Influence of Substrate to Biomass Ratio (F/M) and pH On Microbial Stability of Activated Sludge

By

Muhammad Afif bin Mohd Said

(10803)

Dissertation submitted in partial fulfillment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

JANUARY 2012

Universiti Teknologi PETRONAS
Bandar Seri Iskandar
31750 Tronoh
Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

Influence of Substrate to Biomass Ratio (F/M) and pH On Microbial Stability of Activated Sludge

by

Muhammad Afif bin Mohd Said

A project dissertation submitted to the
Chemical Engineering Programme
Universiti Teknologi PETRONAS
in partial fulfilment of the requirement for the
BACHELOR OF ENGINEERING (Hons)
(CHEMICAL ENGINEERING)

Approved by,

(AP Ir Abdul Aziz bin Omar)

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK
September 2011

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

MUHAMMAD AFIF BIN MOHD SAID

ABSTRACT

Influence of Substrate to Biomass Ratio (F/M) and pH On Microbial Stability of Activated Sludge

Activated sludge contains biological flocs composed of bacteria that degrade the organic matter in the waste water for its growth. The microbial stability of the activated sludge represented by the characteristics of the flocs. Project is focused on activated sludge used by most operating wastewater treatment plant owned by PETRONAS. Bacteria chosen will be BIOCHEM (BCI-103) brand. The bacteria are taken from a gas processing plant located in Kerteh, Terengganu.

The main objective of this project to study on the influence of two major parameters in wastewater, which are the substrate to biomass ratio or also known as food to microorganism (F/M) and pH, to the characteristics of the bacteria flocs. Optimum value of F/M and pH obtained from this project will be recommended to further enhance the wastewater process control at the processing plant. Besides that, the characteristics of flocs that obtained at different F/M and pH can be served as informative data to further enhance the understanding of the bacteria used.

Nowadays, with the increasing industrial activity, usage of hazardous chemicals escalates thus endangering the environment. News raised on the environment pollution especially the water pollution effecting the seas and rivers. One of the main causes of this pollution is the ineffective operation of wastewater treatment plant. Many microbially-related activated sludge solids separation problems occurred such as dispersed growth, filamentous bulking and foam formation. Existing design parameter such as sludge age, Chemical Oxygen Demand (COD), and Sludge Volume Index (SVI) can't predict the stability of the bacteria flocs. These design parameters are more oriented to the final goal of releasing effluent below the standard limit set by the government. Thus, the project will focus on the study of F/M and pH as design parameters and their influence to the bacteria flocs.

The scope of study for this project contains three elements which are the F/M, pH, and the microbial stability of the activated sludge. With others parameters such as temperature, Mixed Liquor Suspended Solids (MLSS), and sludge retention time (SRT) set constant, F/M and pH are manipulated to obtained the results. Microbial stability of activated sludge is judged by the characteristics of bacteria flocs. Activated sludge is stable when microorganisms to stick to each other and to nonbiological particles as sludge flocs (Jenkins et al.,2004). Increase size of floc diameter indicates the sludge is stable and at optimum condition. Activated sludge is populated by all kinds of bacterias. However, to study the microbial stability for this experiment, six species were chosen which are amoebae, flagellates, free swim ciliates, crawling ciliates, stalked ciliates, and rotifer. The abundance of these species are found and analyzed as each species has its own function and indication for the condition of sludge.

Research starts by searching for any other relevant journal or article published by other researches and from there, the results would be review and useful information will be summarize and taken as an information to this research. Next, contacts are made with the industry personnel in order to obtain the sample bacteria and knowledge of the activated sludge system used in the plant. Then, research progress experimentally in the laboratory and the results gained are discussed and documented.

Findings from this project show that the microbial stability of activated sludge is influenced by F/M and pH. Under different F/M and pH conditions, bacteria flocs particles and sizes are different. Under microscope, bacteria population is analyzed and documented. Efficiency which is calculated by the removing percentage of the (COD) is plotted. Further findings are discussed in latter part of this dissertation.

ACKNOWLEDGEMENT

Alhamdulillah Praise to Allah, the Most Gracious, Most Mercy, Most Wise for His Blessings, giving me the opportunity to still live in this world and oblige my duties as a Muslim. Blessings by Allah S.W.T to Prophet Muhammad (peace be upon him) for his sacrifices in his duty, spreading knowledge in the name of Allah S.W.T and teaching us the beauty of Islam. First of all, I would like to express my feel of gratitude to the Almighty Allah S.W.T as His permission allows me to complete this task successfully. This dissertation is prepared due to the requirement for the requirement for the Bachelor Of Engineering (Hons)(Chemical Engineering). I would like to thank to my supervisor, AP Ir Abdul Aziz bin Omar for giving me the knowledge and guidance through all the process of completing this report. Next I would like to thanks the lab technicians for helping me with the equipments needed for this project. Then, I would like to thanks my friends who have provided me with numerous comments and suggestions. I also like to thank Mr Zukefeli Bin Jusoh, IETS Competent Person, for his knowledge and information of wastewater treatment plant. Last but not least, I would like to thank my beloved parents, En Mohd Said Bin Abdul Hamid and Norizan Binti Hamid for their never ending support that helps me completed the project. Thank you.

Table of Contents

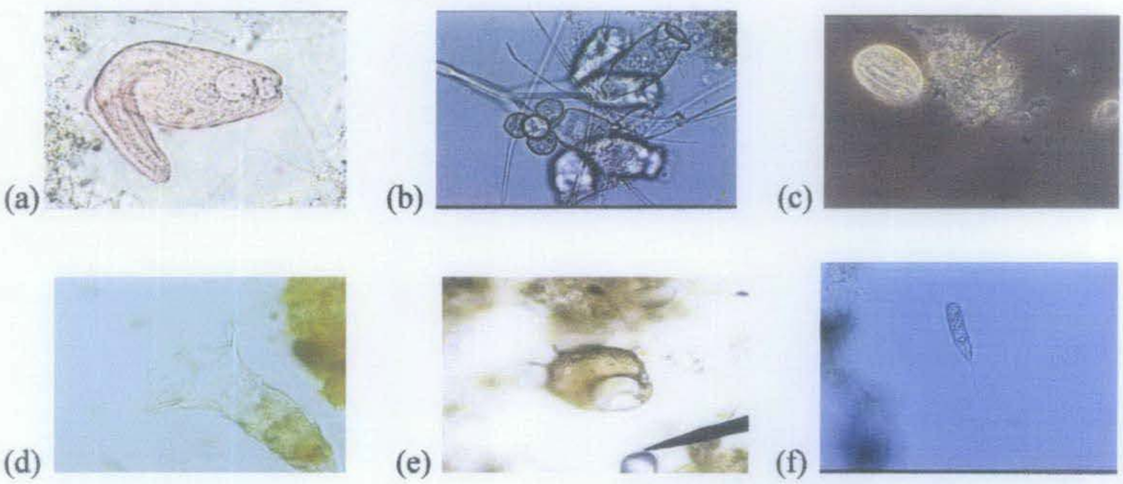
List of figures

CHAPTER 1: INTRODUCTION

1.0 Background of study

Activated sludge system has been used to treat municipal and industrial waste water since 1913. The main purpose of activated sludge is to oxidize carbonaceous and nitrogenous matter to remove the contaminants from waste water. The system involves introducing oxygen into the wastewater to create biological flocs. The flocs composed of biological components and nonbiological components. The biological component consists of a wide variety of bacteria, fungi, protozoa and some metazoa. The nonbiological component is made up of organic and inorganic particulates. There are six main types of bacteria in the effluent of the studied industrial wastewater treatment plant. The six species are illustrated in the figure below:

Figure 1: Main type of bacteria in industrial WWTP: (a)Free-swimming Ciliates (b)Stalked Ciliates(c) Crawling Ciliates (d)Rotifer (e) Amoebae (f)Flagellates



Commonly, activated sludge is used in conventional wastewater treatment plant. In the conventional plant, the main components are aeration basin and clarifier. Wastewater is treated at the basin where activated sludge is stored and aerated then transferred to clarifier to clarify the water from sludge and recycled the sludge back to the basin. However, nowadays, the Sequencing Bach Reactor (SBR) is beginning to be widely used.

Compared to the conventional method that runs continuously, SBR system run in batch which save a lot of operating cost and reducing the need of many equipments such as pump for the recycling process. Therefore, for this research, SBR system is used because of its simplicity and ease of operating besides feasible for the experiment to be done in the university laboratory.

There are many parameters that can affect the stability of activated sludge. The studied parameters are F/M ratio and pH. Those parameters are the main challenges to control in the wastewater plant. F/M ratio is food to microorganism ratio or also known as substrate to biomass. F/M ratio is the most suitable design parameter to indicate the microbial activity of the sludge floc as it indicates the amount of substrate supplied per day per unit of biomass in the biological reactor. When COD is used as substrate parameter, the F/M ratio should be expressed in terms of the influent biodegradable COD. Formula below shows the F/M calculation:

$$\frac{F}{M} = \frac{QC}{VrXh}$$

Where, Q = wastewater flowrate , C = influent biodegradable COD, Vr = volume of reactor, Xh = active heterotrophic biomass(mixed liquor suspended solids(MLSS))

Meanwhile, pH is a measure of the acidity or basicity of an aqueous solution. pH is measured using pH indicator. From manipulating these two parameters, microscopic examination is done on the sludge to analyze the stability in terms of the bacteria flocs.

1.1 Problem statement

In early industrial design, McKinney (1962) introduced reactor hydraulics as an important design parameter. Then, sludge age was defined as the major parameter for design (Jenkins and Garrison 1968). A major milestone for a better system was the adoption of COD as substrate parameter (Grady and Wiliams, 1975). However, these approaches might not be accurate as the microbial stability of the activated sludge can't be predicted. The design parameters are more oriented towards the final effluent discharge to comply with the regulation limit set by the governmet. There are many microbially-related activated sludge solids separation problems. Such as dispersed growth,filamentous bulking and foam formation. Therefore, research is done on influence of F/M ratio and pH parameters on micrology of sludge. The significant of the project is to promote the use of F/M and pH as design parameters by studying the effect of these parameters to the stability of the bacteria flocs.

1.2 Objective of report

The objectives of this project are:

1) To study the effect of F/M ratio and pH to the activated sludge in detail
2) To obtain optimum value of F/M and pH for a specific bacteria brand used in WWTP in Malaysia
3) To do a microscopic experiment on the sludge stability by characterizing the bacteria species that exists in the flocs by means of manipulating the F/M and pH value

1.3 Scope of study

The scope of study would be on the effect of food to microorganism ratio (F/M) and pH parameters to the sludge. Other parameters are set constant. First, the source of the sludge is identified from a gas processing plant and is produced from rice husk. In order to make it feasible to be done in the university laboratory, a suitable oxidation system is chosen and equipments are setup based on the oxidation system theory. While research is going, daily controlling and monitoring of the sludge sample are done such as the pH monitoring so that the bacteria will be in good conditions and the filamentous characteristics are not affected. The results are documented and the microscopic images can benefit in the process control of wastewater plant in the near future.

1.4 Relevancy and feasibility of the project

From statistic of Malaysia Water Industry Guide, 2007, effluent discharge to the river and sea water consists of 45% of manufacturing industry, 5% from animal farm (pig farm), 3% from agro-based industry, and 47% of sewage treatment plants. Most of the manufacturing industry and sewage treatment plants use conventional biological treatment by activated sludge to treat the effluent water. This study is relevant and practical for the situations in Malaysia as it studies new strategy of improving process performance and stability of biological treatment by manipulating the parameters of F/M ratio and pH of sludge. Besides that, this study is also feasible as there is environmental research lab in UTP and the facilities can be used by the student. This study also provides an opportunity to widen knowledge on wastewater operations and problems specifically regarding the microbial of the activated sludge.

CHAPTER 2: LITERATURE REVIEW

2.1 Substrate to biomass ratio (F/M)

The first related literature is ***Influence of operational conditions on the performance of a mesh filter activated sludge process*** reported in 2005 by W.Fuchs, C.Resch, M.Kernstock,M.Mayer,P. Schoeberl, and R. Braun. The objective of the research is to discuss on effect of suspended solids concentration, flux rate and aeration rate to mesh filter process. A model mesh filter reactor created and the parameters manipulated to obtain the result. From result, F/M ratio has significant effect on floc characteristics

The second literature is ***Sequencing versus continuous membrane bioreactors: Effect of substrate to biomass ratio (F/M) on process performance*** reported in 2008 by J.Lobos, C. Wisniewski, M. Heran, and A. Grasmick. The aim of the project is to study effect of substrate/biomass ratio(F/M) on performance of the two immersed membrane bioreactors. High and low F/M conditions are created by differencing the feeding period.

The third literature is ***Effect of food to microorganism ratio on biohydrogen production from food waste via anaerobic fermentation*** written in 2008 by J. Pan, R. Zhang, H. Mashad, H. Sun, and Y. Ying. The objective is to study effect of different F/M ratio on hydrogen production of fermentation at mesophilic and termophilic temperature. Different amount of food waste added to existing volatile solids in inoculum reactor to give different F/M ratio. Controlling F/M ratio at proper level was a feasible approach to enhance H_2 production

From these literatures, useful informations are extracted for the use of this project. For example, information on the methodology of the experiments. It can be concluded that to maintain the conditions of bacteria, continuous supply of oxygen must be provided and mixing activity must be done. Thus, this experiment will be done in a jar test apparatus to provide the mixing activity and used air pumps to provide oxygen for bacteria.

2.2 pH

The first literature is the ***effect of pH on anaerobic fermentation of primary sludge at room temperature*** reported in 2009 by HaiyanWu, Dianhai Yang, Qi Zhou, and Zhoubing Song. The objective of this research is to investigate the effect of pH in the range of 3.0–11.0 on anaerobic fermentation of primary sludge (PS) at room temperature. In order to investigate the effect of pH on anaerobic fermentation of sludge, 10 identical reactors, each with working volume of 3 L of sludge were used. All the reactors were mixed with a mechanical stirrer operated at a rate of 70rpm (revolutions per minute) to sustain homogenous mixing during fermentation The pH was adjusted using 2M NaOH or 2M HCl stock solution.

The second literature is the ***production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent*** written in 2003 by Adeline S.M. Chua, Hiro Takabatake, Hiroyasu Satoh, and Takashi Mino. The objective of this paper is to study the effect of three operational factors, which are the acetate concentration in influent, pH, and sludge retention time (SRT). In PHA production batch experiments, pH value influenced significantly the PHA accumulation behavior of activated sludge. When pH of batch experiments was controlled at 6 or 7, a very low PHA production was observed. The production of PHA was stimulated when pH was kept at 8 or 9.

From these literatures, knowledge was gained on the operating procedure and methodology. For this project, SBR system with a 24-h cycle will be set and the mixing speed will at 60 rpm for each 1L beaker. Besides that, information on the optimum value of pH for the sludge in China and Japan are extracted. It shows that different sludge have different optimum pH. The informations will later be compared with the resulting optimum pH from this project.

2.3 Microbial stability of activated sludge

In this project, microbial stability of activated sludge is represented by the composition of six protozoan species which are amoebae, flagellates, free swimming ciliates, crawling ciliates, stalked ciliates, and rotifer. These species indicates the healthiness of the sludge. Summary on the indications of the six species is shown in table below:

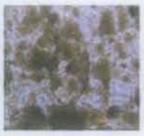
Species	Amoebae	Flagellates	Free swim ciliates	Crawling ciliates	Stalked ciliates	Rotifer
Diagram						
Indicates	A lot of floating nutrient available	Large amount of soluble organic nutrients	Feed on increased number of bacteria	Healthy sludge.Can find food within floc	Appear in mature sludge	Sludge is stable and floc structure is developing

Table 1: Wastewater species indications

The main literature for analyzing the bacteria flocs is the ***manual on the cause and control of activated sludge bulking, foaming, and other solid separation problems*** written in 2003 by David Jenkins and Glen Daigger. From the book, informations on the methods for bacteria flocs characterization are shown. The book also promotes the use of protozoa as indicator organisms. Satisfactory activated sludge performance occurs when there is a balance among free-swimming and ciliates and rotifers.

2.4 Sequential Batch Reactor(SBR) System

Comparison is made between conventional wastewater treatment plant system and sequential batch reactor (SBR) system. In conventional system, two main equipments are used which are the aeration tank and clarifier. In aeration tank, oxygen is introduced into the system to create an aerobic environment that meets the needs of the biological community and that keeps the activated sludge properly mixed. To ensure enough bacteria are available to consume waste product, sludge must be returned to the tank from the clarifier as return activated sludge. In clarifier, activated-sludge solids separate from the surrounding wastewater by the process of flocculation. Next, return activated sludge must be collected from the clarifier and pumped back to the aeration tank.

The main literature for SBR is ***sequencing batch reactor design and operational considerations***, written in 2005 by New England Interstate Water Pollution Control Commission. Sequential batch reactor system is a new system compared to conventional sytem. It only use one main equipment which is the aeration tank. Costs are saved by using SBR as there is no need for additional pump and electricity for the clarifier. SBR system also needs oxygen that is supplied through bubble diffusers. The operation of an SBR is based on fill-and-draw principles, which consists of five steps—fill, react, settle, decant, and idle. These steps can be altered for different operational applications. During the fill phase, the basin receives influent wastewater which brings food to the microorganisms in the activated sludge. Reaction phase allows for reduction of wastewater parameters mainly the COD and the Biological Oxygen Demand(BOD). During settle phase, activated sludge is allowed to settle in order to form a distinctive interface with the clear supernatant. At decanter phase, a decanter is used to remove the clear supernatant effluent. Idle phase occurs between the decant and the fill phases and during this phase, wasting process occur.

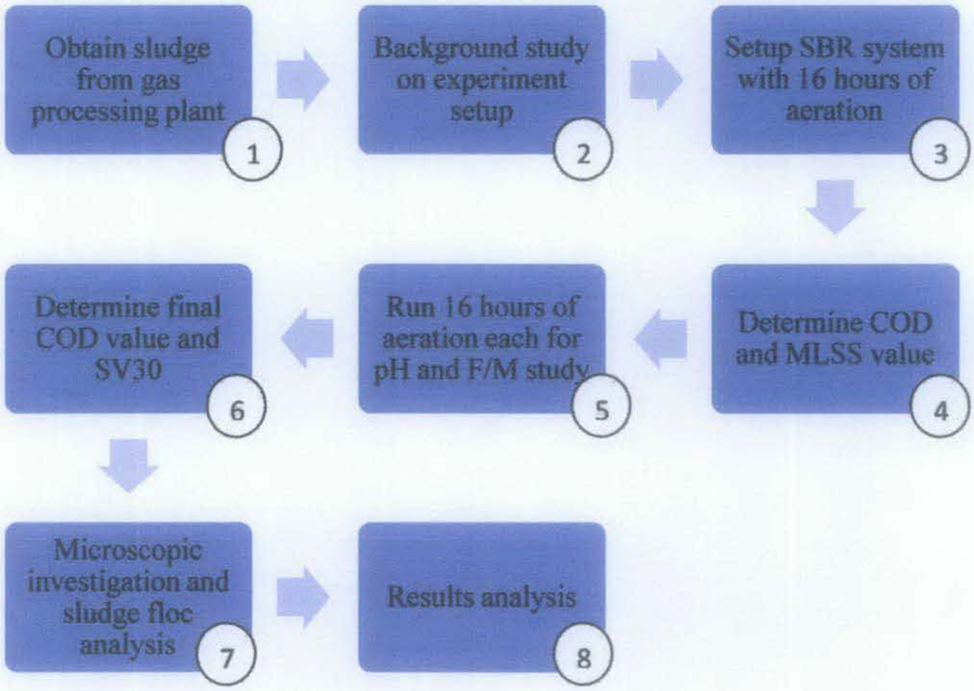
CHAPTER 3: RESEARCH METHODOLOGY

3.1 Methodology flowchart

Figure 2: Flowchart of the research methodology and its descriptions

3.2 Project activities

Figure 3: Experiment methodology



3.2 .1 Sample preparation

1. *Introduction*

The source of the sludge is from aeration basin in WWTP in Gas Processing Plant, Terengganu. Microscopic examination will generally yields information related to activated sludge behavior in solids separation processes because the physical properties of the activated sludge revealed during microscopic examination determine its settling and compaction characteristics.

2. *Sampling points*

Take mixed liquor sludge at points of good missing either from effluent end of an aeration basin or from mixed liquor channel between aeration basin and clarifier. Take samples from below the surface to exclude any foam or other floating material. If foam or scum present, take separate sample of foam and scum from one of the following points: (1) surface of the effluent of the aeration basin, (2) surface of mixed liquor channel, (3) surface of clarifier.

3. *Sample transport and storage*

5L of sludge will be taken from the aeration basin. The sample will be transported from Terengganu to the chemical lab in UTP in plastic containers. The containers must not be filled more than half full to prevent samples from being septic. Since the journey from the WWTP to UTP will be more than 8 hours, the sample is stored in ice chests throughout the journey to lower the bacteria mechanism and to avoid bacteria death. In UTP, the sample will be stored in cool room before the F/M ratio and pH experiments are done. Sample conditions are monitored and controlled daily such as the pH and the amount of foods available.

3.2.2 Background study on experiment setup

From the literature review, SBR system has been chosen as the treatment method for this project. Main elements for SBR system are the aeration tank where reaction occur, oxygen supply and the mixer.

3.2.3 Setup SBR system with 16 hours of aeration

To setup the SBR system, jar test equipment, 1L beaker and aquarium air pump were used. Jar test equipment were used to provide the mixing action. The beaker served as the reactor tank and the air pump is used to provide oxygen to the microorganisms. 24 hour cycle was used for this SBR system, which 30 minutes are for fill phase, 3 hours of anaerobic stirring which is still in fill phase, 16 hours of react phase with aeration process, 2 hours of sludge settling to create layers of supernatant and sludge, and lastly idle phase of 90 minutes for microscopic examination. The setup of the equipment is shown in figure:

Figure 4: Experiment Setup

3.2.4 Determine Chemical Oxygen Demand (COD) and Mixed Liquor Suspended Solids (MLSS) value

COD experiment

1. Samples to be used are raw sewage and effluent.
2. Take 2mL of solution and put into high range COD reagent.
3. Heat under 150°C for 2 hours.
4. Leave to room temperature.
5. Put into COD reader and record the COD value.

Figure 5: COD reader

MLSS experiment

1. Samples to be used are from the activated sludge.
2. Obtain the tare weight of an aluminium dishes containing a glass fibre filter.
3. Assemble filtering apparatus, position the filter and begin suction. Wet filter with a small volume of distilled water to seat it.
4. Stir the samples contents and then rapidly (so that it does not settle) measure 10 ml of sludge.Record the total volume filtered. Rinse the graduated cylinder with small amounts of distilled water and add to filter.
5. Carefully remove filter from filtration apparatus and transfer it back to the aluminium dish. Pinch sides of dish in a bit to protect the filter from oven drafts. Place the aluminium dish into the 103°C oven to dry for at least one hour (leave drying overnight).
6. Transfer dish to dessicator, cool and weigh. Calculate the MLSS in terms of mg/L.

Figure 6: Filtering Apparatus

3.2.5 Run 16 hours of aeration each for pH and F/M study

Experiment on pH

1. Set equipment as figure 4 above. Connect air pump and use six beakers to store the sludge.
2. Label the six beakers with A, B, C, D , E, and F.
3. Fill in 300mL of activated sludge into each beaker
4. Fill in 700mL of effluent water into each beaker.
5. Measure the MLSS and COD.
6. Ensure that the MLSS and COD are even for every beaker.
7. Arrange the pH so that beaker A will have ph of 2, beaker B with ph of 4, beaker C with ph of 6, beaker D with pH of 7, beaker E with ph of 8, and beaker F with ph of 10 using hydrochloric acid and calcium hydroxide.
8. Start the air pump and mixer for aeration and mixing process of the activated sludge.
9. The reaction process of the SBR system is set running for 16 hours.
10. After aeration, stop aeration and mixing for 3 hours and 30 minutes for decanter and idle phase.
11. Measure the SV30 for each samples.
12. Measure the COD level of the effluent after aeration and calculate the removal efficiency.
13. Plot graph of pH versus COD removal efficiency.
14. Record the floc characteristics using microscope.

Experiment on F/M ratio

1. Set equipment as figure above.
2. Label the five beaker with A, B, C, D and E.
3. Fill in 300mL of activated sludge into each beaker
4. Fill in 700mL of effluent water into each beaker.
5. Measure the MLSS

6. After obtaining the MLSS value, add in glucose as source of COD to the effluent until desired F/M value is calculated using formula.
7. Add COD in beaker A until F/M is 0.2.
8. Add COD in beaker B, C, D and E until the F/M value is 0.4, 0.6, 0.8 and 1.0 respectively.
9. Start the air pump and mixer for aeration and mixing process of the activated sludge.
10. The reaction process of the SBR system is set running for 16 hours.
11. After aeration, stop aeration and mixing for 3 hours and 30 minutes for decanter and idle phase.
12. Measure the SV30 for each sample.
13. Measure the COD level of the effluent after aeration and calculate the removal efficiency.
14. Plot graph of F/M ratio versus COD removal efficiency
15. Record the floc characteristics using microscope

3.2.6 Determine final COD value and SV30

1. After aeration, transfer the activated sludge into 1L Imhoff cone.
2. Let the sludge settle for 30 minutes and measure the settled sludge quantity.
3. Take 50 mL of clear supernatant at the top of the sludge layer and measure the final COD value using the steps mentioned in COD experiment.

Figure 7: SV30 test

3.2.7 Microscopic investigation and sludge floc analysis

Floc Microscopic Observation

1. Spread 1 drop of sample evenly over the area of microscope slides.
2. Place a glass cover slip on the drop and press down gently on the cover slip with a blunt object.
3. Place prepared sample on paper towel to expel excess liquid
4. Examine the wet mount using electron microscope under phase contrast illumination at 10x, 20x and 40x magnification.
5. Determine floc characteristics. Observe whether flocs are round or irregular and compact or diffuse and whether the texture is firm or weak.

Figure 8: Optical Microscope

3.3 Gantt Chart

No	Project Activities	FYP I														FYP II														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Selection of Project Topic																													
2	Topic Introduction with SV																													
3	Research on Relevant References																													
4	Preparation on Work Schedule																													
5	Study on F/M ratio and pH																													
6	Study on parameters effect on sludge floc																													
7	Confirmation of Equipments and Material at Lab																													
8	Preparing Complete Methodology																													
9	Work on Literature Review																													
10	Proposal Preparation																													
11	Submission of Extended Proposal Defence																													
12	Proposal Defence																													
13	Preparing Standards & Procedure																													
14	Preparation of Lab Scale equipments																													
16	Travelling to industry to get sludge specimens																													
17	Study industrial wastewater plant practices																													
18	Preparation on Interim Report																													
19	Sumission of Interim Draft Report																													
20	Submission of Interim Report																													
21	F/M experiments																													
22	pH experiments																													
23	Microscopic inverstigation and photo recording																													
24	Analysis of sludge flocs																													
25	Verify Result with Previous Result																													
26	Establish Pattern of microbial stability vs F/M & pH																													
27	Preparation on Progress Report																													
28	Submission of Progress Report																													

29	Pre-EDX																								●					
30	Preparation on Final Report & Technical Paper																					■	■	■	■					
32	Submission of Draft Report																									●				
33	Submission of Soft Bound & Technical Paper																										●			
35	Preparation on Oral Presentation																									■	■			
36	Oral Presentation																											●		
37	Submission of Hard Bound																												●	

Table 2: Gantt Chart

● Key Milestone

3.4 Equipments/Tools involved

No.	Tool / Equipment	Description
1.	Jar test apparatus	For mixing activity and experiments workplace
2.	1L beaker	To culture/store bacteria where the biological reaction happens
3.	Industrial Specimens(BIOCHEM)	Activated sludge from industry
4.	Glucose	Source of COD for activated sludge
5	Air Pump	To supply oxygen to bacteria
6.	pH indicator	To specify the pH value in samples
7.	COD test apparatus	To measure COD value in samples
8.	MLSS test apparatus	To calculate the amount of MLSS in sample for F/M calculation
9.	Optical microscope (OM)	Scanning specimen with a light beam to view the bacteria flocs and record the images

Table 3: List of equipments/tools involved

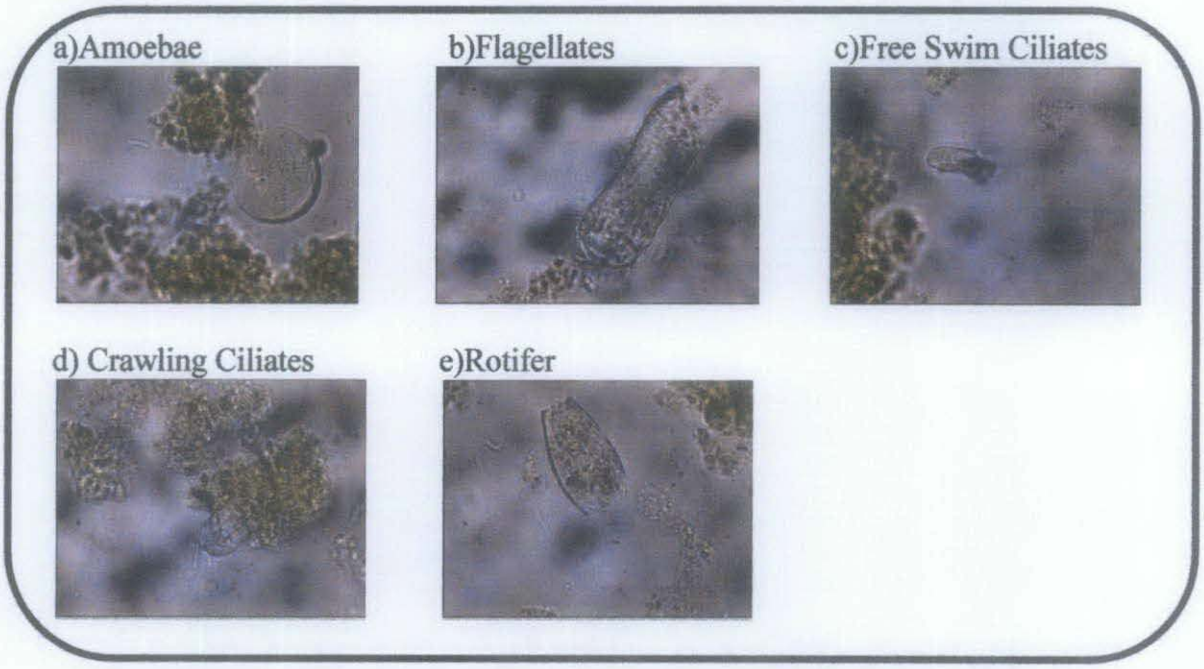
CHAPTER 4: RESULT AND DISCUSSION

4.1 Result

4.1.1 pH

Microscopic observation

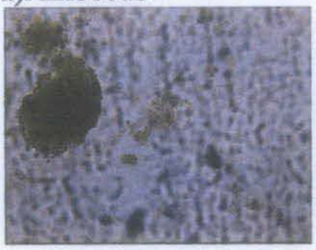
pH2



Species	Abundace Ranking(Highest to Lowest)
Amoebae	1
Flagellates	2
Free-swim ciliate	4
Crawling ciliate	5
Stalked ciliate	6
Rotifer	3

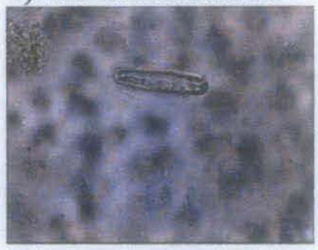
pH4

a)Amoebae



b)Flagellates

c)Free Swim Ciliates



d) Crawling Ciliates

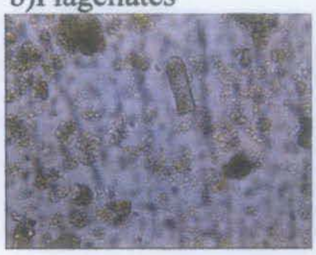
e)Rotifer

Species	Abundace Ranking(Highest to Lowest)
Amoebae	1
Flagellates	4
Free-swim ciliate	2
Crawling ciliate	5
Stalked ciliate	6
Rotifer	3

pH6

a)Amoebae

b)Flagellates



c)Free Swim Ciliates

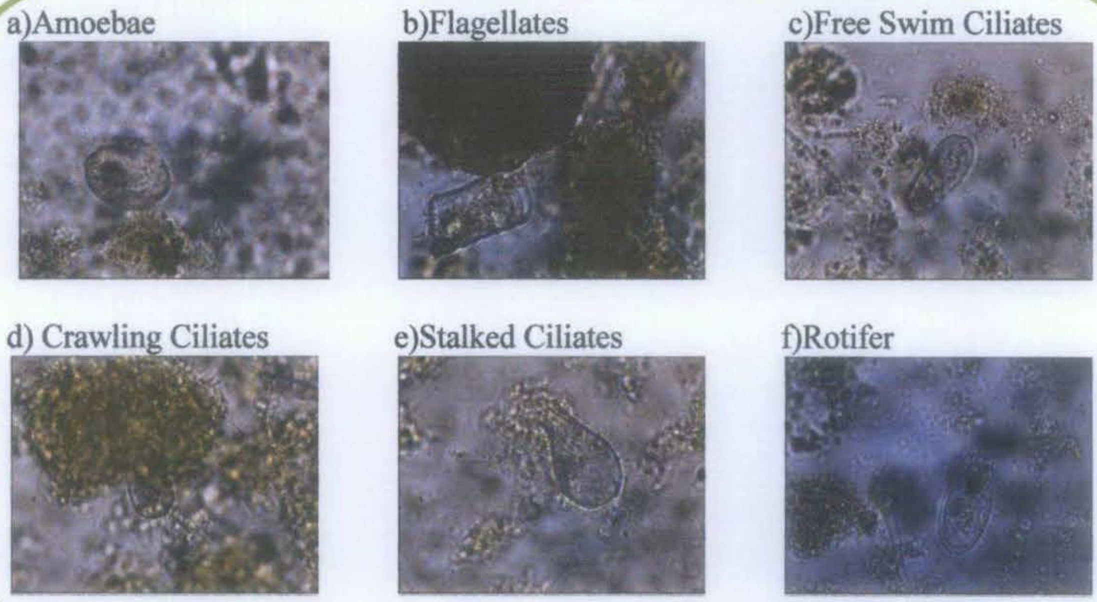
d) Crawling Ciliates

e)Stalked Ciliates

f)Rotifer

Species	Abundace Ranking(Highest to Lowest)
Amoebae	5
Flagellates	4
Free-swim ciliate	1
Crawling ciliate	2
Stalked ciliate	6
Rotifer	3

<u>pH7</u>



Species	Abundace Ranking(Highest to Lowest)
Amoebae	5
Flagellates	6
Free-swim ciliate	2
Crawling ciliate	1
Stalked ciliate	4
Rotifer	3

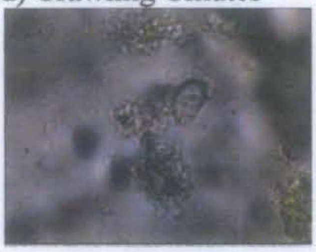
pH8

a)Amoebae

b)Flagellates

c)Free Swim Ciliates

d) Crawling Ciliates



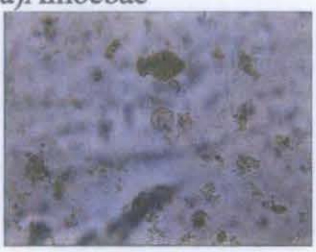
e)Stalked Ciliates

f)Rotifer

Species	Abundace Ranking(Highest to Lowest)
Amoebae	4
Flagellates	5
Free-swim ciliate	1
Crawling ciliate	2
Stalked ciliate	6
Rotifer	3

pH10

a)Amoebae



b)Flagellates

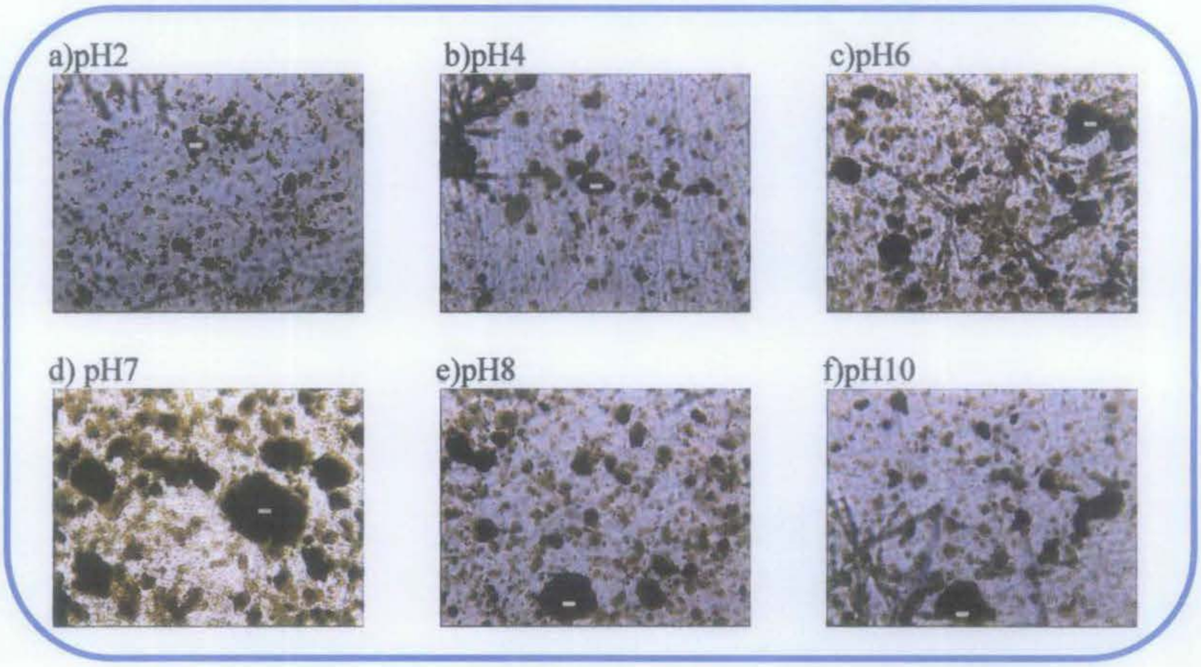
c)Free Swim Ciliates

d) Crawling Ciliates

e)Rotifer

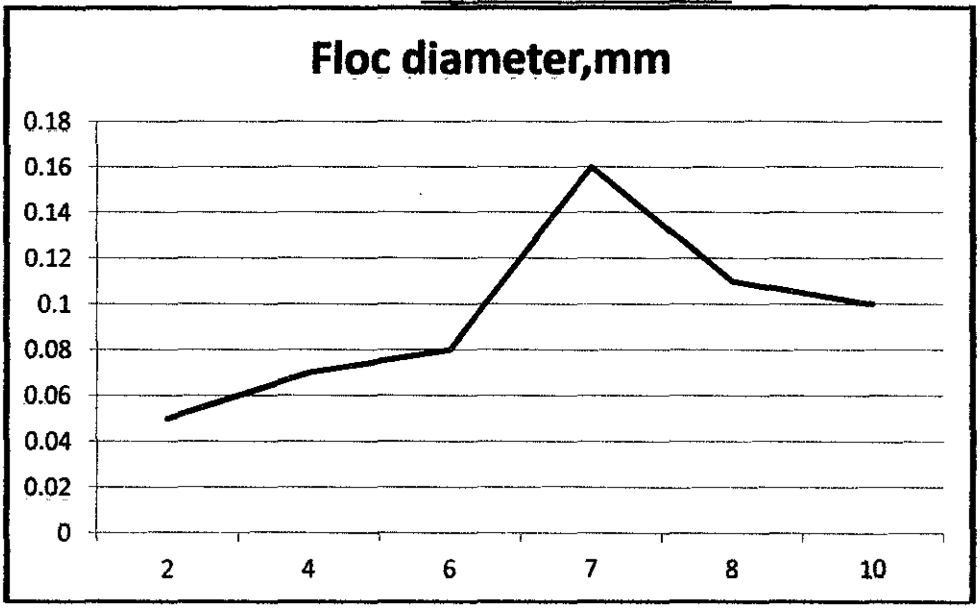
Species	Abundace Ranking(Highest to Lowest)
Amoebae	1
Flagellates	4
Free-swim ciliate	2
Crawling ciliate	5
Stalked ciliate	6
Rotifer	3

Floc characteristics



pH	Floc diameter	Floc arrangement	Texture
2	0.05	Diffuse	Weak
4	0.07	Diffuse	Weak
6	0.08	Compact	Firm
7	0.16	Compact	Firm
8	0.11	Compact	Firm
10	0.1	Diffuse	Firm

Figure 9: Floc Diameter



COD removal efficiency

$$\frac{F}{M} = \frac{QC}{VrXh}$$

Where, Q = wastewater flowrate (80 m^3/h) , C = influent biodegradable COD(20,000 ppm), Vr = volume of reactor (2250m^3), Xh = MLSS(4455 ppm)

*wastewater flowrate and volume of reactor data obtained from gas processing WWTP

COD Removal efficiency = (Initial COD-Final COD) x 100%

Initial COD

pH	Initial COD(ppm)	Final COD(ppm)	Efficiency	SV30
2	22500	16731	25.64	310
4	22500	18707	16.85778	260
6	22500	20466	9.04	300
7	22500	10030	55.42222	340
8	22500	15969	29.02667	260
10	22500	20538	8.72	300

Table 4: COD Removal Efficiency

Figure 10: pH COD Removal Efficiency

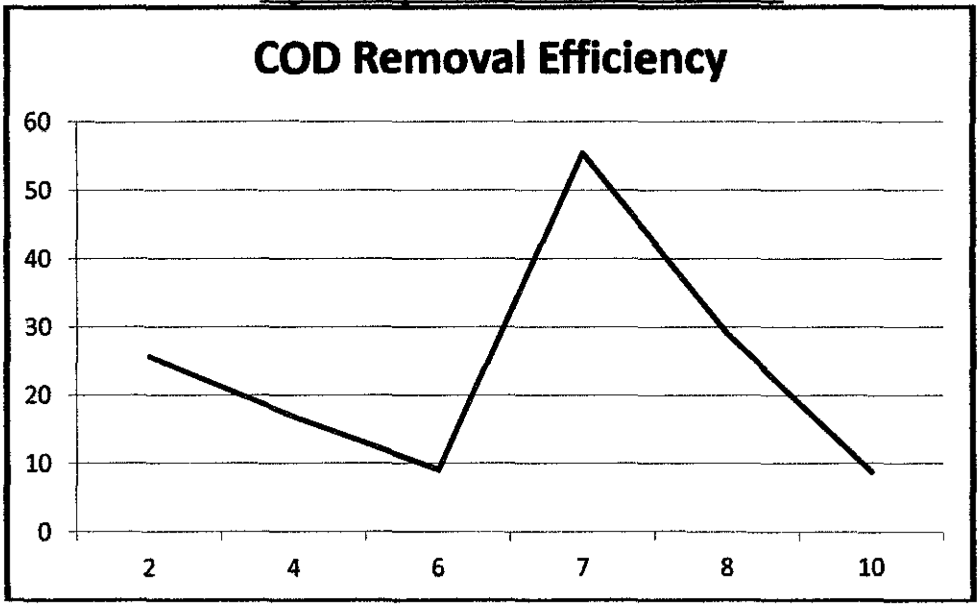
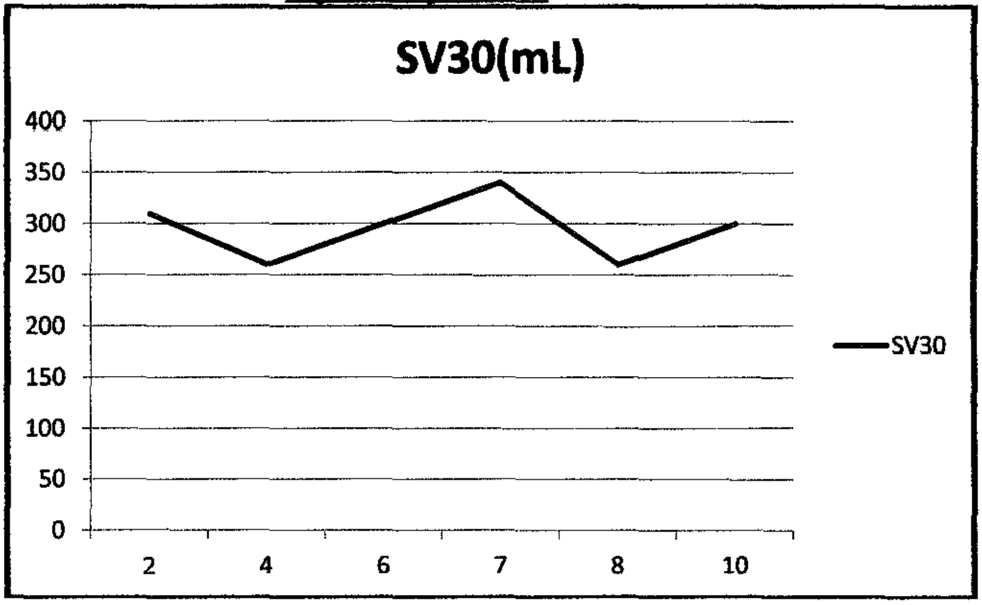


Figure 11: pH SV30



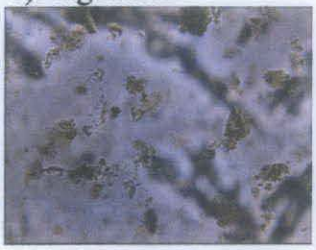
4.1.2 F/M

Microscopic observation

F/M = 0.2

a)Amoebae

b)Flagellates



c)Free Swim Ciliates

d) Crawling Ciliates

e)Stalked Ciliates

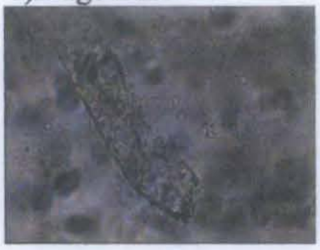
f)Rotifer

Species	Abundace Ranking(Highest to Lowest)
Amoebae	5
Flagellates	6
Free-swim ciliate	2
Crawling ciliate	1
Stalked ciliate	4
Rotifer	3

F/M = 0.4

a)Amoebae

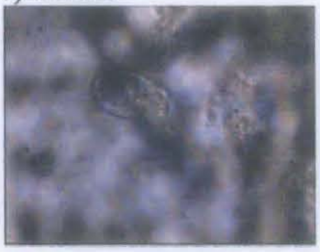
b)Flagellates



c)Free Swim Ciliates

d) Crawling Ciliates

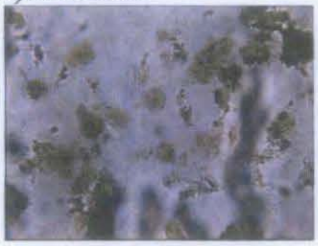
e)Rotifer



Species	Abundace Ranking(Highest to Lowest)
Amoebae	5
Flagellates	4
Free-swim ciliate	1
Crawling ciliate	2
Stalked ciliate	6
Rotifer	3

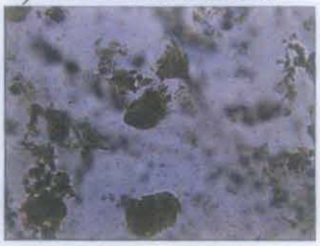
F/M = 0.6

a)Amoebae



b)Flagellates

c)Free Swim Ciliates



d) Crawling Ciliates

e)Rotifer

Species	Abundace Ranking(Highest to Lowest)
Amoebae	1
Flagellates	4
Free-swim ciliate	2
Crawling ciliate	5
Stalked ciliate	6
Rotifer	3

F/M 0.8

a)Amoebae

b)Flagellates

c)Free Swim Ciliates

e)Rotifer

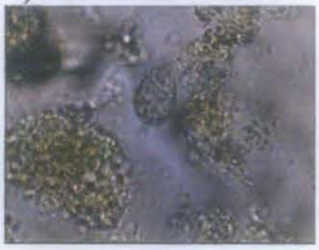
Species	Abundace Ranking(Highest to Lowest)
Amoebae	1
Flagellates	2
Free-swim ciliate	3
Crawling ciliate	5
Stalked ciliate	6
Rotifer	4

F/M = 1.0

a)Amoebae

b)Flagellates

c)Free Swim Ciliates

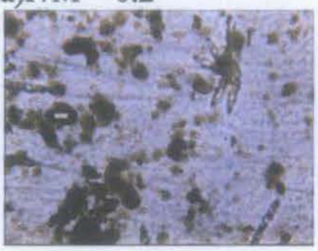


e)Rotifer

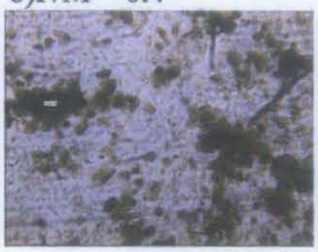
Species	Abundace Ranking(Highest to Lowest)
Amoebae	1
Flagellates	2
Free-swim ciliate	3
Crawling ciliate	5
Stalked ciliate	6
Rotifer	4

Floc characteristics

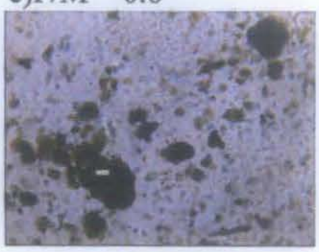
a)F/M = 0.2



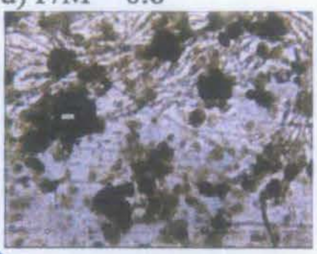
b)F/M = 0.4



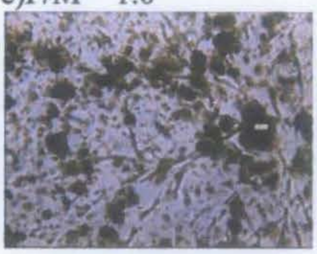
c)F/M = 0.6



d) F/M = 0.8

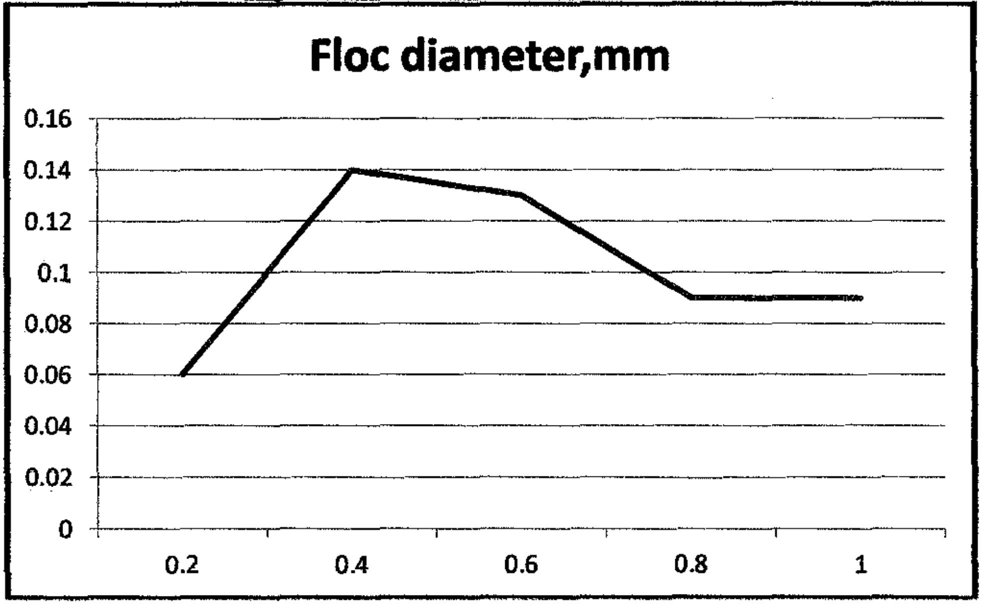


e)F/M = 1.0



F/M	Floc diameter	Floc arrangement	Texture
0.2	0.06	Compact	Firm
0.4	0.14	Compact	Firm
0.6	0.13	Compact	Weak
0.8	0.09	Compact	Weak
1.0	0.09	Compact	Weak

Figure 12: F/M Floc Diameter



COD removal efficiency

F/M	Initial COD (ppm)	Final COD (ppm)	Efficiency(%)	SV30(mL)
0.2	22500	8195	63.57778	310
0.4	45000	20664	54.08	260
0.6	67500	51356	23.91704	300
0.8	90000	63223	29.75222	340
1	112500	80200	28.71111	260

Table 4: F/M COD Removal Efficiency

Figure 13: F/M COD Removal Efficiency

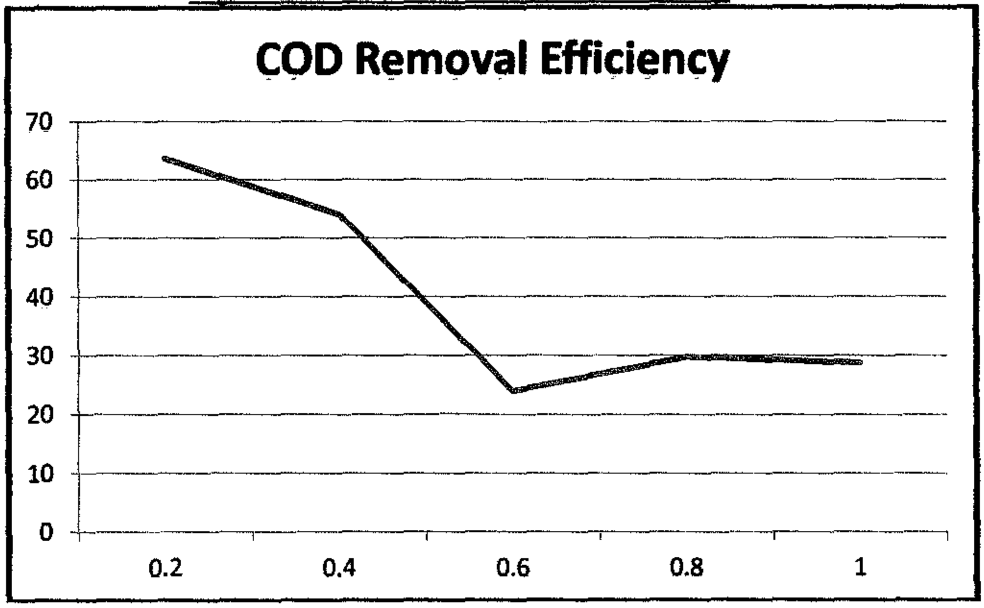
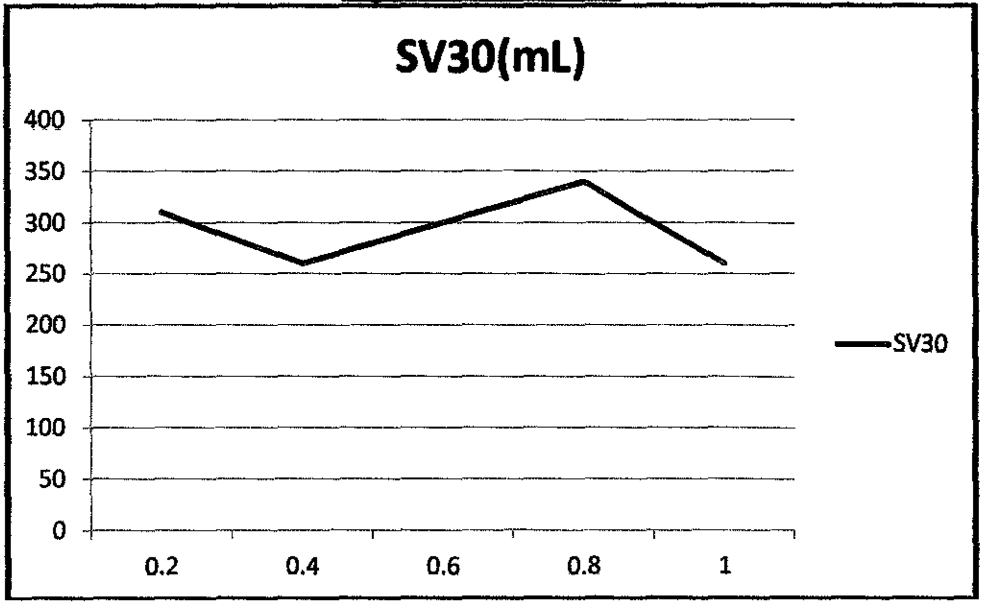


Figure 14: F/M SV30



4.2 Discussions

4.2.1 pH

From the results, the experiment will be analyzed in two parts which are in terms of floc characteristics and the COD removal efficiency. Floc characteristics is analyzed through the species found in the sludge at the specific pH. Firstly at pH 2, the flocs flocs arrangement are diffuse and the texture of the flocs are weak. From the abundance ranking, it shows that the bacteria growth is not efficient as the highest species found is amoebae. Amoebae indicate that a lot of floating nutrient available, meanings the contaminants are not digested by the bacteria. It shows that strong acidic condition is not favored by the bacteria for its growth. Same characteristics are also shown by bacteria flocs in the pH 4. Overall it shows that bacteria don't prefer acidic condition. Moving on the the pH 6, flocs arrangement are compact and the texture of the flocs are firm. The highest amount of species found is free swim ciliates. It indicates that sludge is in stable condition as the ciliates feed on the increased number of bacteria in the water. Bacteria flocs in pH 8 exhibit same nature, showing that the bacteria prefer pH near to the natural conditions. At natural pH7, healthy sludge exist as the highest amount of species found is crawling ciliates. Crawling ciliates indicate the population of floc is stable thus the ciliates can find food within floc. Lastly at pH 10, flocs arrangements are diffuse and the texture are firm. The population of species found is as pH4, showing that bacteria also didn't prefer alcalic condition. Further evidence can be found in the floc diameter trend as the biggest diameter of floc is at pH7. The bigger the flocs, the higher the amount of crawling ciliates, indicating the sludge is healthy.

In terms of COD removal efficiency, pH2 shows slightly high removal efficiency at 25.64%. Although the efficiency is high, acidic conditions are not suitable for bacteria growth as shown in the microscopic view of the bacteria flocs. The high efficiency reading is because bacteria have died and formed flocs leaving the water clear but still full with amount of constituents. Less amount of dissolved bacteria in the water, thus in the COD reader, less particle will impacted with the ray resulting in low COD reading. This is proven by the SV30 reading where At pH of 4,6, 8, and 10, the removal efficiency are 16.86, 9.04, 29.03 and 8.72 respectively. The findings show that even though in unsuitable conditions, some bacteria still live and bacteria synthesis happen while some bacteria hibernate or die. The highest removal efficiency at 55.42% occurred at pH 7. At this pH, bacteria activities are the highest resulting in low final effluent COD value. SV30 test represent the sludge age of the sample. The recommended range of SV30 is from 300 to 500 mL. However, from the graph, it shows that at acidic and alcalic condition, the SV30 is still in favorable range. Thus, it proves the problem statement that sludge age can't be used as design parameter to indicate microbial stability of activated sludge.

In summary, from the two analyses, pH7 is the most suitable for the BIOCHEM bacteria brand. Source from the gas processing plant personnel where the bacteria originated informed that the operating range of the pH control in the plant is from 6.5-8.5. Therefore, based on the results of this experiment, it is suggested that the wastewater treatment plant at the company be operated at the pH7 for optimum removal efficiency and bacteria growth.

4.2.2 F/M

Different conditions of F/M ratio is created by manipulating the amount of initial COD into the water and taking constant the value of bacteria. At F/M of 0.2, the highest species found is crawling ciliates, which indicates that the sludge is healthy and this value is preferable by bacteria. On the other hand, at F/M of 0.4, the highest species population is free swimming ciliates. Free swimming ciliates shows that the system is stable as the amount of bacteria is high, which the bacteria is feed on by the ciliates. At F/M of 0.2 and 0.4, flocs arrangement are compact and the texture is firm, indicating that these values are optimum for bacteria growth. Meanwhile, F/M of 0.6, 0.8, and 1.0 show similar trend where the highest amount of species is amoebae. It indicates the condition is not good for the system as a lot of floating nutrient still available. Comparing F/M of 0.2 and 0.4, F/M of 0.4 is more optimum for this bacteria at this aeration period of 16 hours. This is because the floc diameter at this condition is the biggest. Optimum F/M value differs according to the period of aeration of a system. Therefore, this prove the objective of this project where F/M ratio effect the mictobial stability of activated sludge.

With regards to COD removal efficiency, the highest efficiency is at F/M of 0.2 with 63.58%. While at F/M of 0.4, 0.6, 0.8, and 1.0, the efficiency is 52.08%, 23.92%, 29.75% and 28.71%. The results shows that the graph is on a decreasing trend with the increase of value of F/M. Slight error occur at F/M of 0.6 because of there still exist bacteria in the water, thus effecting the COD reading when more particle impacted with the light ray. This problem exist because of the clarity of supernatant left at the top the sludge during settling phase is different. SV30 represents the sludge age. The SV30 for all samples are in favorable range, thus proving again that sludge age is not suitable as design parameter for stability of bacteria flocs.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

In the nutshell, F/M ratio and pH do influence the microbial stability of activated sludge. Recap back the objectives which are to study the effect of F/M ratio and pH to the activated sludge in detail, to obtain optimum value of F/M and pH for a specific bacteria brand used in WWTP in Malaysia (BIOCHEM), and to do a microscopic experiment on the sludge stability by characterizing the bacteria species that exists in the flocs by means of manipulating the F/M and pH value it can be concluded that the objectives are achieved.

From this research, findings show that at different pH, bacteria flocs changes in characteristics and thus affecting the COD removal efficiency. The optimum value of pH obtained from the experiment is the value of 7. Therefore, it is suggested for the wastewater treatment plant to control the pH value to that range to maximize the efficiency of the plant.

In the journal ***Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: Effect of pH***,the optimal conditions for WAS fermentation observed in this study was pH 9.0. Besides that, in ***Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH***, the researcher demonstrated that pH 8 was beneficial for PHA production by using activated sludge acclimatized with synthetic wastewater while in ***the effect of pH on anaerobic fermentation of primary sludge at room temperature***, at fermentation time of 5 days, the average Short-chain fatty acids (SCFA)s concentration increased from 968 to 3511mg COD/L with the increase of pH from 3.0 to 10.0. However, further increasing pH to 11.0 resulted in the decrease of SCFAs. Comparing to these three researches, the value of pH 7 obtained from the experiment is feasible. It explains that neutral pH benefited the stability of the activated sludge and its efficiency.

From the results of the study of the influence of F/M ratio, optimum F/M ratio with biggest floc and high COD removal efficiency is F/M of 0.4. It can be concluded that the acceptable range of F/M that can be provided for efficient performance is until 0.4. F/M ratio over that range causes too much nutrient thus effecting the performance of bacteria to degrade the organic matter. In addition, from this study, it is suggested for the wastewater treatment plant to control the sludge retention time in accordance with the value of F/M ratio to optimum efficiency. Low F/M ratio or organic loading rate need shorter retention time and high F/M ratio need longer retention time. As conclusion, the research is relevant to the objectives and has academic values that can help improve the process control of a wastewater treatment plant.

Lastly, it is recommended that the plant operator control the operation conditions of pH and F/M as close to the proposed value as possible. Besides that, it is also recommended to use F/M ratio and pH as there parameters can predict the microbial stability of activated sludge better that other parameter such as sludge age. For future work to improve the wastewater treatment plant operation, continuous monitoring of these parameters must be done plant operates effectively thus releasing only clear effluent water to the environment.

REFERENCES

Adeline S.M. Chua, Hiroo Takabatake, Hiroyasu Satoh, Takashi Mino,(2003), Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent, Water Research 37, 3602–3611

Curds,C.R. and Fey, G. J. (1969) "The Effect of Ciliated Protozoa on the Fate of Escherichia coli in the Activated-Sludge Process," Water Research. : Vol.3, 853-867.

Curds, C. R. and Cockburn, A. (1970) "Protozoa in Biological Sewage-Treatment Processes—I. A Survey of the Protozoan Fauna of British Percolating Filters and Activated-Sludge Plants," Water Research, Vol. 4, : 225-236

Davis RD (1997) Production, treatment and disposal of wastewater sludge in Europe from a UK perspective, Hall JE,7(2),9-17

Grady, C.P.L. Jr. and Williams, D.E(1975)Effects of influent substrate concentrations on the kinetics of natural microbial pollutions in continuous culture.Water Res. 9.171-180

HaiyanWu, Dianhai Yang, Qi Zhou, Zhoubing Song, (2009) The effect of pH on anaerobic fermentation of primary sludge at room temperature, Journal of Hazardous Materials 172, 196–201

J.Lobos, C. Wisniewski, M. Heran, and A. Grasmick,(2008) Sequencing versus continuous membrane bioreactors: Effect of substrate to biomass ratio (F/M) on process performance, Journal of Membrane Science 317,71–77

J. Pan, R. Zhang, H. Mashad, H. Sun, and Y. Ying, (2008) Effect of food to microorganism ratio on biohydrogen production from food waste via anaerobic fermentation, international journal of hydrogen energy 33, 6968 – 6975

Jenkins, D. and Garrison, W.E. (1968) Control of activated sludge by mean cell residence time. J. Wat. Pollut. Control Fed. 40, 1905

Jenkins D., Richard M.G., Daigger G.T., (2003) Manual on the causes and control of activated sludge bulking, foaming, and other solids separation problems, Lewis Publishers, 4(3),78-79

Jusoh Zukefeli, (2007) Effectiveness of Return Activated Sludge on Secondary Treatment, Bac. Of Chem. Eng. Thesis, UITM

Jusoh Zukefeli, IETS Competent Person, PGB Kerteh, Personal Interview, Jun.21.2011

Malaysia Water Industry Guide(2007) <http://www.span.gov.my>

McKinney, R.E. (1962) Mathematics of complete-mixing activated sludge. Proc. Am. Soc. Civil Eng. 88(SA3), 87-113

New England Interstate Water Pollution Control Commission, (2005) Sequencing Batch Reactor Design And Operational Considerations,< www.neiwpcc.org>

Orhon D, Babuna F.G, Karahan (2009). Industrial Wastewater treatment by activated sludge, IWA Publishing, 7(2),182-186

Peng Zhang, Yinguang Chena,Qi Zhoua,(2009), Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: Effect of pH, water research 43, 3735 – 3742

Richard I. Dick and P. Aarne Vesilind(1969), The Sludge Volume Index: What Is It?, Journal (Water Pollution Control Federation), Vol. 41, No. 7 ,pp. 1285-1291

R. Trussel, R. Merlo, S. Herwanowicz, D. Jenkins,(2005), The effect of organic loading on process performance and membrane fouling in a submerged membrane bioreactor treating municipal wastewater, Water Research, Volume 40, Issue 18, October 2006, Pages 3479-3482,

W.Fuchs, C.Resch, M.Kernstock,M.Mayer,P. Schoeberl, and R. Braun,(2005) Influence of operational conditions on the performance of a mesh filter activated sludge process, Biochemical Engineering Journal 40, 253–261