INFLUENCE OF MAJOR OPERATIONAL PARAMETERS ON AEROBIC GRANULATION FOR THE TREATMENT OF WASTEWATER

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By

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Ph.D. Thesis

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Certificate

This is to certify that the thesis entitled ***Influence of Major Operational Parameters on Aerobic Granulation for the Treatment of Wastewater*** *is an authentic original work done by* ***Bindhu B. K.*** *under my supervision and guidance in the School of Engineering, Cochin University of Science and Technology. No part of this thesis has been presented for any other degree from any other institution.*

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Declaration

I hereby declare that the work presented in the thesis entitled ***Influence of Major Operational Parameters on Aerobic Granulation for the Treatment of Wastewater*** *is based on the original work done by me under the supervision and guidance of Prof. (Dr.) G. Madhu, Division of Safety and Fire Engineering, School of Engineering, Cochin University of Science and Technology. No part of this thesis has been presented for any other degree from any other Institution.*

Bindhu B.K.

21st December 2013.

Dedicated to

The Little Wonders of Nature

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Abstract

Effective solids-liquid separation is the basic concept of any wastewater treatment system. Biological treatment methods involve microorganisms for the treatment of wastewater. Conventional activated sludge process (ASP) poses the problem of poor settleability and hence require a large footprint. Biogranulation is an effective biotechnological process which can overcome the drawbacks of conventional ASP to a great extent. Aerobic granulation represents an innovative cell immobilization strategy in biological wastewater treatment. Aerobic granules are self-immobilized microbial aggregates that are cultivated in sequencing batch reactors (SBRs). Aerobic granules have several advantages over conventional activated sludge flocs such as a dense and compact microbial structure, good settleability and high biomass retention.

For cells in a culture to aggregate, a number of conditions have to be satisfied. Hence aerobic granulation is affected by many operating parameters. The organic loading rate (OLR) helps to enrich different bacterial species and to influence the size and settling ability of granules. Hence, OLR was argued as an influencing parameter by helping to enrich different bacterial species and to influence the size and settling ability of granules. Hydrodynamic shear force, caused by aeration and measured as superficial upflow air velocity (SUAV), has a strong influence and hence it is used to control the granulation process. Settling time (ST) and volume exchange ratio (VER) are also two key influencing factors, which can be considered as selection pressures responsible for aerobic granulation based on the concept of minimal settling velocity. Hence, these four parameters - OLR, SUAV, ST and VER- were selected as major influencing parameters

for the present study. Influence of these four parameters on aerobic granulation was investigated in this work.

A laboratory scale column type SBR with a capacity of 2 litres was designed and fabricated. Three values for each parameter (OLR - 3, 6 and 9 kg COD m^{-3} d^{-1}, SUAV - 2, 3 and 4 cm s^{-1}, ST - 3, 5 and 10 min and VER - 25, 50 and 75%) were attempted in nine trials. All the other operating conditions except the studied parameters were kept constant throughout the study. Performance of the reactor was observed in terms of formation and development of aerobic granules, settleability of the sludge and COD removal.

The influence of the studied parameters (compared parameters) on important performance characteristics (reference parameters) of aerobic granulation was analyzed using grey system theory (GST). Sludge volume index (SVI), time taken for the appearance of aerobic granules, size and specific gravity of granules and COD removal efficiency were taken as the reference parameters. Using grey relational coefficients (GRCs) and grey entropy relational grade (GERG), the impact of the compared parameters on reference parameters was estimated. A ranking based on the order of importance was also made. The optimal values of the compared parameters were estimated as 6 kg COD m^{-3} d^{-1} for OLR, 3 to 4 cm s^{-1} for SUAV, 5 min for settling time, and 50% for VER.

Rubber is one of the main agro-based industrial sectors that play an important role in Kerala’s economy. The rubber latex processing units generate wastewater with high organic content during the various stages of processing. The treatment and disposal of this wastewater is a major problem. Where availability of land is a constraint, treatment of latex

processing wastewater by aerobic granulation is thought to be a viable option. Hence wastewater from the rubber latex coagulating units was selected as a real wastewater for the present study. Latex effluent was subjected to treatment by aerobic granulation using the optimized values of the operational parameters studied. Excellent performance was observed in the study in terms of aerobic granule formation, settleability of sludge, COD removal (97%), and nitrogen removal (90.9%).

This study has established the suitability of aerobic granulation technology for the treatment of high strength wastewater under controlled operating conditions. Prioritization of major influencing factors is necessary for the design of pilot plants and for scaling-up of SBRs. GST was found to be an effective tool for arriving the order of importance. The optimized values of operational parameters were proved successfully for the treatment of a real wastewater.

Key words: Aerobic granulation; sequencing batch reactor; organic loading rate; hydrodynamic shear force; superficial upflow air velocity; settling time; volume exchange ratio; grey system theory; latex processing effluent.

Abbreviations

ASP	Activated Sludge Process
BOD	Biochemical Oxygen Demand
CMTR	Continuous Mixed Tank Reactor
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
GERG	Grey Entropy Relational Grade
GRA	Grey Relational Analysis
GST	Grey System Theory
HRT	Hydraulic Retention Time
IA	Image Analysis
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
OLR	Organic Loading Rate
RBC	Rotating Biological Contactor
SBR	Sequencing Batch Reactor
SEM	Scanning Electron Microscope
SF	Shear Force
SOUR	Specific Oxygen Utilization Rate
SRT	Sludge Retention Time
SS	Suspended Solids
ST	Settling Time
SUAV	Superficial Upflow Air Velocity
SVI	Sludge Volume Index
UASB	Upflow Anaerobic Sludge Blanket
VER	Volume Exchange Ratio

Nomenclature

A	Area of cross section of the reactor
BOD_u	Ultimate BOD
COD_e	Effluent COD
COD_{in}	Influent COD
COD_{rem}	COD removal efficiency
COD_s	Soluble COD
COD_T	Total COD
D	Diameter of the reactor
d	Diameter of the particle
E_{ij}	GERG of j on i
H	Effective height of the reactor
L	Length of travel to the discharge port
p_{ij}	Map value of the GRCs
S_{ij}	Grey relational entropy of j on i
S_{max}	Sequence maximum entropy
SS_e	Effluent suspended solids
SVI_{30}	SVI after 30 min settling
V	Volume of the reactor
V_e	Volume of the discharged effluent
V_s	Settling velocity
V_d	Volume of the discharged mixed liquor in each cycle
V_{smin}	Minimum settling velocity
X	MLSS in the reactor
X_e	SS concentration in the effluent
X_i^0	Reference sequence

X_j^*	Compared sequence
Y_{ob}	Observed yield
μ	Viscosity of the medium
μm	Micrometre
ρ	Distinguishing coefficient
ρ_m	Density of the medium
ρ_p	Density of the particle

Contents

List of Tables

List of Figures

Chapter 1

INTRODUCTION

Contents

1.1 History and Development of Wastewater Treatment

Treatment of wastewater is relatively a modern practice. While sewers to remove foul-smelling water were common in ancient Rome, it was not until the 19th century that large cities began to realize the necessity of reducing pollutants in the used water. Despite large supplies of fresh water and the natural ability of water to cleanse itself over time, population had become so concentrated by the second half of the 19th century that outbreaks of life-threatening diseases were traced to bacteria in the polluted water. Since that time, the practice of wastewater collection and treatment has been developed and perfected, using some of the most technically sound physical, chemical, and biological techniques available.

The principal objective of wastewater treatment is generally to allow domestic and industrial effluents to be disposed off without danger to human health or unacceptable damage to the natural environment. The impurities in water vary in size and form, ranging from large floating matter to dissolved solids. It contains inorganic and organic pollutants which can add pollution load to the natural ecosystems (Ng, 2006). Solid-liquid separation is the basic idea of all types of water and wastewater treatment systems. Basic wastewater treatment facilities reduce organic and suspended solids to limit pollution to the environment. Advancement in needs and technology has necessitated better treatment processes that remove dissolved matter and toxic substances.

1.2 Types of Wastewater Treatment

Conventional wastewater treatment consists of a combination of physical, chemical, and biological processes. It includes operations to remove solids, organic matter and sometimes, nutrients from wastewater. General terms used to describe different degrees of treatment, in order of increasing treatment level, are preliminary, primary, secondary, and tertiary and/or advanced wastewater treatment.

1.2.1 Preliminary Treatment

The objective of preliminary treatment is the removal of coarse solids and other large materials often found in raw wastewater. Removal of these materials is necessary to enhance the operation and maintenance of subsequent treatment units. Preliminary treatment operations typically include coarse screening, grit removal and in some cases, comminution of large objects. In grit chambers, the velocity of the water through the chamber is maintained sufficiently high, or air is used, so as to prevent the settling of most organic solids. Grit removal is not included as a preliminary treatment step in most small wastewater treatment plants. Comminutors are sometimes adopted to supplement coarse screening, which serve to reduce the size of large particles. Flow measurement devices, often standing-wave flumes, are always included at the preliminary treatment stage.

1.2.2 Primary Treatment

The objective of primary treatment is the removal of settleable organic and inorganic solids by sedimentation, and the removal of materials that will float (scum) by skimming. Approximately 25 to 50% of the incoming biochemical oxygen demand (BOD), 50 to 70% of the total suspended solids (SS), and 65% of the oil and grease are removed during primary treatment. Some organic nitrogen, organic phosphorus, and heavy metals associated with solids are also removed during primary sedimentation, but colloidal and dissolved constituents are not affected. The effluent from primary sedimentation units is referred to as primary effluent.

1.2.3 Secondary Treatment

Primary treatment provided a good start, but it is inadequate to protect water quality as required by the regulatory agencies. The objective of secondary treatment is to further treat the effluent from primary treatment to remove the residual organics and suspended solids. In most cases, secondary treatment follows primary treatment and involves the removal of biodegradable dissolved and colloidal organic matter using aerobic biological treatment processes. Aerobic biological treatment is performed in the presence of oxygen by aerobic microorganisms (principally bacteria) that metabolize the organic matter in the wastewater, thereby producing more microorganisms and inorganic end-products (principally CO_2, NH_3, and H_2O). Several aerobic biological processes are used for secondary treatment differing primarily in the manner in which oxygen is supplied to the microorganisms and in the rate at which organisms metabolize the organic matter.

High-rate biological processes are characterized by relatively small reactor volumes and high concentrations of microorganisms compared with low rate processes. Consequently, the growth rate of new organisms is much greater in high-rate systems because of the well controlled environment. The microorganisms must be separated from the treated wastewater by sedimentation to produce clarified secondary effluent. The sedimentation tanks used in secondary treatment, often referred to as secondary clarifiers, operate in the same basic manner as the primary clarifiers described previously. The biological solids removed during secondary sedimentation, called secondary or biological sludge, are normally combined with primary sludge for sludge processing.

Common high-rate processes include the activated sludge processes, trickling filters or biofilters, oxidation ditches, and rotating biological contactors (RBC). A combination of two of these processes in series (e.g., biofilter followed by activated sludge) is sometimes used to treat municipal wastewater containing a high concentration of organic material from industrial sources.

1.2.4 Tertiary/ Advanced Treatment

Tertiary and/or advanced wastewater treatment is employed when specific wastewater constituents cannot be removed by secondary treatment. It is sometimes referred to as tertiary treatment, because advanced treatment usually follows high-rate secondary treatment. However, advanced treatment processes are sometimes combined with primary or secondary treatment (e.g., chemical addition to primary clarifiers or aeration basins to remove phosphorus) or used in place of secondary treatment (e.g., overland flow treatment of primary effluent).

1.3. Biological Treatment of Wastewater - An Overview

Biological treatment of wastewater employs the use of microorganisms in the processing and cleansing of wastewater. Many microorganisms are able to metabolize a variety of organic and inorganic substances that are present in wastewater. Biological wastewater treatment takes advantage of this property and supports it with various nutrients and aeration. The overall objectives of the biological treatment of wastewater are to (i) transform dissolved and particulate biodegradable constituents into acceptable end products, (ii) capture and incorporate suspended and non settleable colloidal solids into a biological floc or biofilm, (iii) transform or remove nutrients such as nitrogen and phosphorus, and (iv) in some cases, remove specific trace organic constituents and compounds (Metcalf and Eddy, 2003).

The basic mechanisms of biological treatment are the same for all treatment processes. Microorganisms, principally bacteria, metabolize organic materials and inorganic ions present in wastewater during its growth, which brings us to the fundamental differences between catabolic and anabolic processes. Catabolic processes are those biochemical processes involved in the breakdown of organic products for the production of energy or for use in anabolism. Catabolic processes are dissimilar and they involve the transfer of electrons resulting in the generation of energy to be used in cell metabolism. In contrast, anabolic processes are the biochemical processes involved in the synthesis of cell constituents from simpler molecules. These processes usually require energy and are assimilatory.

The amount of contaminant in the wastewater is rated based on the biochemical oxygen demand (BOD) or chemical oxygen demand (COD).

The biological treatments are designed to remove or reduce the BOD or COD of the raw wastewater to the limits prescribed for the end use. With appropriate analysis and environmental control, almost all wastewaters containing biodegradable constituents with a BOD/COD ratio of 0.5 or greater can be treated easily by biological means (Metcalf and Eddy, 2003). In comparison to other methods of wastewater treatment, it also has the advantages of lower treatment costs with no secondary pollution (Sponza and Ulukoy, 2005).

1.3.1 Attached Growth Processes

In attached growth system, the microorganisms responsible for the conversion of organic matter and nutrients are attached to inert packing media. These packing media may be sand, gravel, rock, or a wide range of synthetic materials. Microorganisms form a biofilm over the media. As the wastewater flows past the biofilm, the organic matter and nutrients are removed by the action of microorganisms. The most common examples of biological treatment which employs aerobic attached growth process are different variants of trickling filter and rotating biological contactors.

1.3.2 Suspended Growth Processes

In suspended growth processes, the microorganisms responsible for the biodegradation are maintained in suspension by various mixing methods, either natural or mechanical. In most processes, the required volume is reduced by returning bacteria from the secondary clarifier in order to maintain a high suspended solids concentration. The most common suspended growth process used for municipal wastewater treatment is activated sludge process, which involves the production of activated mass of microorganisms capable of stabilizing organic matter and nutrients present in wastewater.

1.3.3 Aerobic and Anaerobic Treatment Processes

The types of bacteria utilized in wastewater processing can be categorized based upon their necessity or intolerance of oxygen to survive. Those bacteria that require oxygen to convert food into energy are called aerobic bacteria, those that will perish in the presence of oxygen are anaerobic bacteria, and finally facultative anaerobes may thrive in either the presence or absence of oxygen. Aerobic biological processes are commonly used in the treatment of organic wastewaters for achieving high degree of treatment efficiency, while in anaerobic treatment, considerable progress has been achieved in anaerobic biotechnology for waste treatment based on the concept of resource recovery and utilization while still achieving the objective of pollution control (Yeoh, 1995; Seghezzo et al., 1998). In general, aerobic systems are suitable for the treatment of low strength wastewaters (biodegradable COD concentrations less than 1000 mg l^{-1}) while anaerobic systems are suitable for the treatment of high strength wastewaters (biodegradable COD concentrations over 4000 mg l^{-1}). According to Cakir and Stenstrom (2005), there exist cross over points of ultimate BOD (BOD_u) of influent wastewater ranging from 300 to 700 mg l^{-1}, which are crucial for effective functioning of aerobic treatment systems. The advantages of anaerobic treatment outweigh the advantages of aerobic treatment when treating influents in higher concentrations than the cross over values, and generally anaerobic treatment requires less energy with potential bio-energy and nutrient recovery. However, compared to anaerobic systems, aerobic systems achieve higher removal of soluble biodegradable organic matter material and the produced biomass is generally well flocculated, resulting in lower effluent suspended solids concentration (Leslie et al., 1999). As a result, the effluent quality from an aerobic system is generally higher than the anaerobic system.

Highly polluting industrial wastewaters are preferably treated in an anaerobic reactor due to the high level of COD, potential for energy generation and low surplus sludge production. However in practical applications, anaerobic treatment suffers from the low growth rate of the microorganisms, a low settling rate, process instabilities and the need for post treatment of the noxious anaerobic effluent which often contains ammonium ion (NH_4^+) and hydrogen sulfide (HS^-) (Heijnen et al., 1991).

In aerobic treatment, microbial biomass, after having adsorbed and partly metabolized the soluble and colloidal organics, flocculates and settles out, so that a clear effluent is obtained. The concepts of "filament-strengthened sludge flocs" (Segzin et al. 1978) and "feast/famine sludge flocs" (Rensink et al., 1982; Slijkhuis, 1983) now make it possible to operate activated sludge units with a fair degree of insight and control (Verstraete and Van Vaerenbergh, 1986).

Another factor hampering aerobic wastewater biotechnology is the relatively low density of the microbial biomass in the reactor. Due to the settling problems, the amount of biomass in the mixed liquid was to be kept in the range 3 - 5 kg volatile suspended solids (VSS) m^{-3}. The most obvious solution to this problem is to allow the biomass to anchor to a heavy carrier. Biomass densities up to 30 kg m^{-3} can be attained and volumetric loading rates surpassing those of conventional activated sludge by a factor of 10 can be reached accordingly.

A major asset of the aerobic systems is their capacity to handle all kinds of wastewaters, especially those with extremely variable composition and even, from time to time, toxic pulses. Yet, although robust, these systems cannot cope with everything. Berthouex and Fan (1986) reported that even well attended aerobic wastewater treatment plants, facing no major shocks or toxic pulses, are currently not meeting the discharge standards around 20% of the time. Better and controlled design and operation will undoubtedly improve the attractiveness of aerobic treatment in general, and of variable industrial waste-streams in particular.

In contrast to aerobic degradation, which is mainly a single species phenomenon, anaerobic degradation proceeds as a chain process, in which several sequent organisms are involved. Overall anaerobic conversion of complex substrates therefore requires the synergistic action of the micro-organisms involved. Another factor of fundamental importance has been the identification of new methanogenic species, and the characterization of their physiological behaviour. Of particular interest were the determination of the substrate affinity constants of both hydrogenotrophic and acetotrophic methanogens.

If anaerobic processes treat dilute wastewater consistently and reliably, it would be a highly significant development in wastewater treatment. Anaerobic fermentation results in a lower cellular yield and hence lower sludge handling costs. In addition, lower energy requirements would result, since aeration would not be necessary, and methane would be produced as a byproduct. Thus, the treatment of wastewater might be a net energy producer. As anaerobic granular systems are capable of handling high organic loadings associated with high strength wastewater and short hydraulic retention time, they could render much more carbon credits than other conventional anaerobic systems (Wong et al., 2009).

A comparison between aerobic and anaerobic treatment can arrive at the following conclusions:

- Aerobic microbial communities have several specific advantages. They have large free energy potentials, enabling a variety of often parallel biochemical mechanisms to be operated. These communities are therefore capable of coping with low substrate levels, variable environmental conditions and multitudes of different chemicals in the influent. They have some very useful capabilities such as nitrification, denitrification, phosphate accumulation, ligninase radical oxidation, etc. which make them indispensable in waste treatment.
- Anaerobic microbial communities are specifically advantageous at high temperatures and high concentrations of soluble organic matter. They also have special physiological traits, such as reductive dechlorination.
- In the near future, important progress can be expected with regard to the optimal linkage between anaerobic and aerobic processes. Aerobic treatment needs to be specifically focused on the removal of the soluble pollutants.
- Both in aerobic and anaerobic treatment there is an urgent need for better control and regulation. Particularly online monitoring of the biologically removable load and of the possible presence of toxicants

is necessary, to improve both types of processes as well as their combined application.

- It is evident that a long solids residence time (SRT) is necessary for the treatment of sewage by anaerobic processes, because of the low specific growth rates associated with anaerobic bacteria.

A viable alternative to expensive conventional aerobic systems for domestic wastewater treatment is the sequential anaerobic–aerobic systems due to low energy consumption, low chemical consumption, low sludge production, vast potential of resource recovery, less equipment requirement and high operational simplicity (Chan et al., 2009; Kassab et al., 2010).

1.4. Activated Sludge Process

In activated sludge process, treatment of wastewater is based on providing intimate contact between wastewater and biologically active sludge. The activated sludge is obtained by settling sewage in presence of abundant oxygen so as to be supercharged with favourable aerobic microorganisms (Punmia, 2010). The effluent from the primary treatment is mixed with a dose of activated sludge from the secondary sedimentation unit and aerated in an aeration tank for sufficient period (usually 4-8 hours). During the period of aeration, the microorganisms multiply by assimilating the organic matter present in the influent. A part of this assimilated organic matter is synthesized to new cells and a part is oxidized to derive energy. The assimilation, synthesis, oxidation and the biomass separation together form the basic mechanisms for reduction of organic load in activated sludge process.

The activated sludge which is made to settle in the secondary sedimentation tank is flocculant in nature. These biosolids have relatively poor settling characteristics, which in turn demand large area for the settling units. Hence conventional wastewater treatment plants based on activated sludge process require a large footprint. To overcome the disadvantages of a conventional wastewater treatment plant, biomass has to be grown in a compact form, like granular sludge. This eliminates the use of the large settling tanks and allows much higher biomass concentrations in the reactors. This leads to the concept of biogranulation.

1.5. Biogranulation

The performance of a biological system for wastewater treatment depends significantly on the active biomass concentration. Successful wastewater treatment depends upon the selection of metabolically capable microorganisms and the efficient separation of those organisms from the treated effluent. For the conventional activated sludge process, separation efficiency of biosolids from liquid phase has a significant implication for the design of biological wastewater treatment systems (Tay et al., 2002a). Because of the relatively poor settling characteristics, conventional sewage treatment plant based on activated sludge technology requires a large footprint (de Bruin et al., 2004). Much research has focused on reducing the settling time required for activated sludge by forming dense flocs or by using biofilm reactors. Biogranules are a kind of condensed biofilm formed through self-immobilization. Biogranulation can be classified as aerobic or anaerobic granulation. These granules are dense packets of bacteria of different species.

Formation of anaerobic granules has been extensively studied and reported. The most widely known system for anaerobic granulation is upflow anaerobic sludge blanket (UASB) reactor. Anaerobic granulation technology has already been applied in many wastewater treatment plants (Alves et al., 2000). In granular sludge reactors, the anaerobic granules with high density settle rapidly, which reduces the separation time of the treated effluent from the biomass. Anaerobic granular systems are designed in wastewater treatment to maximize biomass retention and methane yield. These reactor systems provide efficient and stable operational performance with high biomass concentration and rich microbial diversity (Wong et al., 2009). Anaerobic granular sludge technology is suitable for high-strength wastewater treatment. However, the anaerobic granulation technology has some drawbacks, such as a long start-up period, a relatively high operation temperature and unsuitability for low strength organic wastewater and nutrient removal (Adav et al., 2008). In order to overcome these weaknesses, research has been concentrated to the development of aerobic granulation technology.

Aerobic granulation represents an innovative cell immobilization strategy in biological wastewater treatment and it is attracting research interests (Beun et al., 1999; Beun et al., 2002). Aerobic granules were first developed in early 90's by Mishima and Nakamura (1991). Since then research has been intensively made in the area of aerobic granulation, exploring its various potentials. Aerobic granules can be regarded as compact and dense microbial aggregates with a spherical outer shape. The aggregation of microorganisms into compact aerobic granules also has additional benefits such as protection against predation and resistance to chemical toxicity (Jiang et al., 2004).

Compared with conventional activated sludge, aerobic granules have a regular, compact and strong structure and good settling properties, and they contain a high biomass and can handle high organic loading rates (Zheng et al., 2005, 2006; Li et al., 2008a). All the studies so far reported revealed that aerobic granules were successfully cultivated in sequencing batch reactors (SBRs) only, while the reason is not yet clearly understood. SBR works in a cyclic mode, which consists of filling, aeration, settling, and decanting. In an SBR, at the end of every cycle, settling of biomass takes place before effluent is withdrawn, to retain the biomass in the reactor. It is clearly understood that the basis of granulation is the continuous selection of sludge particles that occur in the reactor (Arrojo et al., 2004).

Granulation was reported to be affected by a number of parameters such as seed sludge, reactor configuration, nature of the substrate, feeding strategy, organic loading rate (OLR), intensity of aeration, settling time (ST), volume exchange ratio (VER) (Adav et al., 2008; Wan and Sperandio, 2009). To date significant researches were focused on various parameters influencing aerobic granulation. Experimental evidences show that formation of aerobic granules is a gradual process from seed sludge to compact aggregate, and then to matured granular sludge (Tay et al., 2001a). A number of conditions have to be fulfilled for the successful formation of the granules from the seed sludge. Although a number of parameters are there to influence the granulation process in general, only a few parameters can really contribute to the formation of aerobic granules. These parameters are called selection pressures. Several researchers worked on the influence of various parameters on aerobic granulation and proved the influence of these parameters with experimental evidences.

In spite of the intense research, the mechanism behind the formation of aerobic granules is still not clear. The basic question is whether the aerobic granulation is constitutive or inducible. If the capacity for aerobic granulation is constitutive (i.e. wherever the cell is, with regard to its cell cycle or its life cycle), aerobic granulation will be present, provided the environmental conditions allow it to occur. In contrast, if the capacity for aerobic granulation is inducible, then it will be present only when the cells are physiologically competent (Liu et al., 2005a). As there are evidences that granulation was possible by different species such as methanogens, acidifying bacteria, nitrifying bacteria and denitrifying bacteria, it can be stated that aerobic granulation is species independent and could be inducible rather than constitutive.

1.6. Need for the Study

Different theories were proposed by the researchers about the concept of selection pressures for aerobic granulation in SBR. Liu et al. (2005a) suggested that *settling time* and *volume exchange ratio* are the two key influencing factors, which can be considered as selection pressures responsible for aerobic granulation based on the concept of minimal settling velocity. Later *discharge time* was also reported as a selection pressure along with settling time and VER (Liu, 2008). But studies also show that hydraulic selection pressure and substrate loading rate are the two key factors that influence the formation, structure, and stability of aerobic granules (Kim et al., 2008; Moy et al., 2002; Zheng et al., 2006). *Hydraulic shear force,* caused by aeration and measured as superficial surface upflow air velocity (SUAV), has a strong influence and used to control granulation process (Liu and Tay, 2002; Tay et al., 2004a; Gao et al., 2011). The *OLR* helps to enrich different bacterial species and to influence the size and settling ability of granules, hence it was argued as a selection pressure (Chen et al., 2008). It was also reported that stable granules were developed under moderate OLR (Tay et al., 2004b, 2004c). All the above suggestions and conclusions regarding the selection pressure were based on experimental studies which were performed under different laboratory conditions. Different substrates were used to study the influence of shear force and settling time. One cannot compare the influence of these major parameters unless the experiments are conducted under similar operating conditions. Individual studies cannot provide a holistic understanding of the effects of these parameters. A comprehensive study to analyze the influence of these parameters is lacking. Under these circumstances, it was decided to find the influence of the important operational parameters, in terms of selection pressures, on the formation and development of aerobic granules.

1.7. Objectives of the Present Study

The objectives of the present study were identified as:

- To investigate the influence of organic loading rate on aerobic granulation and pollutant removal efficiency.
- To investigate the influence of superficial upflow air velocity (hydrodynamic shear force) on aerobic granulation and pollutant removal efficiency.
- To investigate the influence of settling time on aerobic granulation and pollutant removal efficiency.
- To investigate the influence of volume exchange ratio on aerobic granulation and pollutant removal efficiency.

- To analyze all the above parameters for the successful development of aerobic granules
- To analyze and compare these influencing parameters as selection pressures of aerobic granulation using grey system theory (GST) method
- To apply the optimized experimental values for the treatment of real wastewater (natural latex processing wastewater) by aerobic granulation technology.

1.8. Summary and Organization of Thesis

Wastewater treatment has been in practice since 19th century. Biological treatment of wastewater is attractive because of efficiency and economy. Activated sludge process is a prominent aerobic treatment technology, but it has the disadvantages of longer settling time for sludge and large plant area requirement. Biogranulation may be a solution to this problem. Aerobic granulation is influenced by a number of parameters. The influences of four major parameters (OLR, SUAV, ST and VER) on aerobic granulation were identified as the objectives of the present study. It is proposed to make a relational analysis of these parameters and to apply the optimal values for the treatment of a real wastewater to ascertain the research findings.

The thesis is divided into five major chapters. Chapter 1 introduces the statement of research problem and research objectives. The second chapter is devoted for the review of literature. A review of earlier investigations in the related topics is made in this chapter. The third chapter discusses the methodology adopted for the study. The experimental set-up required for the study and various methods for the analysis are also described in this chapter. The results obtained from the experiments are reported and discussed in chapter 4. The fifth chapter gives summary and conclusion. The references are listed at the end.

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Chapter 2

LITERATURE SURVEY

Contents

2.1 Introduction

With proper analysis and environmental control, almost all wastewaters containing biodegradable constituents can be treated biologically. Biological treatment systems are living systems which rely on mixed biological cultures to break down the organic matter present in wastewater and to remove them from solution. A treatment unit provides a controlled environment for the desired biological process. The removal of organic matter is achieved by microorganisms in the biological treatment systems – attached or suspended. The effective separation of biomass from the treated effluent leads to the success of any biological treatment process. Thus separation efficiency of biosolids from liquid phase has a significant implication for the design of biological wastewater treatment systems.

2.2 Development of Biogranulation Technique

Conventional sewage treatment plants based on activated sludge technology requires large footprint which is mainly because of the poor settling characteristics of the bioflocs. Bioflocs formed in activated sludge process need longer time for separation as they are not in compacted form and are light in weight. This again leads to the low permissible dry solids concentration in the aeration tank and the low maximum hydraulic load of secondary sedimentation tanks (de Bruin et al., 2004). Thus for the conventional activated sludge process, separation efficiency of biosolids from liquid phase has a significant role in the design of biological wastewater treatment systems (Tay et al., 2002a). Hence researches have been done to improve the settleability of the biosolids which lead to the concept of biogranulation.

Biogranules can be considered as aggregates of bacteria which are much more compact than conventional bioflocs. These are formed by self-immobilization of microorganisms. These granules are dense microbial consortia packed with different bacterial species and typically contain millions of organisms per gram of biomass (Liu and Tay, 2004). Studies regarding sludge granulation have focused on upflow anaerobic sludge blanket (UASB) reactors since the 1980s (Lettinga et al., 1980, Schmidt and Ahring, 1996). Granulation of anaerobes has been well documented for decades. Many wastewater treatment plants apply anaerobic granulation technology (Alves et al., 2000). It has proved to be capable of treating high-strength wastewater containing soluble organic pollutants. Various modifications of UASB have been successfully demonstrated for the treatment of municipal and industrial wastewater (Lettinga et al., 1980; Schmidt and Ahring, 1996). Reports on granulation by methanogens, acidifying bacteria, nitrifying bacteria and denitrifying bacteria indicate that granulation process would not be strictly restricted to some specific species (Tay et al., 2002a.).

In spite of the wide scale applications, anaerobic granulation technology shows some drawbacks like a long start-up period, a relatively high operation temperature and unsuitability for low strength organic wastewater (Morgenroth et al., 1997). To overcome the above drawbacks, the research has been devoted to the development of aerobic granulation technology.

2.3 Aerobic Granulation Process

During the last 20 years, intensive research in the field of biological wastewater treatment and other applications demonstrated that biofilms were often more efficient for water purification than suspended activated sludge (Adav et al., 2008). Successful development of aerobic granules was first reported by Mishima and Nakamura (1991) in a continuous aerobic upflow sludge blanket reactor. Later many researchers cultivated aerobic granules in sequencing batch reactors (SBRs) using a wide range of substrates under various operating conditions (Morgenroth et al., 1997; Beun et al., 1999; Peng et al., 1999; Etterer and Wilderer, 2001; Tay et al., 2001a; Liu and Tay, 2002).

When compared with conventional activated sludge flocs, the advantages of granular activated sludge are compactness and strength of the structure. It also has good settleability, high capacity for biomass retention and is able to withstand high organic loading rates. Because of their ability to retain biomass, aerobic granules are capable of significantly higher organic loading rates compared to conventional activated sludge systems (Moy et al., 2002). Since granules are compact in structure, usage of bulky settling devices will be not needed (Beun et al., 1999; Jiang et al., 2002; Liu et al., 2006). Granulation facilitates the accumulation of high amounts of active biomass and the separation of this biomass from the wastewater liquor. The aggregation of microorganisms into compact aerobic granules also has additional benefits such as protection against predation and resistance to chemical toxicity (Jiang et al., 2004). Because of the excellent settling capacity of aerobic granules, the land area needed for a municipal wastewater treatment plant could be reduced by 80%, and accordingly the construction investment would be cut down, when a granular sludge system, instead of a conventional activated sludge system, was applied (de Bruin et al., 2004).

Biogranulation involves cell-to-cell interactions in which biological, physical and chemical phenomena occur to form biogranules of spherical shape through self-immobilization of microorganisms. Biogranules are dense microbial consortia packed with different bacterial species. These bacteria perform different roles in degrading the complex industrial wastes (Liu and Tay, 2004). Aggregation of microorganisms is the most important activity in granule formation. It is a multi-step process in which many physical and biochemical factors play. Hence for the successful development of the aerobic granules, a number of conditions have to be met, but the mechanisms of aerobic granulation were not well understood (Liu et al., 2004a).

2.4 Aerobic Granulation in SBR

Wastewater treatment by sequencing batch reactor (SBR) has received considerable attention because of its compact nature and easiness in operation and maintenance. Almost all researches on aerobic granulation have been conducted and proved to be successful in SBRs, while no aerobic granulation has been reported in continuous reactors. SBRs work in a cyclic mode, thereby the cycle time and its fractions for various stages can influence the granulation process. The sequential steps of feeding, aeration, settling and discharge of supernatant fluid in a SBR were conducted in the same tank (Metcalf and Eddy, 2003). The working of the SBR is flexible as time for each stage can be easily varied according to operational needs including hydraulic loading, economic efficiency of power requirements, or treatment levels for target contaminants (Lee et al., 2008). The schematic diagram of the SBR is shown in Fig. 2.1.

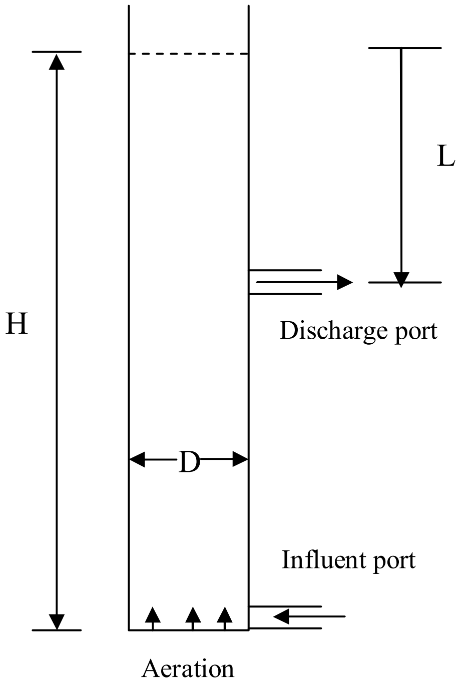


Fig. 2.1 Schematics of a column SBR

2.5 Mechanism of Aerobic Granulation

To date, majority of research on aerobic granulation has been with SBRs; however, the mechanisms responsible for the formation of aerobic granules are still unclear (Qin et al., 2004a).

Beun et al., (1999) experimented aerobic granulation in a SBR with a small amount of suspended, non-settling cells with a short settling time. Based on the observations, a mechanism for the granulation process was proposed as given in Fig.2.2. During the initial period after seeding the reactor, fungi become dominating. Fungi form mycelial pellets which have good settleability. Shear in the reactor detach the filaments, and pellets become more compact. In due course of time, pellets grow in size and lyse due to oxygen limitation in the inner part. Then these mycelial pellets act as an immobilization matrix, in which bacteria can grow and develop colonies, which further lead to aerobic granules.

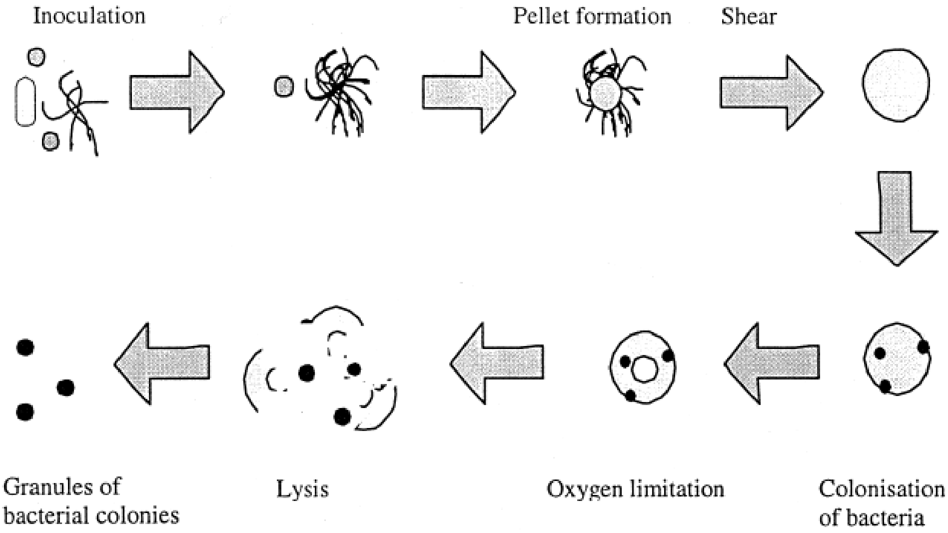


Fig 2.2 Proposed mechanism of granulation after the start up of a SBR reactor with a short settling time (*Source*: Beun et al., 1999)

Liu and Tay (2002) proposed a model for the aerobic granulation consisting of four steps.

Step 1. Physical movement to initiate bacterium-to bacterium contact or bacterial attachment on a solid surface. The forces involved in this step are hydrodynamic force, diffusion force, gravity force, thermodynamic forces like Brownian movement, and cell mobility.

Step 2. Stabilization of the multicell contacts resulting from the initial attractive forces. Physical forces like Van der Waals force, opposite charge attraction force, thermodynamic forces, hydrophobicity, chemical forces like Hydrogen liaison, formation of ionic pairs and triplets, inter-particulate bridging, and biochemical forces like cellular surface dehydration and cellular membrane fusion play for the stabilization.

Step 3. Maturation of cell aggregation through production of extracellular polymer, growth of cellular clusters, metabolic change.

Step 4. Shaping of the steady state three-dimensional structure of microbial aggregate by hydrodynamic shear forces.

Aerobic granulation may be initiated by microbial self-adhesion. Each aerobic granule is an enormous group of microbes containing millions of individual bacteria. Liu and Tay, (2004) reported that aerobic granules are developed by self-adhesion of microbes. Natural aggregation is less possible because of the repulsive electrostatic forces and hydration interactions among them. It appears certain that aerobic granulation is a gradual process involving the progression from seed sludge to compact aggregates, then further to granular sludge and finally to mature granules (Liu and Tay, 2004). No carrier material is used in the process of granulation; instead, granulation results from the progressive densification and growth of an initial flocculated biomass (Wan and Sperandio, 2009).

Many mechanisms and models for granulation process have been developed by researchers, but none of them provides a complete picture.

2.6 Factors Affecting Aerobic Granulation

The development of aerobic granules from seed sludge is a gradual process. Granulation process can be influenced by many parameters, of which a few can trigger the process. Compact granules with sufficient density achieve a good settling velocity; thereby the settling time required can be reduced. Hence it is worth analyzing the important parameters contributing to the development of compact granules.

Factors like seed sludge, substrate composition, feeding strategy, reactor configuration, especially the height to diameter ratio, support material to initiate the granulation process, augmentation by certain elements can influence the aerobic granulation process. But comparing the influence of these parameters, certain other parameters like OLR, superficial upflow air velocity (SUAV) in terms of shear force, settling time and volume exchange ratio have a critical impact on granulation process. Hence the parameters which can develop a selection pressure on granulation process are discussed in detail.

2.6.1 Seed Sludge

Aerobic granules were cultivated usually with activated sludge seed. Linlin et al., (2005) successfully demonstrated the possibility of aerobic granulation in SBR by seeding anaerobic granules. The anaerobic granules experienced a process of disintegration—recombination—growing up. It was assumed that the disintegrated anaerobic sludge may play a role of nucleus for the granulation of aerobic sludge. Hydrophobic bacteria have more affinity to attach to the flocs, than hydrophilic bacteria (Adav et al., 2008). Seed sludge with a higher number of hydrophobic bacteria can form granules with good settleability (Wilen et al., 2008). When seed sludge (activated sludge flocs) along with chlamydospores of *Phanerochaete* sp. HSD inoculum was used, the rate of aerobic granulation was accelerated considerably (Hailei et al., 2011).

Sheng et al. (2010) carried out a study to investigate the effects of seed sludge properties and selective sludge discharge in aerobic granulation process. It was found that aerobic granules were successfully developed from two different types of seed sludge - small-loose flocs and larger-denser flocs, which were separated from actual activated sludge by sedimentation. The initial washout of small and slow-settling sludge during SBR start-up did not appear to be a crucial factor for aerobic granulation. The key operation was the daily discharge at a ratio of 10% of relatively slow-settling sludge flocs from the reactors. Microbiological analysis revealed that there were no differences between the bacterial communities of the granular sludge in the two reactors with different types of seeding sludge. It was found from experiments that when crushed granular sludge was used as the seed sludge, granulation time could be considerably reduced (Pijuan et al., 2011; Verawaty et al., 2012). This can be adopted as an effective strategy in aerobic granulation when dealing with real wastewaters, where long start up period is the hurdle.

2.6.2 Substrate Composition

Aerobic granulation was tried with a wide verity of substrates including glucose, acetate, phenol, starch, ethanol, molasses, sucrose and other synthetic wastewater components (Adav et al., 2008; Yun et al., 2006; Wang et al., 2007a; Carucci et al., 2008; Usmani et al., 2008). Real wastewater was also experimented for granulation process by many investigators (Arrojo et al., 2004; de Bruin et al., 2004; Schwarzenbeck et al., 2005; Hailei et al., 2006; Wang et al., 2007b; Abdullah at al, 2013; Rosman et al., 2013). Granules could be developed in all cases, however, it is found that the type of carbon source closely affect the granule microstructure and species diversity. The granules derived from acetate substrate appeared to be more compact and less filamentous than those derived from glucose as substrate (Tay et al. 2001a.).

Tay et al. (2001a.) cultivated granules in two reactors, one fed with glucose and other with acetate. Even though the seed sludge had a very loose and irregular structure, dominated by filamentous bacteria, compact aggregates were appeared after operation in SBR for one week. The filamentous bacteria gradually disappeared in the acetate-fed reactor; however, in the glucose-fed reactor, filamentous bacteria still prevailed. The granular sludge with clear round outer shape was formed in both reactors two weeks after the start-up which was converted to matured aerobic granules after three weeks. Compared to acetate-fed granules, glucose-fed granules had a fluffy outer surface because of the predominance of filamentous bacteria (Fig.2.3). Scanning Electron Microscope (SEM) observations revealed that the glucose-fed mature aerobic granules indeed had a filamentous dominant outer surface, while the acetate-fed aerobic granules had a very compact microstructure in which cells were tightly linked together and rod-like bacteria were predominant (Fig. 2.4). It was concluded by Tay et al. (2002b) that substrate component is not a key factor for the formation of aerobic granules, but the microbial density of aerobic granules is closely associated with the carbon sources.

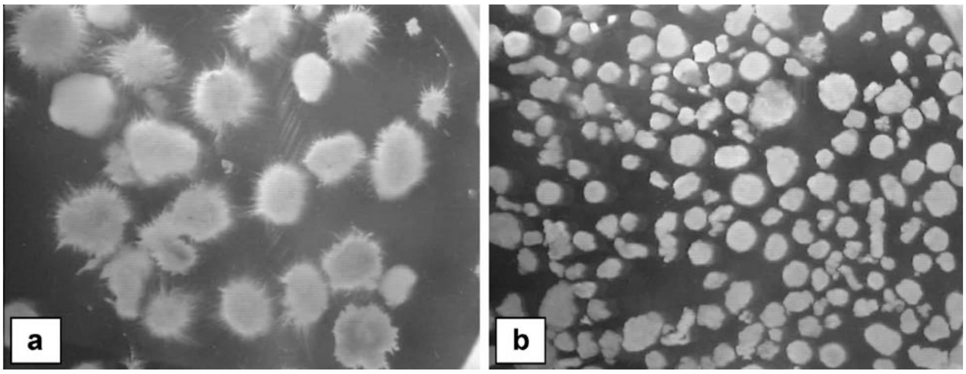


Fig. 2.3 Macrostructures of glucose-fed (a) and acetate-fed (b) aerobic granules (*Source:* Tay et al., 2001a).

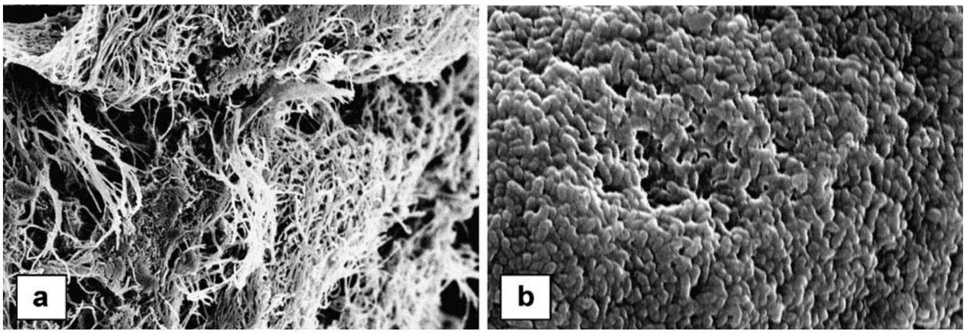


Fig. 2.4 Microstructures of glucose-fed (a) and acetate-fed (b) aerobic granules (*Source:* Tay et al., 2001a).

2.6.3 Feeding strategy

SBR works in a cyclic mode and each cycle consists of feeding, aeration, settling and discharge. During the aeration period, as a first phase, the substrate is depleted to a minimum and in the second phase, no substrate is available for the microorganisms. It leads to periodic feast and famine condition. Thus as intermittent feeding strategy is practiced in an SBR, it causes periodic starvation of microorganisms (Tay et al., 2001a). It is experimentally proved that under periodic feast and famine condition, bacteria become more hydrophobic and this increased hydrophobicity facilitates microbial aggregation (Bossier and Verstraete, 1996). It seems that intermittent feeding strategy and alternate feast and famine regimen may influence the characteristics of aerobic granulation, but there are no solid experimental evidences that it can act as a trigger force for aerobic granulation.

2.6.4 Reactor Configuration

In almost all cases, successful aerobic granulation was reported in column type upflow SBRs. Column type upflow sequencing batch reactor and completely mixed tank reactor (CMTR) have different hydraulic behaviour. In CMTRs the aggregates move stochastically with dispersed flow in all directions. But in the case of upflow SBRs, air or liquid upflow reactors can create a relatively homogeneous circular flow and localized vortexing along the reactor's axis and microbial aggregates are constantly subjected to hydraulic attrition (Liu and Tay, 2004). The flow patterns in an upflow column reactor and CMTR are shown in Fig. 2.5 (a) and (b) respectively. According to the thermodynamics, the circular flow could force microbial aggregates to be shaped as regular granules that have a minimum surface free energy, provided those aggregates could be kept in the reactors under given dynamic conditions. Thus this circular flow helps the aggregates to attain a regular granular shape. Longer circular trajectory in a column reactor can be achieved by a higher ratio of reactor height (H) to diameter (D). A higher H/D ratio can ensure a circular flow trajectory and more effective hydraulic attrition of aggregates. Apart from that, a high H/D ratio can improve the oxygen transfer and result in a reactor with a smaller footprint. Pan (2003) could develop aerobic granules in SBR under various H/D ratios.

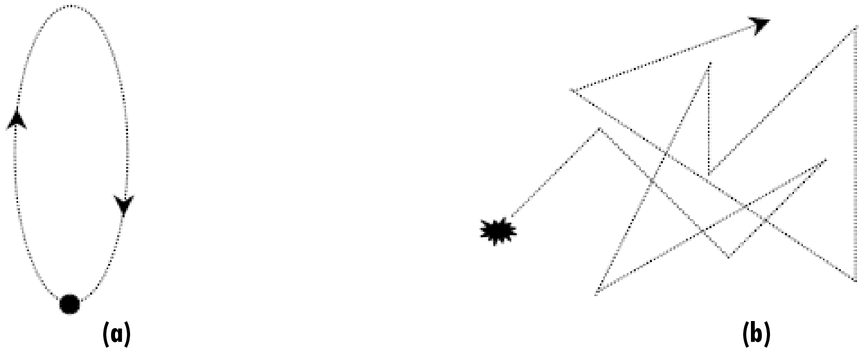


Fig 2. 5. Flow patterns in the (a) upflow column reactor and (b) CMTR (*Source:* Liu and Tay, 2002).

Kong et al (2009) experimented aerobic granulation under four different H/D ratios 24, 16, 8, and 4 and aerobic granules were successfully developed in four reactors corresponding to a minimal settling velocity of 12, 8, 4 and 2 m h^{-1}, respectively. Generally, the most important design parameter for bubble column reactor is H/D ratio and the minimal settling velocity is closely related to the absolute height of reactor. However, the results with reactor H/D ranging from 24 to 4 demonstrated that microbial aggregation and microbial selection do not depend on reactor H/D in the studied range. Reactor H/D ratio thus can be very flexible in the practice, which is important for the application of aerobic granulation technology. Thus reactor H/D ratio cannot be considered as a selection pressure for aerobic granulation.

2.6.5 Sludge Retention Time

Sludge retention time (SRT) represents the average period of time during which the sludge has remained in the system. SRT is one of the important factors which can change the state of biomass in an activated sludge system and the concentration of the mixed liquor suspended solids (MLSS) increases with SRT. Basically an SRT of 2 days is often required for the formation of flocculated activated sludge with good settling ability, while the optimum SRT for good bioflocculation and low effluent COD was found to be in the range of 2 to 8 days (Rittmann, 1987). As per Metcalf and Eddy (2003), in conventional activated sludge process, for BOD removal, SRT values may range from 3 to 5 days depending on the mixed liquor temperature. When the influence of SRT was studied in membrane bioreactor, it was found that COD removal in the bioreactor slightly decreased with shortened SRT (Han et al., 2005).

Li et al. (2008b) investigated the role of SRT in aerobic granulation under negligible hydraulic selection pressure. Results showed that no successful aerobic granulation was observed at the studied SRTs in the range of 3–40 days. When SRTs of 5, 10, 15, and 20 days were studied, with all other working conditions same, granulation was successful in all the cases, but only slight changes in the COD removal (95 to 96%) were observed (Hajiabadi et al., 2009). A comparison analysis revealed that hydraulic selection pressure in terms of the minimum settling velocity would be much more effective than SRT for enhancing heterotrophic aerobic granulation in sequencing batch reactor. It was shown that SRT would not be a decisive factor for aerobic granulation in SBR. This finding may have a great engineering implication in the design, optimization and operation of full scale aerobic granular sludge SBR.

2.6.7 Dissolved Oxygen

The dissolved oxygen content in the reactor liquid can influence the aerobic granulation process. Evidences are there to show that aerobic granules can be formed at DO concentration as low as 0.7-1.0 mg l^{-1} (Peng et al., 1999; Tokutomi, 2004) while they can also be developed at relatively high DO concentrations from 2 – 6 mg l^{-1} (Tay et al. 2002b; Yang et al., 2003; Tsuneda et al., 2003; Qin et al., 2004a). It shows that DO concentration is not a decisive factor for the formation of aerobic granules. But DO diffusion into the interior parts of the granule is affected by the availability of DO in the mixed liquor. Li et al. (2008a) studied DO diffusion profiles in aerobic granules with different sizes under substrate-free and substrate-sufficient conditions. Results showed that DO only partially penetrated through 500 μm from the granule surface under substrate sufficient condition. On the contrary, no DO limitation was found in aerobic granules with a radius less than 2.2 mm under substrate-free condition, i.e., aerobic condition could be maintained in the entire aerobic granules.

2.6.8 Temperature and pH

Temperature changes can influence biological processes considerably. de Kreuk et al. (2005) investigated the effect of temperature changes on the conversion processes and stability of aerobic granular sludge. It was found that the start-up of a reactor at low temperatures led to the presence of organic COD in the aerobic phase and therefore to instable granules that aggregated during settling and biomass washout. Once a reactor is started up at higher temperatures, it is possible to operate a stable aerobic granular sludge system at lower temperatures. But aerobic granules could be successfully cultivated at temperatures varied from 25^{o}C to 35^{o}C (Zhiwei et al., 2009). This study demonstrates that temperature is not a determining factor in the granulation process, whereas it can influence the morphology, settling properties and treatment efficiency as well as the community structure. Concerning the role of reactor pH on aerobic granulation, detailed studies are lacking.

2.6.9 Augmentation techniques

Jiang et al. (2003) reported that addition of Ca^{2+} accelerated the aerobic granulation process. With addition of 100 mg Ca^{2+} l^{-1}, the formation of aerobic granules took 16 days and 32 days in the culture without Ca^{2+} added. The Ca^{2+}-augmented aerobic granules also showed better settling and strength characteristics and had higher polysaccharides contents. But high calcium content in the form of calcium carbonate was reported even when Ca^{2+} is not used for augmentation. It was shown that the size of the acetate-fed aerobic granule would indeed play an essential role in the $CaCO_3$ formation, and provided experimental evidence that a crystal $CaCO_3$ core was not necessarily required for granulation (Wang et al., 2007c). Ren et al., (2008) found that compared with the granules without calcium accumulation, the Ca-rich granules had more rigid structure and a higher strength. Similar to Ca^{2+}, Mg^{2+} ions also can enhance aerobic granulation, but Ca^{2+} was proved to be more effective than Mg^{2+} in augmentation (Li, et al., 2009; Liu, et al., 2010a). Ca^{2+} augmentation was also proved to be very effective in cultivating denitrifying granules in SBR (Liu and Sun, 2011). Support materials like bivalve shell carrier can enhance granulation even at higher OLRs, and the granules formed showed very good settleability, compactness and resistance to shock loading (Thanh et al., 2009).

2.6.10 Organic loading rate

It was found that aerobic granulation is possible under a wide range of chemical oxygen demand (COD) concentrations and organic loading rates (OLRs) (Liu et al., 2003; Tay et al., 2004a). Liu et al. (2003) cultivated aerobic granules with influent COD concentrations from 500 to 3000 mg l^{-1} corresponding to OLR from 1.5 to 9.0 kg COD m^{-3} d^{-1}. The observations showed that high substrate concentration favoured a fast increase in granule size. The granule size was 1.57 mm at 500 mg l^{-1} while it increased to 1.79 mm at 1000 and 2000 mg l^{-1}, and further to 1.89 mm at 3000 mg l^{-1}. But as the size of the granule was increased, the roundness of the granule was decreased, with loose structure for granules developed at higher substrate concentrations. The granule strength, expressed as integrity coefficient was found decreased from 97% with influent COD 500 mg L^{-1} to 87% with influent COD as 3000 mg l^{-1}. But Tay et al. (2004b) reported that aerobic granulation was not successful for an OLR below 2 kg COD m^{-3} d^{-1}.

Granulation was found to be more efficient over a broader range of OLR from 2.5 to 15 kg COD m^{-3} d^{-1} when bivalve shell carrier was used as support material (Thanh et al., 2009). However, at the higher OLR (>10 kg COD m^{-3} d^{-1}) where no famine condition exists, a part of the organic substrate remained in the reactors (effluent COD was around 100 mg l^{-1}), thus reducing cell hydrophobicity. Hence it can be said that though fast settleable granules are formed at higher OLRs, the efficiency of the process in terms of pollutant removal is low.

Chen et al. (2008) studied the combined effects of hydraulic shear force and organic loading rate on granulation process at superficial upflow air velocity of 3.2 and 2.4 cm s^{-1} under an OLR range of 6.0–15.0 kg COD m^{-3} d^{-1}. Good granule characteristics were achieved in a wide OLR range from 6.0 high up to 15.0 kg COD m^{-3} d^{-1} at 3.2 cm s^{-1}, while under the upflow air velocity of 2.4 cm s^{-1}, stable operation was limited in the OLR range of 6.0–9.0 kg COD m^{-3} d^{-1} and failed to operate with granule deterioration under further higher OLRs. Table 2.1 summarizes some major experimental findings by various researchers revealing the effect of OLR in the aerobic granulation process.

Table 2.1 Effect of OLR on aerobic granulation

OLR, kg COD m^{-3} d^{-1}	Major Experimental Findings	Reference
6 - 15	Acetate-fed granules disintegrated at 9 kg COD m^{-3} d^{-1} Glucose-fed granules sustained at maximum OLR	Moy et al. (2002)
1.5	Granule size was 1.57mm Integrity coefficient 97%	Liu et al. (2003)
3.0	Granule size was 1.79 mm	
6.0	Granule size was 1.79 mm	
9.0	Granule size was 1.89 mm Integrity coefficient 87%	
2	Granulation not found successful	Tay et al. (2004b)
6	Unstable granules with filamentous growth	Zheng et al. (2006)
6 - 15	Good granulation at shear force 3.2 cm s^{-1} Limited granulation at shear force 2.4 cm s^{-1}	Chen et al. (2008)
2.5 - 15	Granulation found efficient with support material	Thanh et al., 2009

2.6.12 Hydrodynamic Shear Force

Formation, shaping and densification of aerobic granules are enhanced by proper hydrodynamic shear force. In SBR, aeration causes hydrodynamic turbulence and hydraulic shear force. Hydraulic shear force caused by aeration can be a direct indicator of hydraulic selection pressure and quantified by superficial upflow air velocity (SUAV). A higher shear force would result in a stronger and compact biofilm, whereas biofilm tends to become heterogeneous, porous and weaker when the shear force is weak. It appears that a certain shear force is necessary to produce a compact biofilm structure, that is, higher shear force favours the formation of a smoother and denser biofilm (Chen et al., 2008, Liu, 2008, Chang et al., 1991).

In a hydrodynamic sense, column-type upflow reactor and CMTR have very different hydrodynamic behaviours in terms of interactive patterns between flow and microbial aggregates. The air or liquid upflow pattern in column reactors can create a relatively homogeneous circular flow along the reactor height, and microbial aggregates are constantly subjected to such a circular hydraulic attrition. However, in CMTR, microbial aggregates stochastically move with dispersed flow in all directions. Thus, microbial aggregates are subjected to varying localized hydrodynamic shear force, flowing trajectory and random collision (Refer Fig 2.5)

The effect of shear force on aerobic granulation was studied by Tay et al (2001b) where hydrodynamic turbulence caused by upflow aeration served as the main shear force in the systems. Aerobic granulation was experimented with superficial upflow air velocities from 0.3 to 3.6 cm s^{-1}. Compact and regular aerobic granules were formed in the reactors with a superficial upflow air velocity higher than 1.2 cm $s^{–1}$, whereas, only typical weak bioflocs were observed in the reactor with a superficial upflow air velocity of 0.3 cm s^{-1}. Hence it can be concluded that a minimum shear force is necessary for aerobic granulation.

It can be expected that the size of aerobic granules would be a net result of interaction between biomass growth and detachment. The balance between growth and detachment would lead to a stable size. It is known that high hydrodynamic shear force would lead to more collision and attrition among granules or particles, and then high detachment. Further studies by Tay et al, (2004a) in this line proved the effect of upflow air velocity, ranging from 1.2 to 3.6 cm s^{-1}, on the size, morphology, settleability, physical strength and polysaccharide production of granules. The results indicated that the size of aerobic granules tends to decrease with the increase of upflow air velocity, showing granule sizes of 0.39 mm, 0.37 mm and 0.33 mm corresponding to 1.2, 2.4 and 3.6 cm s^{-1}. Rounder aerobic granules obtained in the reactors with high upflow air velocity could also be attributed to the more frequent collision and attrition created by upflow aeration. The sludge volume index (SVI) and biomass density of the granules also showed that the physical structures of the granule become more compact and settling ability increase at high upflow air velocity.

It was found that the shear force has a positive effect on the production of polysaccharide, specific oxygen utilization rate (SOUR), hydrophobicity of cell surface and specific gravity of granules. Cell polysaccharides can help in the cohesion and adhesion of cells and thereby maintain the structural integrity of biofilms (Tsuneda et al., 2001). The hydrophobicity of granular sludge is much higher than that of bioflocs. Therefore, it appears that hydrophobicity could induce and further strengthen cell–cell interaction and might be the main force for the initiation of granulation. The shear-stimulated production of polysaccharides favours the formation of a stable granular structure (Chen et al., 2008).

Studies on the combined effect of hydrodynamic shear force (2.4 and 3.6 cm s^{-1}) and organic loading rate (6 – 15 kg COD m^{-3} d^{-1}) showed that under the upflow air velocity of 2.4 cm s^{-1}, stable operation was limited in the OLR range of 6–9 kg COD m^{-3} d^{-1} and failed to operate with granule deterioration under further higher OLRs. But good reactor performance and well granule characteristics were achieved in a wide OLR range from 6.0 high up to 15.0 kg COD m^{-3} d^{-1} at 3.2 cm s^{-1} (Chen et al., 2008). Hence it can be seen that sufficient shear force in terms of upflow air velocity can help in the aerobic granulation at a wider range of OLR.

The high value of shear force required in terms of upflow air velocity results in high energy consumption. For example, 400 m^3 of air should be supplied per kilogram of COD removed, with an OLR of 6 kg COD m^{-3} d^{-1} and upflow air velocity of 2.4 cm s^{-1}. This is very high as compared to the air requirement of 20 to 50 kg COD m^{-3} d^{-1} for conventional activated sludge process (Liu, 2008). This means that the operation cost for aeration in an aerobic granular sludge reactor would be several times higher than that of a conventional activated sludge process. To overcome the high operation cost, effective counter measures have to be adopted in aerobic granular sludge reactors. Research has to be advanced to optimize the air supply for the minimum requirement of shear force.

There are examples of better settleability in terms of SVI with higher values of SUAV. Liu, (2008) reported that when the SUAV was 0.3 cm s^{-1}, there was no successful granulation and the SVI of the bioflocs was 170 mL g^{-1}. But settleability was found to increase with better SVI values of 62, 55, and 46 mL g^{-1}correseponding to SUAV 1.2, 2.4, and 3.6 cm s^{-1}. Though shear force stimulates the production of extracellular polysaccharides, the content and composition of these polymeric substances are not affected by hydrodynamic shear force (Di Iaconi et al., 2005). The results of certain relevant studies showing the effect of shear force on aerobic granulation is summarized as shown in Table 2.2.

Table 2.2 Effect of Shear Force on aerobic granulation

SUAV, cm s^{-1}	Major Experimental Findings	Reference
0.3 to 3.6	Good granulation above SUAV 1.2 cm s^{-1} No granulation at SUAV 0.3 cm s^{-1} SVI decreases with increasing SF	Tay et al (2001b)
1.2 to 3.6	Granule size decreases with increase in SF (size 0.39 to 0.33 mm)	Tay et al, (2004a)
2.4	Stable operation was limited in the OLR range of 6–9 kg COD m^{-3} d^{-1}	Chen et al., (2008)
3.6	Good reactor performance from 6.0 to 15.0 kg COD m^{-3} d^{-1}	

Thus it can be concluded that higher shear force leads to more compact, denser, rounder and smaller granules. It can increase the stability of granules by enhancing the production of polysaccharides. Liu (2008) commented that it is reasonable to consider hydrophobicity as an inducing force for cell immobilization, while shear stimulated extracellular polysaccharide production may play an important role in building up and maintaining the architecture of the granules. He believed that the structure of mature aerobic granules is determined by hydrodynamic shear force, but there is no concrete evidence to show that shear force is a primary inducer of aerobic granulation in SBRs.

2.6.13 SBR Cycle Times and Settling Time

The main feature of SBR is its cyclic mode of operation, which consists of filling, aeration, settling and discharging. The cycle time can be considered as an influencing parameter because it is related to the solid washout frequency, and hence to hydraulic retention time (HRT). Many studies have been carried out to assess the efficiency of granulation and the performance of the reactor under various cycle times. Different cycle times from 3 to 24 hours were tried in SBRs to develop nitrifying granules by Tay et al., (2002c). It was observed that complete solids were washed out with a cycle time of 3 hours and granules were not at all formed with cycle time 24 hours. But granules of reasonable sizes were formed in SBRs operated at cycle times 12 hours and 6 hours, with smoother and denser granules in the latter case. Wang et al. (2005) cultivated aerobic granules with cycle times 3 and 12 hours, in which sucrose was the substrate and concluded that in order to achieve a rapid aerobic granulation in SBR, cycle time needs to be controlled at a relatively low level. Results of the studies by Pan et al. (2004) and Liu and Tay (2008) also reinforced the above conclusion and excellent sludge with very low sludge volume index (SVI) could be cultivated with lower cycle time.

Among the various phases of the cycle time, settling time (ST) is the most important fraction that can exert a selection pressure. Particles that can settle within the allowed settling time will be retained in the reactor, and particles with poor settleability will be washed out. This continuous selection process may lead to the formation of aerobic granules slowly from the easily settleable bioflocs. Qin et al. (2004b) operated four SBRs with settling times 20, 15, 10 and 5 minutes and investigated the effects of settling time on granulation. Fastest development of granules and best performance in terms of sludge settleability (measured as SVI), cell surface hydrophobicity and microbial activity were observed in reactor with lowest settling time of 5 minutes. In a similar study with settling times 2 minutes and 10 minutes, sludge with SVI 47 ml g^{-1} and 115 ml g^{-1} were obtained respectively (Mc Swain et al., 2004). These studies support the fact that at a longer settling time, poorly settleable flocs cannot be effectively removed and they may outcompete granule forming bioparticles, leading to the failure of granulation. Thus settling time is proved to be a strong selection pressure in aerobic granulation.

Settling time seems to induce changes in cell surface hydrophobicity, which can strongly influence the efficiency of granule formation and its settling behaviour. A shorter settling time or a stronger hydraulic selection pressure results in a more hydrophobic cell surface. Microbial activity, measured in terms of specific oxygen utilization rate (SOUR), was found to be inversely related to the settling time (Qin et al., 2004b). Shorter settling times seem to stimulate the respirometric activity of the bacteria. It shows that microorganisms may regulate their energy metabolism in response to the changes in hydraulic selection pressure exerted on them (Liu, 2008). Mc Swain et al. (2004) observed entirely different consortium of bacteria in granules developed with settling time 2 minutes and 10 minutes. It shows that settling time could select species during the granulation (Adav et al., 2009). Table 2.3 summarizes the major experimental findings by various researchers and demonstrates the effect of settling time in the aerobic granulation process.

Table 2.3 Effect of Settling Time on aerobic granulation

Settling Time	Major Experimental Findings	Reference
20, 15, 10, and 5 minutes	Fastest development of granules and best settleability at settling time of 5 minutes	Qin et al. (2004b)
2 and 10 minutes	SVI 47 ml g^{-1} and 115 ml g^{-1} respectively	Mc Swain et al., (2004)
10, 7, and 5 minutes	Simpler microbial community at settling time 5 min	Adav et al. (2009)

In SBR, particles those cannot reach the discharge point within the allowable settling time are washed out from the reactor, and particles with good settleability are retained in the reactor. If the distance for the sludge particle to reach the discharge port is L, the time taken for travelling by the particle is:

$$\text{Travelling Time} = \frac{L}{Vs} \tag{2.1}$$

Where Vs is the settling velocity, and it can be estimated by Stoke’s law as

$$Vs = \frac{g(\rho_p - \rho_m)\,d^2}{18\mu} \tag{2.2}$$

d = diameter of the particle

ρ_p and ρ_m = density of the particle and media respectively

μ = viscosity of the media

As the equation (2.2) shows, settling velocity is a function of size and density of the particles. Bioparticles with low settling velocity and hence longer settling time than the designed value will be washed out of the reactor. Thus a minimum settling velocity $(Vs)_{min}$ is needed for the particles to be retained in the reactor. This velocity can be determined as:

$$(Vs)_{\min} = \frac{L}{SettlingTime} \tag{2.3}$$

The applied settling times in aerobic granulation studies mostly range from 2 min to 30 min, in which better results were obtained with shorter settling time. All the studies supported settling time as a decisive parameter for the formation of aerobic granules in SBRs and it can apply a strong selection pressure in the granule formation.

2.6.14 Volume Exchange Ratio (VER)

Volume exchange ratio (VER) is defined as the ratio of volume of the effluent withdrawn in a cycle to the total volume of the reactor. If a uniform cross section is maintained throughout the height H for the column reactor, VER can be found as:

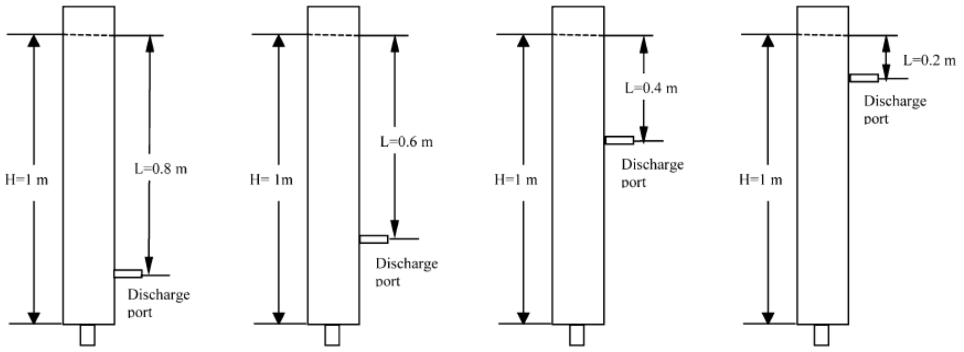
$$VER = \frac{L}{H} \tag{2.4}$$

Where L = distance to the discharging port from the top level of the solution.

L and H are shown in Fig. 2.1.

Since height of the reactor is fixed, VER depends solely on L, which in turn with settling time decides the settling velocity as explained by equation (2.3). Thus to obtain a minimum settling velocity for the bioparticles to remain in the reactor, settling time and distance to the discharging port should be carefully selected so that efficiency and economy also may be attained.

Wang et al. (2006) studied the effect of VER on aerobic granule formation, by applying various VERs, 20%, 40%, 60% and 80%. The schematics of various VER is shown in Fig 2.6. Results revealed that larger and more spherical granules were formed at highest VER of 80%, whereas bioflocs were dominated at VER of 20%. Lowest SVI was also observed in the reactor with VER 80%.



(a) (b) (c) (d)

Fig. 2.6 Operation of SBR at various VER (a) 80% (b) 60% (c) 40% (d) 20%) (*Source*: Wang, 2004)

Thus it can be understood that VER has an influencing role in aerobic granulation and high VER supports a rapid granulation. Investigations on minimum settling velocity also support the above conclusion, since length of travel and settling time are related. Large bio-particles of 1 to 2 mm were developed with minimum settling velocity 0.7 m hr^{-1}, while only small bio-particles of size 0.5 mm could be developed when minimum settling velocity was set at 0.6 m hr^{-1} (Kim et al., 2004).

These studies revealed the importance of volume exchange ratio in efficient granulation.

2.7 Applications of Aerobic Granular Technology

High Strength organic wastewater can be effectively treated by aerobic granulation technology. Moy et al. (2002) demonstrated the feasibility of treatment of synthetic wastewater containing glucose by gradually increasing the OLR and found that aerobic granules were able to sustain the maximum organic loading rate of 15.0 kg COD m^{-3} d^{-1} while removing more than 92% of the COD. Aerobic granulation technology is proven to be efficient in treating toxic pollutants also. Phenol is a toxic pollutant which often comes from fossil fuel refining, pharmaceutical, and pesticide industries. Studies by Jiang et al. (2002, 2004) proved that aerobic granulation is successful with phenol containing wastewaters and that the phenol-degrading aerobic granules displayed an excellent ability to degrade phenol. Adav et al. (2007) reported a specific rate of 1.18 g phenol g^{-1} Volatile Suspended Solids (VSS) d^{-1} for phenol. Pyridine and its derivatives were also subjected to treatment using aerobic granules with excellent results. Compact and well-settling aerobic granules could be successfully developed for the degradation of 2,4-Dichlorophenol adopting glucose as a co-substrate with a removal efficiency of 94-95% (Wang et al., 2007a). Carucci et al. (2008) experimented a novel strategy of using aerobic granules grown on acetate as carbon source for the degradation of 4-chlorophenol which resulted in very good removal rate. When SBR was fed with phenol and p-cresol together, aerobic granules could remove 97% of phenol (Usmani et al., 2008).

The coexistence of heterotrophic, nitrifying, and denitrifying populations in aerobic sludge granules makes it possible to remove nitrogen (N) and phosphorus (P) simultaneously from wastewaters. Use of aerobic granules for nitrification and denitrification were also reported by Beun et al. (2001). Lemaire et al. (2007) studied simultaneous nitrification, denitrification, and phosphorus removal process in a laboratory-scale SBR by alternate aerobic and anaerobic period for 450 days and reported promising results.

Many researchers tried aerobic granulation technology for the treatment of real wastewaters. When low strength municipal wastewater was subjected to treatment, after operation of 300 days, the reactor was predominated with granular sludge (85%) (Ni et al., 2009). Liu et al. (2010b) also obtained similar results when real wastewater (40% domestic wastewater and 60% industrial wastewater) was experimented in a pilot plant for 400 days. It should be noted that when real wastewater is treated in a pilot plant, it takes longer period (around one year) for granulation, instead of 1-2 months in a laboratory scale. But many times aerobic granulation was found difficult for the treatment of low strength wastewater. When fine granular activated carbon was added to the sludge mixture, complete granulation was achieved in a shorter span (Li et al., 2011).

Granules with good settling properties were obtained when wastewater from a dairy analysis laboratory was used as influent (Arrojo et al., 2004). Aerobic granular sludge was successfully cultivated when wastewater from the malting process with a high content of particulate organic matter was treated in an SBR (Schwarzenbeck et al., 2004). At an organic loading rate of 3.4 kg COD m^{-3} d^{-1} and an influent particle concentration of 0.9 g l^{-1}, total suspended solids (TSS), an average removal of 50% in total COD and 80% in soluble COD could be achieved. Apart from synthetic wastewaters, Schwarzenbeck et al. (2005) used wastewater from dairy plant, which contained significant concentrations of nutrients, in investigating the performance of aerobic granules. The removal efficiencies of 90% of total COD, 80% of total N and 67% of total P were reported at a volumetric exchange ratio of 50%. Wastewaters from pharmaceutical industry, abattoir (slaughterhouse), Soybean processing, brewery and paper industry were successfully treated with aerobic granular sludge (Inizan et al., 2005; Cassidy and Belia, 2005; Su and Yu, 2005; Hailei et al., 2006; Wang et al., 2007b). Fish canning effluent characterized by high salt content (30 g NaCl l^{-1}) was also treated successfully by aerobic granulation method (Figueroa et al., 2008). Latest researches are in the treatment of agro-based wastewater and rubber processing wastewater which give promising results (Abdullah at al, 2013; Rosman et al., 2013).

2.8 Selection Pressure Theory of Aerobic Granulation

In SBR operation, only particles that settle within a given time frame could be retained in the reactor, while those with poor settleability were washed out from the system. Apparently, borrowed from biological evolution theory, this physical screening step was considered to provide a “selection pressure” to the biomass in the reactor, and only those which adapted to this challenge (to become big and dense enough to settle fast) would survive and be retained in the reactor (Adav et al., 2008). The physical settling–washing out action was a pure screening step without a demand for the microbes to respond to or to make changes upon the fluid carryout, hence having different intended meaning by the biological evolution theory (Adav et al., 2008).

Aerobic granulation is a process, culminated by a number of influencing parameters like reactor configuration, substrate composition, organic loading rate, feeding strategy, hydraulic retention time, hydrodynamic shear force, settling velocity, volume exchange ratio, cycle time and solids retention time. Selection pressures for aerobic granulation are trigger forces that play a crucial role in granulation process and further influence the granular characteristics and reactor performance (Qin et al., 2004a; Wang et al., 2007d). Even though many parameters can influence the granule formation, only a few can be considered as selection pressures.

Different theories regarding the selection pressures for aerobic granulation were proposed by different researchers in the light of their experimental works. Liu et al. (2005a) suggested that settling time and exchange ratio are the two selection pressures responsible for aerobic granulation based on the concept of minimal settling velocity. But later, discharge time was also reported as a selection pressure along with settling time and VER (Liu, 2008). Qin et al. (2004b) experimentally proved that settling time is the selection pressure for aerobic granulation because a short settling time can induce microbial changes favouring the formation of aerobic granules. Chen et al. (2008) considered selection pressures for aerobic granulation as hydraulic shear force and organic loading and investigated the feasibility of granulation process under various values for these parameters. Substrate concentration was investigated as the selection pressure by Wang et al. (2007d) also and proved to be a successful strategy when it was applied with stepwise increase.

Aerobic granules could be developed under a wide range of chemical oxygen demand (COD) concentrations and organic loading rates (OLRs) with various substrates as discussed earlier, even though the type of carbon source closely affect the granule microstructure, species diversity and kinetic behaviour. Hence it can be concluded that aerobic granulation is independent of substrate type and composition and that substrate concentration and organic loading rate can be considered as a selection pressure for aerobic granulation in SBR.

Superficial upflow air velocity (SUAV) mainly accounts for the hydrodynamic shear force in column type SBR. As discussed earlier, hydrodynamic shear force has a positive effect on granule formation and shaping, density and structure of the granules, production of polysaccharides etc. Granulation is not possible in the absence of sufficient hydrodynamic shear force. Even though there is no concrete evidence to demonstrate hydrodynamic shear force as the primary inducer of granulation, Liu (2008) remarked hydrophobicity as an inducing force for cell immobilization. In the light of studies so far conducted, hydrodynamic shear force can be regarded as a selection pressure for aerobic granulation in SBRs.

The cycle time will decide the hydraulic retention time in an SBR, but settling time is the most important fraction that can exert a selection pressure on the microbial community. Longer settling time will retain the poorly settling bioflocs in the reactor, and the selection of the best particles will not be effective. This will hinder the formation of aerobic granules. Shorter settling time favours aerobic granulation. Thus settling time turns to be a decisive factor and considered as to derive the most important selection pressure in granulation process. Moreover, those tiny flocs those were washed out initially had no chance to evolve with the environmental changes hence the selection is not fair. Nonetheless, the screening step successfully cultivated aerobic granules in SBR (Beun et al., 2002, Liu et al., 2005a).

Hydraulic retention time is also a function of volume exchange ratio. Studies have proved that the fraction of granules in the sludge is directly proportional to the volume exchange ratio. Hence VER also exerts a selection pressure on granulation. The required selection pressures had been created by keeping the constant column height and varying the discharge port height (Wang et al., 2006).

Tay et al. (2002c) studied nitrifying bacterial granulation at different selection pressures and concluded the need of strong selection forces for granulation. Wang et al., (2007d) noted that the stability of the granule could be enhanced with stepwise increased selection pressure.

2.9 Grey System Theory (GST)

Grey system theory is an interdisciplinary scientific area that was first introduced in early 1980s by Deng (1982). Since then, the theory has become quite popular with its ability to deal with the systems that have partially unknown parameters. As compared to conventional statistical models, grey models require only a limited amount of data to estimate the behaviour of unknown systems (Deng, 1989). During the last two decades, the grey system theory has been developed rapidly and caught the attention of many researchers. It has been widely and successfully applied to various systems such as social, economical, financial, agricultural, industrial, transportational, ecological, hydrological, and medical systems (Kayacan et al, 2010).

The grey system theory (GST) method proposed by Deng Julong in 1982 included grey relational analysis (GRA) and grey models. GRA, denoted by the grey relational coefficients (GRCs) and grey entropy relational grade (GERG), could investigate the relationship between reference sequences and compared sequences. The GRA approach could quantify the obscure and complex relationship among multi-parameters. It distinguishes the impact order of the chosen parameters on a certain reference parameter, which is especially suitable for cases that contain a small amount of representative data. Generally speaking, as a powerful analysis technique, the GST method is able to cope up with complex, uncertain or fragmented systems by means of a reliable calculation process (Zhang and Zhang, 2013).

Although the parameters that affect the characteristics of aerobic granular sludge are complicated, GST provides an alternative to quantitatively describe their relationships. Zhang and Zhang (2013) applied GST in aerobic granulation technology to assess the relational grade of the compared parameters on reference parameters. GERG was generated from a series of correlation function calculations which helped to distinguish the key impact factors and its optimal scope. The data used for this study was obtained from studies by different researchers. A similar attempt is proposed for the present study also.

2.10 Treatment of Wastewater from Natural Rubber Processing

Rubber is one of the important plantation crops in India. Presently, India is the fourth largest rubber producer in the world after Thailand, Indonesia and Malaysia. Rubber is one of the main agro-based industrial sectors that play an important role in Kerala’s economy.

Rubber generally occurs in plants as milky white latex but the chemical composition however, varies from species to species. Chemically, rubber is a polyterpene consisting of a long chain of isoprene units joined together end to end to form giant molecules called polymers which are coiled up like tiny springs (Iyagba et al., 2008). Raw material products from natural rubber processing sector provide huge benefits to human beings as they are exploited to manufacture many kinds of important rubber goods. However, environmental damages generated from this sector could become big issues.

Verhaar (1973) mentioned that rubber content in the latex from the trees is approximately 30 to 40%. Latex, which is a kind of biotic liquid, will be deteriorated if it is not preserved by ammonia or sodium sulphite which is called anticoagulant. Anticoagulants prevent latex from pre-coagulation. By centrifugation, field latex is converted to latex concentrate containing about 60% dry rubber and skim latex with 4-6% of dry content. The skim latex is de-ammoniated, coagulated with acid, creped and dried. Processing of rubber sheets, crepe rubber and crump rubber are also important sections of rubber processing industry. Natural rubber processing sector consumes large volumes of water and energy and uses large amount of chemicals as well as other utilities. It also discharges massive amounts of wastes and effluents containing high concentration of organic matter, suspended solids and nitrogen (Rakkoed et al., 1999).

The wastewater from the latex processing units typically contains a small amount of uncoagulated latex, serum with substantial quantities of proteins, carbohydrates, sugars, lipids, carotenoids, as well as inorganic and organic salts and also includes wash water from the various processing stages (Mohammadi et al., 2010). Industries face the problem of disposal of wastewater from their latex processing units.

Several methods have been described in the scientific literatures for industrial effluent treatment, however in recent years microbes have been drawing tremendous attention due to their ability to degrade waste materials and thereby improving water quality (Boominathan et al., 2007). For latex effluents also, both chemical and biological methods have been in practice.

Widely adopted biological methods are conventional activated sludge process, extended aeration version of ASP and oxidation ditches. Combined pond system - anaerobic followed by aerobic - also has been in practice. Where there is no constraint of land, all the systems mentioned above could be successful.

Treatment of latex processing effluents by aerobic granulation is thought to be a viable option when land is a constraint. Rosman et al., (2013) investigated the treatment of standard Malaysian rubber (SMR) wastewater using aerobic granulation technology and achieved removal efficiencies of COD, ammonia and total nitrogen as 96.6%, 94.7% and 89.4% respectively. The applied OLR was 3.7 kg COD m^{-3} d^{-1}.

At this point, it is thought to be worth studying the treatment of natural rubber in the laboratory using the optimized operational parameters of aerobic granulation.

2.11 Summary

This chapter has presented a detailed survey of literature in the area of aerobic granulation. Intensive research during the past 20 years has proved that aerobic granulation technology is a viable option for the treatment of wastewater. Previous works to investigate the influencing parameters were discussed. Four major influencing factors which can act as selection pressures on aerobic granule formation and development were identified. The concept of grey relational analysis was discussed. The magnitude of the problem associated with the treatment of rubber latex wastewater was also discussed. The next chapter will explain the methodology adopted for the present study.

* * *

Chapter 3

MATERIALS AND METHODS

Contents

3.1 Introduction

For the best performance of any wastewater system, the major influencing factors are to be identified and optimized. Although there are many operational parameters that can influence the granulation process, the parameters that can hydraulically select the sludge particles are called selection pressures in aerobic granulation. The major selection pressures in aerobic granulation are identified as organic loading rate (OLR), hydraulic shear force in terms of superficial upflow air velocity (SUAV), settling time (ST) and volume exchange ratio (VER). The objective of this study is to investigate the influence of these four key operational parameters on aerobic granulation in a laboratory set-up of sequencing batch reactor.

3.2 Reactor Configuration and Design

Almost all successful developments of aerobic granules have been done in sequencing batch reactors (SBRs), while reason is not yet clear. An SBR of column type is more effective in the case of aerobic granulation as it offers vortex air movement (Liu and Tay, 2004). Moreover a column type

SBR offers a smaller footprint for wastewater treatment (de Bruin et al, 2004). The present study has been done in SBR. The design details of the column SBR are as follows:

Internal diameter (D) = 6.5 cm

Effective height (H) = 60.3 cm

Free board = 10 cm

Overall height = 75 cm (including base)

Number of ports = 5

H / D ratio = 9.3

Effective volume = 2 litres

Each port is of 2.5 cm length and 10 mm external diameter with outer-threaded end. All the five ports are distributed at equal interval (centre to centre distance) along the effective height of the column. The first (topmost) port may work as an overflow port, while the bottommost one as the feeding port. The second, third and the fourth port from top may be used as effluent withdrawal ports for 25%, 50% and 75% VER respectively. Samples can be collected through any of the ports as and when required. The column reactor was fabricated of borosil glass, with thick flat bottom for vertical stability. The design of the reactor is shown in Fig. 3.1 and the fabricated reactor is shown in Fig. 3.2. The general equations used for the study are given in section 3.8 (Equation 3.1 to 3.13).

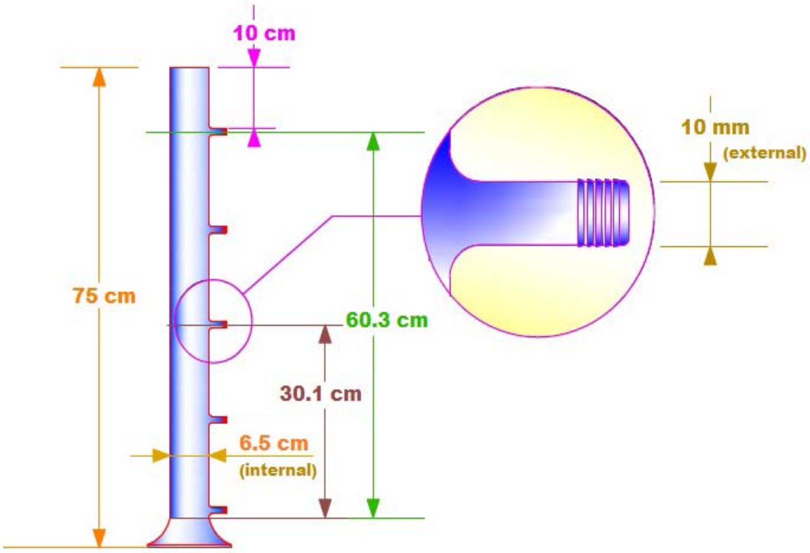


Fig. 3.1 Design details of the column reactor

Fig. 3.2 Column reactor for the experimental study

3.3 Operating Conditions

A detailed survey of the influencing parameters on aerobic granulation was made in literature. The major influencing factors which can act as selection pressures on granulation process were identified as OLR, shear force in terms of SUAV, ST and VER. For the complete experimental studies, for each parameter a certain possible range of values was selected from the available literature as follows:

- Organic loading rate (OLR) - In the present study OLR was measured and expressed in kg COD m^{-3} d^{-1}. Three values - 3, 6, and 9 kg COD m^{-3} d^{-1} were selected for the present study to investigate the influence of OLR on aerobic granulation.
- Shear force (SF) - Air flow in terms of SUAV is the main cause of shear force in SBR. Three values of air flow - 4, 6, and 8 l min^{-1} were applied so that the corresponding SUAVs were 2, 3, and 4 cm s^{-1}.
- Settling time (ST) - Settling time can select the particles to be retained in the reactor, thereby can control the formation of aerobic granules. Experimental values of 10 min, 5 min, and 3 min were attempted to study the influence of settling time.
- Volume exchange ratio (VER) - VER is the ratio of volume of the effluent withdrawn in a cycle to the total volume of the reactor. If the cross section of the reactor is uniform throughout the height H, and if L is the distance to the discharging port from the top level of the solution, VER = L/H. As H is fixed for a particular column, VER can be changed by changing the value of L. The values of VER selected for the present study were 25%, 50% and 75%. This was

achieved by withdrawing effluent from the second, third, and fourth port respectively (The ports are counted from the top).

- Temperature - It was evident from the previous research that temperature does not have much influence on the granulation process (Liu and Tay, 2004). Hence no special control method was adopted for the temperature adjustment. The reactor was kept at room temperature for the entire operation period, and the room temperature was monitored daily to see whether there were any abnormal or extreme changes in temperature.
- pH - Concerning the role of pH in aerobic granulation, detailed studies are lacking. However the pH of the reactor content was monitored daily and maintained in the range of 6.5 to 8 with the help of 0.1N HCl and 0.1N NaOH.
- SRT is the ratio of amount of solids present in the system to the amount of solids leaving the system per day. It was reported in previous studies that SRT would not be a decisive factor for aerobic granulation in SBR. SRT was not controlled during the present experiment, thus varying depending on sludge properties.

3.4 Experimental Set Up

3.4.1 Feeding and Discharge

Feeding of the influent and discharge of the effluent are the two components in the cycle of operation. Feeding and withdrawal were achieved by two peristaltic pumps (Model ENPD 300 Victor, Flow rate – 7 ml min^{-1} to 2100 ml min^{-1}, RPM – 20-160, Input supply – 230V AC, DC Motor, 2 Rollers, Digital display, Variable speed, with Timer mode, Volume mode and Calibration mode). The peristaltic pumps used for the study are shown in Fig. 3.3.

The SBR works in an up-flow mode, hence the influent was fed to the bottom of the reactor through the lowermost port of the column reactor. The withdrawal was from the intermediate ports according to the VER applied.

3.4.2 Aeration

An aerator (air compressor) with a flow meter (Flow Point, FP/ABR-221) which can measure 0 to 10 l min^{-1} was used for providing aeration for the entire study. Air was admitted at the bottom of the column reactor as fine bubbles using porous diffuser stones. The rising air bubbles provide necessary shear force in terms of SUAV which can be controlled by the flow meter. Fig. 3.4 shows the air compressor with flow meter.

3.4.3 Cycle of Operation

Cyclic operation is the main feature of SBR. Each cycle consists of four sequential operations viz filling (feeding), aeration (reaction or degradation of substrate), settling (solid-liquid separation), and discharge (effluent withdrawal or decanting). The cycle time for the present study was selected as 4 hours. The basic cycle, except in trials to study the influence of settling time, the cycle time was splitted as follows:

➢ Feeding	–	5 min
➢ Aeration	–	225 min
➢ Settling – idle	–	5 min
➢ Effluent withdrawal	–	5 min
➢ Total one cycle	–	240 min

Fig. 3.3 Peristaltic Pump: Model ENPD 300 Victor

Fig. 3.4 Air compressor with flow meter

For trials in which settling time was 10 min and 3 min, the aeration time was adjusted to 220 min and 227 min respectively, keeping the filling time and discharge time constant (5 min each).

The cyclic operation was achieved by a micro-controller AT89C51, which is a member of 8051 micro-controller family. The micro-controller is programmed in such a way that power supply to socket 1 (attached to feeding pump) will be available for 5 minutes and socket 2 (attached to aerator) will be given for 225 minutes. Then the power supply to all sockets will be switched off for 5 min and then power supply to socket 3 (attached to discharge pump) will be given for 5 min. The cycle repeats. The circuit diagram for the Micro-controller AT89C51 is given as Fig. 3.5 and the micro-controller assembly used for the study is shown in Fig 3.6. The programme used in the micro-controller AT89C51 is shown as Annexure I.

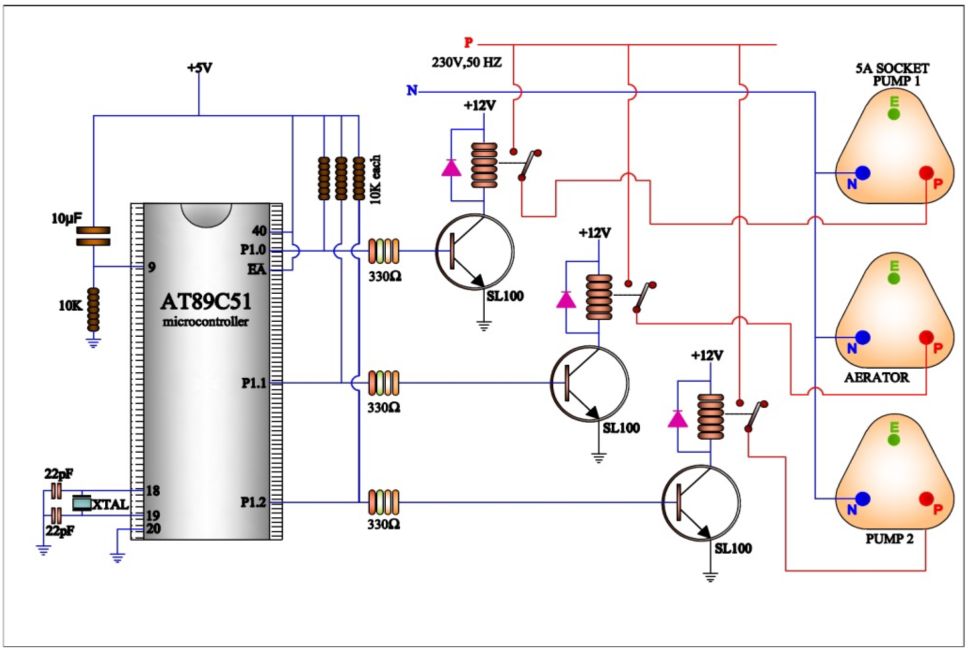


Fig. 3.5 Micro-controller AT89C51 – Circuit Diagram

Electromagnetic relays are used to control power supply (230 V, 50 Hz AC supply) to three sockets. The operation of electromagnetic relays is controlled by programme using 8051 micro-controller.

Fig 3.6 Micro-controller AT89C51 assembly for the experimental set-up

3.4.4 Laboratory Set up

Two separate tanks of 50 litres capacity each were used to store the influent and effluent. The influent was fed to the bottom of the reactor and the effluent was discharged from the reactor with the help of the peristaltic pumps. The speed of the pumps was adjusted in such a way to supply or withdraw sufficient quantity in specified time. The air compressor with flowmeter supplied air in required flow rate at the bottom of the reactor. The air was released at the bottom using porous stone dispensers. The column reactor was held vertical using clamps fixed on wall. The cycle of operation was controlled electronically using the micro-controller AT89C51. The experimental set-up assembled is shown in Fig.3.7.

Fig 3.7 Laboratory Experimental Set-up

3.5 Feed Composition

The synthetic wastewater consisted of sodium acetate as the sole carbon source was used for the study. The composition of the feeding solution including micro-nutrients was adopted from Tay et al. (2002a). It gives a total COD of 2000 mg l^{-1}. The composition of the influent is given in Table 3.1. Micro-elements were added as solutions at the rate of 1 ml l^{-1}. Microelement solution contained: H_3BO_3 - 0.05 g l^{-1}, $ZnCl_2$ - 0.05 g l^{-1}, $CuCl_2$ - 0.03 g l^{-1}, $MnSO_4.H_2O$ - 0.05 g l^{-1}, $(NH_4)_6$ $Mo_7O_{24}.4H_2O$ - 0.05 g l^{-1}, $AlCl_3$ - 0.05 g l^{-1}, $CoCl_2.6H_2O$ - 0.05 g l^{-1}, $NiCl_2$ - 0.05 g l^{-1}.

Table 3.1 Composition of the feed used for the study

Constituent	Concentration
CH_3COONa	2.93 g l^{-1}
NH_4Cl	350 mg l^{-1}
K_2HPO_4	30 mg l^{-1}
KH_2PO_4	25 mg l^{-1}
$CaCl_2.2H_2O$	30 mg l^{-1}
$MgSO_4.7H_2O$	25 mg l^{-1}
$FeSO_4.7H_2O$	20 mg l^{-1}
Micro-elements solutions	1 ml l^{-1}

3.6 Seed Sludge

Two samples of seed sludge were used during trial runs. Sample 1 was taken from the activated sludge processing unit of a dairy industry at Kottayam, Kerala (MILMA, Vadavathur). The colour of the sludge was dark grey. The mixed liquor suspended solids (MLSS) and sludge volume index (SVI) of the seed sludge were 2760 mg l^{-1} and 286 ml g^{-1} respectively. The reactor was first put in operation by seeding with the sludge from the dairy industry. As severe washout and bulking of sludge were observed in 3 trials, attempts to continue with the dairy seed sludge were discontinued.

As a second attempt, the seed sludge was collected from the activated sludge processing unit of the Petrochemical Division of Fertilizers And Chemicals Travancore (FACT) Limited, Cochin, Kerala. The sludge had a grey colour with SVI of 245 ml g^{-1} and MLSS of 5050 mg l^{-1}. 750 ml of seed sludge was used to start each trial. Satisfactory performance was shown by all trials.

3.7 Analytical Methods

The influent concentration of COD (COD_{in}) was fixed as 2000 mg l^{-1} as per the feed composition given in Table 3.1. Still each stock of the feed prepared was checked for total COD (COD_T) for accuracy. Sampling ports provided on the column reactor were used to collect samples of effluent and the mixed liquor. For sampling, the ports were carefully opened and samples were collected after discarding initial 15-20 ml. The effluent sample was collected daily and analyzed for COD_T, soluble COD (COD_S) and suspended solids (SS_e). During the reaction phase (aeration) grab samples of reactor contents were drawn daily and analyzed for mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). Sampling of the reactor content was done towards the end of aeration period in the operation cycle, at same time every day. Temperature, pH and dissolved oxygen (DO) of the reactor contents were measured and monitored regularly. Sludge volume index based on 30 min settling (SVI_{30}) was determined once in 2 days for each trial. COD removal efficiency (COD_{rem}), specific oxygen utilization rate (SOUR), observed yield coefficients (Y_{ob}) and sludge retention time (SRT) were calculated from the observed values as per Eqn. 3.9, 3.10, 3.11 and 3.12 respectively. SOUR, Y_{ob}, and SRT were calculated during the steady state only in each trial.

Wastewater from natural rubber latex centrifuging unit contains nitrogen and ammonia along with high amount of organic matter. Hence, total nitrogen, ammoniacal nitrogen, and sulphides were also estimated for the raw wastewater and treated wastewater.

The appearance and development of granules were monitored daily in each trial. Once appeared in the reactor, samples of granules were collected and analyzed for morphological characteristics like size, shape, specific gravity and settling velocity as and when required. The methods adopted for the analysis of each parameter are described below.

3.7.1 COD and Suspended Solids

COD and SS were analyzed using methods described in S*tandard Methods for the Examination of Water and Wastewater* (APHA, 1998). Always duplicate samples were analyzed for reducing the error in estimation. COD_T and COD_S were analyzed by closed reflux method (Section 5220 C). Digestion for COD determination was done in a COD digester (Hanna make, Model HI 83099). Suspended solids (SS) and volatile suspended solids (VSS) were quantified using gravimetric methods (Section 2540 D and 2540 E respectively). Glass fibre filter disc was used for the determination of SS. Total nitrogen, ammoniacal nitrogen and sulphides of the wastewater were analyzed using APHA, 1998.

3.7.2 Temperature, pH and DO

Temperature of the mixed liquor and that of the feed were measured using a standard Mercury filled Celsius thermometer of 0.1^oC accuracy (Section 2550 B of APHA, 1998). Measurement of pH and DO of the reactor content was done by pH meter (Eutech Instruments, Cyberscan pH-510, pH/mV/^{o}C) and a handheld DO meter (Eutech Instruments, Cyberscan DO-110, DO/^{o}C/^{o}F) respectively. The analytical instruments used for the estimation of pH, DO, COD and SS are shown in Fig. 3.8 (a-d).

3.7.3 SVI_{30}

Determination of SVI_{30} was done as specified in Standard Methods (Section 2710 D). Each time the mixed liquor was withdrawn and poured in a

100 ml graduated measuring cylinder and allowed to settle for 30 min. SVI_{30} was estimated from the settled volume after 30 min and the MLSS concentration. The measurement of SVI in the laboratory is shown in Fig 3.9.

3.7.4 SOUR

For the determination of specific oxygen utilization rate, the granules were carefully washed with tap water and placed in a BOD bottle containing pre-aerated substrate and nutrient solution. In order to avoid breaking the granules, the bottle was gently mixed with a magnetic mixer at 50 rpm. The temperature was kept at 25°C. The decrease in dissolved oxygen concentration was recorded using DO meter at a time interval of 30 s. SOUR was then calculated according to the change in dissolved oxygen concentration over time and the MLVSS in the bottle and expressed in mg O_2 (g VSS)$^{-1}$ hr^{-1} as per equation 3.10.

3.7.5 Y_{ob}

Sludge observed yield coefficient is defined as the mass of cells formed to mass of substrate consumed (Metcalf & Eddy, 2003). It is measured as the ratio of observed daily sludge production rate (mg MLVSS per day) to mass of substrate consumed per day (mg COD per day). The calculation of Y_{ob} was done as per equation 3.11.

3.7.6 SRT

Sludge retention time represents the average period of time during which the sludge has remained in the system. The SRT was calculated by dividing the amount of biomass in the reactor by the amount of biomass removed with the effluent per day. SRT was calculated as per equation 3.12.

3.7.7 Size, Shape, Specific Gravity and Settling Velocity of Aerobic Granules

Seed sludge and sludge developed in the reactor were closely examined using micrography.

3.7.7.1 Image Analysis

Morphological characteristics like size, shape and regularity of the seed sludge and the evolution of granular sludge were followed by an Image Analysis (IA) system. The IA system consists of an Olympus Zoom Stereomicroscope SZ40, system microscope BX41 (Olympus Ltd., Japan), Nikon 4500 Zoom digital camera and Image-pro plus 4.0 Analysis Software (Media Cybernetics Ltd., USA). Image analysis was also done with Leitz – Diaplan microscope attached with Leica Q-WIN 3 image analyzing system and Olympus bx 51 fluorescent microscope. The systems used for the image analysis are shown in Fig 3.10 (a-b).

3.7.7.2 Scanning Electron Microscopy (SEM)

Morphology and micro-structure of typical aerobic granules were observed qualitatively with SEM (JEOL Model JSM – 6390 LV).

3.7.7.3 Specific Gravity

The specific gravity of aerobic granules was measured by method explained by Zheng et al., 2006. This method is based on the correlation between the density and concentration (v/w) of sucrose solution. Sucrose was used to make a series of solutions with densities of 1.003, 1.004, 1.005, 1.006, 1.007, 1.008, 1.009, 1.010 and 1.011, 1.012 g l^{-1}. Ten granules or 1 ml flocs were added into each of the 100 ml tubes filled with the sucrose solutions of different densities. Under a quiescent condition, the granules or sludge flocs moved up or down in the tubes, depending on the solution density. In this way, the wet specific gravity of the granules and flocs was estimated.

3.7.7.4 Settling Velocity

The settling velocity was measured by recording the time taken for individual granules to fall from a certain height in a measuring cylinder.

Fig. 3.8 Analytical instruments used for the study (a) pH meter (Cyberscan pH-510) (b) DO meter (Cyberscan DO-110) (c) COD digester (Hanna make, Model HI 83099) (d) vacuum pressure pump assembly for SS determination

Fig 3.9 Laboratory set-up for estimating SVI

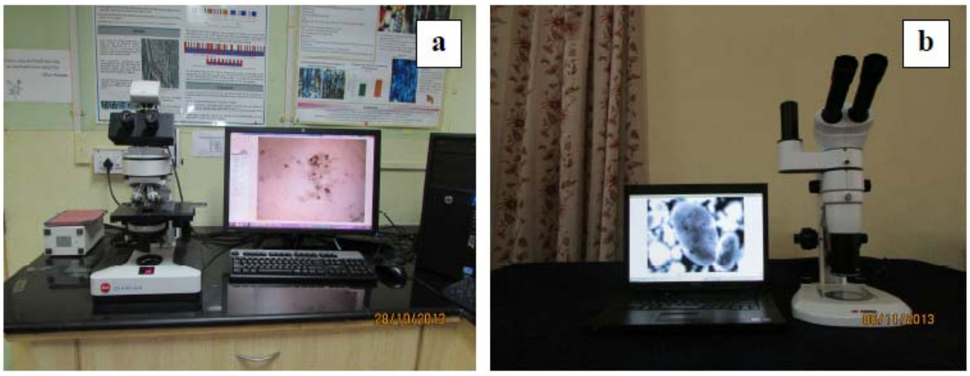


Fig. 3.10 Image Analysis systems (a) Leitz – Diaplan microscope attached with Leica Q-WIN 3 image analyzing system (b) Olympus Zoom Stereomicroscope SZ40 Image-pro plus 4.0 Software

3.8 General Equations Used for the Study

The general equations used for the study are given in this section.

Given the dimensions of the column reactor as: (Refer Fig.2.1)

Diameter (internal) = D

Effective height = H

Distance to the discharging port from the top level of the solution

= L

then,

$$\text{Cross sectional area of the reactor (A)} = \frac{\pi D^2}{4} \quad (3.1)$$

$$\text{Working Volume of the reactor (V)} = \left(\frac{\pi D^2}{4}\right) \times H \quad (3.2)$$

$$\text{Volume Exchange Ratio (VER)} = \frac{\mathrm{L}}{\mathrm{H}} \quad (3.3)$$

Given the details as:

Cycle Time = T

Influent COD = $\mathrm{COD_{in}}$

Effluent COD = $\mathrm{COD_e}$

Airflow rate = F,

then,

$$\text{Number of cycles per day (N)} = \frac{24}{T} \quad (3.4)$$

$$\text{Discharge (Q)} = \mathrm{V * VER * N} \quad (3.5)$$

$$\text{Hydraulic Retention Time(HRT)} = \frac{V}{Q} \quad (3.6)$$

$$\text{Organic Loading Rate (OLR)} = \frac{(COD_{in} \times Q)}{V} \quad (3.7)$$

$$\text{Shear Force (or SUAV)} = \frac{F}{A} \quad (3.8)$$

$$\text{COD removal Efficiency (\%)} = \frac{\left(COD_{in} - COD_{out}\right)\times 100}{COD_{in}} \qquad (3.9)$$

Specific Oxygen Utilization Rate (SOUR)

$$SOUR = \frac{(oxygen\ consumption\ rate, mg/l\ \text{min})}{(volatile\ suspended\ solids, g/l)} \times \frac{60\ \text{min}}{h} \qquad (3.10)$$

Sludge Observed Yield Coefficient (Y_{OB})

$$Y_{OB} = \frac{\Delta X}{\Delta S} \qquad (3.11)$$

Where, ΔX = observed daily sludge production rate (mg MLVSS per day)

ΔS = mass of substrate consumed per day (mg COD per day)

Sludge Retention Time (SRT)

$$SRT = \frac{VX}{6(V_e X_e + V_d X)} \qquad (3.12)$$

Where, V = Volume of the reactor

X = MLSS in the reactor (or SS in the discharged mixed liquor)

X_e = SS concentration in the effluent

V_e = Volume of the discharged supernatant after settling

V_d = Volume of the discharged mixed liquor in each cycle

To achieve a desirable SRT, V_s can be determined by rearranging the above equation as:

$$V_s = \frac{V}{6SRT} - \frac{V_e X_e}{X} \qquad (3.13)$$

3.9 Operational Parameters Selected for the Study

The major parameters selected for the present study were OLR, SUAV, ST and VER. Three values for each parameter were attempted in various trials. The values were selected based on literature survey. Total nine trial runs were performed to investigate the influence of these parameters on aerobic granulation.

3.10. Grey System Theory (GST) Method

Grey relational analysis (GRA) and grey models are the two sections in grey system theory. As the name indicates, GRA provides a relational grading or ranking of concerned parameters. Grey relational coefficients (GRCs) and grey entropy relational grade (GERG) are the components of GRA. Using these two indices one can investigate the relationship between reference sequences and compared sequences.

3.10.1 Reference Parameters and Compared Parameters

For the process of an aerobic granular sludge system, the appearance of the granules in the system is an important indication of the successful functioning of the reactor. Once the granules appear, their growth and development are measured in terms of size and specific gravity. SVI could effectively indicate the degree of granulation and the settling ability of the granular sludge. Both SVI and settling velocity of the granular sludge would be eventually influenced, whether directly or not, by the operational impact factors. The ultimate efficiency of a treatment system depends on its capacity to remove pollutants. Hence, SVI, granule appearance time, size and specific gravity of the granules, and COD removal efficiency were chosen as the reference parameters.

Influence of four major parameters on aerobic granulation in SBR was investigated. The influence of each parameter on granulation can be compared and a relational grading can be achieved by GRA. These four operational parameters *viz* OLR, SUAV, settling time and VER were taken as the compared parameters for the present study.

3.10.2 Theory of GRA

In grey relational space, a system contains many series with *k* entities:

$$X_i^{0*} = \left\{ X_i^{0*}(k) \middle| i = 1,2,...,m, k = 1,2,...,n \right\} \tag{3.14}$$

$$X_j^{*} = \left\{ X_j^{*}(k) \middle| j = 1,2,...,r, k = 1,2,...,n \right\} \tag{3.15}$$

where, X_i^{0*} is the reference sequence, X_j^{*} is the compared sequence, *m*, *r* and *n* stand for the number of the reference parameter, compared parameter, and total experiments, respectively. In this study, granule appearance time, SVI, size and specific gravity of the granules, settling velocity, and COD removal efficiency were chosen as the reference parameters and OLR, SUAV, settling time and VER were chosen as compared parameters. The data acquired from all nine trials were used for the analysis. Thus, $m = 5$, $r = 4$ and $n = 9$.

Since the units of the sequences varied widely from each other, the series data were subjected to pre-processing normalization as shown below before calculating the GRCs as follows:

$$X_i^{0}(k) = \frac{X_i^{0*}(k)}{\frac{1}{n}\sum_{k=1}^{n} X_i^{0*}(k)} \tag{3.16}$$

$$X_j(k) = \frac{X_j^*(k)}{\frac{1}{n}\sum_{k=1}^{n} X_j^*(k)} \tag{3.17}$$

Now the reference sequence and compared sequence are transformed to the following forms for the present study.

$$X_i^0 = \left\{ X_i^0(k) \middle| i = 1,2,\ldots5,.k = 1,2,\ldots,9 \right\} \tag{3.18}$$

$$X_j = \left\{ X_j(k) \middle| j = 1,2,\ldots,4, k = 1,2,\ldots,9 \right\} \tag{3.19}$$

Then the GRC between reference sequence $Xi^0(k)$ and compared sequence $Xj(k)$ at point k is calculated as:

$$\zeta_{ij}(k) = \frac{\min_j \min_k \left| X_i^0(k) - X_j(k) \right| + \rho \max_j \max_k \left| X_i^0(k) - X_j(k) \right|}{\left| X_i^0(k) - X_j(k) \right| + \rho \max_j \max_k \left| X_i^0(k) - X_j(k) \right|} \tag{3.20}$$

where, ρ, the coefficient of $\max_j \max_k \left| X_i^0(k) - X_j(k) \right|$ is known as the distinguishing coefficient and its value varies from 0 to 1. Since $\max_j \max_k \left| X_i^0(k) - X_j(k) \right|$ is used to describe the integrity of a system, ρ is typically taken as 0.5 to control the resolution between $\max_j \max_k \left| X_i^0(k) - X_j(k) \right|$ and $\min_j \min_k \left| X_i^0(k) - X_j(k) \right|$.

GRC is used to indicate the relational grade between the reference sequence Xi^0 and the compared sequence Xj^* at point *k*. To make full use of the abundant information supplied by GRCs and to avoid being misled by larger GRCs, GERG is used. GERG helps to determine the key operational impact factors and rank them by relational grade. The map value of the GRCs distributed map is the relational coefficient distribution density (p_{ij}), which is given as:

$$p_{ij}(k) = \frac{\zeta_{ij}(k)}{\sum_{k=1}^{n} \zeta_{ij}(k)} \tag{3.21}$$

The grey relational entropy of the operational impact factor *j* on the reference parameter *i* can be calculated as:

$$S_{ij} = -\sum_{k=1}^{n} \left[p_{ij}(k) \ln p_{ij}(k) \right] \tag{3.22}$$

The GERG (E_{ij}) for the operational impact factor j on the reference parameter i is defined as:

$$E_{ij} = \frac{S_{ij}}{S_{\max}} \tag{3.23}$$

Where, $S_{\max}$ is the sequence maximum entropy, which is a constant ($\ln n$) that only associates with the number of the element when the value of elements contained in each series equals to each other. A larger E_{ij} value represents a stronger relevance between the reference sequence and compared sequence. The key operational impact factor and the influence order can be acquired by comparing the calculated GERG of each compared sequence.

3.11 Natural Rubber Latex Processing Wastewater - Methodology of Treatment

Natural latex and rubber processing industries are very common in Kerala. Kottayam is the hub of all the activities connected with cultivation, marketing and processing of rubber. Raw latex is collected and processed in latex processing factories. Centrifuging is the major step in the latex processing by which the dry rubber content (DRC) in the latex is increased from 30% to 60%. The wastewater generated from this unit contain high amount of organic matter and ammoniacal nitrogen.

Samples of wastewater from the centrifuging unit of a natural rubber latex processing industry were collected and analyzed for pH and COD, suspended solids, total nitrogen, ammoniacal nitrogen, and sulphides. As the pH and COD_T were found 7.5 ± 0.5 and 2000 ± 300 mg l^{-1} respectively, the wastewater was considered suitable to replicate the feed characteristics adopted for the laboratory study. Samples were collected from the industry once in three days. Dilution or concentration of the feed was done to adjust the COD_T to 2000 mg l^{-1}. A quantity of 6 litres was required daily to run the reactor to maintain the OLR as 6 kg m^{-3} d^{-1}. Seed sludge was taken from FACT, Cochin. A settling time of 5 min, VER of 50% and a SUAV of 3 cm s^{-1} were applied for the laboratory study with latex processing wastewater.

The overall methodology adopted for the study was schematically represented in Fig.3.11.

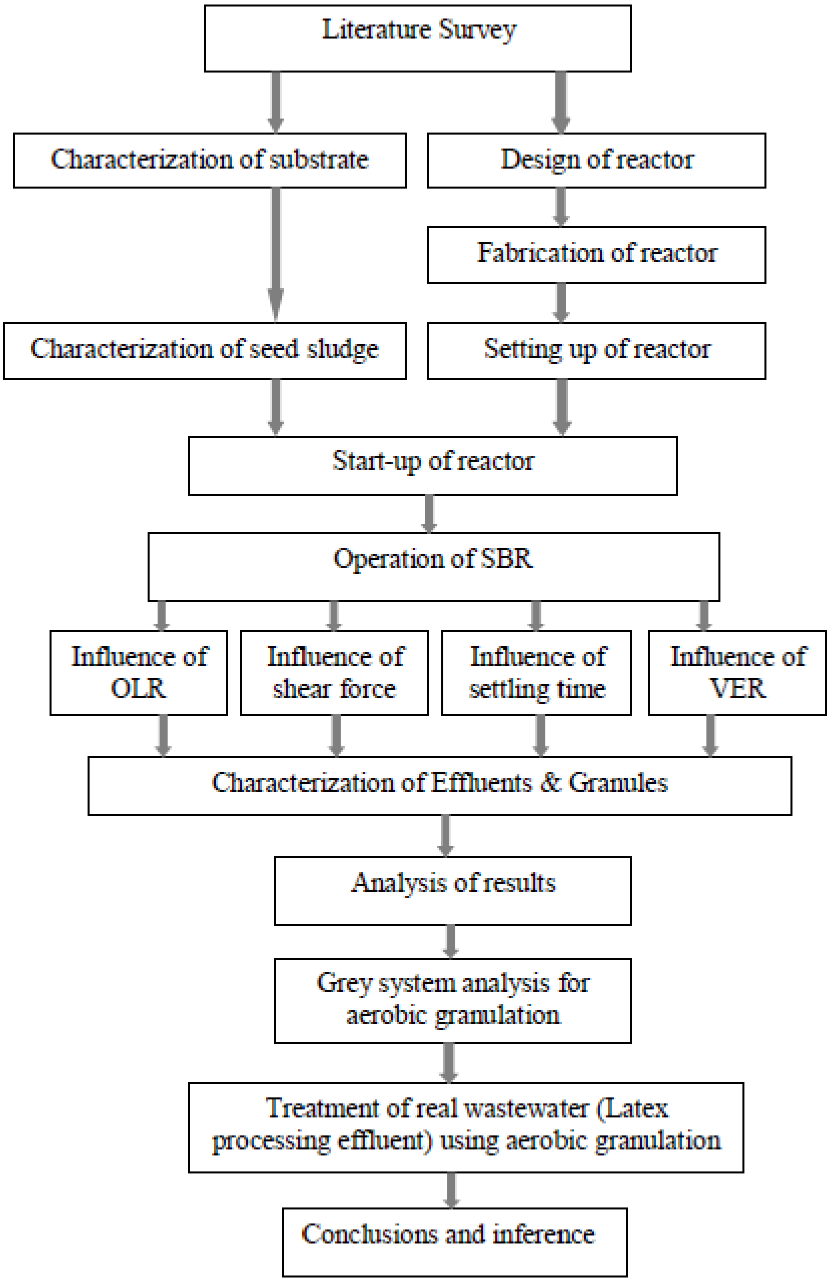


Fig. 3.11 Methodology adopted for the aerobic granulation study

3.12 Summary

The methodology adopted for the experimental study is given in this chapter. The design and configuration of the column reactor and the accessories for the experimental set-up are explained with illustrations. The cycle of operation and description of the seed sludge and feed are also included. All the analytical methods adopted in the laboratory including image analysis techniques are explained. This chapter also explains the theory of grey relational analysis. The next chapter gives a detailed report and analysis of the results of the present study.

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Chapter 4

RESULTS AND DISCUSSION

Contents

4.1 Introduction

Aerobic granulation was reported to be affected by a number of operational parameters. Four parameters which may critically influence the process were selected for this study. They are organic loading rate, hydraulic shear force, settling time and volume exchange ratio. The significance of these parameters was described in Chapter 2 and the methodology of the experimental studies and analysis was explained in Chapter 3. Experiments were conducted in the laboratory and results are reported and discussed in this chapter. When different parameters influence a process, it is necessary to prioritize their importance. Relative grading or ranking of the influencing factors may be helpful for the optimum performance of the reactor, especially while designing pilot plants. Grey relational analysis is an effective ranking tool. Grey relational coefficients and grey entropy relational grades are used to rank the influence of compared sequence on reference sequence. Optimal scopes are also necessary for the successful design and working of any treatment system. These optimal values can be accepted only when they are demonstrated successfully for the treatment of a real wastewater. Effluent from the natural latex centrifuging unit was selected for the treatment by aerobic granulation, using the optimal values obtained from the experimental trials.

4.2 Trial Runs

Seed sludge from the activated sludge treatment unit of a Dairy industry in Kottayam district (MILMA, Vadavathur) was collected and used for the trial runs of the experiment. In all the three trials conducted, foaming of sludge and severe wash-out were observed in first week. Hence it was decided to use sludge from another source. Seed sludge was collected from the activated sludge processing unit of the Petrochemical Division of Fertilizers And Chemicals Travancore (FACT) Limited, Cochin, Kerala. As trial runs showed good performance, it was decided to continue the experimental study with the seed sludge from FACT. The average characteristics of both sludges are given in Table 4.1.

Table 4.1 Characteristics of seed sludge used for the study

Characteristics	Seed Sludge from MILMA	Seed Sludge from FACT
Physical Appearance	Dark grey in colour	Grey in colour
MLSS (mg l^{-1})	2760	5050
MLVSS (mg l^{-1})	1635	2927
SVI (ml g^{-1})	286	245
pH	7.78	7.89

4.3 Operational Parameters

The parameters selected for the study were OLR, SUAV, ST and VER. Nine trial runs were performed to investigate the influence of these parameters on aerobic granulation. Literature was surveyed to select the values for the above operational parameters. Different researchers conducted experiments with OLR ranging from 1.5 to 9.0 kg COD m^{-3} d^{-1} yielding different COD removal efficiency. Hence a moderate value of OLR 6 kg COD m^{-3} d^{-1} was selected as a basic value for the present study. It was observed from the experiments by various researchers that a SUAV less than 1.2 m s^{-1} is not suitable for granulation and SUAV of 2.4 – 3.6 m s^{-1} is favourable for good granulation. Hence a SUAV of 3 m s^{-1} was selected for the present investigation as a basic value. A settling time 5 min was also selected based on the literature survey. Most of the previous studies employed a VER of 50% for aerobic granulation in SBR. Hence in the present study also, a VER of 50% was adopted as a base value. Thus the basic values selected for OLR, SUAV, ST and VER were 6 kg COD m^{-3} d^{-1}, 3 cm s^{-1}, 5 min and 50 % respectively.

Two more values, one higher than the basic value and one lower than the basic value were also selected to study the influence. Thus OLR selected were 3, 6 and 9 kg COD m^{-3} d^{-1}, SUAV selected were 2, 3 and 4 cm s^{-1}, settling time selected were 3, 5 and 10 min, and VER selected were 25%, 50% and 75% respectively. Thus total number of trials was 9, which are listed in Table 4.2.

Table 4.2 Operational conditions of various trials

Operational Parameters	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
OLR, kg COD m^{-3} d^{-1}	3	**6**	9	6	6	6	6	6	6
SUAV, cm s^{-1}	3	**3**	3	2	4	3	3	3	3
ST, min	5	**5**	5	5	5	3	10	5	5
VER, %	50	**50**	50	50	50	50	50	25	75

Second trial being a common trial,
trials 1, 2 and 3 were used to study the influence of OLR,
trials 4, 2 and 5 were used to investigate the influence of SUAV,
trials 6, 2 and 7 were used to study the influence of ST and trials 8,2 and 9 were used to investigate the influence of VER on aerobic granulation.
A set of sample calculations is given as Annexure II

4.4 Influence of OLR on Aerobic Granulation

Organic loading rate is thought to be a strong influencing parameter on aerobic granulation in SBR. Three trials (trial1, trial 2 and trial3) were conducted with OLR 3, 6 and 9 kg COD m^{-3} d^{-1} respectively.

4.4.1 Trial 1 (OLR = 3 kg COD m^{-3} d^{-1})

For trial 1, influent COD concentration (COD_{in}) was adjusted to 1000 mg l^{-1} to obtain the OLR of 3 kg COD m^{-3} d^{-1}. The reactor was seeded with 750 ml of sludge having a MLSS of 5050 mg l^{-1} which was taken from activated sludge processing unit of Petrochemical division of FACT, Cochin. The pH of the mixed liquor was adjusted to a range of 6.5 to 8 using 1 N HCl and 1 N NaOH. The reactor was put in operation with a cycle time of 4 hours (5 min for feeding, 225 min for aeration, 5 min for settling and 5 min for effluent withdrawal). Air was admitted at the bottom at the rate of 6 litres in 1 min, so that the shear force in terms of SUAV was 3 cm s^{-1}.

The effluent COD (COD_e) was monitored daily. MLSS and MLVSS were measured daily by taking samples from the middle of the column reactor. Samples were taken and analyzed in duplicate to minimize error. SVI of the reactor content was determined on alternate days by taking 100 ml sample and observing the settled volume after 30 min. The sample was poured back in the reactor after SVI determination.

High fluctuations in effluent COD (COD_e) were observed during the start-up period, which was reflected in COD removal efficiency. The growth rate of MLSS was slow for the initial 2 weeks, which varied from 1750 mg l^{-1} at the beginning of operation to 2245 mg l^{-1} at the end of 2nd week. From third week onwards the growth of MLSS was found to improve and reached an average value of 5000 mg l^{-1} by 6th week. At these times, the MLVSS/MLSS ratio reached an average value of 0.8, maximum being 0.85 on day 37. A reduction in the SVI values was observed, which was at a rapid rate during the first three weeks, and then at a slower rate.

The activated seed sludge has a mean floc size of 0.1 mm. The evolution and growth of granules were monitored by image analysis. During the initial fluctuations and disturbances, sludge particles with very low density were washed out of the reactor. Tiny granules appeared in the reactor by 28th day of operation. At this stage the settling of the sludge became faster. The granules slowly grew in size and reached an average size of 1.8 mm. The reactor was operated for 45 days, in which the last 10 days showed a COD removal efficiency of 95% to 96%. The constancy in MLSS values and effluent COD values was taken as a criterion for steady state.

The settling velocity of granules was measured by selecting 10 granules randomly and recording the time taken by these particles to fall from a certain height in a measuring cylinder. Settling velocity of the matured granules was found as 45 m hr^{-1}. These granules were with an average specific gravity of 1.0055. The SVI of the mixed liquor reduced to a minimum of 31 ml g^{-1} from the start-up value of 280 ml g^{-1}. The trial run was stopped after 45 days.

4.4.2 Trial 2 (OLR = 6 kg COD m^{-3} d^{-1})

In trial 2, the reactor was fed with influent of COD_{in} 2000 mg l^{-1} so that the OLR was 6 kg COD m^{-3} d^{-1}. Even though the initial fluctuations in

MLSS were similar to the previous trial, a more steady growth in MLSS and MLVSS was observed in trial 2. By the end of 4th week, steady state condition was achieved in terms of MLSS values. At this stage a good value for MLVSS/MLSS ratio of 0.91 was achieved. The average values of MLSS and MLVSS for the last one week were 7900 mg l^{-1} and 7200 mg l^{-1} respectively.

The COD_e was reduced gradually, but not steadily till day 22, and then reduced at a faster rate. The average value of COD_e for the last one week was 51 mg l^{-1} and a minimum value of 43 mg l^{-1}. The corresponding COD removal efficiency achieved were 97.4 % and 97.9 % respectively. As the steady state was achieved in an earlier period compared the previous trial, the reactor was stopped after 36 days.

Small granules appeared on 17th day and they got matured in the 4th week of operation. Towards the end of operation, the granules reached an average size of 2.4 mm with a specific gravity of 1.007. At this stage the compact dense granules exhibited a fairly good settling velocity of 55.2 m hr^{-1}. The SVI of the reactor content was dropped to a value <100 ml g^{-1} after 10 days and continued to decrease smoothly to reach the lowest value of 25.1 ml g^{-1} during the trial run.

4.4.3 Trial 3 (OLR = 9 kg COD m^{-3} d^{-1})

In the next attempt (trial 3) OLR was increased to 9 kg COD m^{-3} d^{-1} by feeding the substrate with COD_i 3000 mg l^{-1}. A drastic reduction in the suspended solids was observed in the reactor during the first week of operation. This may be due to the inability of the microorganisms to cope up with the high OLR. But gradually the MLSS and MLVSS concentrations were increased, but not in proportion with the growth as in trial 2 with OLR 6 kg COD m^{-3} d^{-1}. After reaching a high concentration of about 12000 mg l^{-1} by day 33, the system could not maintain this concentration further. It dropped to a value around 9900 mg l^{-1} towards the end of operation. The MLVSS/MLSS ratio also decreased from 0.88 in 5th week to 0.82 in 7th week.

Compared to trial 1 and trial 2, the initial fluctuations in the COD_e were very high in trial 3. Unexpected ups and downs were noticed in the effluent quality. This may be due to the inability of the microorganisms to adjust suddenly with the high OLR. By the end of 5th week only, a fairly good COD removal efficiency of 95% was achieved. But the effluent quality in the days after that, showed that the system was losing the purification capacity. The COD removal efficiency was dropped to 89% by day 45. As negative growth in MLSS and increase in COD_e were observed, the reactor was stopped by day 45.

The first appearance of granules happened to be on day 34. But these granules appeared to be less dense compared to those developed in previous trials. Specific gravity of these granules was measured as 1.004 and the settling velocity as 33.6 m hr^{-1}. There was a sudden increase in SVI during the initial 2-3 days, but gradually it reduced and reached a value of 38.7 ml g^{-1} towards the closing of the trial run.

The daily variations in effluent COD, COD removal efficiency, MLSS, MLVSS, MLVSS/MLSS ratio, and SVI in all the three trials are shown in Fig 4.1 to 4.6.

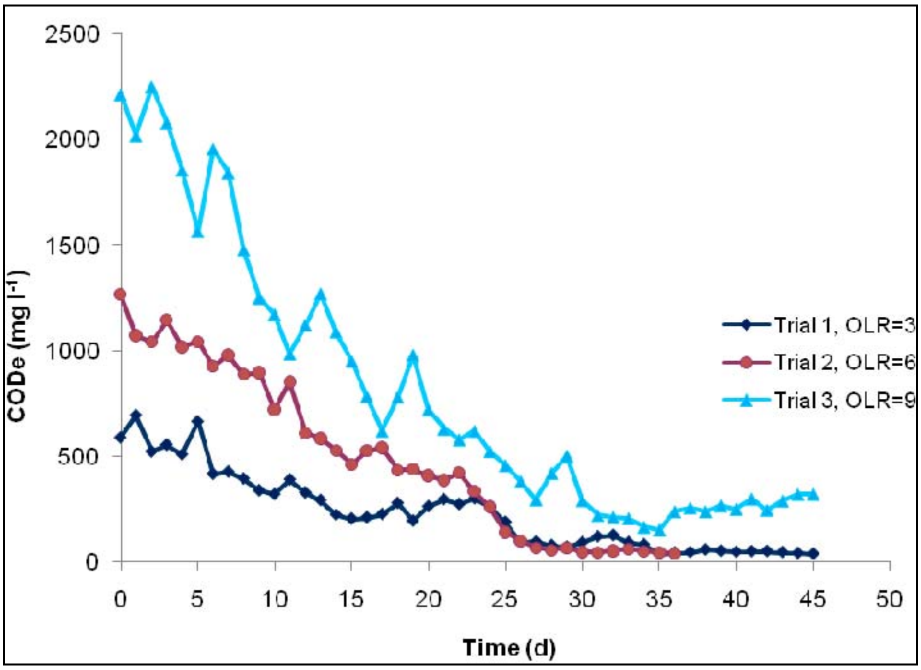

Fig. 4.1 Variation of COD_e at different OLRs (3 - 9 kg COD m^{-3} d^{-1}) with time

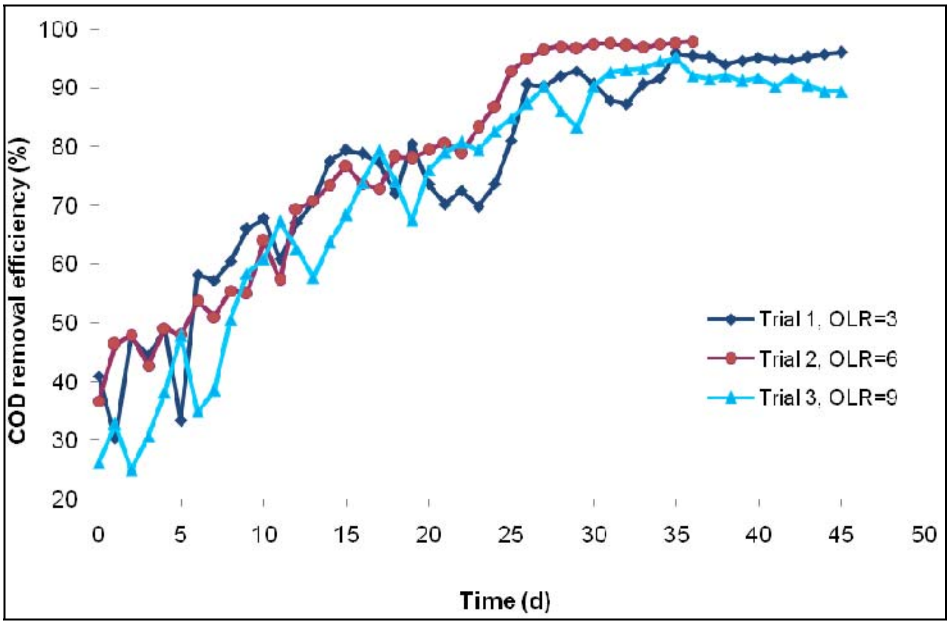

Fig. 4.2 Variation of COD removal at different OLRs (3 - 9 kg COD m^{-3} d^{-1}) with time

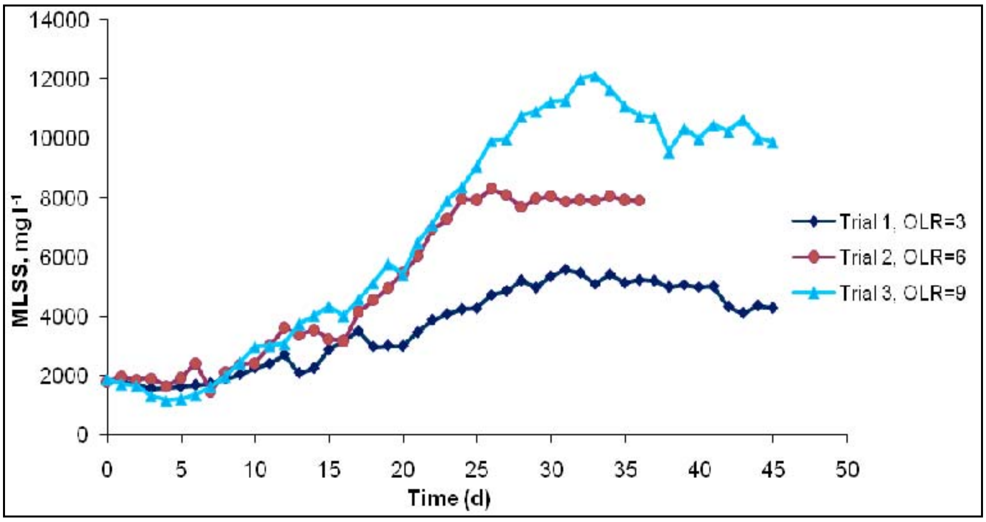

Fig. 4.3 Variation of MLSS at different OLRs (3 - 9 kg COD m^{-3} d^{-1}) with time

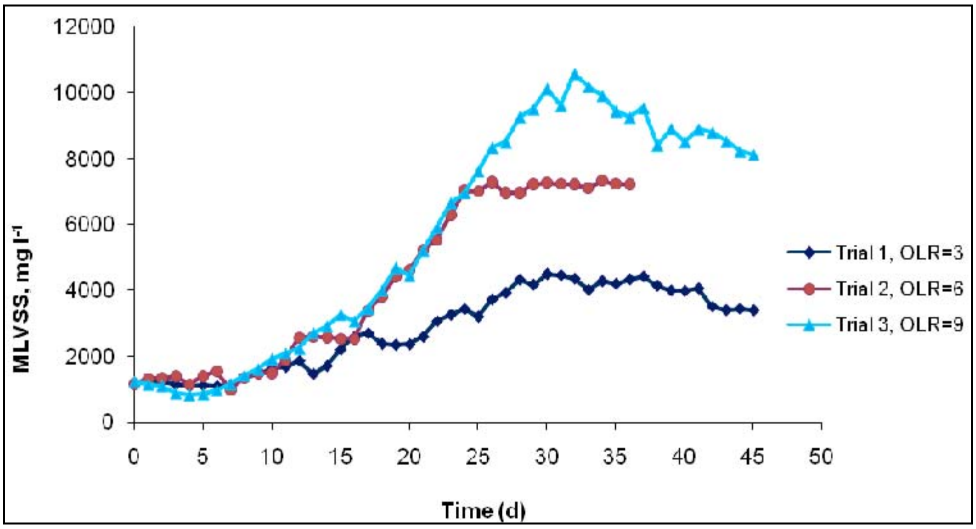

Fig. 4.4 Variation of MLVSS at different OLRs (3 - 9 kg COD m^{-3} d^{-1}) with time

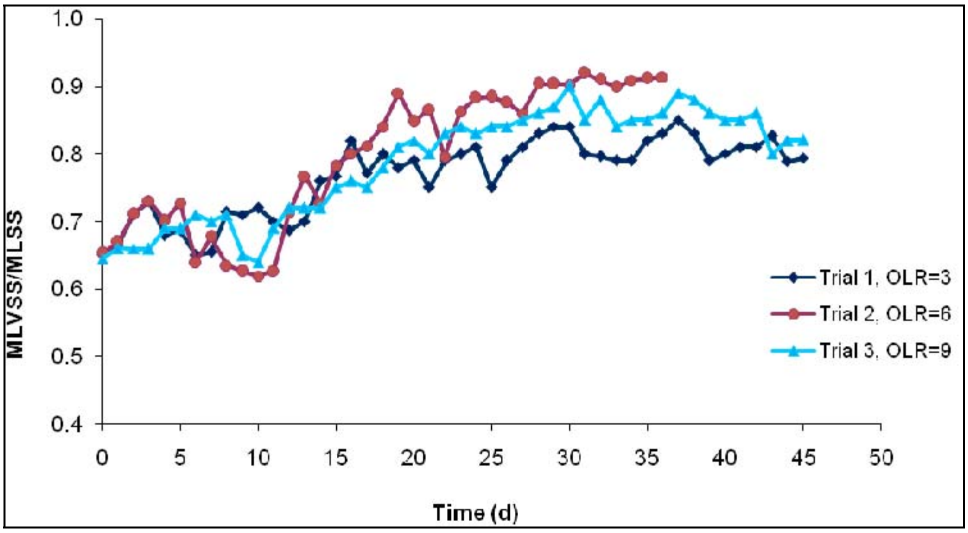

Fig. 4.5 Variation of MLVSS/MLSS at different OLRs (3 - 9 kg COD m^{-3} d^{-1}) with time

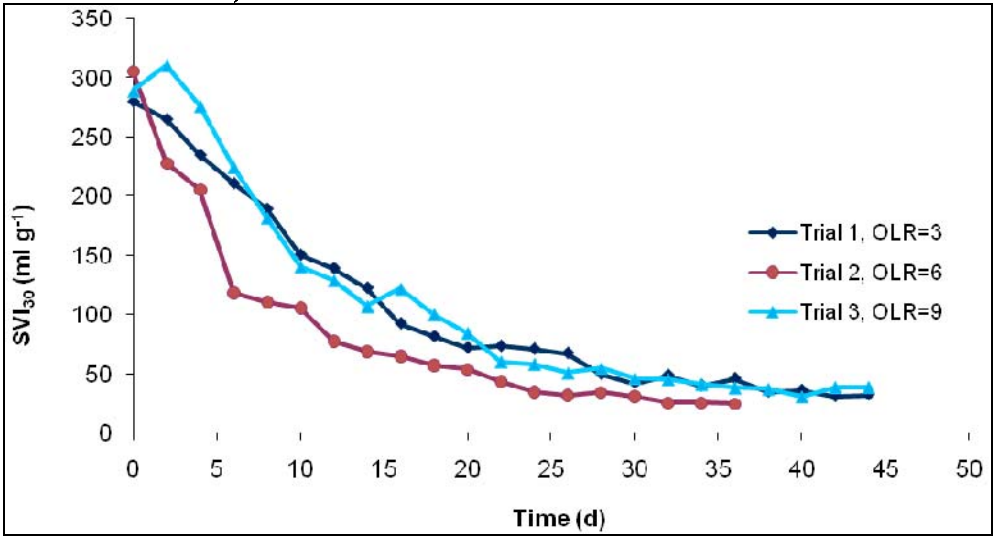

Fig. 4.6 Variation of SVI_{30} at different OLRs (3 - 9 kg COD m^{-3} d^{-1}) with time

The ultimate aim of any treatment system is its efficiency of pollutant removal. Here, the COD removal efficiency at various OLRs is compared (Fig. 4.7). In trial 1 COD removal attained was 96% and in trial 2 it was 97.9%. But still higher OLR (9 kg COD m^{-3} d^{-1}) could not improve or maintain the situation, but reduced the removal efficiency to 89.3%. It is a known fact that MLSS concentrations will be increased along with concentrations of the substrate. At higher OLRs, more amounts of SS and VSS can be expected in the reactor. The variation of MLSS and MLVSS, and the ratio of MLVSS/MLSS with OLRs are represented in Fig 4.8 and 4.9 respectively.

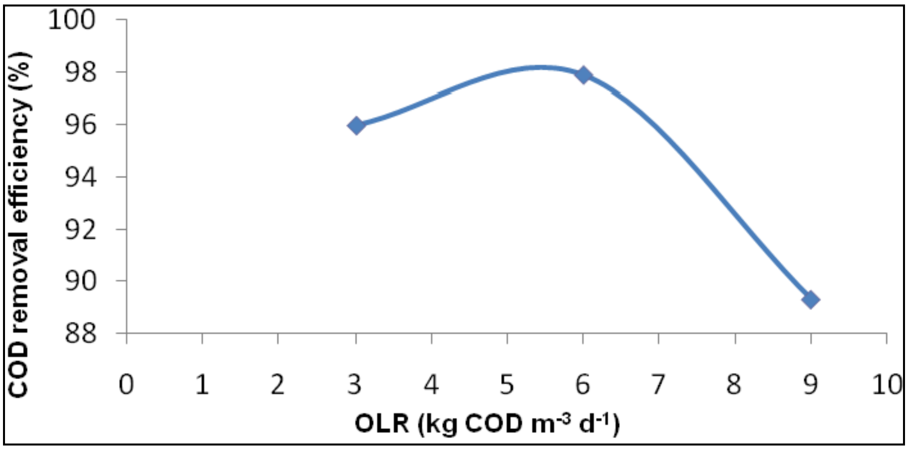

Fig. 4.7 Variation of COD removal efficiency with OLR

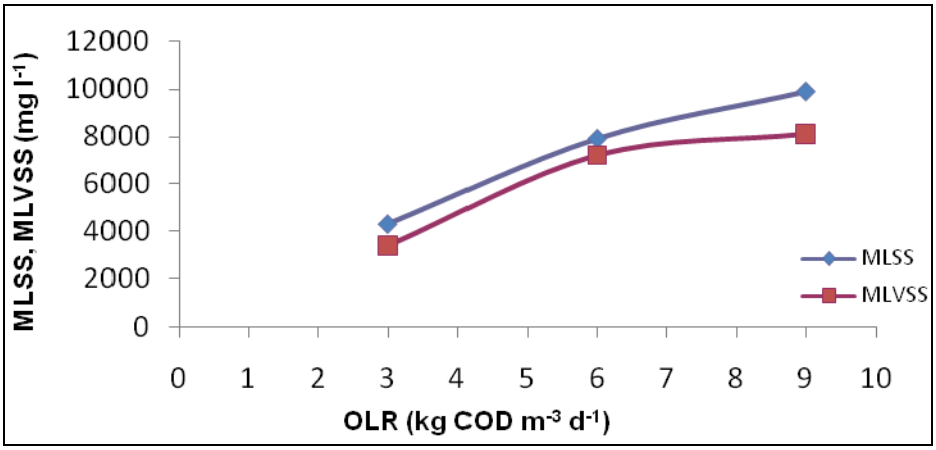

Fig. 4.8 Variation of MLSS & MLVSS with OLR

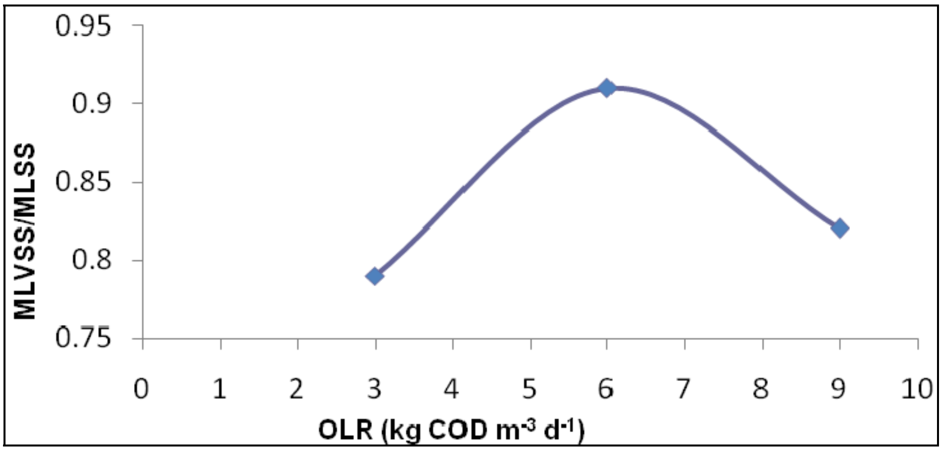

Fig. 4.9 Variation of MLVSS/MLSS with OLR

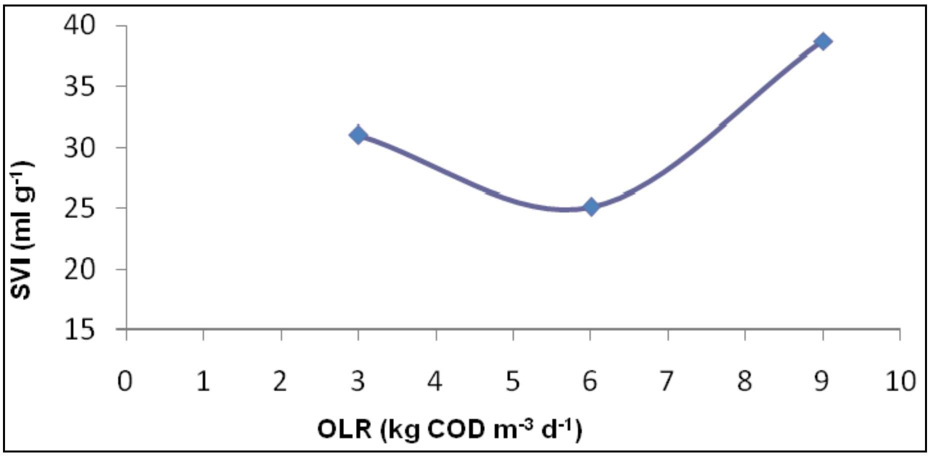

Fig. 4.10 Variation of SVI_{30} with OLR

The SVI was minimum in the second trial (25.1 ml g^{-1}). Trial 2 also showed the best performance in terms of specific gravity and the settling velocity of the granules developed. The variation of SVI with OLR is represented in Fig 4.10.

Morphological variations of the sludge during the experimental period in trial 2 and trial 3 are shown in Fig 4.11 and Fig 4.12 respectively. The variations in the size and density of the sludge were rapid and clear in trial 2, while it was slow and poor in trial 3 with high OLR (9 kg COD m^{-3} d^{-1}). Even on day 21, the granules appeared to be less dense and compact.

Fig 4.13 and 4.14 show the microstructure of the granular sludge developed in trial 2 on day 14 and day 28, observed by SEM at various magnifications. It is evident from the figures that the tiny cluster of cocci bacteria (marked by a circle in Fig. 4.13) developed on day 14 has grown to thick patches of bacteria (Fig 4.14).

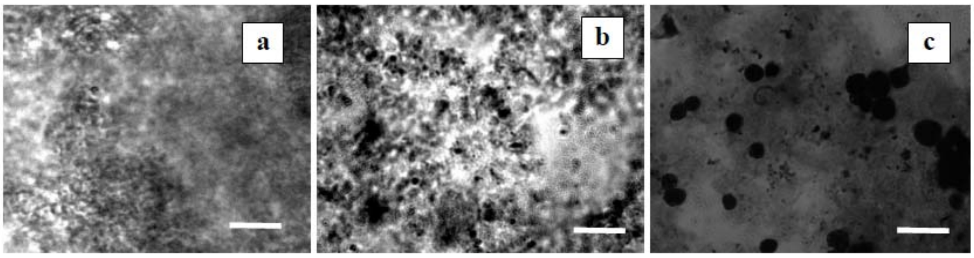

Fig 4.11 Morphological variation of the sludge during the experimental period in trial 2 (OLR=6 kg COD m^{-3} d^{-1}) (a) seed sludge at start-up (b) on 7th day (c) on 14th day (bar = 2 mm)

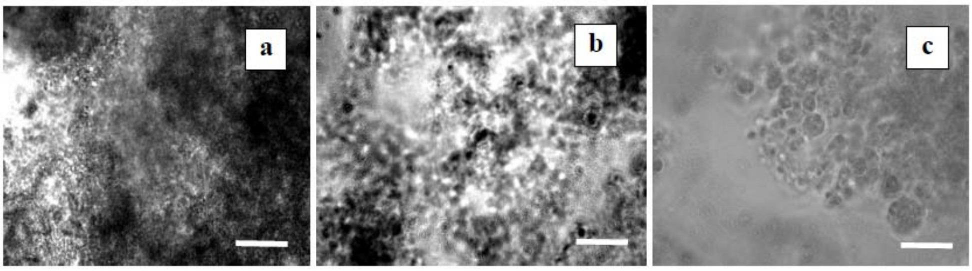

Fig 4.12 Morphological variation of the sludge during the experimental period in trial 3 (OLR=9 kg COD m^{-3} d^{-1}) (a) seed sludge at start-up (b) on 7th day (c) on 21st day (bar = 2 mm)

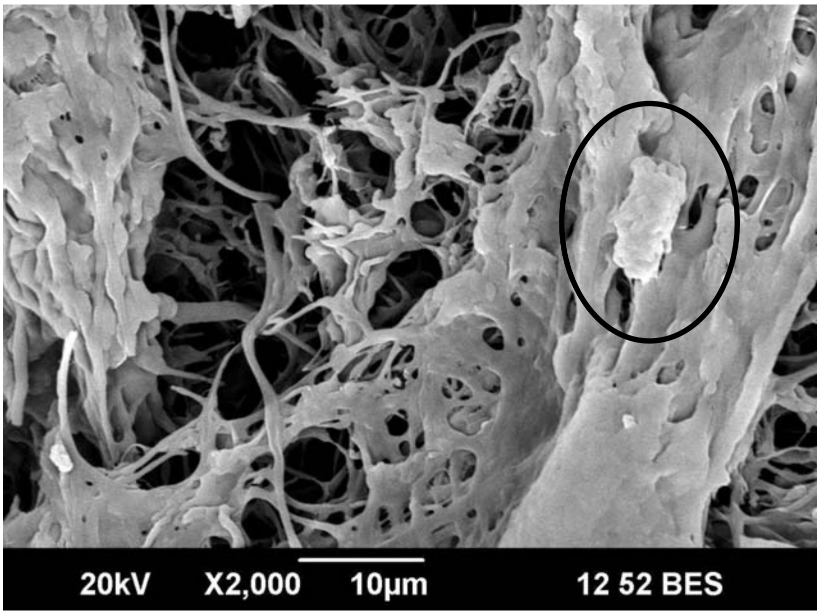

Fig. 4.13 Microstructure of aerobic granules developed in trial 2 (OLR=6 kg COD m^{-3} d^{-1}), observed by SEM at a magnification of 2000 on 14th day. Tiny cluster of cocci bacteria is marked by a circle.

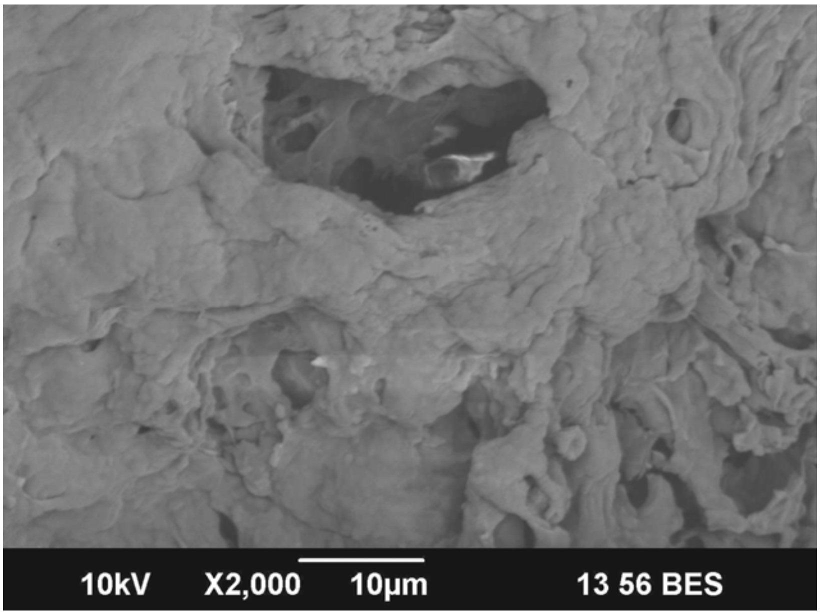

Fig 4.14 Microstructure of aerobic granules developed in trial 2 (OLR=6 kg COD m^{-3} d^{-1}), observed by SEM at a magnification of 2000 on 28th day

The previous studies on the influence of OLR showed varied results with respect to the granulation process and characteristics of the granules developed. Moy et al. (2002) cultivated aerobic granules feeding with glucose and acetate individually, applying gradual increase in OLRs from 6 to 9, 12 and 15 kg COD m^{-3} d^{-1} and found that acetate-fed granules could not sustain high OLRs and disintegrated when the OLR reached 9 kg COD m^{-3} d^{-1}. Even though granules of size range 2 - 4.2 mm were developed, these granules were disintegrated and washed out of the reactor within a week. The COD removal efficiency showed a decreasing trend as OLR was increased. These findings are comparable with the results of the present study. But SVI of the acetate fed granules was reported as improved, as the OLR increased from 6 to 9 kg COD m^{-3} d^{-1}. The SVI in the present study was more in trial 3 with 9 kg COD m^{-3} d^{-1} (25 ml g^{-1}) than that in trial 2 with 6 kg COD m^{-3} d^{-1} (38 ml g^{-1}).

Image analysis of the acetate-fed granules developed in the present study showed that the granules are a complex community dominated by spherical shaped bacteria. The glucose-fed granules developed by Moy et al. (2002) had a more filamentous structure and could sustain a still higher OLR of 15 kg COD m^{-3} d^{-1} successfully. At higher OLRs, the glucose-fed granules developed an irregular appearance characterized by folds, crevices and depressions. These irregularities allowed for shorter diffusion distances and better penetration of nutrients into the granule interior compared to spherical-shaped granules. Diffusion was also enhanced by the higher substrate concentration that existed in the bulk solution at higher OLRs. These factors probably enabled the glucose-fed granules to sustain the high OLR of 15 kg COD m^{-3} d^{-1}, eventhough they had a lower SVI and were more compact than the acetate-fed granules that disintegrated at the intermediate OLR of 9 kg COD m^{-3} d^{-1}.

When four different OLRs (1, 2, 4 and 8 kg COD m^{-3} d^{-1}) were attempted by Tay et al. (2004b), it was found that best performance was achieved in the reactor with OLR 4 kg COD m^{-3} d^{-1} with a COD removal rate of 99%, an observed yield coefficient (Y_{ob}) of 0.10 mg MLVSS mg^{-1} COD, and a sludge volume index 24 ml g^{-1} MLVSS. This study also supports the fact that optimal choice of OLR favours the cultivation and retention of well-settling granules and enhanced the overall ability of the reactor to remove COD.

Liu et al. (2003) reported that aerobic granule formation is independent of substrate concentration after experimenting with four different concentrations (500, 1000, 2000 and 3000 mg l^{-1}) of acetate. In all cases granules were successfully developed and the size of the granules showed a meager increase in size with increase in substrate concentration (1.57 mm to 1.89 mm). The variations in SVI and the specific gravity of sludge were very small. The COD removal efficiency was > 95%, being maximum (97 ±1.1%) in the trial with substrate concentration 2000 mg l^{-1}. In the present study also the COD removal efficiency with substrate concentration 2000 mg l^{-1} was 97.9%, but it fell to 89.3 % with substrate concentration 3000 mg l^{-1}. Zheng et al. (2006) also observed that the granules formed at high OLR (6 kg COD m^{-3} d^{-1}) were unstable because of the wild filamentous growth over the granules. Based on the SVI and microbial density, Kim et al. (2008) arrived at a low value of OLR (2.52 kg COD m^{-3} d^{-1}) as the optimum OLR for aerobic granulation.

Chen et al. (2008) attempted the feasibility of aerobic granulation under combined selection pressures of hydraulic shear force and substrate loading. It was concluded that the reactor could perform successfully at higher OLR of the order of 15 kg COD m^{-3} d^{-1} when hydraulic shear force also increased sufficiently (from 2.4 to 3.6 cm s^{-1}). In the present study, upflow air velocity was fixed as 3 cm s^{-1} in all the three trials. The best performance under the applied shear force was in trial 2, where the OLR was 6 kg COD m^{-3} d^{-1}. The performance with 9 kg COD m^{-3} d^{-1} would have been improved, if higher shear forces were applied. Thus it can be concluded that OLR is a decisive parameter for the aerobic granulation process, but its effects can be varied depending upon the shear force applied.

4.5 Influence of SUAV on Aerobic Granulation

Aerobic granulation in SBR can be highly influenced by hydrodynamic turbulence. Aeration is the sole source of hydrodynamic turbulence and hydraulic shear force. The air bubbles moving upward will create superficial upflow air velocity. The shear force caused by this upflow velocity is thought to be the main cause for the shaping and densification of granules. Three trials (trial 4, trial 2 and trial5) were conducted with SUAV 2 cm s^{-1}, 3 cm s^{-1} and 4 cm s^{-1} respectively. These velocities were achieved by admitting air flow at the rate of 4 l min^{-1}, 6 l min^{-1} and 8 l min^{-1} respectively. Sample calculation is shown in Annexure II. Throughout the three trials, the OLR was kept as 6 kg COD m^{-3} d^{-1} by feeding with the influent of COD concentration 2000 mg l^{-1}. The settling time was maintained as 5 min and the VER as 50 %.

4.5.1 Trial 4 (SUAV = 2 cm s^{-1})

It was noted that the MLSS and MLVSS growth rates were low in the beginning of the reactor run. A slight improvement was observed only after 3 weeks of operation. Then a steady increase was noted until the MLSS reached a value above 5500 mg l^{-1}. Observing constancy in MLSS and MLVSS values, the system was assumed to reach a steady state. At this stage the volatile fraction of the suspended solids was only 0.82 to 0.83 %. During an operation period of 36 days, the COD concentration could be reduced from 2000 mg l^{-1} to 124 mg l^{-1}, attaining a removal rate of 93.8 %.

Even after the operation for 2 weeks with upflow air velocity 2 cm s^{-1}, the sludge remained in flocculent form. Tiny granules were appeared in trial 4 on 21st day and grown gradually to an average size of 2.6 mm towards the end of operation. Eventhough the size of the granule was increased, it appeared to have a loose structure. The specific gravity and the settling velocity of the matured granules were measured as 1.005 and 38.4 m hr^{-1} respectively. The SVI was reduced to 40.2 ml g^{-1}.

4.5.2 Trial 2 (SUAV = 3 cm s^{-1})

Trial 2 was conducted to investigate the performance of the reactor under a shear force of 3 cm s^{-1} in terms of SUAV. Faster granulation was observed in this trial. Performance of the trial in terms of MLSS growth, COD removal, sludge settleability and granule formation has been described in Section 4.4.2.

4.5.3 Trial 5 (SUAV = 4 cm s^{-1})

As the next phase to investigate the influence of SUAV on aerobic granulation, the air flow was increased to 8 l min^{-1} in the next run, so that the shear force was 4 cm s^{-1}. The suspended solids and volatile suspended solids were dropped down during the first week, but good growth rate was observed in the second week. After 4 weeks of operation MLSS and MLVSS values overtook the corresponding values in trial 4 and trial 2. Observing consistent values for 4-5 days, the trial run was stopped on day 36. Volatile fraction of the solids was 0.91 at that time. A good reduction in the effluent COD was noted (97.1%) with effluent quality <60 mg l^{-1} COD.

Among the three trials to study the effect of shear force, earliest appearance and development of aerobic granules were in trial 5, which run at the maximum air flow. Granules were visible first on day 14 and appeared to be matured by day 22. They grew to an average size of 1.5 mm with a specific gravity of 1.008. At this stage the settling velocity was measured as 63.6 m hr^{-1}. A very good SVI of 24 ml g^{-1} showed the excellent settleability of the granular sludge formed.

The daily variations in effluent COD, COD removal efficiency, MLSS, MLVSS, MLVSS/MLSS ratio, and SVI with time at different SUAV (trial 4, 2 and 5) are shown in Fig 4.15 to 4.20. The variation of COD removal efficiency, MLSS & MLVSS, MLVSS/MLSS and SVI with respect to SUAV are shown in Fig 4.21 to 4.24

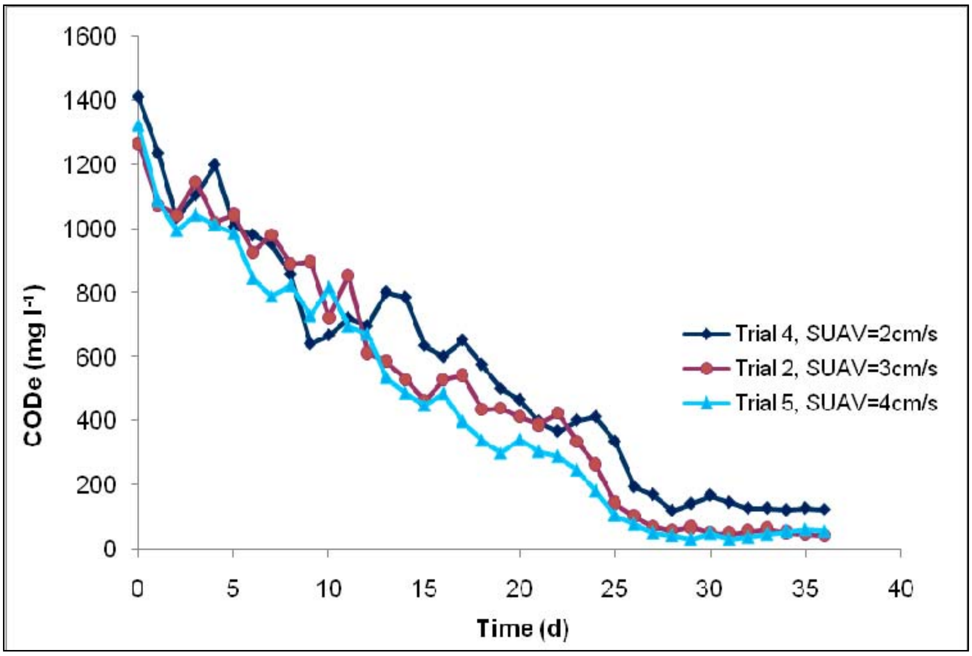

Fig 4.15 Variation of COD$_e$ at different shear forces (SUAV 2 - 4 cm s^{-1}) with time

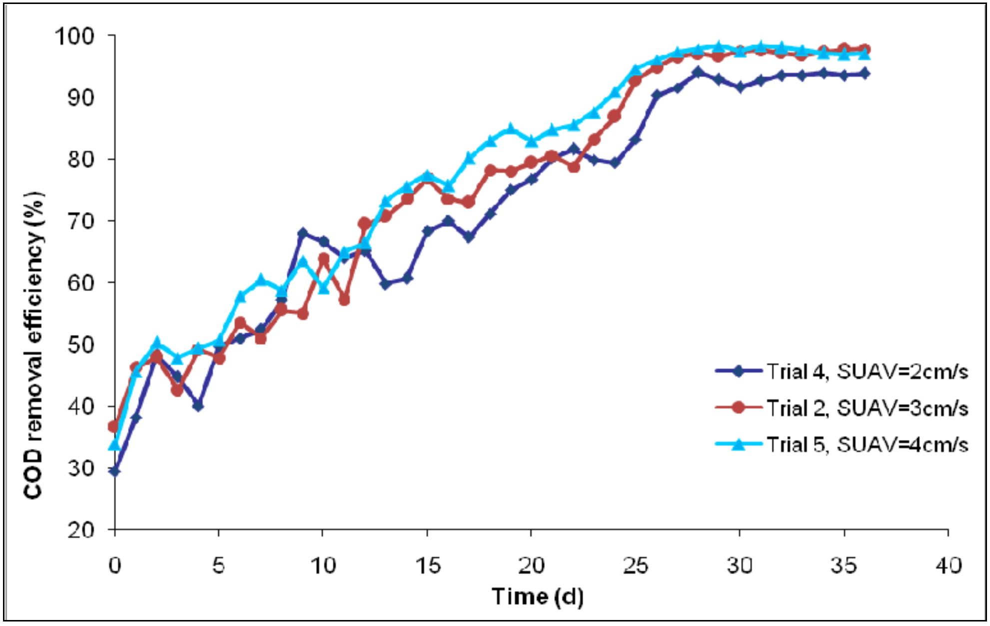

Fig 4.16 Variation of COD removal at different shear forces (SUAV 2 - 4 cm s^{-1}) with time

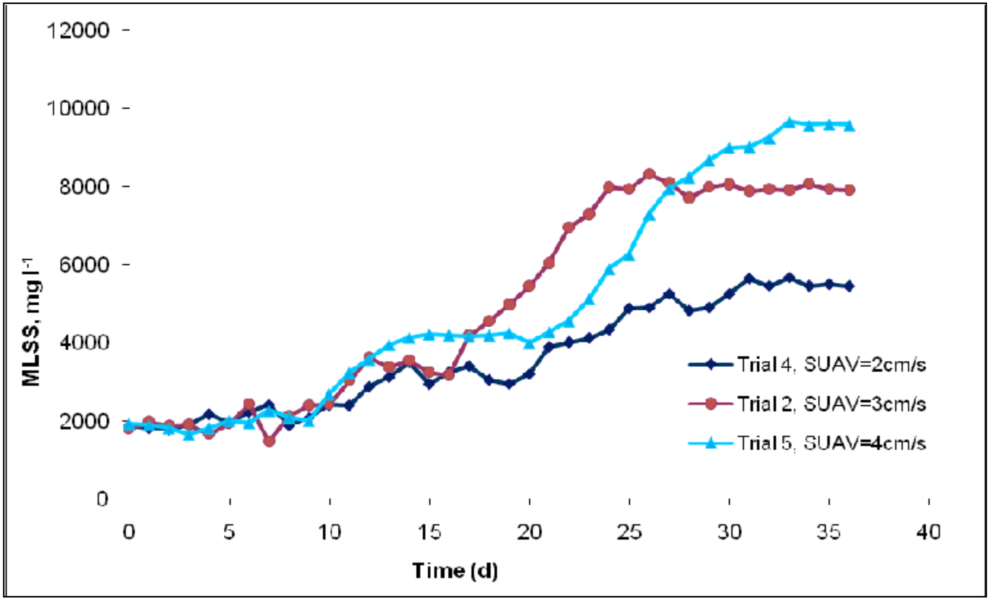

Fig 4.17 Variation of MLSS at different shear forces (SUAV 2 - 4 cm s^{-1}) with time

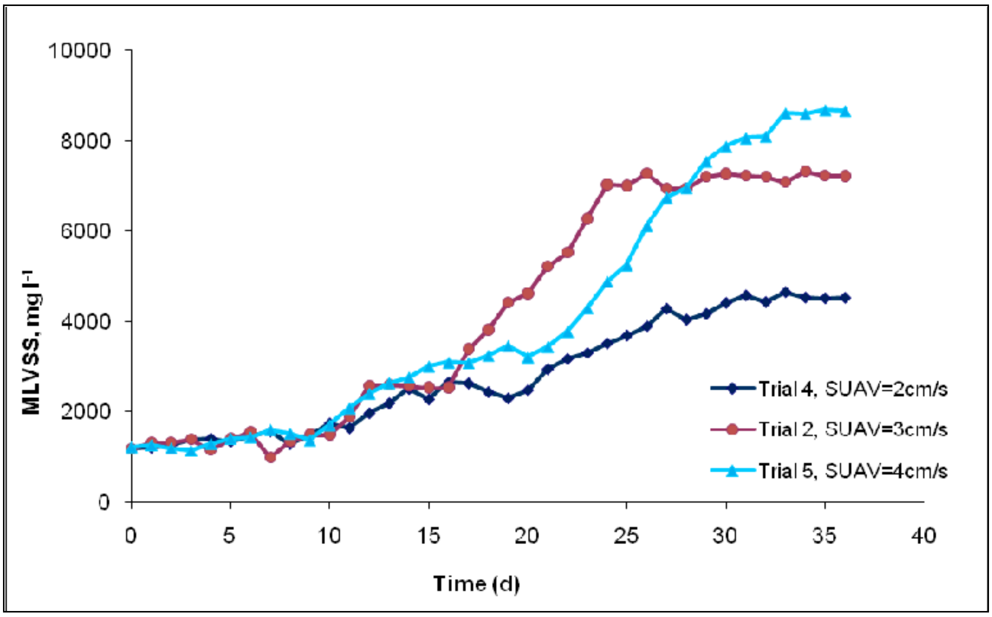

Fig 4.18 Variation of MLVSS at different shear forces (SUAV 2 - 4 cm s^{-1}) with time

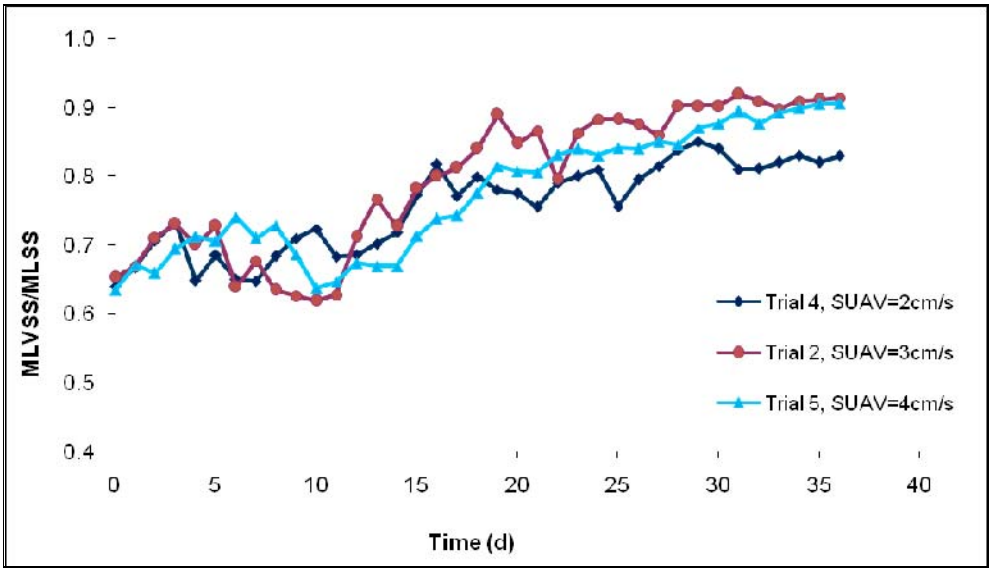

Fig 4.19 Variation of COD_e at different shear forces (SUAV 2 - 4 cm s^{-1}) with time

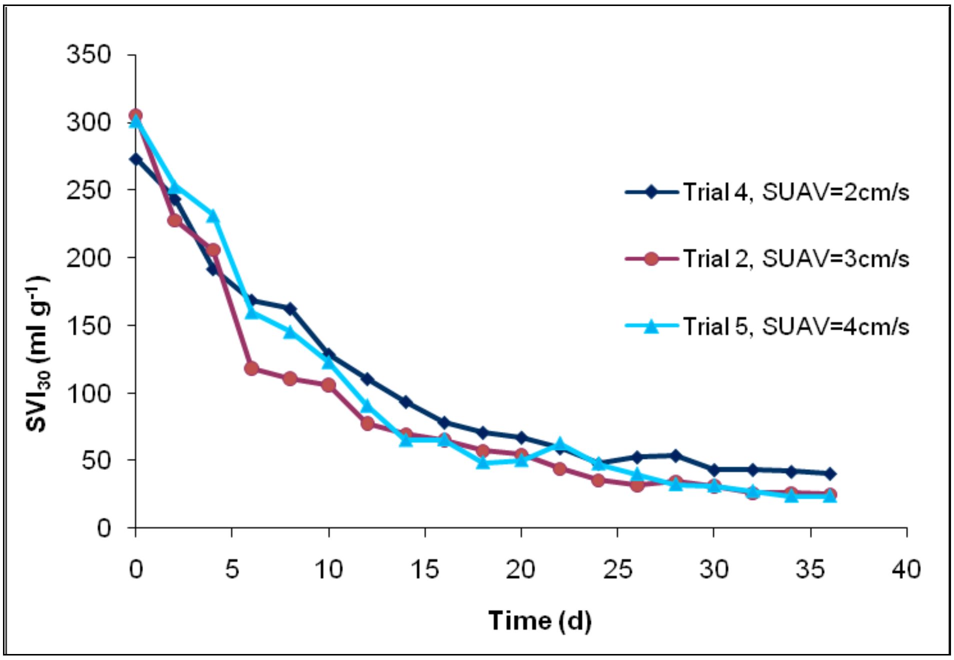

Fig 4.20 Variation of SVI_{30} at different shear forces (SUAV 2 - 4 cm s^{-1}) with time

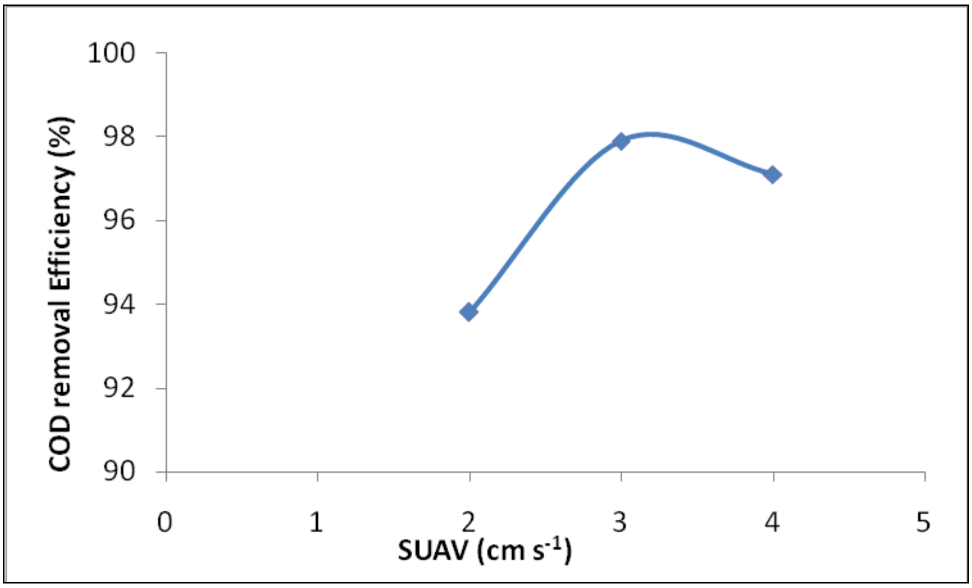

Fig. 4.21 Variation of CO D removal efficiency with SUAV

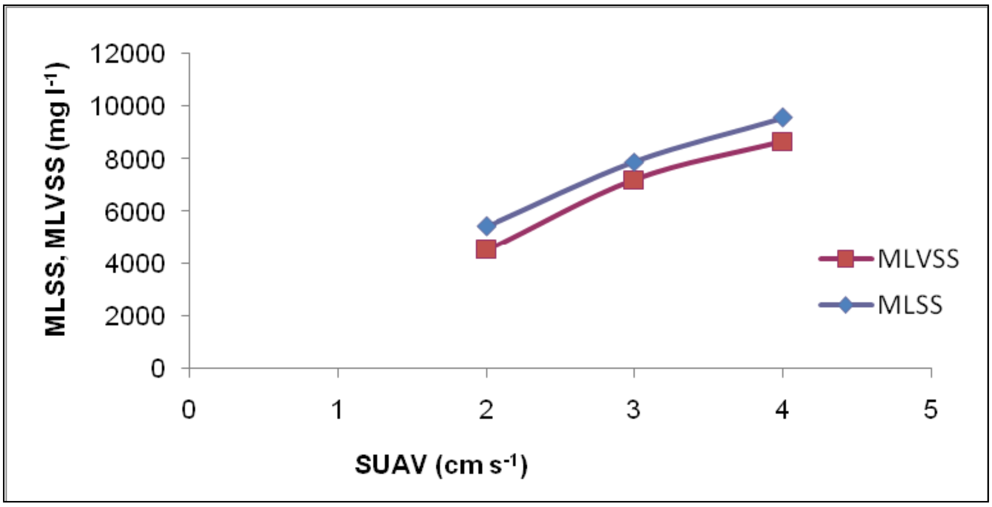

Fig. 4.22 Variation of MLSS & MLVSS with SUAV

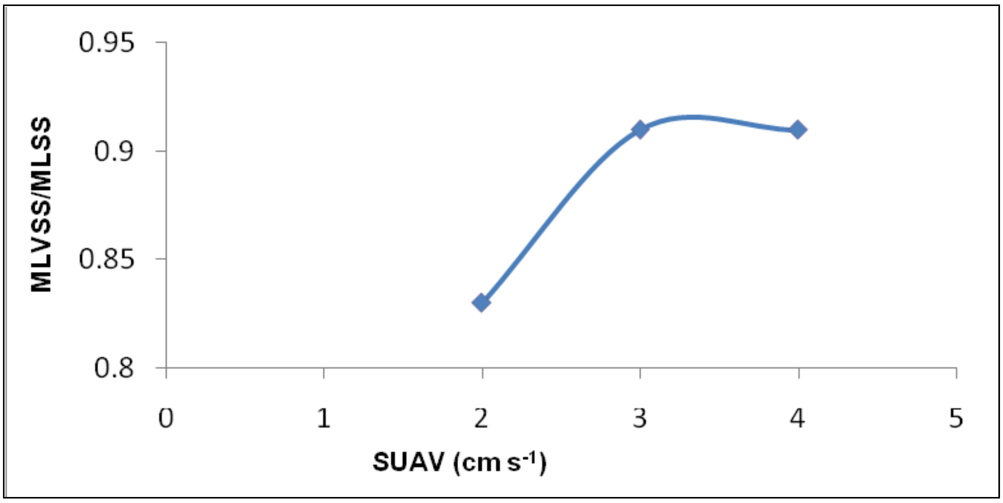

Fig. 4.23 Variation of MLVSS/MLSS with SUAV

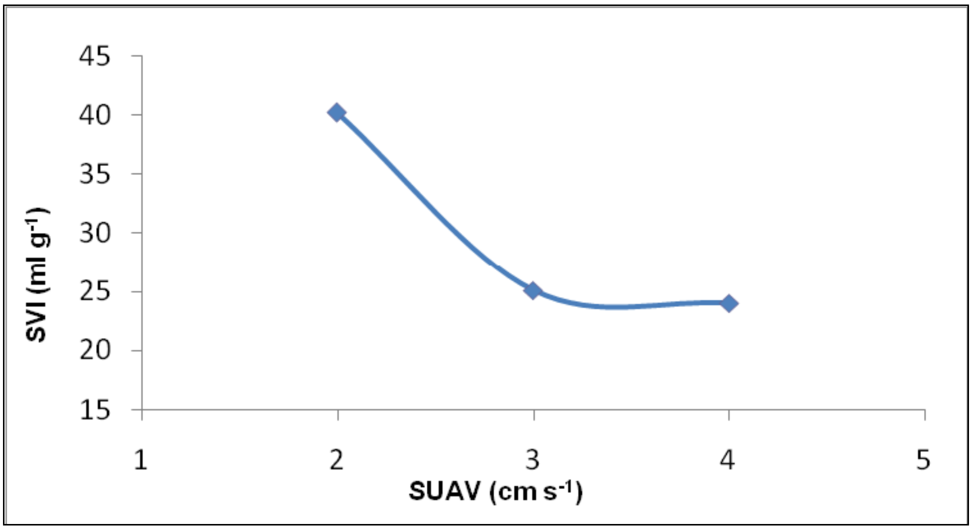

Fig. 4.24 Variation of SVI with SUAV

Images of granular sludge formed under various shear forces (trial 4, 2 and 5), on 35th day are shown in Fig. 4.25 (a-c). Fig. 4.26 (a-b) also shows a comparative picture of granules developed at low (2 cm s^{-1}) and high (3 cm s^{-1}) SUAVs. The lack of a smooth outer surface and shape for granules at low shear force (trial 4) is clearly seen in both figures. The compactness of such granules was much lower than that of the granules developed at higher shear forces.

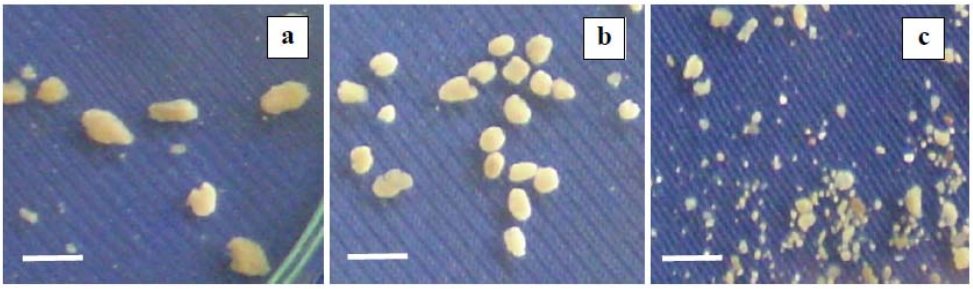

Fig.4.25. Granular sludge formed under various shear forces, on 35th day (a) trial 4 (b) trial 2 (c) trial 5 (bar = 5 mm)

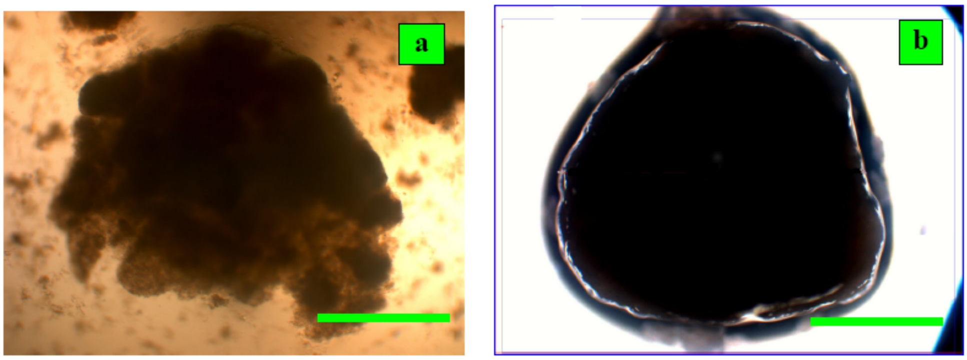

Fig. 4. 26 Granular sludge formed in (a) trial 4 (SUAV=2 cm s^{-1}) (b) trial 2 (SUAV=3 cm s^{-1}) (bar = 1 mm)

The performance of the SBR shows that the development of biomass is efficient only with higher upflow air velocities. But Chen et al. (2008) in an attempt to study the effect of combined hydraulic and loading selection pressures got results contradicting the above statement. The results showed higher biomass 7.16 ± 0.93 mg l^{-1} at lower shear force (SUAV = 2.4 cm s^{-1}) and lower biomass 4.69 ± 0.42 mg l^{-1} at higher shear force (SUAV = 3.2 cm s^{-1}) at same OLR of 6 kg m^{-3} d^{-1}.

The experimental results showed that the size of the granule decreased with increase in shear force, but the specific gravity of the granule was found increased with increasing shear force. The specific gravity of sludge represents the compactness of a microbial community. More compact and dense granules were formed at higher upflow velocity. Similar results have been reported in aerobic granulation in SBRs (Tay et al., 2001b, Tay et al., 2002d; Chen et al., 2007).

In these studies, it was reported that the production of more compact and dense granules was because of the stimulated production of extracellular polysaccharides (EPS) at higher shear force. EPS is a sticky material secreted by bacterium which constitutes of proteins, polysaccharides, humic acids and lipids. It is clear that extracellular polysaccharides can mediate both cohesion and adhesion of cells and play a crucial role in maintaining structural integrity of a community of immobilized cells (Liu et al., 2004b, 2005b). But the exact mechanism by which cell to cell communication is happened, and granulation is initiated and developed is still under research. The microstructure of the granules developed in trial 2, observed by scanning electron microscope (SEM) on 14th day and 28th day is shown in Fig 4.13 to 4.14. It is evident from the figure that EPS dominant structure developed in 2 weeks had turned to microbial rich biomass after 4 weeks.

The influence of hydrodynamic shear force on the production of EPS and the structure of granules can be understood by the SEM images, taken at various magnifications (Fig 4.27 to 4.29). All images were taken in the third week of operation. As per the Fig. 4.27 it is clear that the development of EPS and the microbial density were poor in the case of trial 4 (SUAV = 2 cm s^{-1}). Such a low shear force is not sufficient to form compact granules. Fig 4.28 and Fig. 4.29 show the ample production of EPS, which can influence the structural integrity of the granules in trial 2 (SUAV = 3 cm s^{-1}) and 5 (SUAV = 4 cm s^{-1}) respectively.

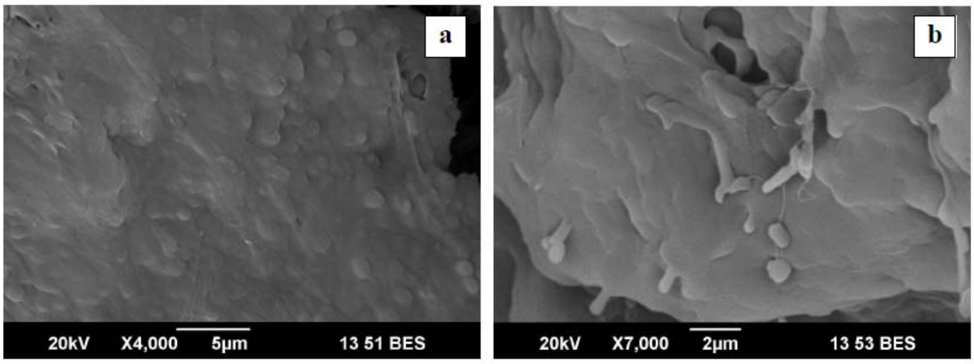

Fig. 4.27 Microstructure of aerobic granules developed in trial 4 (SUAV=2 cm s^{-1}), observed by SEM at a magnification of (a) 4000 and (b) 7000

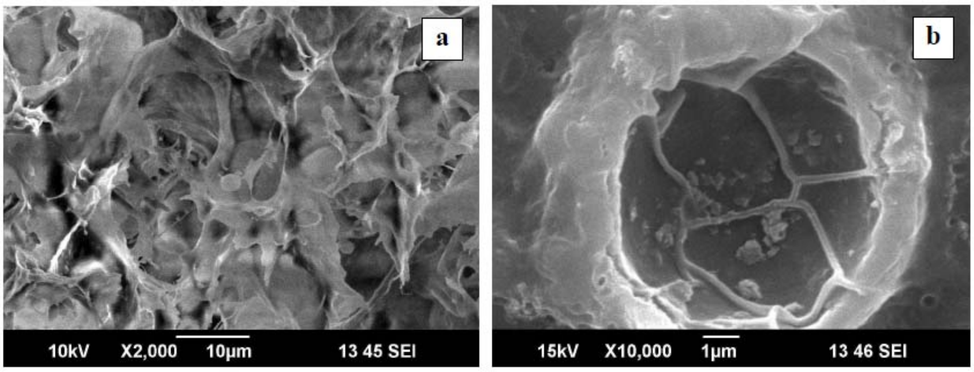

Fig. 4.28 Microstructure of aerobic granules developed in trial 2 (SUAV=3 cm s^{-1}), observed by SEM at a magnification of (a) 2000 and (b) 10000

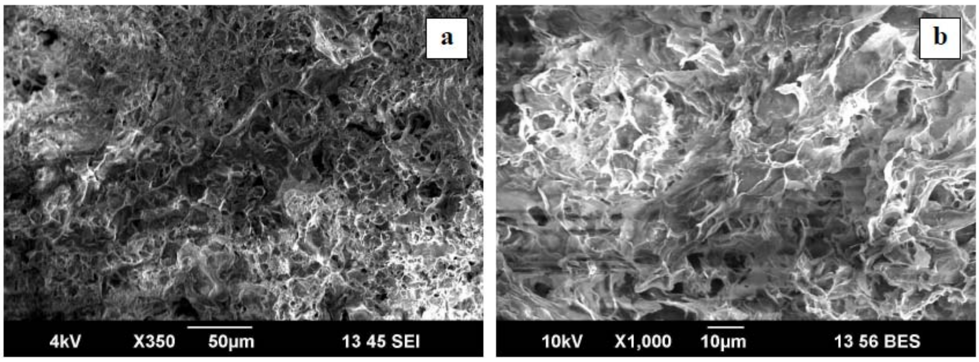

Fig. 4.29 Microstructure of aerobic granules developed in trial 5 (SUAV=4 cm s^{-1}), observed by SEM at a magnification of (a) 350 and (b) 1000

Efficiency of a wastewater treatment system depends on the pollutant removal potential. In the present study the COD removal efficiency when the SUAV is 4 cm s^{-1} is 97.1% only, while it was about 97.9% at a lower SUAV (3 cm s^{-1}). A higher shear force favoured the development of more dense and compact granules at an earlier date, but increase in COD removal efficiency was not in proportional to the increase in shear force. The researchers pointed out that despite their low density and poor settling properties, the granules developed at low shear force show better pollutant degradation performance than dense and compact granules because of their low mass transfer resistance (Ji et al., 2010). As per the results of the present study, it can be concluded that optimum performance was observed in trial 2 with shear force in terms of SUAV 3 cm s^{-1}. Even though specific gravity and the settling velocity were more when shear force was 4 cm s^{-1}, it cannot be recommended in real situations due to the higher energy investment involved. Hence, amongst the various shear forces attempted in the present study, a shear force in terms of SUAV 3 cm s^{-1} is considered as optimal for the performance of aerobic granulation.

A higher shear force favours better compactness and better settling velocity, which results in a lower SVI. COD removal efficiency also is less at lower shear forces. The high value of shear force required in terms of upflow air velocity results in high energy consumption. For example, 400 m^3 of air should be supplied per kilogram of COD removed, with an OLR of 6 kg COD m^{-3} d^{-1} and upflow air velocity of 2.4 cm s^{-1}. This is very high as compared to the air requirement of 20 to 50 kg COD m^{-3} d^{-1} for conventional activated sludge process (Liu, 2008). This means that the operation cost for aeration in an aerobic granular sludge reactor would be several times higher than that of a conventional activated sludge process unit. To overcome the high operation cost, effective counter measures have to be adopted in aerobic granular sludge reactors. Along with efficiency, economy also should be considered for the selection of optimum hydraulic shear force. Research has to be advanced to optimize the air supply for the minimum requirement of shear force.

4.6 Influence of Settling Time on Aerobic Granulation

So far aerobic granules have been cultivated in SBR. The speciality of SBR is its cyclic nature of operation. Filling, aeration, settling and discharge are the steps involved in any SBR. A single unit can act as an aeration tank and settling tank, hence no separate settling devises are required. Thus an SBR operates sequentially in time, rather than in space. Settling time can select the particles which can be retained in the reactor, thereby control the nature of the sludge particles. Experimental values of 10 min, 5 min, and 3 min were attempted to study the influence of settling time. Three trials were conducted with settling time as 3 min (trial 6), 5 min (trial 2) and 10 min (trial 7). In all these attempts, OLR, SUAV and VER were kept constant as 6 kg COD m^{-3} d^{-1}, 3 cm s^{-1} and 50% respectively.

4.6.1 Trial 6 (ST = 3 min)

The trial run 6 was started with settling time as 3 min. The feeding time, aeration time and discharge time were set as 5 min, 227 min and 5 min respectively. During the initial period, a considerable reduction in MLSS and MLVSS values were observed. Starting with a suspended solids concentration of 1930 mg l^{-1}, the system could not control the COD removal for a period of two weeks. Within a settling time of 3 min, only sufficiently dense particles could fall to a height lower than the level of the discharging port (middle height). Naturally a severe wash out of the loose structured flocculent sludge particles was observed, which resulted in a reduction of the retained solids. Even on 15th day of operation the MLSS and MLVSS were <1400 mg l^{-1} and <1000 mg l^{-1} respectively. For the next three weeks, a marked improvement in the solids concentration was observed, which showed healthy conditions in the reactor. When the values became almost steady for five consecutive days (10000 mg l^{-1} for MLSS and 9000 mg l^{-1} for MLVSS), the trial run was assumed to reach the steady state and hence stopped on the 40th day.

The severe wash-out of the sludge particles during the initial two weeks was reflected in the effluent COD also. The COD_e could not be reduced well below 1000 mg l^{-1} which yields COD removal efficiency less than 50% only. Since then, a noted improvement in the COD removal was observed which reached an average value of 97.8% during the last week of operation.

Granules which appeared on day 19 were grown to an average size of 2.1 mm during the last week of operation. The image analysis showed that these granules have a dense and compact structure, which was also supported by the specific gravity value (1.011). Excellent settling velocity of 72 m hr^{-1}, was shown by the granular sludge. The SVI at the steady state of trial 6 was estimated as 23 ml g^{-1}.

4.6.2 Trial 2 (ST = 5 min)

Trial 2 was conducted to investigate the performance of the reactor under a settling time of 5 min. The suspended solids concentration reached a value of 7900 mg l^{-1} with 91% of volatile solids at the steady state. The trial resulted in a good COD removal (97.9 %) and SVI (25 ml g^{-1}). Performance of the trial in terms of MLSS growth, COD removal, sludge settleability and characteristics of the granules has been described in Section 4.4.2.

4.6.3. Trial 7 (ST = 10 min)

In trial 7, a longer settling time of 10 min was experimented. Feeding time and discharge time were kept as 5 min each and aeration time was adjusted to 220 min so that the total cycle time would be 4 hours. Both MLSS and MLVSS were found to increase since the beginning of the trial run, which may be due to a longer settling time. Even flocculent and light-weight particles were retained in the reactor because of the longer settling time. The solids content continued to increase for the first two weeks, thereafter it did not improve, but slightly decreased and maintained a low MLSS value between 4000 - 44000 mg l^{-1}. From day 35 onwards, wash-out of the sludge was observed, and the system led to unhealthy conditions.

The rate of growth of COD removal efficiency was good during the first four weeks, but it was reduced considerably in the later days of operation.

Tiny, but not dense granules were developed in the reactor by day 28. They grew in size to reach an average diameter of 1.1 mm by the end of operation. Physical properties of these granules showed that they were not compact, with a specific gravity of 1.004. Poor settling velocity (31.2 m hr^{-1}) and low SVI (75 ml g^{-1}) of the granules showed that long settling time does not favour aerobic granulation.

The daily variations in effluent COD, COD removal efficiency, MLSS, MLVSS, MLVSS/MLSS ratio, and SVI with time at different settling times (3 min, 5 min, 10 min) are shown in Fig 4.30 to 4.35. COD removal was comparable in trials 2 and 6 (with settling times 5 and 3 min) with values 97.9% and 97.8% respectively. But it was only 90.1% at the end of operation in trial 7 with 10 min settling time.

The values of COD removal efficiency, MLSS & MLVSS, MLVSS/MLSS ratio, and SVI at the steady state with settling time are shown in Fig. 4.36 to 4.39.

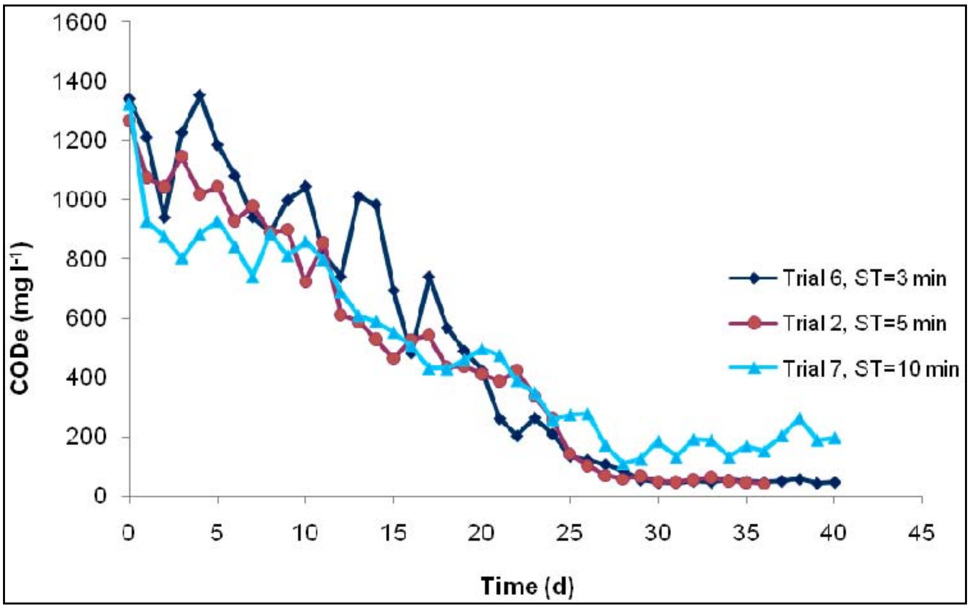

Fig 4.30 Variation of COD_e at different Settling times (3 – 10 min) with time

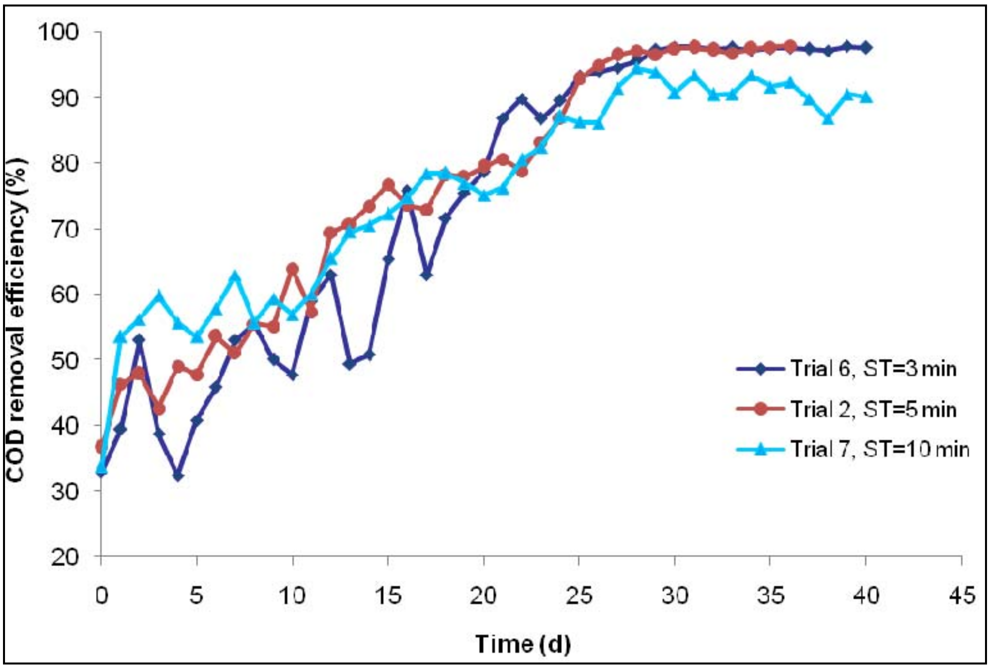

Fig 4.31 Variation of COD removal at different Settling times (3 – 10 min) with time

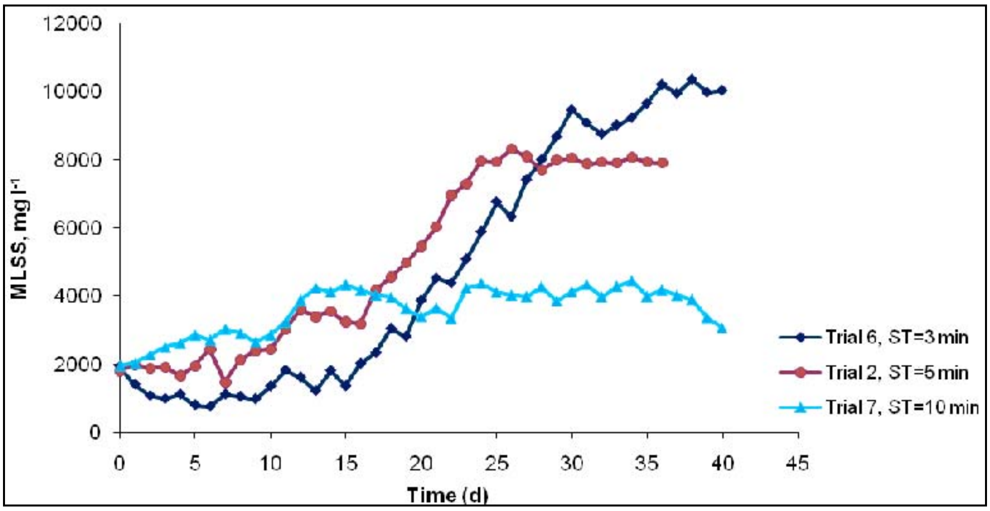

Fig 4.32 Variation of MLSS at different Settling times (3 – 10 min) with time

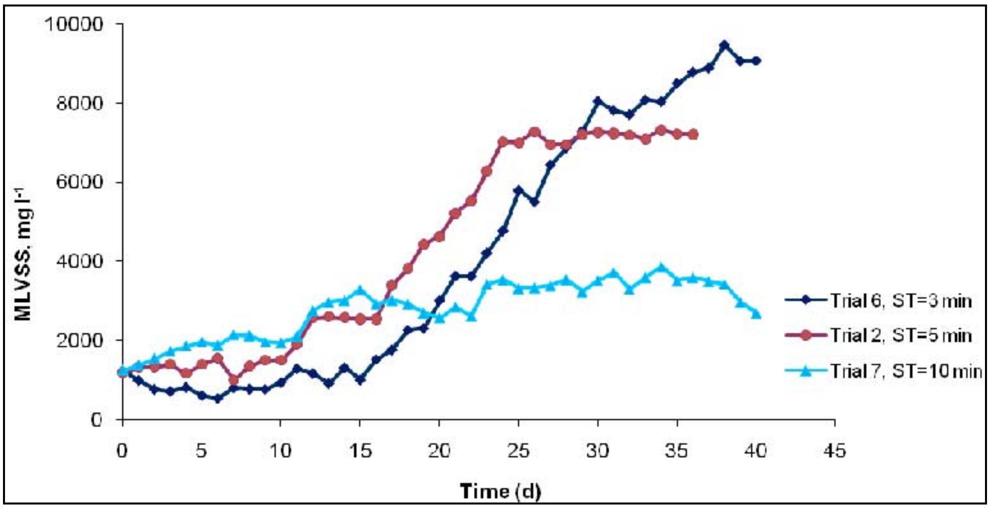

Fig 4.33 Variation of MLVSS at different Settling times (3 – 10 min) with time

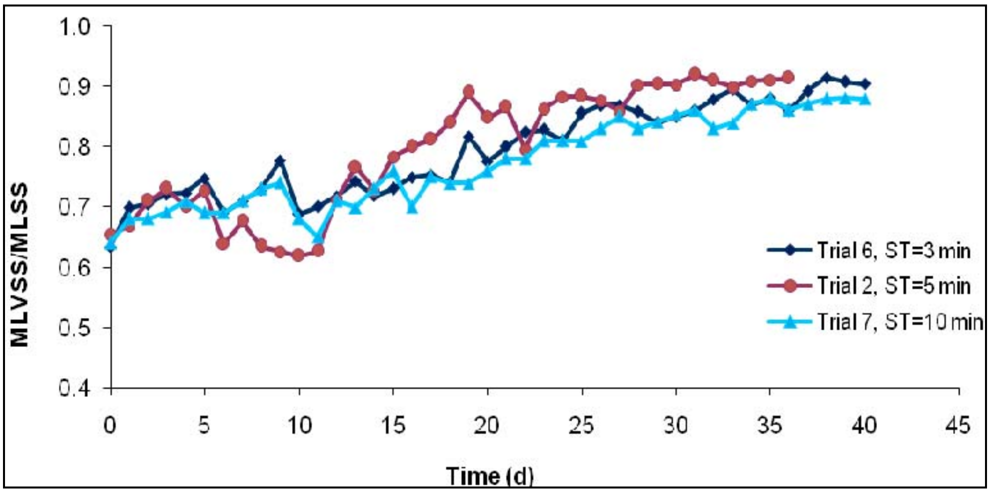

Fig 4.34 Variation of MLVSS/MLSS at different Settling times (3 – 10 min) with time

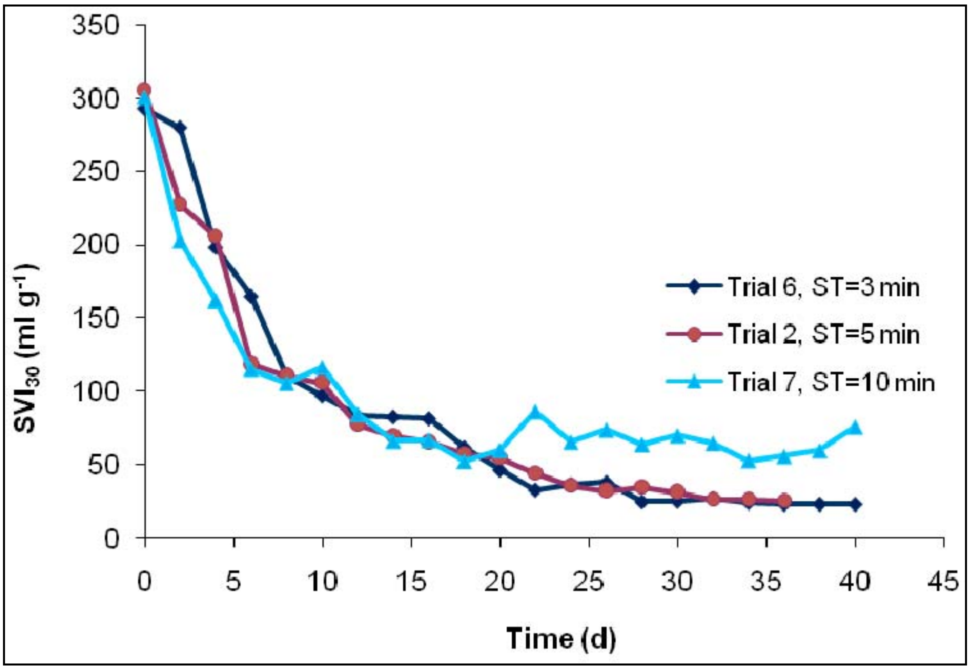

Fig 4.35 Variation of SVI_{30} at different Settling times (3 – 10 min) with time

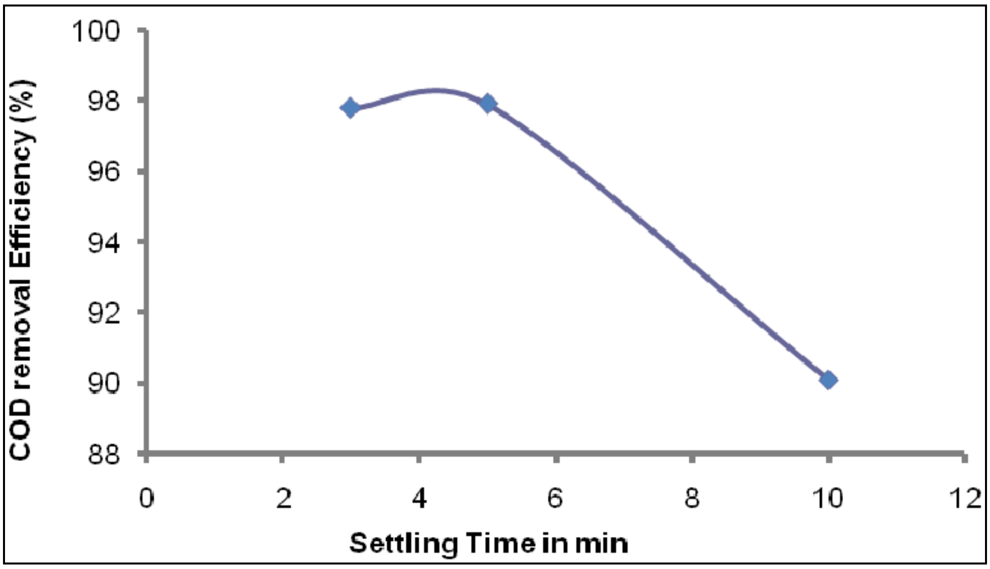

Fig. 4.36 Variation of COD removal efficiency with settling time

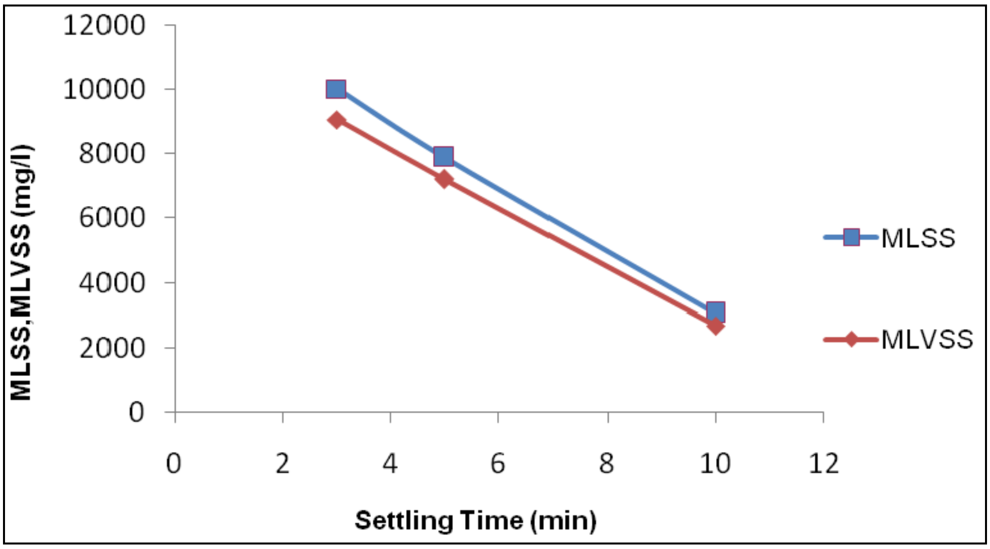

Fig. 4.37 Variation of MLSS & MLVSS with settling time

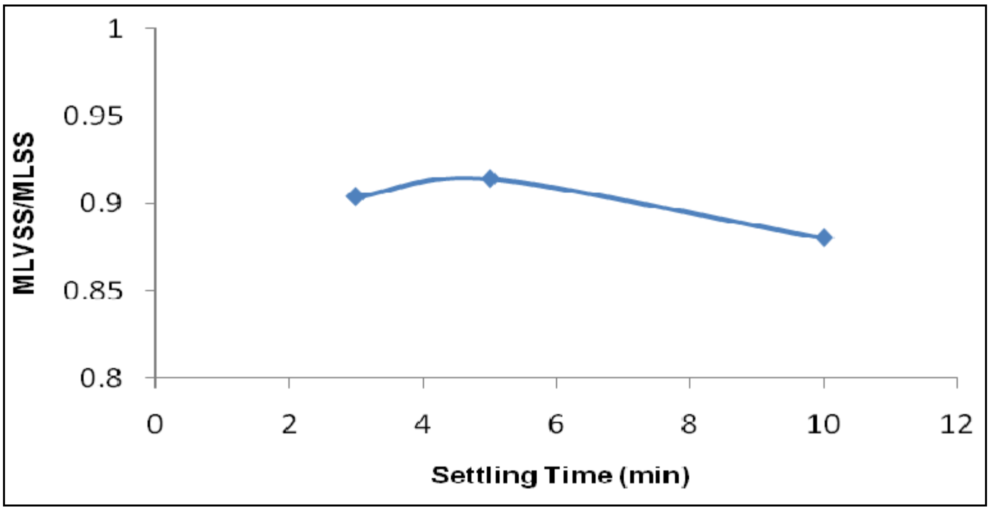

Fig. 4.38 Variation of MLVSS/MLSS with settling time

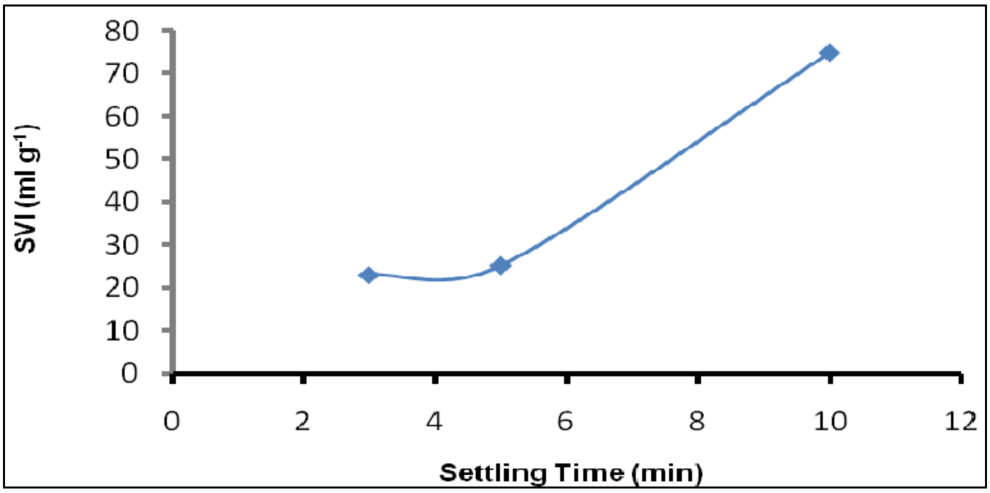

Fig. 4.39 Variation of SVI_{30} with settling time

In these attempts to investigate the influence of settling time on aerobic granulation, it was noticed that the trial 6 experienced the lowest concentrations in suspended solids during the start-up period. Obviously it can be correlated to the wash out of the loose-structured sludge particles due to the low settling time. This selection process which lasted for about 2 weeks helped the system to retain heavier particles, which in turn resulted in an early formation of dense compact granules. This caused a rapid growth of MLSS in the reactor and reached the maximum value in the three trials. The minimum SVI value obtained in trial with 3 min settling time supports the fact that a short settling time favours aerobic granulation. Specific gravity of granules developed with 3 min settling time and 10 min settling time showed variation from 1.011 to 1.004. This is clear in the images of the granules developed in trial 6 and trial 7 as shown in Fig. 4.40 (a-b). Fig. 4.40(a) shows a typical granule formed when a settling time of 3 min was attempted. The granule appeared to have a dense and compact structure with a clear outer boundary. Typical granule developed when the settling time was 10 min, is shown in Fig. 4.40(b). The loose and fluffy nature of the granular sludge is clear from the figure.

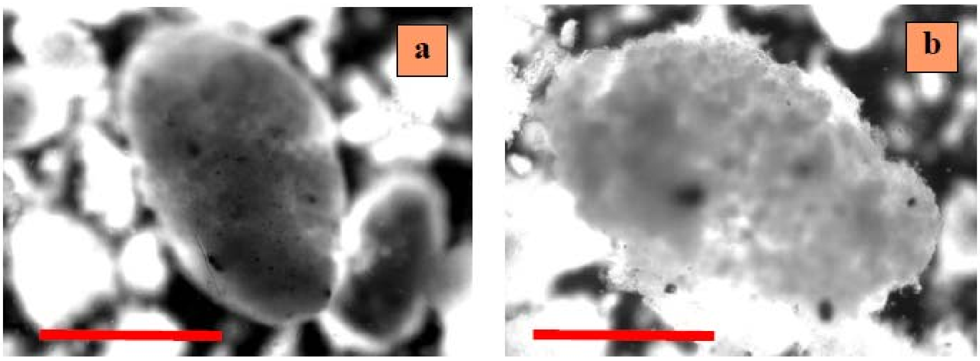

Fig. 4.40 Images of aerobic granules developed (a) in trial 6 (settling time=3min) and (b) in trial 7 (settling time=10 min) (bar = 1 mm)

When Qin et al. (2004b) operated four SBRs with settling times 5, 10, 15 and 20 minutes, fastest development of granules and best performance in terms of sludge settleability (measured as SVI), cell surface hydrophobicity and microbial activity were observed in reactor with lowest settling time of 5 minutes. The cell surface hydrophobicity was found to be inversely proportional to settling time. It was found from the study that SVI is closely related to settling time. SVI around 50 ml g^{-1} and 75 ml g^{-1} were obtained in that study for settling time 5 min and 10 min respectively. In the present study also, the same trend in SVI was obtained.

Mc Swain et al. (2004) studied the effect of settling time on aerobic granulation by applying two different values of 2 min and 10 min, which also showed better results with shorter settling time. Eventhough granules could be developed in both cases, complete granular sludge was formed in reactor with settling time 2 min. It was concluded that the initial mass wash-out and continuous removal of flocs affect species selection during start-up and produce a less diverse but more stable population. In the present study a better SVI (23 ml g^{-1}) was obtained with 3 min, than that obtained (47 ml g^{-1}) with 2 min by Mc Swain et al. (2004). Studies by Adav et al. (2009) also showed similar results when operated with settling times of 10, 7 and 5 min. Comparing the effects of settling times of longer duration (10 min and 20 min), it was found that there was not much variations in the SVI and COD removal (Miao et al., 2011). It can be concluded that considering economy and efficiency, shorter settling times (2-5 min) may be adopted for successful aerobic granulation in sequencing batch reactors.

It can be concluded that a short settling favours faster selection of settleable particles in a column reactor. Thus settling time was proved to be a strong selection pressure in aerobic granulation process. A shorter settling time helps to make the wastewater treatment efficient and economical.

4.7 Influence of VER on Aerobic Granulation

In a column type SBR, VER is the ratio of distance to the discharging port from the top level of the solution to the effective height of the column. In other words, similar to settling time, VER also can decide the particles to be retained in the reactor, based on the minimum velocity concept which was explained in Section 2.6.14. Three values for VER were tried in trial 8 (VER = 25%), trial 2 (VER = 50%) and trial 9 (VER = 75%). In all these trials, OLR (6 kg COD m^{-3} d^{-1}), SUAV (3 cm s^{-1}) and settling time (5 min) were kept constant.

4.7.1 Trial 8 (VER = 25%)

Trial run 8 was started by feeding the influent at the bottom and withdrawing the effluent through the port set at quarter height from the top of the reactor, making the VER 25%. Starting with a suspended solids concentration of about 1900 mg l^{-1}, the system showed a steady growth in solids concentration till day 17. On day 21, minute granules first appeared in the reactor. A more steady growth in MLSS was observed after the formation of granules. It continued till day 31, and maintained a value of 8200 ± 50 for the next 5 days. Hence the system was assumed to attain a steady state and stopped operation on day 36. At this stage the MLVSS concentration was 7200 ± 50, with a MLVSS/MLSS ratio of 0.88.

The effluent COD showed a very good reduction during the initial period, and then the reduction rate decreased. By the end of operation the COD removal reached a value of 94.9 %. The granules formed on day 21 grew to an average size of 0.9 mm only, with a specific gravity of 1.004. Eventhough a fairly good COD removal was achieved at the steady state, the settleability of the sludge was not good. The SVI at this stage was 40 ml g^{-1} and the settling velocity of the grown-up granules was 34.8 m hr^{-1}.

4.7.2 Trial 2 (VER = 50%)

A VER of 50 % was attempted in trial 2. Compared to the trial with VER 25%, trial 2 showed a reduced growth rate during the initial period, but the growth rate was much better after day 16. A better COD removal and a better granulation were observed with 50% VER. Detailed performance of the trial in terms of MLSS growth, COD removal, sludge settleability and characteristics of the granules has been described in Section 4.4.2.

4.7.3 Trial 9 (VER = 75 %)

A higher VER of 75 % was applied in trial 9. This was achieved by withdrawing the effluent from the port set at the 3/4th height (from top) of the reactor. A severe wash-out of sludge was observed during the first week. A good fraction of the light flocculent particles escaped from the reactor as they could not reach the discharge port within the specified settling time (5 min). After first week, a gradual increase in the solids content was observed. By day 14, tiny granules appeared in the reactor and they were seemed to be matured in the 4th week. Eventhough the MLSS content was not grown beyond 5300 mg l^{-1}, the retained granules were of good specific gravity of 1.0075. A good ratio of MLVSS to MLSS (0.93) was achieved in the system.

The average value of COD removal was 98.5% during the steady state. The SVI was dropped to 28 ml g^{-1}. Settling velocity of the matured granules was measured as 66 m hr^{-1}.

The daily variations in effluent COD, COD removal efficiency, MLSS, MLVSS, MLVSS/MLSS ratio, and SVI with time at different VER (25%, 50% and 75%) are shown in Fig 4.41 to 4.46.

The variations of COD removal efficiency, MLSS & MLVSS, MLVSS/MLSS ratio, and SVI with settling time are shown in Fig. 4.47 to 4.50 which are self explanatory.

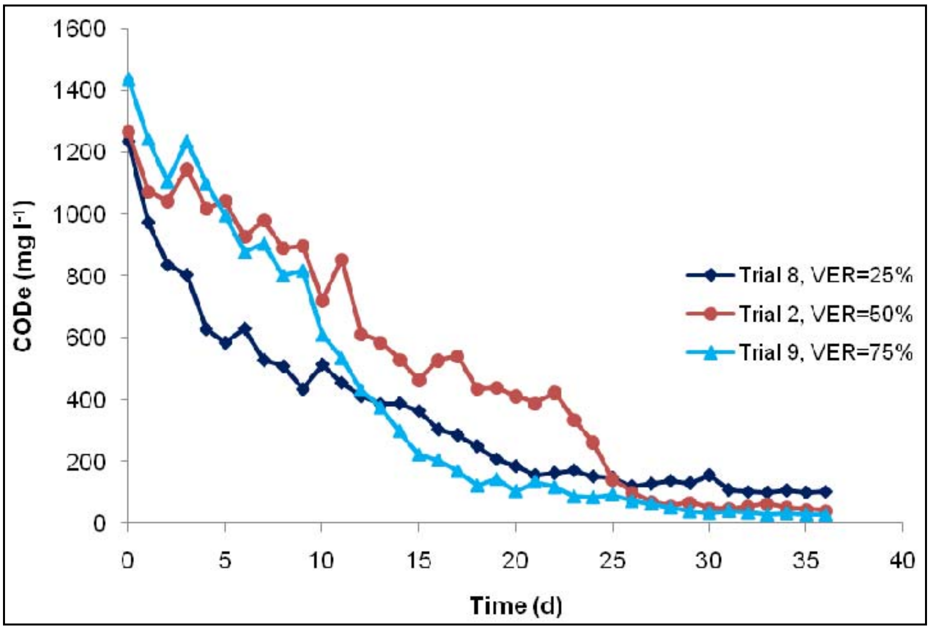

Fig 4.41 Variation of COD_e at different VER (25 – 75 %) with time

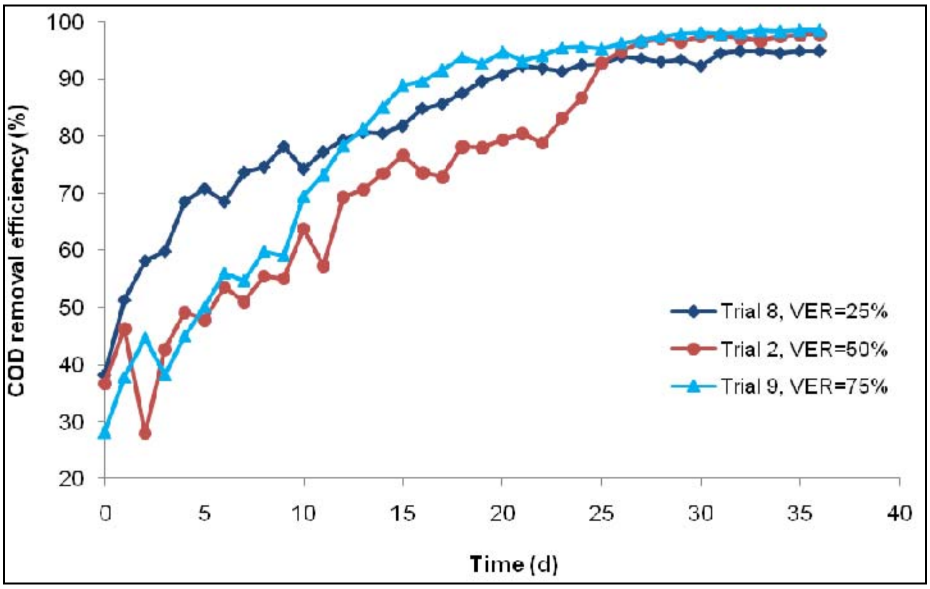

Fig 4.42 Variation of COD removal at different VER (25 – 75 %) with time

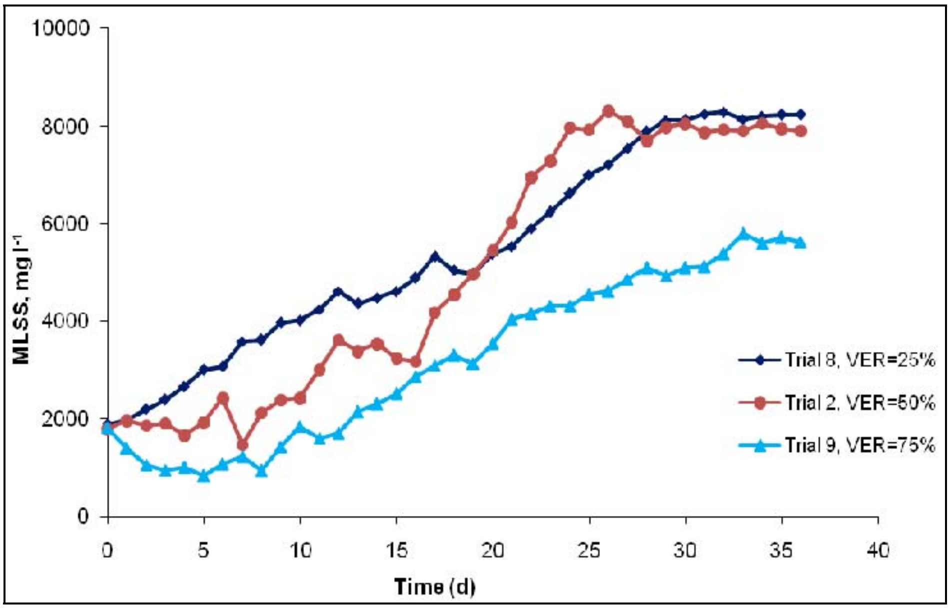

Fig 4.43 Variation of MLSS at different VER (25 – 75 %) with time

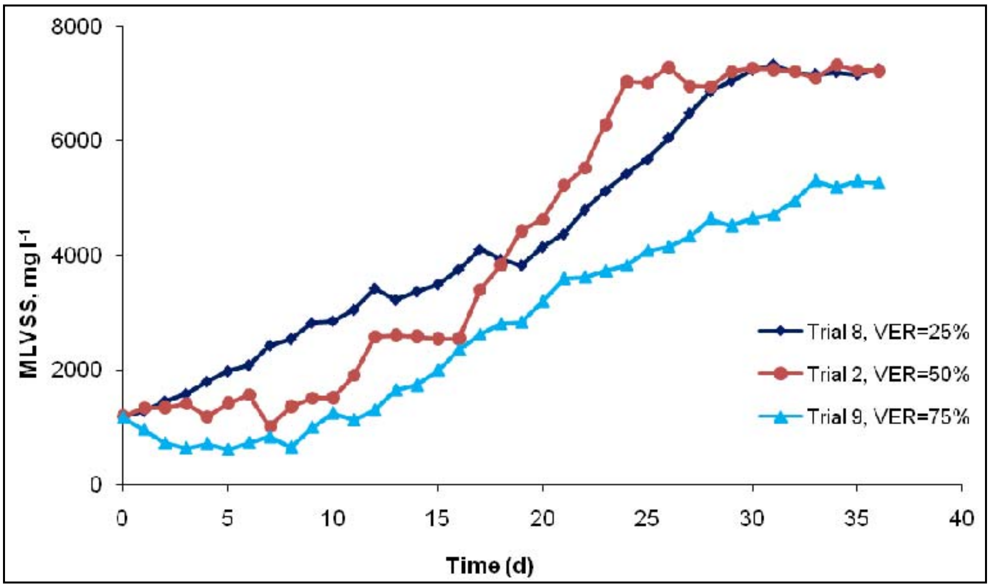

Fig 4.44 Variation of MLVSS at different VER (25 – 75 %) with time

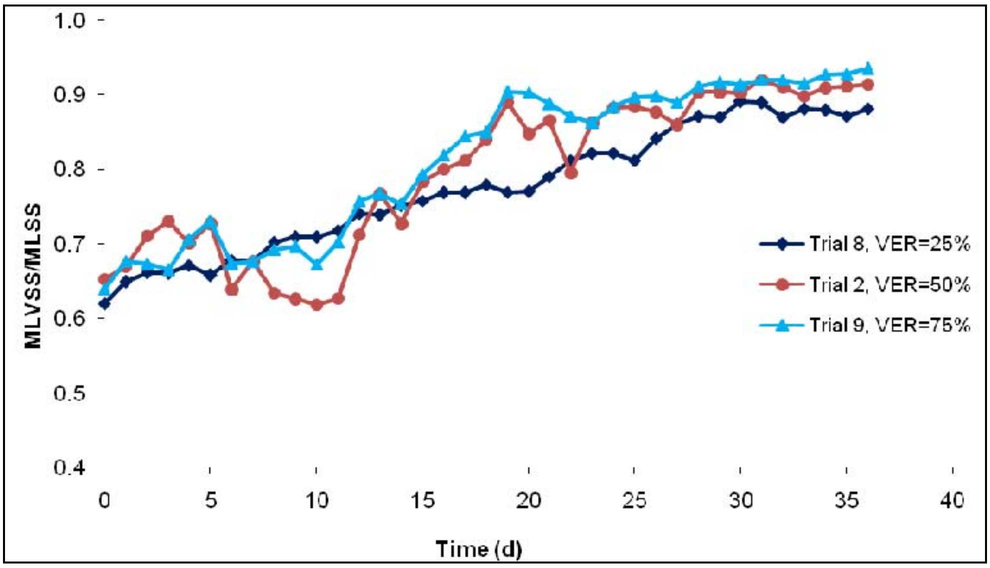

Fig 4.45 Variation of MLVSS/MLSS at different VER (25 – 75 %) with time

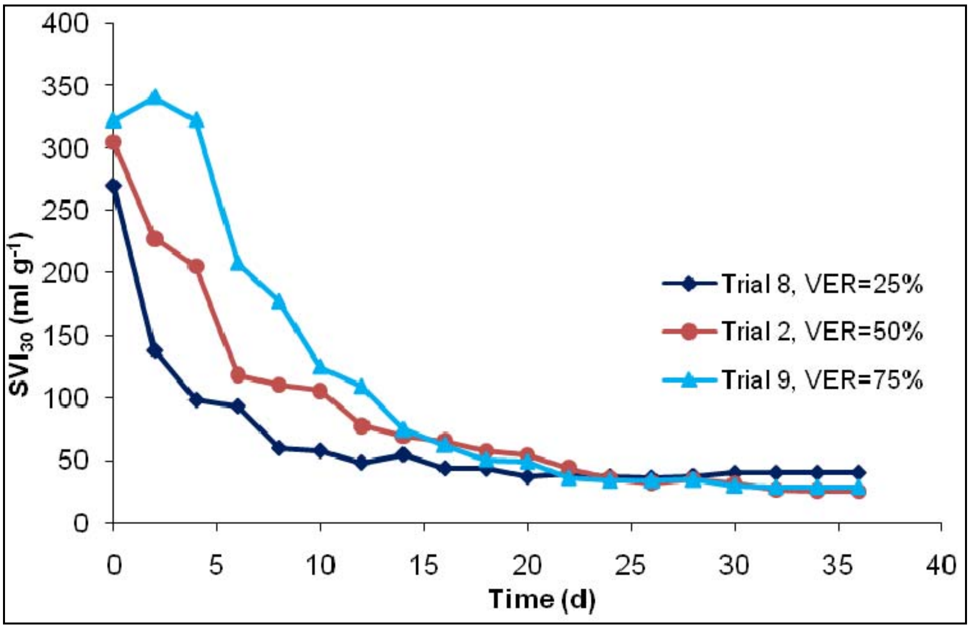

Fig 4.46 Variation of SVI_{30} at different VER (25 – 75 %) with time

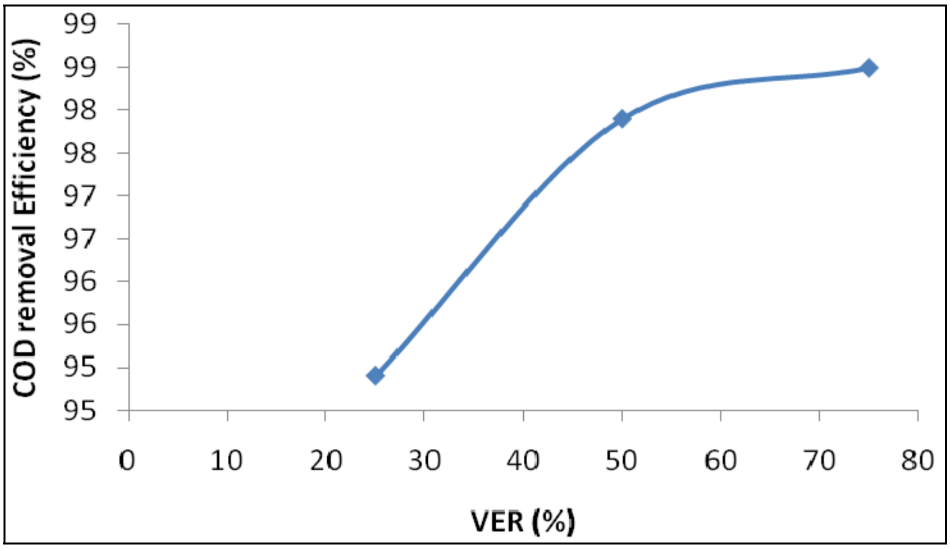

Fig. 4.47 Variation of COD removal efficiency with VER

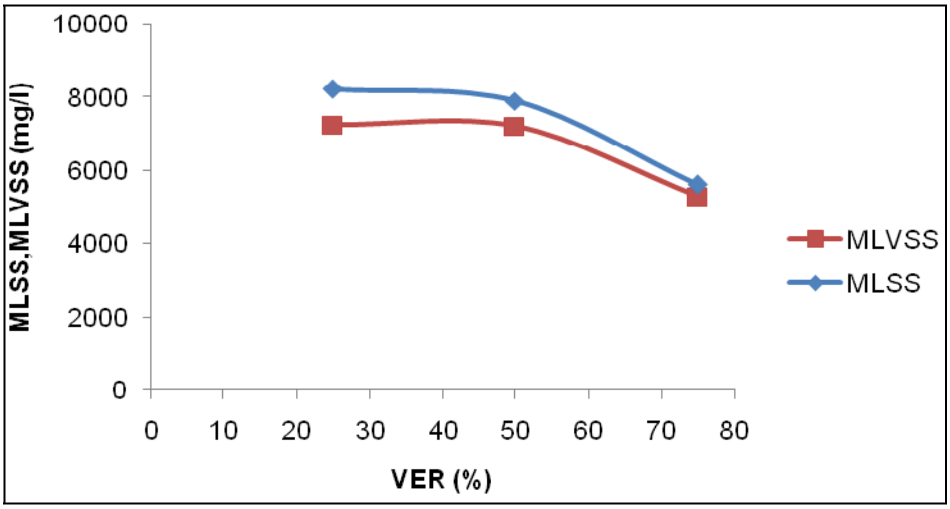

Fig. 4.48 Variation of MLSS & MLVSS with VER

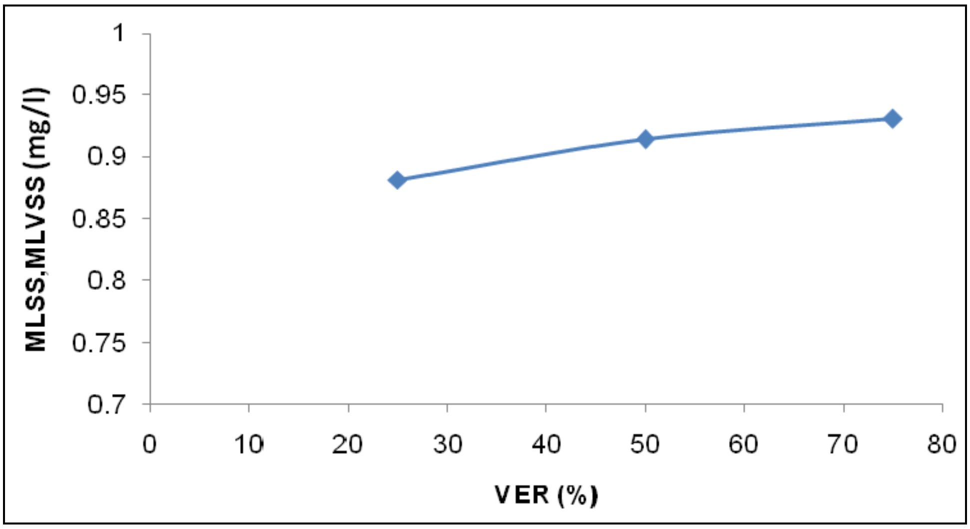

Fig. 4.49 Variation of MLSS/MLVSS with VER

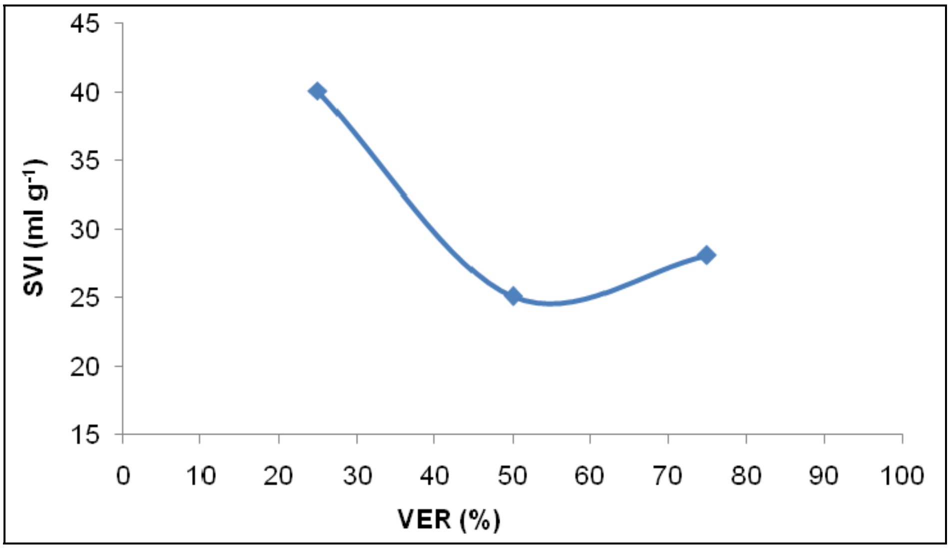

Fig. 4.50 Variation of SVI_{30} with VER

Among three trials that had been done to study the influence of aerobic granulation, the growth of MLSS and MLVSS concentrations was minimum for the trial 9. This can be clearly explained by the washing out of a good fraction of the sludge particles. In each cycle in trial 9 with VER 75%, sludge particles which could not reach (fall) to 3/4th height of the reactor in 5 min escaped from the reactor. Thus only particles sufficiently dense were retained in the reactor. This selection process is very similar to the selection by means of settling time.

As explained in Section 2.6.13, a minimum settling velocity $(Vs)_{min}$ is needed for the particles to be retained in the reactor. This velocity can be determined as (Equation 2.3):

$$(Vs)_{\min} = \frac{L}{SettlingTime}$$

For trial 8, 2 and 9 (VER 25%, 50% and 75%) the L was variable (25%, 50% and 75% of the effective height of the column reactor) and settling time was fixed as 5 min. Hence the $(Vs)_{min}$ was calculated as 3.015, 6.03 and 9.045 cm min^{-1} respectively for trial 8, 2 and 9. All the sludge particles with settling velocity less than 3.015 cm min^{-1} escaped from the reactor in trial 8. The retained fraction was a mixture of light and dense particles, which took about 3 weeks to develop into granules. The morphological variations of the sludge particles in trial 8 are shown in Fig. 4.51. By day 21, granules were visible, but they appeared to be less dense and loose structured.

In trial 9, dense particles whose settling velocity was greater than 9.045 cm min^{-1} retained in the reactor. Obviously early appearance of granules and faster development into dense granules were observed. Lowest SVI was observed in this trial. Wang et al. (2006) when studied the effect of VER on aerobic granulation with different VER (20, 40, 60 and 80%), the lowest SVI was obtained in reactor with VER 80%. Only bioflocs were formed in reactor with 20% VER. Present study also supports the fact that a higher VER favours better granulation and COD removal. No other reports are available in literature for the influence of VER on aerobic granulation.

VER and settling time help to retain sufficiently dense particles in a column type SBR and allow poorly settleable flocs to escape from the reactor. Thus VER along with settling time was proved to be a strong selection pressure for the process of aerobic granulation.

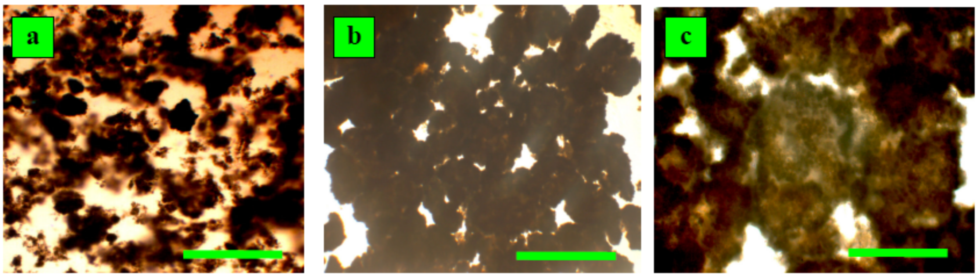

Fig. 4.51 Morphological variation of the sludge during the experimental period in trial 8 (VER=25%) (a) on day 7 (b) on day 14 (c) on day 21 (bar = 0.5 mm)

The typical settling behaviour of granular sludge developed in trial 2 was captured at different timings and shown in Fig. 4.52.

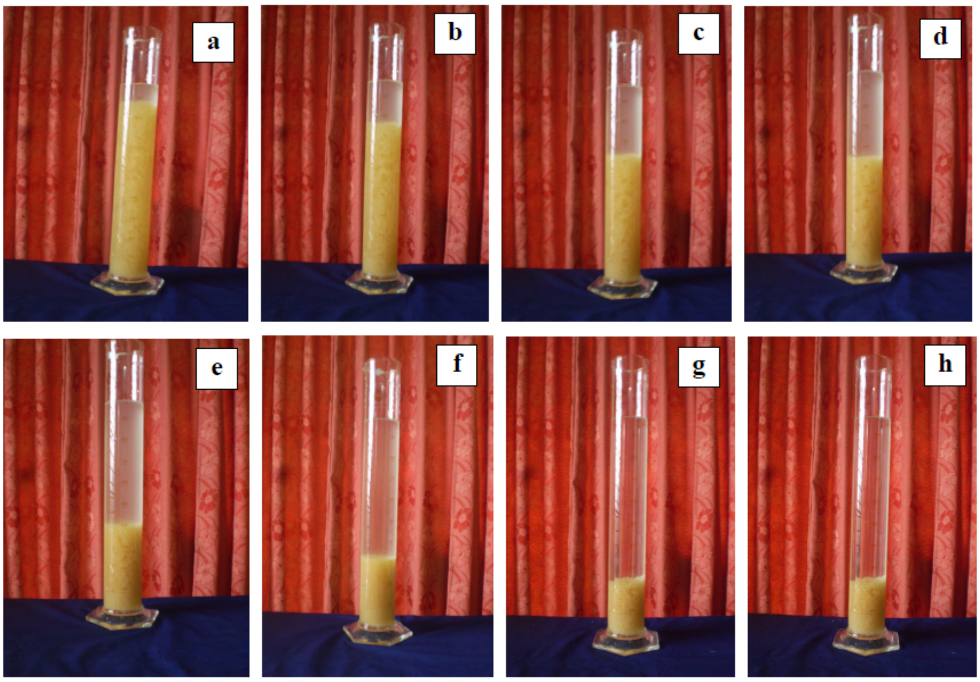

Fig. 4.52 Settling behaviour of granular sludge developed in trial 2, images being taken on day 20, at (a) 10 s (b) 20 s (c) 30 s (d) 1 min (e) 2 min (f) 3 min (g) 4 min (h) 5 min

The SRT in each trial varied according to the sludge properties throughout the trials. During the steady state, it was calculated and found to vary from 5 d to around 20 d. In all the nine trials, the SOUR and Y_{ob} were measured only during the last week of operation. The average values of SOUR was found to vary from 35 to 60 mg O_2 (g VSS)$^{-1}$ hr^{-1}. The variations in Y_{ob} were less in the trials. It varied from 0.28 to 0.40 mg VSS (mg COD)$^{-1}$. The variations in the operating conditions did not appear to have much effect on the oxygen uptake rate and the biomass yield. Hence no attempts were made in that line to correlate SOUR and Y_{ob} to variable operating conditions.

The values of all the parameters obtained at steady state in all the nine trials are summarized in Table 4.3.

Table 4.3 Summary of results obtained at steady state in trial 1 to 9

Trial No	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Operating Parameters									
OLR (kg COD m^{-3} d^{-1})	3	**6**	9	6	6	6	6	6	6
SUAV, cm s^{-1}	3	**3**	3	2	4	3	3	3	3
ST, min	5	**5**	5	5	5	3	10	5	5
VER, %	50	**50**	50	50	50	50	50	25	75
Reactor Performance Characteristics									
Granule appearance, day	28	**17**	34	21	14	19	28	21	14
Matured granules, day	35	**23**	----	28	22	28	---	28	25
Steady state, day	40	**28**	40	32	33	30	40	32	31
Size of granule, mm	1.8	**2.4**	0.9	2.6	1.5	2.1	1.1	0.9	2.0
Specific gravity of granules	1.0055	**1.007**	1.004	1.005	1.008	1.011	1.004	1.004	1.0075
Settling velocity, m hr^{-1}	45.0	**55.2**	33.6	38.4	63.6	72	31.2	34.8	66
MLSS, mg l^{-1}	4200	**7900**	10000	5500	9500	10000	3500	8200	5600
MLVSS, mg l^{-1}	3400	**7200**	8100	4500	8600	9000	2800	7200	5300
MLVSS/MLSS	0.81	**0.91**	0.81	0.82	0.905	0.9	0.8	0.88	0.946
SVI, ml g^{-1}	31	**25**	38	40	24	23	75	40	28
Effluent COD, mg l^{-1}	40	**43**	320	125	60	45	195	102	30
COD removal efficiency,%	96	**97.9**	89.3	93.8	97.1	97.8	90.1	94.9	98.5

From the summary of results, it can be seen that maximum removal of COD was in trial 9 with VER 75%. Good pollutant removal was observed in trial 2 and 6 also. In all these trials, the OLR was 6 kg COD m^{-1} d^{-1}. When the OLR was increased to 9 kg COD m^{-1} d^{-1} (trial 3) and when the settling time was increased to 10 min (trial 7), the COD removal was reduced to a value near 90 %. The overall performance of the reactor with longer settling time (10 min) was poor and it resembled the performance of a typical ASP unit. The compactness and the settling velocity of the granules were maximum in trial 6 with a reduced settling time (3 min).

SUAV has great influence on the formation and the densification of the granules. A higher SUAV (4 cm s^{-1}) caused by increased air flow (8 l min^{-1}) could form granules of good specific gravity (1.008), settling velocity (63.6 m hr^{-1}) and SVI (ml g^{-1}). But better conditions were achieved with a lower air supply (6 l min^{-1}) causing a SUAV of 3 cm s^{-1} in trial 6, which means that economical design is possible with better results.

Several pilot plant studies were conducted by various researchers. Inizan et al. (2005) studied the treatment of synthetic wastewater and industrial wastewater in a pilot plant SBR by aerobic granulation. The settling time, upflow air velocity and VER applied were 3 min, 0.74 cm s^{-1}, and 60% respectively. COD removal efficiency of 95% was achieved in the case of synthetic wastewater, while it was only 80% with real industrial wastewater. In another attempt by Ni et al. (2009), low strength municipal wastewater was treated in a pilot plant of 1 m^3 capacity. A COD removal efficiency of 90% was achieved when a settling time of 15-30 min was applied.

Typical values of OLR in a conventional ASP range from 0.3 to 3 kg COD m^{-1} d^{-1} and the SVI values range between 75 to 100 ml g^{-1}. The settling time allowed in secondary sedimentation tank in an ASP is 1.5 to 2 hrs. A better performance in terms of OLR (3-6 kg COD m^{-1} d^{-1}) and SVI (23-35 ml g^{-1}) was shown by granular sludge process in an SBR with much less requirement of time and space.

4.8 Application of Grey System Theory in Aerobic Granulation

The grey system theory (GST) method includes grey relational analysis (GRA) and grey models. GRA, denoted by the grey relational coefficients (GRCs) and grey entropy relational grade (GERG), is used to investigate the relationship between reference sequences and compared sequences.

In the present study, influence of four major parameters (OLR, SUAV, ST and VER) on aerobic granulation in SBR was investigated. Hence they were chosen as the compared parameters. These parameters were proved to have profound influence on wastewater treatment by aerobic granulation.

SVI of the flocculent sludge developed in activated sludge processing units usually varies from 100 to 250 ml g^{-1}. A decreased SVI is always resulted in SBR with granular sludge. This may be caused by combined stress developed by many factors like high hydraulic shear force, short settling time, high organic loading rate. All these factors can influence the size and specific gravity of the granular sludge. The transformation of flocculent sludge to granular form is achieved by certain selection pressures. Good choice of these selection pressures will discard the fluffy fraction of the sludge and transform the remaining sludge to dense and compact granules. Such granules were proved to have a better COD removal capacity. Thus the reference parameters chosen for this analysis were selected as SVI, granule appearance time, size and specific gravity of the granules, and COD removal efficiency.

Total nine trials were conducted to investigate the influence of OLR, SUAV, ST and VER on aerobic granulation in SBR.

In grey relational space, a system contains many series with *k* entities. Reference sequence (X_i^{0*}) and compared sequence (X_j^*) are given by Equations 3.14 and 3.15.

Now the reference sequence and compared sequence are transformed to the following forms for the present study (Equations 3.18 and 3.19).

$$X_i^0 = \left\{ X_i^0(k) \middle| i = 1,2,...5,.k = 1,2,...,n \right\}$$

$$X_j = \left\{ X_j(k) \middle| j = 1,2,...,4, k = 1,2,...,n \right\}$$

In the present study,

m = number of the reference parameter = 5 (i = 1, 2, 3, 4, 5)

r = number of compared parameter = 4 (j = 1, 2, 3, 4)

n = total number of experiments = 9 (k = 1, 2, 3, 4, 5, 6, 7, 8, 9)

Attributing the entities as:

i1 = SVI, ml g^{-1}

i2 = Granule appearance, d

i3 = Granule size, mm

i4 = Granule specific gravity

i5 = COD removal efficiency, %

j1 = OLR, kg COD m^{-3} d^{-1}

j2 = SUAV, cm s^{-1}

j3 = Settling time, min

j4 = VER, %

The selected values for the parameters from nine trials are listed in Table. 4.4.

Table 4.4 Experimental data used for the Grey System Analysis

		Trials								
		k1	**k2**	**k3**	**k4**	**k5**	**k6**	**k7**	**k8**	**k9**
Reference Parameters	**i1**	31	25	38	40	24	23	75	40	28
	i2	28	17	34	21	14	19	28	21	14
	i3	1.8	2.4	0.9	2.6	1.5	2.1	1.1	0.9	2
	i4	1.0055	1.0069	1.0042	1.005	1.008	1.011	1.004	1.004	1.0075
	i5	96	97.9	89.3	93.8	97.1	97.8	90.1	94.9	98.5
Compared Parameters	**j1**	3	6	9	6	6	6	6	6	6
	j2	3	3	3	2	4	3	3	3	3
	j3	5	5	5	5	5	3	10	5	5
	j4	50	50	50	50	50	50	50	25	75

Since the units of the sequences varied widely from each other, the series data were subjected to normalization prior to the calculation of GRCs using Equations 3.16 and 3.17.

The transformed values are listed in Table 4.5

Table 4.5 Transformed values of the original experimental data

		Trials								
		k1	k2	k3	k4	k5	k6	k7	k8	k9
Reference Parameters	i1	0.8611	0.6944	1.0556	1.1111	0.6667	0.6389	2.0833	1.1111	0.7778
	i2	1.2857	0.7806	1.5612	0.9643	0.6429	0.8724	1.2857	0.9643	0.6429
	i3	1.0588	1.4117	0.5294	1.5294	0.8823	1.2352	0.6470	0.5294	1.1764
	i4	0.9992	1.0006	0.9979	0.9987	1.0017	1.0047	0.9977	0.9977	1.0012
	i5	1.0100	1.0300	0.9395	0.9869	1.0216	1.0289	0.9479	0.9984	1.0363
Compared Parameters	j1	0.5	1	1.5	1	1	1	1	1	1
	j2	1	1	1	0.6667	1.3333	1	1	1	1
	j3	0.9375	0.9375	0.9375	0.9375	0.9375	0.5625	1.8750	0.9375	0.9375
	j4	1	1	1	1	1	1	1	0.5	1.5

Then the GRC between reference sequence $Xi^0(k)$ and compared sequence $Xj(k)$ at point k was calculated as per Equation 3.20, taking ρ as 0.5.

GRCs of each operational factor on SVI, granule appearance time, size and specific gravity of the granules, and COD removal efficiency are calculated and summarized in Table 4.6.

According to the GST, the GRCs reflect the relational grade of the reference sequence and compared sequence at point *k*. A large GRC corresponds to a high relational grade. As GRCs supply abundant information and sometime higher GRCs may lead to confusion, GERG is adopted to determine the key operational impact factors and rank them by relational grade. Relational coefficient distribution density (*pij*) and grey relational entropy (S_{ij}) were calculated by Equations 3.21 and 3.22. The GERG (E_{ij}) for the operational impact factor j on the reference parameter i was estimated by Equation 3.23.

Table 4.6 GRCs of SVI, Granule appearance, Granule size, granule specific gravity and COD removal efficiency

	k1	k2	k3	k4	k5	k6	k7	k8	k9
SVI									
OLR	0.7528	0.7882	0.7053	0.9437	0.7701	0.7528	0.4752	0.9437	0.8481
SUAV	0.5929	0.7528	0.6505	0.5630	0.7701	0.7882	0.3545	0.5630	0.7053
ST	0.9781	0.8323	0.9371	0.8874	0.8121	0.9781	0.8590	0.8874	0.8993
VER	0.9178	0.7882	1.0000	0.9437	0.7701	0.7528	0.4752	0.6262	0.5826
Granule appearance									
OLR	0.4358	0.7527	0.9445	0.9850	0.6396	0.8533	0.6937	0.9850	0.6396
SUAV	0.3493	0.5823	0.3617	0.4925	0.6746	0.5337	0.3878	0.4925	0.6746
ST	0.6459	0.8184	0.4955	1.0000	0.6864	0.6743	0.5104	1.0000	0.6864
VER	0.6937	0.7527	0.5231	0.9850	0.6396	0.8533	0.6937	0.5727	0.4139
Granule size									
OLR	0.5486	0.6272	0.4060	0.5627	0.8794	0.7576	0.6654	0.5932	0.8140
SUAV	0.4272	0.3802	1.0000	0.3555	0.5532	0.4244	0.6935	0.7943	0.4415
ST	0.8750	0.5912	0.6295	0.5335	0.9615	0.5000	0.3493	0.6295	0.7543
VER	0.9563	0.6272	0.5932	0.5627	0.8794	0.7576	0.6654	1.0000	0.6863
Specific gravity									
OLR	0.4684	1.0000	0.4670	0.9987	0.9975	0.9908	0.9965	0.9965	0.9986
SUAV	0.3456	0.3972	0.4690	0.3979	0.3968	0.3957	0.3982	0.3982	0.3970
ST	0.8779	0.8755	0.8802	0.8788	0.8736	0.4987	0.3338	0.8805	0.8744
VER	0.9998	1.0000	0.9969	0.9987	0.9975	0.9908	0.9965	0.4691	0.4686
COD removal efficiency									
OLR	0.4777	0.9422	0.4542	0.9757	0.9586	0.9442	0.9020	1.0000	0.9303
SUAV	0.3558	0.4008	0.5149	0.4163	0.4037	0.4012	0.4313	0.4120	0.3986
ST	0.8675	0.8363	0.9989	0.9066	0.8492	0.5000	0.3344	0.8866	0.8269
VER	0.9820	0.9422	0.8875	0.9757	0.9586	0.9442	0.9020	0.4834	0.5016

A large Eij shows a strong relevance between reference sequence and compared sequence. By comparing the GERG, the influence order and the key operational impact factor were calculated.

Table 4.7 summarizes the GERG of the compared factors on the reference factors. Table 4.8 and Table 4.9 give their importance order and optimal scope respectively.

Table 4.7 GERG (E_{ij})of compared parameters on reference parameters

Parameter	SVI	Granule appearance	Granule size	Granule specific gravity	COD removal efficiency
OLR	0.99289	0.98742	0.98954	0.98358	0.98493
SUAV	0.98981	0.98774	0.97109	0.99879	0.99794
ST	0.99913	0.98717	0.98241	0.98292	0.98207
VER	0.98887	0.98701	0.99080	0.98366	0.98710

Table 4.8 Order of importance of compared parameters on reference parameters

Parameter	SVI	Granule appearance	Granule size	Granule specific gravity	COD removal efficiency
OLR	2	2	2	3	3
SUAV	3	1	4	1	1
ST	1	3	3	4	4
VER	4	4	1	2	2

Table 4.9 Optimal values of compared parameters on reference parameters

Parameter	SVI	Granule appearance	Granule size	Granule specific gravity	COD removal efficiency
OLR, kg COD m^{-3} d^{-1}	6	6	6	6	6
SUAV, cm s^{-1}	3 - 4	4	3	3	3
ST, min	3-5	5	5	5	5
VER, %	50	50	25-50	50	50

4.8.1 Analysis of sludge volume index

In SBRs, the SVI of active sludge flocs mainly fluctuated between 25 and 100 ml g^{-1}. A decrease in SVI, driven by the combined stress supplied by all the factors, indicates that the structure of the aggregates becomes more dense and compact. In other words, lower SVI means settling ability is being effectively improved. Therefore, SVI is an effective measure indicator to describe the degree of granulation in the process.

In the present analysis, for SVI, maximum GERG value is for ST (0.99913), which means settling time can influence the granulation process and help to develop granular sludge with maximum settleability. Therefore the order of importance for settling time on SVI is 1. The light flocs could be effectively washed out when the ST was short. Long ST is unfavorable for the granulation as it could not supply sufficient biological selection pressure to screen heavy aggregates with good settleability. Actually, short ST was the decisive factor responsible for aerobic granulation. Qin et al. (2004b) indicated that during an aerobic granulation process, SVI was indeed determined by the degree of aerobic granulation in the process. Moreover, SVI was closely related to the settling time, and a relative high hydrodynamic selection pressure supplied by short ST was necessary in particular.

OLR was listed as the second place as per the GERG value (0.99289) and the optimal value was 6 kg COD m^{-3} d^{-1} illuminating the fact low OLR is not suitable for SVI reduction. The reason may be attributed to the fact that a low OLR corresponds to a short starvation time and eventually frustrates the granulation process. For OLR, a high rate favored the growth of heterotrophic microbes, which contributed to irregular structure (Moy et al., 2002), and further impeded the granulation process. Therefore, to obtain better conditions for granulation, OLR needed to be adjusted to certain levels.

The GERG values of SUAV and VER were also very high (0.98981 and 0.98887 respectively) which shows that these two compared parameters also have strong influence on SVI. Under high hydrodynamic shear force, more polysaccharides will be secreted which helps granulation. Since SVI was closely related to the formation and granulation grade, SUAV was considered as a key impact factor. VER helps to retain sufficiently dense sludge particles in the reactor, thereby controls the trend of SVI. It could be concluded from the value of GERGs that the operational impact factors' influence order on SVI was ST > OLR > SUAV > VER.

4.8.2 Analysis of granulation (appearance, size and specific gravity)

Appearance of the granule in the SBR marks the beginning of the successful functioning of the system. Once the granule formation is initiated, further adhesion and growth of granules may be enhanced. Hence first appearance of granules would be an effective indication of the reactor efficiency, which is influenced by other operating conditions.

The aerobic granulation process experiences the adhesion phase first, and then the bacteria metabolism. SUAV provides the essential environment for this phase as the air introduced at the bottom of the reactor would enhance the turbulence of the mixed liquid, and strengthen the interaction between the gas and liquid phases. Additionally, under high hydrodynamic shear force, cells also secrete more sticky extracellular polysaccharides (EPS) (Tay et al., 2001b). In short, all of the conditions created by SUAV played positive roles in facilitating the adhesion of the aggregates. Table 4.7 shows that shear force in terms of SUAV has a profound influence on the granule formation (GERG = 0.98774), with importance order 1. The remaining factors have the importance order as OLR > ST > VER.

After the initiation of the granules formation, further development and growth of granules are also influenced by the operational parameters. They are in the order of importance as VER > OLR > ST > SUAV.

Specific gravity of the granular sludge determines the settleability and hence the SVI of the sludge. The specific gravity of the granules along with the size have the roles in determining the settling velocity of the granules. A high GERG value of SUAV on specific gravity (0.99879) reinforces the concept that high air flow can shape and densify the granules by the high shear force developed. The highest GERG value places SUAV as the most important impact factor on specific gravity of the granules. VER, OLR and ST have the importance order 2, 3 and 4 respectively.

4.8.3 Analysis of COD removal efficiency

Pollutant removal is the ultimate objective of the wastewater treatment. The importance of the operational parameters on COD removal capacity was analyzed. As the E_{ij} values of all the four compared parameters were greater than 0.98, it can be indicated that for COD removal, all these operational parameters are key impact factors. It was found from the analysis that among the experimented parameters, SUAV formed by the air supply was the most important operating parameter (GERG = 0.99794) influencing the COD removal. For the appearance and specific gravity of granules also, the order of importance of SUAV was 1. An early formation of dense and compact aerobic granules associates with effective biodegradation of organic matter, which in turn yields a good COD removal. Hence, selection of proper air supply can control the efficiency of the granulation and well as the COD removal. VER, OLR and ST have importance in the decreasing order after SUAV.

4.8.4. Optimal Values of the Compared Parameters

The optimal scopes of all the operational parameters were identified. An OLR of 6 kg COD m^{-3} d^{-1} was proved to be an optimal value for aerobic granulation in SBR when acetate is used as the substrate. SUAV has an optimal value of 3 cm s^{-1} for a good COD removal and optimum size and specific gravity of granular sludge. Still higher shear force of the order 4 cm

s^{-1} is suitable for an earlier formation of granules. Shear force from 3 to 4 cm s^{-1} produces the lowest SVI. The optimal scope of settling time and VER were identified as 5 min and 50% respectively.

The results generated by this analysis mainly focused on the aerobic granules which were cultivated under certain conditions. As the aerobic granular sludge was mainly cultivated under such conditions, it was worth distinguishing the priority and the optimal values of the major operational factors for aerobic granulation process. To acquire granules with certain functions, the result generated from this study could act as a reference. For instance, much attention should be paid to SUAV, OLR, ST, and VER when SVI, granule characteristics and/or COD removal are concerned. Thus grey system theory was shown to be suitable for assessing the priority and providing the optimal values of each operational impact factor with respect to aerobic granulation process indicators.

4.9 Treatment of Latex Processing Wastewater by Aerobic Granulation

The wastewater from the latex centrifuging unit of natural rubber processing industry was collected and analyzed for various parameters. The characteristics of the effluent are listed in Table 4.10. The wastewater was subjected to treatment in SBR with optimal operating parameters, as obtained from the experimental studies conducted. The OLR was kept as 6 kg COD m^{-3} d^{-1} and the airflow as 6 l min^{-1} so that the SUAV was 3 cm s^{-1}. A cycle time of 4 hours was used in which the settling time was kept as 5 min. The VER applied was 50 %. Feeding of influent, withdrawal of effluent, application of air and the control of cycle time were done exactly same as were done in all nine trials previously.

The effluent from the middle port was collected and analyzed for the COD. The reactor content was tested for MLSS, MLVSS, and SVI periodically. The formation and development of the granules were monitored by image analysis.

Table 4.10 Characteristics of latex processing effluent used for the study

Characteristics	Values
pH	7.5 ± 0.5
COD, mg l^{-1}	2000 ± 50
Suspended Solids, mg l^{-1}	74 ± 10
Ammoniacal Nitrogen, mg l^{-1}	91 ± 5
Total Nitrogen, mg l^{-1}	209 ± 18
Sulphides, mg l^{-1}	Trace

A negative growth in MLSS and MLVSS was observed during the first week of operation, which obviously may be due to the time delay for the microorganisms to adapt to the new substrate. Effluent COD also showed fluctuation during this period. From 2nd week onwards a fairly good growth in suspended solids and reduction in effluent COD were observed. Fine granules were visible during the third week of operation. Performance of the reactor showed marked improvement since the appearance of the granules. MLSS grew at a steadier rate to reach a maximum value of 8100 mg l^{-1} with a MLVSS/MLSS ratio of 0.9. The settleability of the sludge was found to improve and reached a value of 28 ml g^{-1} for SVI and it remained for the last 10 days of operation. Based on the constancy in the values of effluent COD, MLSS, MLVSS and SVI, the system was assumed to reach the steady state. Hence the operation was stopped on day 35. The experimental set-up and the performance of the reactor during aeration phase and settling phase are shown in Fig. 4.53 (a-b). The variations in COD removal, SVI, MLSS & MLVSS and MLVSS/MLSS ratio are shown in Fig. 4.54 (a-d).

Fig 4.53 Experimental set-up for the treatment of latex processing wastewater (a) during aeration (b) during settling

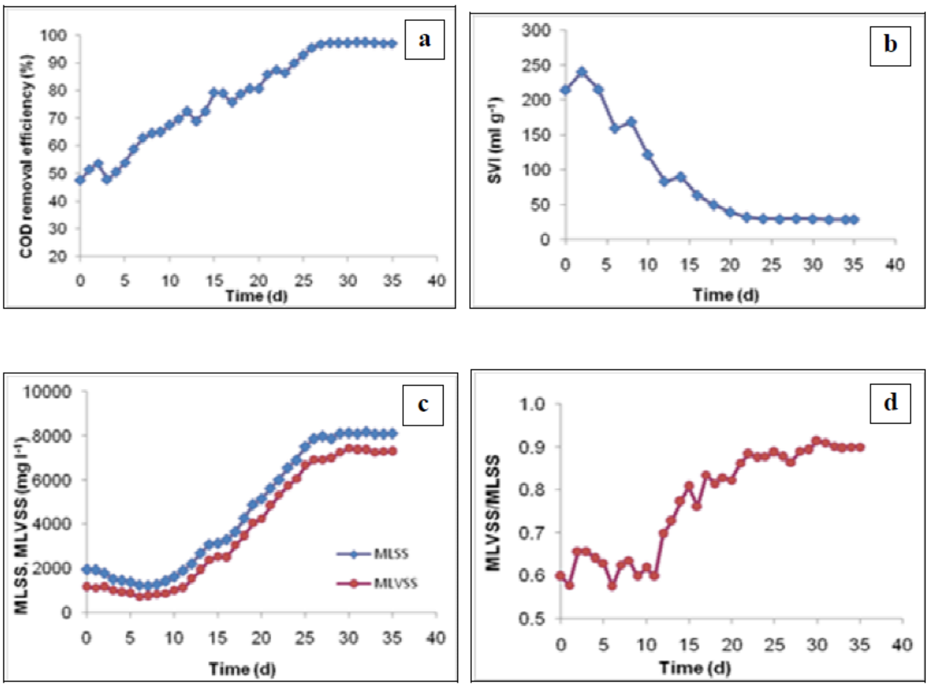

Fig. 4.54 Variation of (a) COD removal efficiency (b) SVI (c) MLSS & MLVSS (d) MLVSS/MLSS with time for the treatment of latex processing effluent

Fig 4.55 (a-b) shows the images of the sludge development during the treatment with latex processing effluent on day 7 and 18. Microstructures of aerobic granules developed during the experiment, observed by SEM at two magnifications on day 22 are shown in Fig. 4.56 (a-b). The SEM images show the thick aggregation of microbes. The average size of the granules was measured as 1.3 mm and average specific gravity as 1.007. The settling velocity of the granules was 45 m hr^{-1} during the last week of operation.

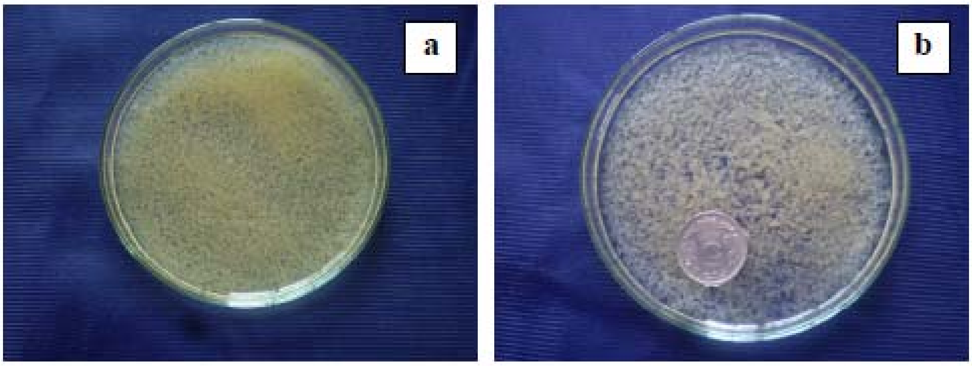

Fig 4.55 Images of sludge during the experiment with Latex processing wastewater (a) on day 7 (b) on day 18

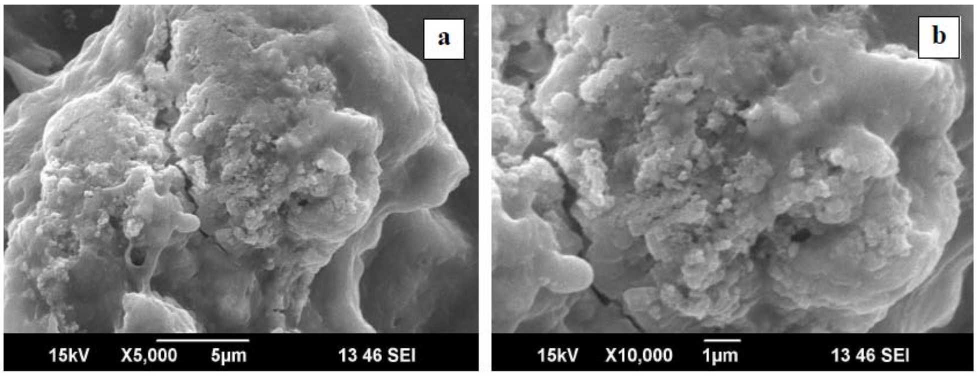

Fig 4.56 Microstructure of aerobic granules developed during the experiment with Latex processing wastewater, observed by SEM at a magnification of (a) 5000 and (b) 10000

The treated effluent was examined for suspended solids and Nitrogen (total nitrogen and ammoniacal nitrogen) also. The performance of the reactor treating latex processing effluent is given in Table 4.11. Table 4.12 shows the average characteristics of the raw latex wastewater and those of treated effluent for comparison. The effluent characteristics were seemed to be satisfactory with respect to the norms of the Kerala State Pollution Control Board.

Table 4.11 Performance of the SBR for the treatment of latex processing effluent

Characteristics	Values at steady state
Granule appearance, day	18
Matured granules, day	26
Steady state, day	30
Size of granule, mm	1.3
Specific gravity of granules	1.007
Settling velocity, m hr^{-1}	45
MLSS, mg l^{-1}	8100
MLVSS, mg l^{-1}	7300
MLVSS/MLSS	0.9
SVI, ml g^{-1}	28
Effluent COD, mg l^{-1}	60

Table 4.12 Comparison of the characteristics of raw latex wastewater and treated effluent

Characteristics	Raw latex wastewater	Treated Effluent	% Removal
pH	7.5	7.32	---
COD, mg l^{-1}	2000	60	97
Suspended Solids, mg l^{-1}	174	11	93.7
Ammoniacal Nitrogen, mg l^{-1}	71	9	87.3
Total Nitrogen, mg l^{-1}	209	19	90.9
Sulphides, mg l^{-1}	Trace	Trace	---

The high COD removal achieved (97%), suspended solids removal (93.7%), nitrogen removal (90.9%) and biomass developed (8100 mg l^{-1}) indicated the good performance of the reactor. Thick consortia of bacteria with excellent sludge settleability also showed the healthy nature of the system. Rosman et al., (2013) when investigated the treatment of standard Malaysian rubber (SMR) wastewater using aerobic granulation technology, achieved removal efficiencies of COD, ammonia and total nitrogen as 96.6%, 94.7% and 89.4% respectively. The applied OLR was 3.7 kg COD m^{-3} d^{-1}. In the present study, with a higher OLR (6 kg COD m^{-3} d^{-1}) comparable results were obtained for the treatment of effluent from the latex centrifuging units. Based on the above results, it can be concluded that aerobic granulation technology is an excellent option for the treatment of effluents from latex processing units.

4.10. Summary

Influence of four major operational parameters – organic loading rate, hydraulic shear force in terms of superficial upflow air velocity, settling time and volume exchange ratio – on aerobic granulation was investigated using a laboratory set up. The results of each experimental trial are presented in this chapter. Characterization of influent, effluent and the reactor contents was done using the methods explained in the previous chapter. The performance of the reactor was thoroughly observed and analyzed. The aerobic granules developed were subjected to image analysis. Grey relational analysis, which is a part of the grey system theory, was done using the results obtained to find the order of importance of the operational parameters. The optimal values of all the four compared parameters were identified based on different reference parameters. A real wastewater (natural rubber latex processing wastewater) was treated using aerobic granulation process applying the optimal values, and excellent performance was observed.

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Chapter 5

SUMMARY AND CONCLUSIONS

5. 1 Summary

Conventional sewage treatment plants based on activated sludge process require large area. This is mainly because of the poor settling characteristics of the flocculant sludge which demand large area for the settling unit. Biogranulation was proven as an effective technology to improve the settleability of the sludge particles. Biogranules are formed through the self-immobilization of microorganisms. Biogranulation may be classified as aerobic and anaerobic granulation. Anaerobic granulation has been extensively studied and best recognized in the upflow anaerobic sludge blanket reactors (UASB). But anaerobic granulation technology has some drawbacks like long start-up period, relatively high operating temperature, unsuitability for low-strength wastewater, and unsuitability for the removal of nutrients (N and P) from wastewater. In order to overcome these weaknesses, research has been devoted to aerobic granulation technology.

Aerobic granulation is a promising biotechnology for the treatment of wastewater. The granulation process is influenced by a number of parameters. Influence of four major parameters on aerobic granulation in SBR was studied in laboratory in nine trials. These parameters were organic loading rate, hydrodynamic shear force in terms of superficial upflow air velocity, settling time and volume exchange ratio. Three values for each parameter (OLR - 3, 6 and 9 kg COD m^{-3} d^{-1}, SUAV - 2, 3 and 4 cm s^{-1}, ST - 3, 5 and 10 min and VER - 25, 50 and 75 %) were selected based on the literature survey. The performance of the reactor was observed and characteristics of the influent, treated effluent and mixed liquor were monitored. The aerobic granules developed were observed by image analysis. All the results obtained were analyzed. Grey system theory was used for analysis and for arriving at optimal values for the operational parameters. A real wastewater (wastewater from natural rubber latex processing unit) was subjected to treatment by aerobic granulation in SBR using optimized values of operational parameters.

5.2 Conclusions

Following conclusions were arrived from this research work.

- Hydraulic selection pressures in terms of OLR, SUAV, ST and VER are decisive parameters in the formation of aerobic granules in SBR. Very weak selection pressures such as low shear force, long settling time and low VER did not favour aerobic granulation.
- OLR played an important role in the formation and development of aerobic granules. Though aerobic granules were formed at high OLRs (9 kg COD m^{-3} d^{-1} in the present study), they were not showing stability and hence the COD removal was considerably reduced.
- Among the three OLRs attempted, aerobic granules with maximum size and specific gravity were developed in trial 2 with OLR 6 kg COD m^{-3} d^{-1}, which resulted in a high settling velocity (55.2 m hr^{-1})

and very low SVI (25 ml g^{-1}). A COD removal of 97.9% was achieved in trial 2.

- High shear force enhances the biomass growth, reduces the SVI and improves the sludge settleability. An increase in SUAV from 2 cm s^{-1} to 3 cm s^{-1}, considerably increased the COD removal efficiency and sludge settleability. But further increase from 3 cm s^{-1} to 4 cm s^{-1} could not make a marked improvement in SVI and COD removal. Hence optimum shear force has to be selected based on the performance as well as from the economic point of view.

- Shorter settling time can select denser and more compact granules to retain in the reactor and lighter particles to wash out from the reactor. A long settling time of the order of 10 min could not yield a fully developed granular sludge with good settling ability. A settling time of 3 min was sufficient to produce well developed granules with excellent settleability and to achieve a COD removal of 97.8%.

- High VER of the order 75% (trial 9) could be an effective operating condition in terms of high COD removal and low SVI. Even though, the biomass concentration was less in trial 9, MLVSS/MLSS ratio was high (0.946) which showed a healthy biological treatment system.

- Impact of the compared parameters on reference parameters was estimated and ranked using GRCs and GERGs. Influence order of operational factors on:

➢ SVI:	ST > OLR > SUAV > VER
➢ Granule appearance:	SUAV > OLR > ST > VER

  - Granule size: VER > OLR > ST > SUAV
  - Granule specific gravity: SUAV > VER > OLR > ST
  - COD removal: SUAV > VER > OLR > ST

- Optimal values of all the compared parameters (OLR, SUAV, ST, VER) were estimated from the nine trials.
- A real wastewater (effluent from natural latex processing) was subjected to treatment by aerobic granulation in SBR using optimized values of operational parameters which resulted in excellent performance in terms of COD removal (97%) and settleability of sludge (SVI = 28 ml g^{-1}).
- Aerobic granulation was proved to be an ideal technology for the treatment of wastewater with the controlled operating conditions.

This study has established the influence of four major operational parameters on aerobic granulation in SBR. A ranking of these influencing factors is necessary for the design of pilot plant SBR. This study has evolved an order of importance of the compared parameters on important reference parameters of aerobic granulation. The optimal values of major operational parameters are proved successfully for the treatment of a real wastewater.

5. 3 Limitations of the Study

The following are the limitations of the study:

- No strict control of temperature was done in the laboratory experiments. The experiments were conducted at room temperature, which was monitored to be in a normal range of 22-28°C.

- The size, specific gravity and the settling velocity of granules were measured as the average of 10 granules taken randomly.
- Each trial run was conducted for 35-45 days only. Steady state conditions were assumed based on the constancy of MLSS and COD removal values for a period of 4-5 days.

5. 4. Scope for Future Research

- Granular bioreactors can be coupled with other treatment units to complement benefits from both processes. The possibility of aerobic granular sludge reactor in combination with a membrane bioreactor can be explored.
- It would be feasible to seed the aerobic granular reactors with engineered species of bacteria to tailor microbial granules for treating specific types of wastewater.
- Pilot plant SBR could be developed for treating real wastewaters using the optimal range of values for the operational parameters. Strategies may be developed to scale up the laboratory performance to pilot plants.

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Annexure i

PROGRAMME FOR MICRO-CONTROLLER AT89C51

```
;*************************************************************************
;Assembly language Program for  AT89C51 Microcontroller.
;*************************************************************************
                        ORG 0
PUMP1_ON                EQU     01
AERATOR_ON              EQU     02
PUMP2_ON                EQU     04

                        MOV TMOD,#01H       ;Timer 0 configured in mode 1
CONTINUE:               MOV A,#PUMP1_ON     ; Switch ON pump 1
                        MOV P1,A
                        MOV R4,#5           ; keep the Pump 1 remains ON for 5 minutes
LOOP1:                  ACALL DELAY_1MIN
                        DJNZ R4,LOOP1
                        MOV A,#AERATOR_ON    ; Switch ON  Aerator
                        MOV P1,A
                        MOV R5,#225         ; keep the Aerator remains ON for 225 minutes
LOOP2:                  ACALL DELAY_1MIN
                        DJNZ R5,LOOP2
                        CLR A
                        MOV P1,A            ; All pumps OFF for 5 minutes
                        MOV R6,#5
LOOP3:                  ACALL DELAY_1MIN
                        DJNZ R6,LOOP3
                        MOV A,#PUMP2_ON     ; Switch ON pump 2
                        MOV R7,#5           ;keep the Pump 2 remains ON for 5 minutes
LOOP4:                  ACALL DELAY_1MIN
                        DJNZ R7,LOOP4
                        SJMP CONTINUE       ; Repeat the cycle.
;***********************************************************************
;SUBROUTINE TO GENERATE 1 MINUTE DELAY
;***********************************************************************
DELAY_1MIN:
                        MOV R2,#60          ; Count for 1 min.  1sec x 60 =1 min.
LOOP6:                  MOV R3,#20          ; count to generate 1 second. 1sec = 20 x 50 msec
LOOP5:                  MOV TH0,#4BH        ; count for 50 millisecond
                        MOV TL0,#0FCH
                        SETB TR0            ; start timer 0
AGAIN:                  JNB TF0,AGAIN       ; timer overlow check
                        CLR TR0             ;
                        CLR TF0
                        DJNZ R3,LOOP5
                        DJNZ R2,LOOP6
                        RET
                        END
```

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Annexure ii
SAMPLE CALCULATIONS

For Trial 2: (Refer Fig 2.1 and 3.1)

Diameter (internal) = D = 6.5 cm

Effective height = H = 60.3 cm

Distance to the discharging port from the top level of the solution = L = 30.15 cm

Cycle Time = T = 4 Hours

Airflow rate = F = 6 l min^{-1}

Influent COD = 2000 mg l^{-1}

then,

$$\text{Cross sectional area of the reactor (A)} = \frac{\pi D^{2}}{4} = \frac{\pi(6.5)^{2}}{4} = 33.183 cm^{2}$$

$$\text{Working Volume of the reactor (V)} = \frac{\pi D^{2}}{4} \times H = \frac{\pi(6.5)^{2}}{4} \times 60.3 = 2000.94 cm^{3} = 21$$

$$\text{Number of cycles per day (N)} = \frac{24}{T} = \frac{24}{4} = 6$$

$$\text{Discharge(Q)} = V \times VER \times N = 2 \times 0.5 \times 6 = 6l d^{-1} = 0.25l hr^{-1}$$

$$\text{Hydraulic Retention Time(HRT)} = \frac{V}{Q} = \frac{2}{0.25} = 8 hours$$

$$\text{Organic Loading Rate (OLR)} = \frac{\left(COD_{in} \times Q\right)}{V} = \frac{(2000 \times 6)}{2} = 6000 mg l^{-1} d^{-1} = 6 kg COD m^{-3} d^{-1}$$

$$\text{Shear Force (or SUAV)} = \frac{F}{A} = \frac{6\left(l \min^{-1}\right)}{33.183} cm^{2} = 3 cms^{-1}$$

$$\text{Volume Exchange Ratio (VER)} = \frac{L}{H} = \frac{30.15}{60.3} = 0.5 = 50\%$$

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LIST OF PUBLICATIONS

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Curriculum Vitae

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Bindhu B.K. was born on 24th April 1968 in Kottayam District, Kerala, India. She obtained her B.Tech Degree in Civil Engineering in 1990 from Mar Athanasius College of Engineering, Kothamangalam. She took a post graduation (M.Sc) in Environmental Studies with first Rank from Cochin University of Science and Technology, Kochi in 1993. She completed her M.Tech Degree in Environmental Engineering from Indian Institute of Technology Madras in 2005. She joined as Lecturer in Civil Engineering in Rajiv Gandhi Institute of Technology (RIT), Kottayam in 2001. Presently she is working as an Assistant Professor in Civil Engineering in RIT, Kottayam. She is a life member of Indian Society for Technical Education (ISTE). Her area of interest includes biological treatment of wastewater, solid waste management, water quality analysis, and environmental impact assessment.