	ASL ID	% C	% H	% N	% S	% ash	% O
SBRA		39.68	3.56	6.44	1.52	0.541771	48.25823
SBRB		41.99	3.63	6.14	1.54	0.578785	46.12121
Avg. (%weight)	(total samples)	40.835	3.595	6.29			47.18972
Avg.(%mole)		3.403	3.595	0.449			2.949
Ratio		1.000	1.056	0.132			0.866715
	Crucible (g)	Cru+sludge	Cru+ash	ash	%		
SBRA	18.458	18.538	18.558	0.100	0.541771		
SBRB	18.487	19.148	18.594	0.107	0.578785		
Summary							
$CH_{1.06}O_{0.87}N_{0.13}$							

Table 5. Experimental data for Figure 1 a.

Day	SV			MLSS			SVI		
	A	B	C	A	B	C	A	B	C
0	0.80	0.80	0.80	1.772	1.772	1.772	452.80	452.80	452.80
5	0.55	0.54	0.54	2.996	2.824	2.224	183.58	191.22	242.81
7	0.51	0.50	0.42	3.448	3.148	2.336	147.91	157.24	179.79
9	0.47	0.48	0.38	3.748	3.86	2.888	125.40	124.35	131.58
13	0.41	0.47	0.31	4.968	4.848	3	83.53	96.95	101.67
16	0.32	0.41	0.31	4.148	4.584	3.728	77.15	89.44	83.15
18	0.28	0.34	0.34	4.444	5.268	4.808	64.13	64.54	70.72
21	0.17	0.24	0.24	3.236	4.424	4.312	54.08	53.12	55.66
27	0.09	0.11	0.18	1.648	3.092	3.412	53.44	35.58	52.75
48	0.06	0.08	0.00	1.104	1.824		54.35	43.86	32.23
62	0.07	0.11	0.12	1.176	1.824	2.836	59.52	60.31	42.31
70	0.08	0.13	0.14	1.468	2.22	3.16	54.50	58.56	44.30
77	0.09	0.13	0.16	1.26	2.308	3.264	67.46	56.33	49.02
83	0.13	0.16	0.17	1.98	2.988	3.756	65.66	53.55	45.26

Table 6. Experimental data for Figure 1 b.

Day	SV		MLSS		SVI	
	A	B	A	B	A	B
0	0.8	0.8	2.52	2.476	317.46	323.10
2	0.3	0.44	1.372	1.516	218.66	290.24
6	0.24	0.27	2.236	2.22	105.10	121.62
13	0.8	0.92	2.036	1.232	392.93	746.75
17	0.8	0.98	0.644	0.804	1242.24	1218.91
21	0.27	0.35	1.048	0.472	257.63	741.53
24	0.25	0.71	1.588	0.864	154.28	821.76
28	0.21	0.39	2.316	1.544	90.67	252.59
35	0.62	0.65	2.536	3.048	244.48	213.25
42	0.69	0.58	0.936	1.756	737.18	330.30
45	0.93	0.92	1.256	1.58	740.45	582.28
48	0.81	0.71	0.824	1.092	983.01	650.18
50	0.63	0.81	0.765	1.204	826.34	672.76
52	0.47	0.78	0.784	1.224	599.49	637.25
65	0.62	0.91	0.798	1.024	780.35	888.67

Table 7. Experimental data for Figure 3 a.

Time (h)	Blank		Sample			Gycogen concentration	
	A1	A2	A1	A2	ΔA	(mg/L)	(C-mmol/L)
0	0.089	0.093	0.103	0.308	0.201	173.594	0.044
0.25	0.089	0.093	0.105	0.441	0.332	286.732	0.073
0.75	0.089	0.093	0.103	0.401	0.294	253.913	0.064

2	0.089	0.093	0.097	0.363	0.262	226.277	0.057
4	0.089	0.093	0.1	0.328	0.224	193.458	0.049
7	0.089	0.093	0.106	0.31	0.2	172.730	0.044

Table 8. Experimental data for Figure 3 b.

Time (h)	Blank		Sample			Gycogen concentration	
	A1	A2	A1	A2	ΔA	(mg/L)	(C-mmol/L)
0	0.038	0.045	0.078	0.086	0.001	0.864	0.000
0.083	0.038	0.045	0.093	0.137	0.037	31.955	0.012
0.75	0.038	0.045	0.065	0.152	0.08	69.092	0.026
1.5	0.038	0.045	0.084	0.112	0.021	18.137	0.007
3	0.038	0.045	0.077	0.095	0.011	9.500	0.004
4	0.038	0.045	0.063	0.078	0.008	6.909	0.003
5	0.038	0.045	0.082	0.094	0.005	4.318	0.002
7	0.038	0.045	0.092	0.101	0.002	1.727	0.001

Table 9. Experimental data for Figure 5 and 6

	type 1851(bulking sludge)		type 1851 (non-bulking sludge)	
($x\ 10^{-9}$)	total	extended	total	extended
well 1	7538.314	4395.28	1958.695	374.4208
well 2	2103.207	1136.724	1523.363	172.4648
well 3	4783.501	3232.098	1112.089	286.9708
well 4	1869.09	1195.917	1241.036	155.6215
Avg. (um)	4073.528	2490.005	1458.796	247.3695
Lv (um/ml)	4.9631	3.0337	1.7774	0.3014
stan. Dev.	3.24	1.95	0.46	0.13
converted Biomass (mg/L)	351.1688	214.6572	125.7592	21.3251
converted biomass/MLVSS	0.1356	0.0829	0.0486	0.0082

	TNI					21N			
	Slide1		Slide2			Slide1		Slide2	
Well 1	Extented	Total	Extented	Total	Well 1	Extented	Total	Extented	Total
Image 1	1807.99	2428.53	510.76	553.15	Image 1	818.2874	1716.744	86.8814	1113.157
Image 2	2745.19	3223.60	420.18	923.43	Image 2	915.9187	1624.203	473.0899	1865.772
Image 3	2018.09	2863.04	703.65	1210.24	Image 3	885.0804	1230.348	103.3754	380.5085
Image 4	824.51	1313.88	992.72	1225.05	Image 4	350.8065	585.0778	375.209	811.5429
Image 5	977.81	1459.72	491.84	815.48	Image 5	724.4809	1294.351	471.4807	1484.468
Sum (um)	8373.60	11288.76	3119.156	4727.34	Sum (um)	3694.57	6450.724	1510.036	5655.448
Avg. (um)	1674.72	2257.75	623.83	945.47	Avg. (um)	738.91	1290.14	302.01	1131.09
Lv (um/ml) (x 10^{-9})	2.0404	2.7508	0.7601	1.1519	Lv (um/ml) (x 10^{-9})	0.9003	1.5719	0.3680	1.3781
converted Biomass (mg/L)	144.3733	194.6351	53.7789	81.5064	converted Biomass (mg/L)	63.7000	111.2201	26.0353	97.5084
converted biomass/MLVSS	0.1000	0.1349	0.0373	0.0565	converted biomass/MLVSS	0.0441	0.0771	0.0180	0.0676
Well 2	Extented	Total	Extented	Total	Well 2	Extented	Total	Extented	Total
Image 1	1094.58	1431.62	574.51	976.49	Image 1	581.7265	921.8428	1121.047	1604.883
Image 2	1843.09	1857.94	1141.86	1373.90	Image 2	529.4206	760.388	668.6884	1635.501
Image 3	1194.68	1514.21	2239.72	3110.67	Image 3	155.3909	379.0491	833.4657	1817.232
Image 4	2512.91	2732.09	988.20	1479.23	Image 4	456.7921	1460.237	830.2072	1852.623
Image 5	1567.47	2038.13	1735.90	2527.62	Image 5	1523.67	2030.056	882.0816	1672.161
Sum (um)	8212.72	9573.99	6680.19	9467.91	Sum (um)	3247.00	5551.573	4335.49	8582.4
Avg. (um)	1642.54	1914.80	1336.04	1893.58	Avg. (um)	649.40	1110.31	867.10	1716.48
Lv (um/ml) (x 10^{-9})	2.0012	2.3329	1.6278	2.3071	Lv (um/ml) (x 10^{-9})	0.7912	1.3528	1.0564	2.0913
converted Biomass (mg/L)	141.5996	165.0700	115.1765	163.2409	converted Biomass (mg/L)	55.9831	95.7174	74.7503	147.9734
converted biomass/MLVSS	0.0981	0.1144	0.0798	0.1131	converted biomass/MLVSS	0.0388	0.0663	0.0518	0.1025

Well 3	Extented	Total	Extented	Total	Well 3	Extented	Total	Extented	Total
Image 1	1561.95	1913.30	900.56	2027.20	Image 1	485.9908	715.3164	1047.857	1971.801
Image 2	1398.53	1559.12	1243.83	1789.09	Image 2	574.4419	1190.759	456.002	829.1517
Image 3	972.94	1215.01	1091.79	1489.42	Image 3	954.1502	1261.899	588.7949	1054.48
Image 4	1493.39	1731.41	571.01	655.14	Image 4	835.6881	1926.513	758.5695	1311.621
Image 5	1159.42	1435.31	662.99	994.45	Image 5	490.8188	1064.218	461.9	984.2963
Sum (um)	6586.23	7854.14	4470.18	6955.29	Sum (um)	3341.09	6158.706	3313.124	6151.351
Avg. (um)	1317.25	1570.83	894.04	1391.06	Avg. (um)	668.22	1231.74	662.62	1230.27
Lv (um/ml) (x 10^{-9})	1.6049	1.9139	1.0893	1.6948	Lv (um/ml) (x 10^{-9})	0.8141	1.5007	0.8073	1.4989
converted Biomass (mg/L)	113.5565	135.4172	77.0726	119.9196	converted Biomass (mg/L)	57.6054	106.1853	57.1232	106.0585
converted biomass/MLVSS	0.0787	0.0938	0.0534	0.0831	converted biomass/MLVSS	0.0399	0.0736	0.0396	0.0735
Well 4	Extented	Total	Extented	Total	Well 4	Extented	Total	Extented	Total
Image 1	922.60	1105.59	843.87	1361.23	Image 1	803.279	1318.712	379.2808	581.0175
Image 2	1114.80	1741.38	1708.79	2438.13	Image 2	733.1424	1124.04	712.9508	930.0578
Image 3	560.38	1090.50	1478.16	1851.67	Image 3	783.4329	920.1036	674.0001	1841.746
Image 4	392.50	1278.02	1434.74	1831.80	Image 4	917.3236	999.6448	490.6882	1619.768
Image 5	1129.58	1654.64	1749.47	2218.65	Image 5	378.3765	1140.547	614.561	1257.213
Sum (um)	4119.86	6870.13	7215.03	9701.48	Sum (um)	3615.55	5503.046	2871.481	6229.802
Avg. (um)	823.97	1374.03	1443.01	1940.30	Avg. (um)	723.11	1100.61	574.30	1245.96
Lv (um/ml) (x 10^{-9})	1.0039	1.6741	1.7581	2.3640	Lv (um/ml) (x 10^{-9})	0.8810	1.3409	0.6997	1.5180
converted Biomass (mg/L)	71.0326	118.4513	124.3979	167.2681	converted Biomass (mg/L)	62.3376	94.8808	49.5086	107.4111
converted biomass/MLVSS	0.0492	0.0821	0.0862	0.1159	converted biomass/MLVSS	0.0432	0.0657	0.0343	0.0744
Well 5	Extented	Total	Extented	Total	Well 5	Extented	Total	Extented	Total
Image 1	502.71	830.84	669.47	1032.82	Image 1	293.5813	1384.312	598.5685	1126.526
Image 2	1087.25	1896.69	506.06	735.03	Image 2	794.9867	2000.72	182.1787	729.6681

Image 3	1559.17	2025.24	1538.75	1796.07	Image 3	1047.986	1671.305	501.2555	789.7639
Image 4	1782.99	3196.94	953.90	1890.06	Image 4	774.0573	1717.478	661.6348	894.7352
Image 5	968.37	1600.27	929.65	1123.97	Image 5	513.0789	1250.437	511.4345	1602.296
Sum (um)	5900.48	9549.99	4597.84	6577.94	Sum (um)	3423.69	8024.25	2455.07	5142.99
Avg. (um)	1180.10	1910.00	919.57	1315.59	Avg. (um)	684.74	1604.85	491.01	1028.60
Lv (um/ml) (x 10^{-9})	1.4378	2.3271	1.1204	1.6029	Lv (um/ml) (x 10^{-9})	0.8343	1.9553	0.5982	1.2532
converted Biomass (mg/L)	101.7332	164.6561	79.2736	113.4135	converted Biomass (mg/L)	59.0295	138.3501	42.3291	88.6728
converted biomass/MLVSS	0.0705	0.1141	0.0549	0.0786	converted biomass/MLVSS	0.0409	0.0959	0.0293	0.0614
Well 6	Extented	Total	Extented	Total	Well 6	Extented	Total	Extented	Total
Image 1	2880.35	1589.30	917.50	1745.36	Image 1	147.3323	727.4419	532.0151	2197.128
Image 2	1042.65	1594.09	637.83	846.36	Image 2	163.2122	1060.364	596.2423	2036.961
Image 3	595.44	1194.17	524.52	720.00	Image 3	599.6938	1180.813	837.8068	2438.193
Image 4	2038.67	821.48	1653.60	2330.86	Image 4	819.4902	1430.392	772.9601	1516.424
Image 5	837.77	1255.14	1046.61	1447.99	Image 5	337.503	978.5229	278.7554	988.3455
Sum (um)	7394.88	6454.18	4780.07	7090.57	Sum (um)	2067.23	5377.53	3017.78	9177.05
Avg. (um)	1478.98	1290.84	956.01	1418.11	Avg. (um)	413.45	1075.51	603.56	1835.41
Lv (um/ml) (x 10^{-9})	1.8019	1.5727	1.1648	1.7278	Lv (um/ml) (x 10^{-9})	0.5037	1.3104	0.7354	2.2362
converted Biomass (mg/L)	127.4987	111.2798	82.4155	122.2520	converted Biomass (mg/L)	35.6422	92.7167	52.0310	158.2261
converted biomass/MLVSS	0.0883	0.0771	0.0571	0.0847	converted biomass/MLVSS	0.0247	0.0642	0.0361	0.1096

Summation

	Bulking sludge											
	TNI			021N			Type 1851			Total		
	extended	total	%	extended	total	%	extended	total	%	extended	total	%
Lv (um/ml) x10^{-9}	1.45	1.95	74.34	0.75	1.58	47.29	3.03	4.96	61.13	5.23	8.50	61.58

% of total filaments		22.96			18.64		58.40		100.00
converted biomass (mg/L)	102.66	138.09		53.01	112.08	214.66	351.17	370.32	601.34
% of MLVSS	7.16	9.64		3.67	7.77	14.98	24.50	25.84	41.96

	Non-bulking sludge		
	Type 1851		
	extended	total	%
Lv (um/ml) $x10^{-9}$	0.30	1.78	16.96
converted biomass (mg/L)	21.33	125.76	16.96
% of MLVSS	0.82	4.86	

Chapter 4

Table 1. Experimental data for Figure 3

COD100				COD 300			
Day	SV	MLSS	SVI	Day	SV	MLSS	SVI
50 rpm				50 rpm			
1	0.15	1.230	121.95	1	0.155	1.550	101.27
2	0.15	1.320	113.65	2	0.17	1.992	85.76
4	0.14	0.955	146.73	3	0.36	2.018	179.05
8	0.47	1.550	303.34	4	0.48	1.690	284.15
9	0.49	1.818	269.54	6	0.23	0.902	254.94
10	0.69	1.658	416.16	8	0.35	1.062	331.66
11	0.75	1.158	647.72	11	0.46	1.317	353.16
13	0.7	1.012	691.97	126 rpm			
15	0.88	0.608	1447.62	14	0.25	1.882	132.91
18	0.81	0.372	2183.73	15	0.25	1.783	140.37
22	0.47	0.186	2534.21	16	0.26	1.860	140.37
30	0.44	0.278	1583.47	18	0.25	1.790	140.06
31	0.41	0.242	1694.33	20	0.27	1.780	152.17
34	0.47	0.202	2326.96	21	0.25	2.102	119.00
35	0.32	0.252	1278.00	23	0.22	1.612	136.53
126 rpm				24	0.22	1.810	121.61
37	0.32	0.252	1277.89	25	0.21	1.635	128.54
39	0.34	0.326	1042.98	28	0.14	1.405	99.64
41	0.17	0.428	398.45	30	0.16	1.928	83.01
45	0.3	1.086	276.66	31	0.17	2.280	74.56
46	0.28	1.018	279.06	35	0.2	2.060	97.23
47	0.28	1.020	274.55	50 rpm again			
52	0.41	0.726	565.70	37	0.23	2.232	103.09
53	0.47	0.522	900.40	38	0.22	1.977	111.35
54	0.44	0.632	697.21	39	0.24	1.857	129.30
55	0.58	0.890	652.10	40	0.25	1.990	125.67
56	0.33	0.498	662.50	41	0.28	2.097	133.64
60	0.48	0.784	612.20	46	0.29	1.627	178.35
61	0.58	0.842	689.40	47	0.18	1.003	179.40
62	0.52	0.758	676.00	48	0.2	1.140	175.44
64	0.56	0.768	697.40	56	0.3	1.413	212.31
67	0.65	0.932	729.20	57	0.27	1.340	201.54

COD 600				COD 1000			
Day	SV	MLSS	SVI	Day	SV	MLSS	SVI
200 rpm				200 rpm			
1	0.19	1.117	170.22	1	0.17	1.200	141.67
2	0.15	1.180	127.12	2	0.14	1.250	112.23
3	0.21	1.367	153.75	3	0.2	1.377	145.29
4	0.13	1.170	120.71	4	0.33	1.827	180.72
5	0.23	0.953	241.26	5	0.27	2.197	122.94
8	0.14	1.163	120.37	8	0.25	1.897	131.81

9	0.18	1.460	123.41	9	0.21	1.937	108.45
11	0.18	1.503	119.87	11	0.19	1.667	114.00
12	0.2	1.760	113.74	12	0.2	2.013	99.34
14	0.21	2.043	102.78	14	0.19	1.863	102.06
16	0.25	2.323	107.82	16	0.24	2.380	100.87
18	0.26	2.907	89.47	18	0.25	2.367	105.85
20	0.24	2.660	90.31	20	0.26	3.263	79.73
23	0.25	2.540	98.49	23	0.27	2.987	90.50
26	0.25	2.557	97.89	26	0.26	2.330	111.59
29	0.26	2.437	106.85	29	0.26	2.440	106.67
32	0.24	2.137	112.50	32	0.27	2.607	103.85
50 rpm				50 rpm			
35	0.3	2.033	147.57	35	0.35	2.563	136.55
43	0.53	2.640	200.76	43	0.52	2.640	197.70
46	0.45	2.560	176.13	46	0.56	2.930	191.13
48	0.44	2.290	193.38	48	0.54	2.513	215.88
50	0.44	2.413	185.05	50	0.57	2.340	247.48
53	0.46	2.180	211.21	53	0.56	2.240	251.28
55	0.46	2.213	211.21	55	0.55	2.510	219.45
60	0.44	2.157	204.32	60	0.54	2.573	210.30
200 rpm again				200 rpm again			
62	0.42	2.547	165.52	62	0.5	2.753	181.91
64	0.39	2.753	142.25	64	0.44	2.450	179.91
66	0.38	3.120	121.81	66	0.4	2.470	161.94
68	0.4	3.127	128.65	68	0.38	2.447	156.04
70	0.39	2.963	132.08	70	0.38	2.477	154.05
72	0.4	3.153	126.91	72	0.42	2.853	147.55
75	0.39	2.973	131.18	75	0.41	2.803	146.26

Program 1. Matlab program for Figure 8

```
% Main Program considering the substrate conc. inside the floc (PDE)
% 1/D*DS/Dt=D2S/Dr2+2/r*DS/Dr-umax*M/D/Y*S/(S+Ks)
% Initial cond. 1) S(r>=R, t=0)=S0; 2) S(r<R, t=0)=0
% Boundary cond. 1) DS/Dr(r=0)=0; 2) DS/Dt(r=R)=-Vf/Vt*3/R*D*DS/Dr(r=R)
deltat=0.01;deltar=12.5;
D=250;
t=60*120;
S0=20;
for R=300:-50:50;
umax=9.2/24/3600;
M=25000;
Y=0.4;
a=umax*M*deltat/Y;
Ks=5;
Vf=0.1;Vt=8;
Sf=1000;Se=20;Q=8*2/3/60/60/2;
DN=D*deltat/deltar^2;
```

```
CN=D*deltat/deltar;
n=R/deltar;
m=t/deltat;
S(1:n+1,1)=0;S(n+2,1)=0;S(n+3,1)=S0;
for k=2:m;
   for i=n+2:-1:2;
      if i==2;
         r=1e-10;
      else
         r=deltar*(i-2);
      end;
      S(i,k)=S(i,k-1)+DN*(S(i+1,k-1)-2*S(i,k-1)+S(i-1,k-1))+CN/r*(S(i+1,k-1)-S(i-1,k-
1))...
         -umax*M*deltat/Y*S(i,k-1)/(S(i,k-1)+Ks);
   end;
      S(1,k)=S(3,k);
   if (mod(t,28800)>=0) && (mod(t,28800)<=7200);
      S(n+3,k)=(Sf-S(n+3,k-1))*Q/(8/3+Q*deltat*k)*deltat+S(n+3,k-1)-
(Vf/(8/3+Q*deltat*k)*3*CN/2/R)*(S(n+3,k-1)-S(n+1,k-1));
   else
      S(n+3,k)=-Vf/Vt*3*CN/2/R*(S(n+3,k-1)-S(n+1,k-1));
   end;
end;
ratio=0:1/n:1;
plot(ratio,S(3:n+3,t/deltat))
hold on;
 end;
hold off;
S(n+3,t/deltat)
```