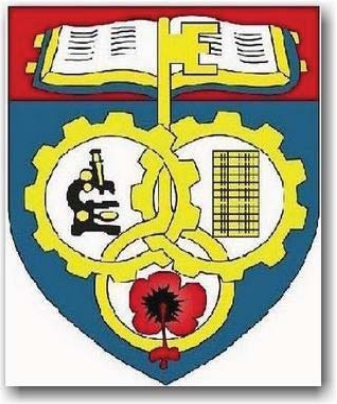
DETERMINATION OF ACETIC ACID IN GARBAGE ENZYME PROPERTY ASSOCIATED WITH IMPROVING WATER QUALITY OF RECREATIONAL LAKE

BY

SOO POEY KEAT



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TUNKU ABDUL RAHMAN COLLEGE

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SOO POEY KEAT

A seminar report submitted to the School of Arts and Science in partial fulfillment of the requirement for the Bachelor of Science, Campbell University, U.S.A. and Advanced Diploma in Science, Tunku Abdul Rahman College

2010/2011

DECLARATION ON PLAGIARISM

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DETERMINATION OF ACETIC ACID IN GARBAGE ENZYME PROPERTY ASSOCIATED WITH IMPROVING WATER QUALITY OF RECREATIONAL LAKE

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LIST OF ABBREVIATIONS

APHA	American Public Health Association
NH_4Cl	Ammonium Chloride
BOD	Biochemical Oxygen Demand
$CaCl_2$	Calcium Chloride
Ca^{2+}	Calcium ions
COD	Chemical Oxygen Demand
cfu	Colony-forming unit
˚C	Degree Celsius (units)
DOE	Department of Environment
K_2HPO_4	Dipotassium hydrogen phosphate
$Na_2HPO_4.7H_2O$	Disodium hydrogen phosphate
DO	Dissolved Oxygen
EPA	Environmental Protection Agency

$FeCl_3$	Ferric Chloride
g	Gram (units)
HCl	Hydrochloric acids
kg	Kilogram (units)
<	Less than
L	Liter
Mg^{2+}	Magnesium ions
$MgSO_4.7H_2O$	Magnesium Sulfate heptahydrate
MIBK	Methyl isobutyl ketone
mg/L	Milligrams per liter (units)
mL	Millimeter
M	moles
>	More than
ppm	Parts per million (units)

%	Percentage (units)
KH_2PO_4	phosphate dihydrogen phosphate
NaOH	Sodium Hydroxide
Na_2SO_4	Sodium Sulfate
Na_2SO_3	Sodium Sulfite
US	United States
v/v	Volume per Volume (units)
w/w	Weight per weight (units)
WRI	World Resources Institute

Abstract

This study was conducted to evaluate the feasibility of using Garbage enzymes in improving recreational lake water quality. The enzyme which was prepared from fruit peeled, water, and brown sugars capable of carry out natural cleaning function. In this study, water sample was collected from recreational lake at Taman Bersatu, Selangor. The water sample collected was analyzed with treatment of various concentration of garbage enzyme for its Dissolved Oxygen (DO) level, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) level, amount of bacteria growth, nutrient availability. At the same time, garbage enzyme's acetic acid concentration was determined which could be the main properties that reflect reason behind the cleaning function and acetic acid simulation experiment was done to observe the possibility of similar cleaning function like garbage enzyme. Based on the result obtained, the optimum concentration of garbage enzymes to act effectively in treating lake water samples was 1:100 (v/v) where it showed greatest and fastest decrement of DO level in 5 days incubation period. While, COD experiment shows better % COD removal of water sample for garbage enzyme in medium without nutrient. In addition, bacteria growth was in proportional relationship to the concentration of garbage enzyme. Furthermore, the garbage enzyme was out to be less effective in catalyzing aerobic respiration of bacteria without nutrient availability. The analysis of acetic acid extraction showed low recovery percentage acetic acid via acid-base separation method. The simulation result of acetic acid proved similar trend of DO uptake but at much slower rate than garbage enzyme.

1.0 Introduction

1.1. Introduction

Recreational lake water is a type of surface water found on the surface of the earth just like rivers, streams, and wetlands. This surface water quality of recreational lake is subject to frequent and dramatic changes as the result of a variety of activities. These activities could be due to human activities or natural occurrence. Typical reasons for poor water quality in recreational lake are such as discharges of municipal raw (untreated) wastewater; treated manufacturing industrial sector, storm water runoff, or other non-point source runoff. However, Botkin and Keller cited in Adlan *et a.l* (2005) state that deterioration in water quality of recreational lake is largely caused by green algae which lead to eutrophication, where a body of water develops a high concentration of nutrients normally in the forms of nitrates and phosphates. Therefore, this kind of pollution of recreational lake leads to severe concern for unsuitable recreational activities as it water quality does not achieve the requirement stated in Interim National Water Quality Standards for Malaysia.

1.2 Problem of Statement

The major problems that wish to be studied are due to the water quality of recreational lake at Taman Bersatu, Rawang which consists of many pollutants inside its water bodies and suspected to directly affect the water quality parameter such as DO level, BOD, and COD value.

1.3 Aim of the study

The purpose of this study is to test out the garbage enzyme as an alternative treatment method to improve water quality in recreational lake. At the mean time, this

study also emphasizes on the effectiveness and efficiency of garbage enzyme in terms of water treatment purposes and its acetic acid property.

1.4 Objectives of the study

The objectives of this study are:

1. To prepare garbage enzyme from fruit peels for recreational lake water treatment
2. To optimize the concentration of garbage enzyme used based on Dissolved Oxygen(DO) uptake
3. Determination of garbage enzyme effectiveness based on Chemical Oxygen Demand (COD)
4. To determine of bacteria growth in garbage enzyme treated recreational lake water sample
5. To investigate the effect of nutrient availability on the garbage enzyme activity
6. To investigate garbage enzyme property through acid-base separation of acetic acid extraction and its method recovery percentage
7. To investigate the effect of the DO uptake for the acidic/basic extracted garbage enzyme compared to control garbage enzyme(non-extracted)
8. To investigate the simulation effect of acetic acid as a replacement for garbage enzyme

1.5 Scope of the Study

To analyze the water quality improvement of recreational lake water sample collected at Taman Bersatu, Rawang using garbage enzyme through the water quality parameters such as DO, BOD, and COD as well as acetic acid extraction from garbage enzyme.

2.0 Literature review

2.1 Water pollution

Water pollution has not been a new issue for Malaysia since the industrial sectors have became the economical priority to achieve the goal of becoming a developed country. However, the growth in industrial sectors has taken a setback of environmental issues such as water pollution in which the wastes of industrial processing have been discharged into the stream, river, or lake as contaminants. Meanwhile, globally, problems with quantity and quality of water supply remain and in some respects are becoming more serious. Therefore, water pollution which contaminate of drinking water due to improperly discarded hazardous wastes not only lead to destruction of wildlife but also human beings (Manahan and Stanley, 2000).

Usually, the pollution of water bodies occur regardless of location (lake, river or ocean) is mainly due to the waste that often contains contaminants. These wastes were mainly discharged from manufacturing industrial sectors and consist substances (chemical compounds) that cannot be processed within the framework of existing technology such as physical (e.g., mechanical), chemical, biological, or combinations among them, or their further treatment is economically inefficient. Therefore, due to the intensification of industry activity in developing country like Malaysia in which is also driven by an exponential growth of population, the wastes have become extremely numerous and without proper channel of treatment prior discharging from industrial sector into stream directly lead to water pollution.

2.2 Sources of water pollution

Water pollution in Malaysia is mainly caused by two categories of sources: point and non-point sources. Point sources are single identifiable localized source while non-point sources are made up of many diffuse sources. A point sources example includes sewage treatment plants, manufacturing and agro-based industries, and animal farms. Non-point source pollution, by contrast, is contamination that occurs when rainwater or irrigation washes off agricultural field. So, as this runoff moves across the land surface, soil particles and pollutants such as nutrients and pesticides are picked up and discharged into water bodies. Originating from numerous small sources, non-point source pollution is widespread, dispersed, and hard to pinpoint compared to point source pollution. It has been estimated that non-point source pollution accounts for more than one-half of the water pollution in the United States (Wisconsin Department of Natural Resources, 2004). Based on the statistic compiled by Department of Environment (DOE) through questionnaires and field surveys in 2009, 20702 cases of water pollution point sources were recorded. These sources of pollution comprise of manufacturing industries (9762; 47.15%), sewage treatment plant (9676: 46.74% inclusive of 736 Network Pump Station), animal farms (769:3.72%) and agro-based industries (495: 2.39%).

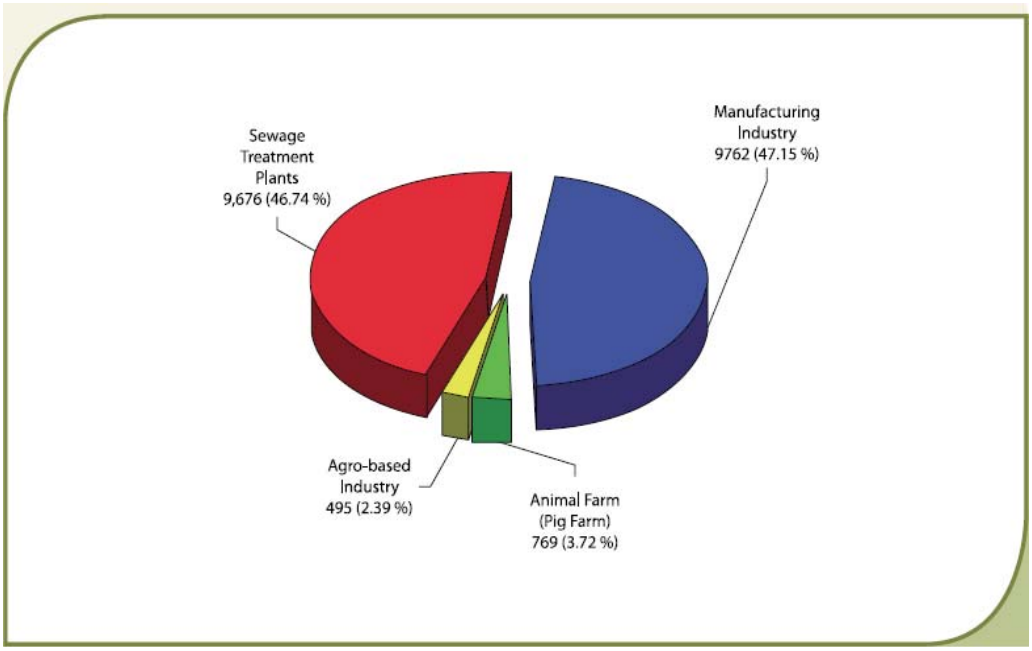

Figure 2.1: Malaysia: Composition of Water Pollution Sources by Sector in 2009.

2.3 Importance of lakes

Lakes serve as important sources of water in Malaysia and can contribute to multipurpose functions. They not only formed part of storage basins for municipal and industrial water supply, but also agriculture and hydropower. Some lakes and reservoir were even purposely constructed as flood control detention storage to buffer the different flow during dry and wet season, even though most of them, currently have versatile functions such as for recreation sites sport or commercial fishing activities (Sharip and Zakaria, 2008).

2.4 Status of lakes in Malaysia

Lakes all over the world experience many different problems. Common problems include eutrophication, sedimentation and weed infestation. Eutrophication of lakes, which is known as a prevalent global concern in lakes, is also a critical issue in Malaysia. Based on the study on the current status of eutrophication of lakes Lakes in Malaysia, there were more than 60% of the 90 lakes in the country is experiencing eutrophication (Sharip and Yusup, 2007). According to Molnar and coworkers (2003), the main cause for eutrophication is mainly due to nutrient rich environment provided by agriculture activities (Sharip and Zakaria, 2008). These nutrients and other pollutants enter lakes from either point sources or nonpoint sources. Thus, to effectively reduce the amount of nutrients available for algae growth, both sources must be reduced (Wisconsin Department of Natural Resources, 2004). Similar trouble of eutrophication struck Europe and North America where agricultural sources are generally documented as the primary contributors to eutrophication, while sewage and industrial sources are secondary pollutants (Priskin, 2008). Meanwhile, in accordance to Abdul Rahim and coworkers (2007), sedimentation of organic matter due to carcasses, plants, and etc. can lead to deprive of the spawning grounds of many species and contributes to the anoxic levels of lake during low flows in which it is serve as part of reason for decrease in the fish population (Sharip and Zakaria, 2008).

2.5 Impact of Pollution on Water quality

The impact of pollution such as eutrophication on water quality usually depends on lake characteristics, intensity and type of pollution, as well as management. However, according to World Resources Institute (WRI) (2008), an excessive introduction of

nutrients by anthropogenic activity has lead to severe eutrophication of certain freshwater systems worldwide (Priskin, 2008). Such condition attributed by excessive growth of phytoplankton and algae, which dramatically changes species abundance and composition, biomass production and dissolved oxygen content. Resulting at certain point lakes can develop into eutrophic which next to reach point of hypoxia because their ecosystem gets completely depleted of oxygen and lead to die off of those living materials in lakes and thus accumulated as organic matter. In addition, eutrophication is also associated with various health risks to humans such as waterborne diseases and a general loss of lake amenity caused by unpleasant smells and reduced water clarity (Larkin and Adams, 2007). Apart from that, once a lake becomes eutrophic, rapid growth of certain harmful algae types were triggered (Larkin and Adams, 2007; Priskin, 2008). Bluegreen algae or cynobacteria are most commonly referred to harmful algae in freshwater lakes, reservoirs, and slow-flowing rivers.

2.6 Water Quality Parameters

The term water quality is used to describe the condition of the water, including its chemical, physical and biological characteristics, with respect to its suitability for a particular purpose such as drinking, swimming or fishing. However, substances like pesticides or fertilizers could affect water quality and aquatic life when present in certain concentrations (Nancy, 2009). Therefore, in order to measure the water quality status, following factors are often used: concentration of dissolved oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD).

2.6.1 Dissolved Oxygen (DO)

Dissolved oxygen (DO) refers to the amount of oxygen that is dissolved in water body. Oxygen usually enters water by direct absorption from the atmosphere, which is enhanced by turbulence. Water also absorbs oxygen released by aquatic plants during photosynthesis. Sufficient DO is essential to growth and reproduction of aerobic aquatic life (US EPA, 2010). As dissolved oxygen levels in water drop below 5.0 mg/l, aquatic life is put under stress while oxygen levels that remain below 1-2 mg/l can kill aquatic life (Lenntech, 2010).

2.6.2 Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand or BOD is a chemical procedure for measuring the rate of dissolved oxygen uptake by the biological organisms in a body of water. It is not a precise quantitative test despite it is widely used as an indication of quality of water. BOD measurement is listed as a conventional pollutant in most of the countries Clean Water Act (Clesceri *et al*., 2005).

2.6.3 Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) is defined as the amount of oxygen consumed to complete chemically oxidise the organic water constituents to inorganic end products. Its application is mostly used to measure the amount of organic pollutants in surface water such as lake and river water. The unit of the mass of oxygen consumed per liter of solution is milligrams per liter (mg/L). However, it is also expressed as parts per million (ppm) in some older references (Clesceri *et al*., 2005).

INTERIM NATIONAL WATER QUALITY STANDARDS FOR MALAYSIA

		CLASSES					
PARAMETERS	UNIT	I	IIA	IIB	III	IV	V
Ammoniacal Nitrogen	mg/l	0.1	0.3	0.3	0.9	2.7	>2.7
BOD	mg/l	1	3	3	6	12	>12
COD	mg/l	10	25	25	50	100	>100
DO	mg/l	7	5 - 7	5 - 7	3 - 5	<3	<1
pH		6.5 - 8.5	6 - 9	6 - 9	5 - 9	5 - 9	-
Colour	TCU	15	150	150	-	-	-
Elec. Conductivity *	umhos/cm	1000	1000	-	-	6000	-
Floatables		N	N	N	-	-	-
Odour		N	N	N	-	-	-
Salinity (%)	%	0.5	1	-	-	2	-
Taste		N	N	N	-	-	-
Total Dissolved Solid	mg/l	500	1000	-	-	4000	-
Total Suspended Solid	mg/l	25	50	50	150	300	300
Temperature (C)	^{o}C	-	Normal $+2^{0}$C		Normal $+2^{0}$C	-	-
Turbidity (NTU)	NTU	5	50	50	-	-	-
Faecal Coliform **	counts/100mL	10	100	400	5000 (20000) a	5000 (20000) a	-
Total Coliform	counts/100mL	100	5000	5000	50000	50000	>50000

Notes

N : No visible floatable materials or debris or No objectionable odour, or No objectionable taste
* : Related parameters, only one recommended for use
** : Geometric mean
a : maximum not to be exceeded

Class Uses
CLASS I : Conservation of natural environment water supply 1 - practically no treatment necessary.
Fishery 1 - very sensitive aquatic species

CLASS IIA : Water Supply II - conventional treatment required
Fishery II - sensitive aquatic species
CLASS IIB : Recreational use with body contact
CLASS III : Water Supply III - extensive treatment required
Fishery III - common, of economic value, and tolerant species livestock drinking
CLASS IV : Irrigation

Table 2.1: Interim National Water Quality Standards for Malaysia. (Adapted from Department of Environment, 2008)

2.7 In-Lake Restoration Techniques

Method	Advantages	Disadvantages
Dilution-Flush with low nutrient water	• Reduces nutrient levels • Washes out surface algae	• Require large volumes of water • Does not eliminate sources of phosphorous
Aluminate sulfate(alum) treatment	• Reduces phosphorous • Inhibits release of phosphorous from sediment • Increases transparency	• Temporary measure potential toxic impacts during application • Increased macrophyte growth due to water clarity
Artificial circulation	• Prevent stratification • Provides aeration/oxygenation • Increases aerobic habitat	• Does not decrease algal biomass • May decrease water clarity • Adverse impact on cold-water fish
Hypolimnetic aeration	• Maintains oxygen in hypolimnion • Limits release of phosphorous from sediments • Increases habitat and food supply	• Difficult to supply adequate oxygen • Potential for destratification and subsequent algae blooms
Dredging	• Controls aquatic vegetation • Deepens lake • Increases lake volume • May improve water quality	• Temporary resuspension of sediments • Temporary destruction of habitat • Disposal concerns • High cost
Water level drawdown	• Controls macrophytes. • Consolidates sediments • Facilitate dredging or excavation • Facilitates dock repairs	• Less effective in wet climates • Short-term benefits • Intensifies algal blooms • Temporary adverse impacts on fish and invertebrates
Biomanipulation: adjust fish species composition	• Encourages growth of zooplankton, which eat algae	• Experimental stage • Not effective where blue-green algae dominate.

Table 2.2: In-Lake restoration Techniques (Sharip and Yusup, 2007)

2.8 Garbage Enzyme

Since there are many drawbacks of the lake restoration in terms of pollution control, an alternative method was studied to treat the water pollution problems. This method involves the use of garbage enzyme which is produced from the food waste such as fruit peeled through fermentation with brown sugar for 3 months time. Then, after 3 months the enzymes are ready to use as a household cleaning liquid, to remove foul odours, toilet, anti-bacterial and anti-viral agent. This garbage enzyme which invented by Dr. Rosukon Poompanvong can be classify as a complex organic substance of protein chains and mineral salts and juvenile hormones. Researchers postulated that this enzyme can functions in four categoteries: decompose, compose, transform and catalysis. However, in this study main emphasis will be on the functions of decomposition of organic matter in water sample and at the same time to investigate its effect in terms of catalysis for this particular decomposition reaction.

Therefore, the aim of this study is to assess effectiveness and efficiency of garbage enzyme to treat the polluted lake water sample in terms DO, BOD and COD level. This enzyme is practically safe to use in treatment of water as it does not lead to any side effects neither to the human being nor environment in addition to its simple and low cost preparation procedure. Basically, in this study the emphasis would be in terms of the optimization of garbage enzyme in order to optimize the standard operating condition of garbage enzyme in treatment of polluted water sample. This optimization study involving the factors that affecting the efficiency and effectiveness of garbage enzyme such as determination of garbage enzyme concentration of 1:10 (v/v), 1:100(v/v), and 1:1000(v/v) in terms of DO, BOD and COD levels. Besides, a study on acetic acid concentration in garbage enzyme was carried out by extraction and then analyzed with GC-MS to determine the garbage enzyme property of acetic acid. Then, a simulation of water sample treatment with acetic acid in terms of DO and COD level will carried out to determine the functions of acetic acid in garbage enzyme property. Meanwhile, the effect of garbage enzyme in various medium upon the bacterial growth will also be observed through bacterial culturing from BOD bottle into agar plate and its growth was counted as well as microscopic view on its shape.

3.0 Materials & Methods

3.1 Collection of recreation lake water sample

In this research, Recreation lake water sample was collected from a man-made recreation lake at Taman Bersatu, Rawang, Selangor. This lake was situated in the settlement area where recreation activity such as fishing becomes the most attraction to both local and visitors. In our observation there was concerned whether the water quality in that particular lake was clean since the number of catch become lesser according to locals fishing lover. Therefore, to clarify this matter whether water quality lead to the death of fishes, the recreation lake water was taken to be treated with garbage enzyme. This water sample was then kept under 5˚C in refrigerator of laboratory until it was used.

3.2 Preparation of Garbage enzyme

The preparation of garbage enzyme required three main materials that are easily obtained and cheap. The main material of the preparation was the food wastes such as peeled fruit skin and raw vegetable waste. According to research, forty-five percent of household waste is organic waste such as fruit peels. Thus, this proved that the main material of the garbage enzyme preparation was easily obtained daily. This food waste was then fermented in a bottle for 3 months along with brown sugar and water in ratio of 3:1:10(w/w). For example, to prepare 10 liters of garbage enzyme: 3kg of food wastes, 1 kg of brown sugars, and 10 liters of water are required. Precautions have to be taken, if the container used is completely air-tight, make sure the container cap was released at least once a day for the first few weeks to let out built-up gas of fermentation in order to avoid any explosion due to high pressure exertion from the fermentation gas released.

3.3 Preparation of oxygen saturated water

Oxygen saturated water was used in the experiment because it is to avoid any unwanted low value of DO measurement due to low dissolved oxygen from air which lead to inability to observe the difference of DO value at the end of the incubation period from initial reading. Besides, the used of this oxygenated also to supply more oxygen for measurement of organic compound in COD measurement. The preparation of oxygenated water required charcoal and glass wool to be heated in Lenton Furnace at 400˚C for 2 hours and then allowed to be cooled down in dessicator to avoid air moisture and contamination. Next, the charcoal was put into air tight charcoal flask with glass wool on top of it. This set up was to ensure that all organic contaminants in the air supplied from bubling motor was filtered prior reaching the Mili-Q Water(>18 MΩ). In order to ensure that the water was fully oxygenated, the bubling process was allowed to occur overnight.

Figure 3.1: Bubbling device used to prepare oxygenated water.

3.4 Preparation of oxygen saturated dilution water

Preparation of oxygen saturated dilution water required a few chemicals which are phosphate dihydrogen phosphate (KH_2PO_4, 99.5%), Disodium hydrogen phosphate ($Na_2HPO_4.7H_2O$, 99% purity), Ammonium Chloride (NH_4Cl, 99.8%), Calcium Chloride ($CaCl_2$, 95%) were purchased from Systerm. Dipotassium hydrogen phosphate (K_2HPO_4, 99% purity) was purchased from Hamburg Chemical. Meanwhile, Magnesium Sulfate heptahydrate ($MgSO_4.7H_2O$, 99.5% purity) was purchased from Merck.

Preparation of oxygenated dilution water required four solutions which phosphate buffer (2.13g KH_2PO_4, 5.437g K_2HPO_4, 4.427g $Na_2HPO_4.7H_2O$, and 0.43g NH_4Cl in 250mL of distilled water), Magnesium Sulfate heptahydrate (5.629g $MgSO_4.7H_2O$ in 250mL of distilled water), Calcium Chloride (6.847g $CaCl_2$ in 250mL of distilled water) and Ferric Chloride(0.15g $FeCl_3.6H_2O$ in 250mL of distilled water). Then, each of these prepared solutions, 1 mL of phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ were transferred into 1L of volumetric flask. After that, it was diluted with oxygen saturated water. This is the oxygen saturated dilution water that will used to dilute the BOD sample (Clesceri *et al*., 2005).

3.5 Calibration of Dissolved Oxygen Meter

The calibration of Dissolved Oxygen (DO) Meter is required to ensure that the measurement not deviated too much from the expected values in addition to prove the validity of measurement and reduced the errors of measurements. This calibration was carried out by preparing a calibration solution of 0.08M Sodium Sulfite, Na_2SO_3 from Merck in a 300mL BOD bottle every time using the DO meter. Standardization icon was clicked and the probe of DO meter was dipped into the prepared 0.08M Sodium Sulfite

solution with stirrer powered until the reading reached < 0.05mg/L. After that, the probe was rinsed and without stirrer powered put it into about 1/3 of distilled water in 300 mL BOD bottle for about 15 minutes. The calibration was done and ready for use if the reading shown values of >7.00mg/L when confirm icon was clicked.

3.6 Optimization of garbage enzyme concentration via Dissolved Oxygen uptake

The purpose of this experiment was to determine the optimum concentration of garbage enzyme that most effective and efficient in aiding the treatment of water. Method used in this experiment was based on APHA Method 5210BOD (Clesceri et al., 2005). Thus, 3 sets of sample were prepared as follows: first set with 10mL of 1:10(v/v) garbage enzyme to distilled water, 50mL of water sample and 240mL of oxygen saturated dilution water. Meanwhile, second set consist of 10mL of 1:100(v/v) garbage enzyme to distilled water, 50mL of water sample and 240mL of oxygen saturated dilution water. For third set sample makeup of 10mL of 1:1000(v/v) garbage enzyme to distilled water, 50mL of water sample and 240mL of oxygen saturated dilution water. At the same time, one set of control without garbage enzyme was prepared with 50mL of lake water sample and 250mL of oxygen saturated dilution water only. These entire samples were prepared in a 300mL BOD bottle. Then, Initial Dissolved Oxygen (DO) for these samples was taken. Next, each BOD bottles was then incubated at 20ºC in LE-519 B.O.D incubator for 5 days and the DO level was measured again each day DO level with Fisher Scientific DO meter. The result obtained was used to plot oxygen uptake curve.

3.7 Determination of garbage enzyme effects based on Chemical Oxygen Demand (COD)

COD test was conducted to investigate whether garbage can assist in reducing the organic compound in lake water samples. Method used in this experiment was in accordance with APHA Method 5220COD (Clesceri *et al*., 2005). Hence, 9 sets of samples were prepared for measurement of COD which consist of 3 sets of sample with 10mL of 1:10(v/v) garbage enzyme to distilled water, 50mL of water sample and 240mL of oxygen saturated dilution water while the other 3 sets of samples were makeup of 10mL of 1:100(v/v) garbage enzyme to distilled water, 50mL of water sample and 240mL of oxygen saturated dilution water. At the same time, 3 sets of control without garbage enzyme were prepared with 50mL of lake water sample and 250mL of oxygen saturated dilution water only. Each BOD bottles was then incubated at 20ºC in LE-519 incubator. Then, COD values for all these samples were measured for initial day, day 4 and day 7, in each measurement 2mL of sample was transferred into COD tube of 25-1500mg/L and heated at 148 ºC using thermoreactor for 2 hours. The COD tube was then allowed to cool overnight and measured by using UV Spectroquant. For the next day measurement, a new BOD samples from the each sets was used.

3.8 Determination of Bacterial Growth in garbage enzyme treated lake water sample

This experiment was conducted to observe the effects of garbage enzyme on inducing bacteria growth through the plate count methods of colonies formed. This experiment was based on Standard Methods for the Examination of Water and Wastewater: APHA 9215 B. According to this method about 10 – 12ml of molten plate count agar of TSA-Tryptic soy

agar from Merck was poured into each petri dish (about 50 plates). The amount required to such number of plates was 60g of nutrient agar powders and dissolved into 1.5L of sterile water. The solution of TSA was then autoclaved at 121˚C for 2 hours using Hirayama Autoclave. It was allowed it to cool down with running tap water and quickly poured into petri dishes. After that, the TSA agar plates were stored in the refrigerator with upside down position to avoid condensation dripping down on the agar surface that may encourage bacterial contamination. Thus, to culture the bacteria in water sample, each day 0.1 mL of BOD samples of each category from Experiment 3.6 was transferred into plates and spread. Then, all plates were incubated using Memmert incubator at 37˚C for 24 hours and the numbers of bacteria growth which appeared as white spot of colonies was observed under naked eye and calculated in the unit of cfu/mL. The purpose of using nutrient agar plate was to culture the bacteria in BOD samples in order to prove that the garbage enzyme which consists of multiple unknown microorganisms can assist in degradation of organic compound by acclimation of bacteria instead of just bacteria in the water sample.

3.9 Microscopy view of microorganisms in recreational lake water sample

The microscopic view of the microorganisms in the water sample was observed using inverted microscope of Leica LMD7000 to prove the present of microorganism that enhance the consumption of DO in samples. However, prior the observation under microscope, a smear preparation on slide was done in accordance Grams stain method. In this method, 2 inoculate drops of water sample from raw lake water sample was transferred onto a sterile slide and then crystal violet dye was applied on it for 1 minute. Next, stain of crystal violet was rinsed off with running tap water. Then, slide was flood with Gram's

Iodine for 1 minute and poured off the extra Iodine from the slide. After that, a slide was decolorized with alcohol (ethanol) in fast manner and washes the remaining with water to remove ethanol. Finally, Safranin counterstain was applied on the slide for 1 minute. Before, view under microscope the counter stain was removed with water and blot dry excess water. The observation of microorganism was taken using low power magnification.

3.10 Effect of nutrient availability on the garbage enzyme activity

This experiment was conducted to prove that the bacteria growth in garbage enzyme was dependent of the nutrient availability. Therefore, to carry out this experiment, 2 sets of parameter samples which consist of different medium of oxygen saturated dilution water (with nutrient) and distilled water (without nutrient) but without lake water sample were used. In first set of parameter (with nutrient), 3 sets of sample were prepared as follows: first set with 10mL of 1:10(v/v) garbage enzyme to distilled water and 290mL of oxygen saturated dilution water. Meanwhile, second set consist of 10mL of 1:100(v/v) garbage enzyme to distilled water and 290mL of oxygen saturated dilution water. For third set sample makeup of 10mL of 1:1000(v/v) garbage enzyme to distilled water and 290mL of oxygen saturated dilution water. At the same time, one sets of control that makeup of only oxygen saturated dilution water was prepared. On the other set of parameter (without nutrient), 3 sets of sample were prepared as follows: first set with 10mL of 1:10(v/v) garbage enzyme to distilled water and 290mL of distilled water. Meanwhile, second set consist of 10mL of 1:100(v/v) garbage enzyme to distilled water and 290mL of distilled water. For third set sample makeup of 10mL of 1:1000(v/v) garbage enzyme to distilled water and 290mL of distilled water. At the same time one sets of control that makeup of

only distilled water was prepared. These prepared samples were then measured for their initial DO using Fisher Scientific DO meter. Next, each BOD bottles was then incubated at 20ºC in LE-519 incubator for 4 days and the DO level was measured again each day DO level with Fisher Scientific DO meter. The result obtained was used to plot oxygen uptake curve. At the same time, the bacteria culture for each of these samples were carried out to prove that the garbage enzyme's bacteria growth in presence of nutrient which directly proportional to the hike in DO uptake measurements.

3.11 Recovery percentage of acetic acid extraction using acid-base separation method

This recovery steps was carried out to find out extraction efficiency of acetic acid from garbage enzyme through extraction of pure acetic acid from PROCHEM in Dichloromethane from R&M Chemicals at concentration of 300ppm. Firstly, the amount of acetic acid of required to obtained 300ppm in 50mL of distilled water was calculated. This volume of acetic acid was then diluted to 50mL of distilled water and acidified with concentrated HCl from R&M Chemicals to pH less than 2 using pH meter. pH of the solution was measured using Metler Toledo pH meter (Model: 320).Then, the subsequent steps were carried out just like the extraction of acetic acid from garbage enzyme. This eluted solvent is known as the final peak area obtained via GC-MS analysis. The final peak area was compared with initial peak area of the sample in which the calculated amount of acetic acid to obtain 300ppm was directly transferred it into 50mL of Dichloromethane in volumetric flask and analyzed with GC-MS. The recovery percentage was then calculated by using following formula:

$$\text{Recovery percentage} = \frac{Final\ \ peak\ \ area}{Initial\ \ peak\ \ area} \times 100\%$$

3.12 Determination of garbage enzyme property through acid-base separation of Acetic acid extraction

This experiment was carried out to determine the property of garbage enzyme whether it made up of acetic acid (vinegar). The extraction was done in which 50mL of garbage enzyme was acidified with concentrated HCl to pH less than 2 using Metler Toledo pH meter (Model: 320) so that acidic compound in the garbage enzyme probably acetic acid could be extracted. Then, the 50mL garbage enzyme was poured into a 250mL separating funnel and extracted with 50mL of organic solvent, Dichloromethane. The extraction was carried out by shaking the separating funnel vigorously for 2-3 minutes to ensure thorough separation of acetic acid into Dichloromethane layer. After the separation into 2 layers, the bottom layer of organic solvent, Dichloromethane, was eluted out. This organic layer was expected to consist of the acetic acid extracted from garbage enzyme. Then, anhydrous Sodium Sulfate, Na_2SO_4 purchased from Hamburg Company was used to dry up any moisture in the solvent for 10minutes. This was to prevent the degradation of column in subsequent steps of injecting the organic solvent into GC-MS for identification. The peak area of acetic acid obtained from GC-MS analysis was then used to calculate the actual concentration of acetic acid in garbage enzyme through the calibration curve of glacial acetic acid in Dichloromethane. The calibration curve was carried out by preparing various concentrations of 100ppm, 200ppm, 300ppm, 400ppm, and 500ppm of acetic acid in 50mL of Dichloromethane.

Gas Chromatograph: Agilent Technologies 7980A			
Analytical Column: DB-WAX (30 m x 0.320 mm x 0.25 μm)			
Injection Port Type: Programmable Splitless			
Injector Temperature: 250 ˚C			
Injection Type: Split (25 mL/min)			
Syringe Volume: 10 μL			
Injection Volume: 1 μL			
Rinse Solvent: Dichloromethane			
Carrier Gas Type: Helium			
Carrier Gas Program:	Flow	Hold Time	
	1.8 mL/min	1.0304 min	
Oven Program:	Temperature	Hold Time	Rate
	35˚C	10 min	-
	250 ˚C	4.625 min	40 ˚C/min

Table 3.1: Gas Chromatograph Conditions

Mass Spectrometer: Agilent Technologies 5975C inert XL MSD
GC Inlet Line Temp: 250 ˚C
Ion Source Temp: 250 ˚C
Function Type: Full Scan
Full Scan Range: *m/z* 42-60
Solvent Delay: 0 min

Table 3.2: Mass Spectrometer Conditions

3.13 Effect of the DO uptake for the acidic/basic extracted garbage enzyme compared to control garbage enzyme (non-extracted)

This experiment was carried out in order to prove that garbage enzyme is made up of acetic acid in which its removal through acid-base separation would hinder the effect of garbage enzyme in DO uptake. Therefore, in this experiment 50mL of garbage enzyme was acidified with concentrated HCl to pH less than 2 using pH meter so that acidic compound in the garbage enzyme probably acetic acid could be extracted. The respective steps were repeated for basic extraction using concentrated NaOH from R&M Chemicals to adjust the pH to >12 so that basic compound can be removed. Next, the garbage enzyme with desired pH was extracted in separating funnel and extracted with 50mL of organic solvent, Dichloromethane. The extraction was carried out by shaking the separating funnel vigorously for 2-3 minutes to ensure thorough separation of acidic and basic compound into Dichloromethane layer. Then, the top layer of garbage enzyme was eluted out and ready to be used in experimental. These respective garbage enzymes were then diluted to 1:100(v/v) with distilled water. After that, 10mL of these diluted garbage enzymes was added with 50mL of water sample and 240mL of oxygen saturated dilution water in a BOD bottle. At the same time, a control parameter of diluted 1:100 (v/v) control garbage enzyme (non-extracted) was prepared. Then, 10mL of control garbage enzymes was added with 50mL of water sample and 240mL of oxygen saturated dilution water in a BOD bottle. Then, Initial Dissolved Oxygen (DO) for these samples was taken. Next, each BOD bottles was then incubated at 20ºC in LE-519 incubator and the DO level was measured again each day DO level with Fisher Scientific DO meter. The result obtained was used to plot oxygen uptake curve.

3.14 Simulation effect of acetic acid on DO uptake

Since, there was suggestion in which garbage enzyme merely nothing but acetic acid (vinegar). According to research done, garbage enzyme is nothing more than vinegar produced from organic wastes. The key ingredient is the sugar that is metabolized by bacteria into alcohol which subsequently is reduced to acetic acid (YSG, 2009). Therefore, this simulation experiment was carried out to verify such statement by repeated the Experiment 3.6 with the garbage enzyme replaced by acetic acid. In this experiment, 3 sets of sample were prepared as follows: first set with 10mL of 1:10(v/v) glacier acetic acid to distilled water, 50mL of water sample and 240mL of oxygen saturated dilution water. Meanwhile, second set consist of 10mL of 1:100(v/v) glacier acetic acid to distilled water, 50mL of water sample and 240mL of oxygen saturated dilution water. For third set sample makeup of 10mL of 1:1000(v/v) glacier acetic acid to distilled water, 50mL of water sample and 240mL of oxygen saturated dilution water. At the same time, one set of control without garbage enzyme was prepared with 50mL of lake water sample and 250mL of oxygen saturated dilution water only. These entire samples were prepared in a 300mL BOD bottle. Then, Initial Dissolved Oxygen (DO) for these samples was taken. Next, each BOD bottles was then incubated at 20ºC in LE-519 incubator for 5 days and the DO level was measured again each day DO level with Fisher Scientific DO meter. The result obtained was used to plot oxygen uptake curve.

4.0 Result & Discussion

4.1 Optimization of garbage enzyme concentration via Dissolved Oxygen uptake

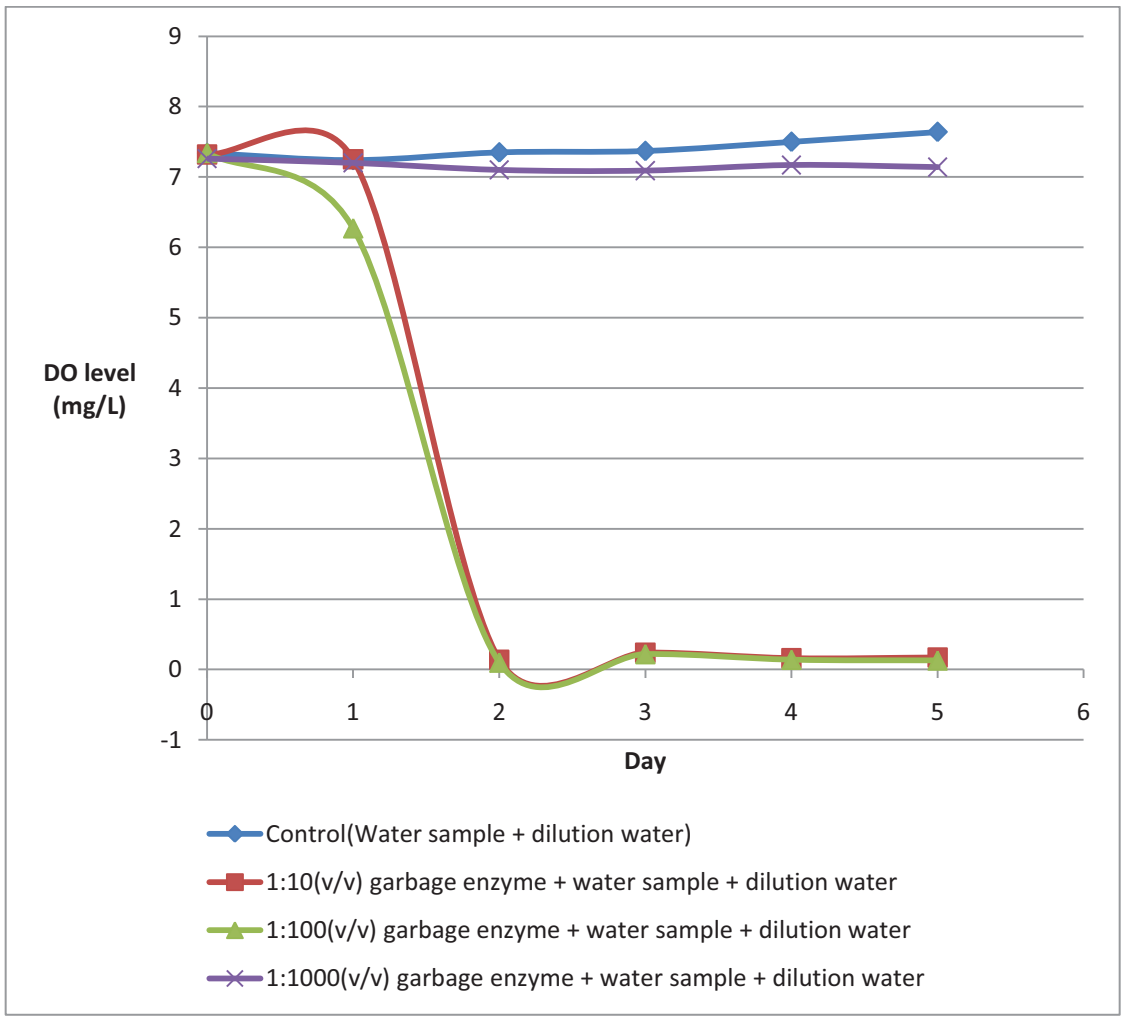

Figure 4.1: DO level versus Day (Effect of various garbage enzyme concentrations on DO uptake).

Figure 4.1, illustrate that DO level of the treated samples especially with 1:100(v/v) and 1:10(v/v) concentrations of garbage enzymes shows significant reduction from initial day to day 2 whereby the DO values drop to reach level of almost zero. These two concentrations were incomparable to the 1:1000(v/v) concentration of garbage enzymes in terms of DO uptake in which the most diluted garbage enzyme shown no significant reduction of DO level as relatively constant results of DO level from initial day until day 5 was observed.

In addition, from the result obtained, the rate of DO uptake was varied with each concentration of garbage enzyme used. The sample treated with 1:100(v/v) of garbage enzyme shown fastest rate of DO uptake compared to 1:10 (v/v) of garbage enzyme and 1:1000(v/v) of garbage enzyme. Despite, 1:10(v/v) of garbage enzyme treated sample also shown increase in DO uptake, however, it does not gave the optimum result as the 1:100(v/v) of garbage enzyme where the rate of DO uptake was faster especially in day 1 where shown significant drop compared to the rest. The rate of DO uptake variation for each concentration can be explained with the fact that garbage enzyme which is an enzyme that work best at its optimum condition. Therefore, the faster rate of DO uptake indicated that 1:100(v/v) concentration of garbage enzyme was the optimum point for desired results of degradation rate compared to the most concentrated garbage enzyme which was expected to show faster rate of DO uptake. Meanwhile, the rate of DO uptake for the most diluted garbage enzyme was slowest among three concentrations can be due to the fact that less concentrated enzyme was out the workable optimum range of enzyme to catalyze the DO uptake in treated sample.

Besides, the increase in DO uptake was observed from the treated samples which also can be explained by the presence of microorganism in water sample that consumed oxygen

for their own aerobic respiration. According to Koumanova (2006), he proposed that bacteria use oxidation-reduction reactions in water to obtain the energy that they need for their own growth and reproduction. These bacteria require oxygen for their metabolic needs and are called aerobic bacteria.

$$O_2 + CH_2O \longrightarrow CO_2 + H_2O \text{ (aerobic respiration)}$$

Therefore, as the bacteria required the oxygen for respiration as the same time degrade the organic matter in water sample which could most probably serve as their food. Apart from that, according to Chapman & Kimstach (1996), sample that is high in organic matter and nutrient content can lead to low concentration of DO and due to increase microbial activity such as respiration for organic matter decomposition.

As the conclusion, the garbage enzyme works best at its optimum concentration of 1:100(v/v) which serves to catalyze the rate of aerobic respiration by microorganism in water sample which suit to our purpose of water treatment by degrading all those organic matter in water sample.

4.1.1 Percentage of BOD removal

Figure 4.2 to 4.4 shows the BOD removal in percentage versus day where the more concentrated garbage enzyme of 1:10(v/v) and 1:100(v/v) give rise to better BOD removal of almost 100 % respectively. Saturation of BOD level at Day 2 was observed due to thrive of bacteria amount whereby aerobic respiration increased significantly. In contrast, most diluted garbage enzyme of 1:1000(v/v) shows ineffectiveness and inefficiency of BOD removal as the percentage was very low. Hence, this particular result further proved that 1:1000 (v/v) garbage enzyme could not be used in treatment of water.

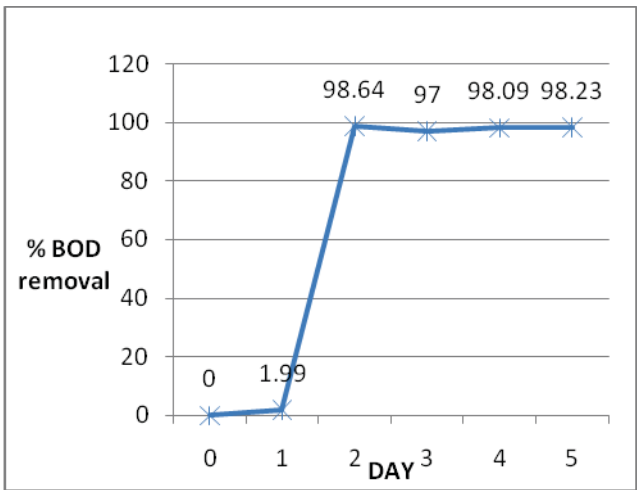

Figure 4.2: Percentage BOD removal of 1:100(v/v) garbage enzyme.

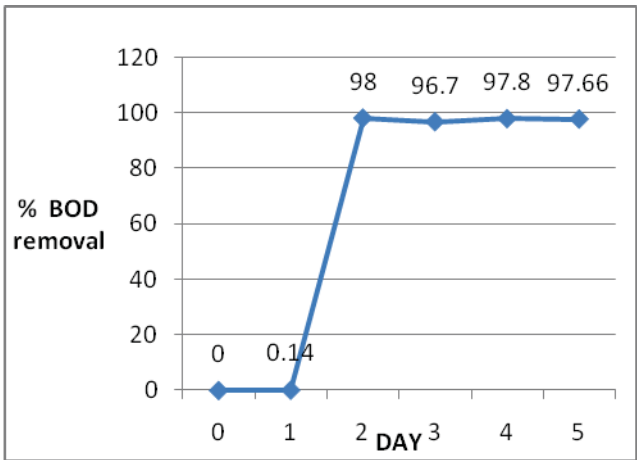

Figure 4.3: Percentage BOD removal of 1:10(v/v) garbage enzyme.

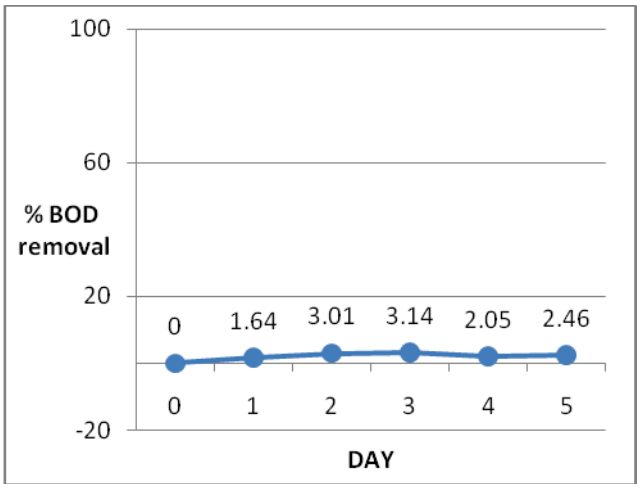

Figure 4.4: Percentage BOD removal of 1:1000(v/v) garbage enzyme.

4.2 Determination of garbage enzyme effectiveness based on Chemical Oxygen Demand (COD)

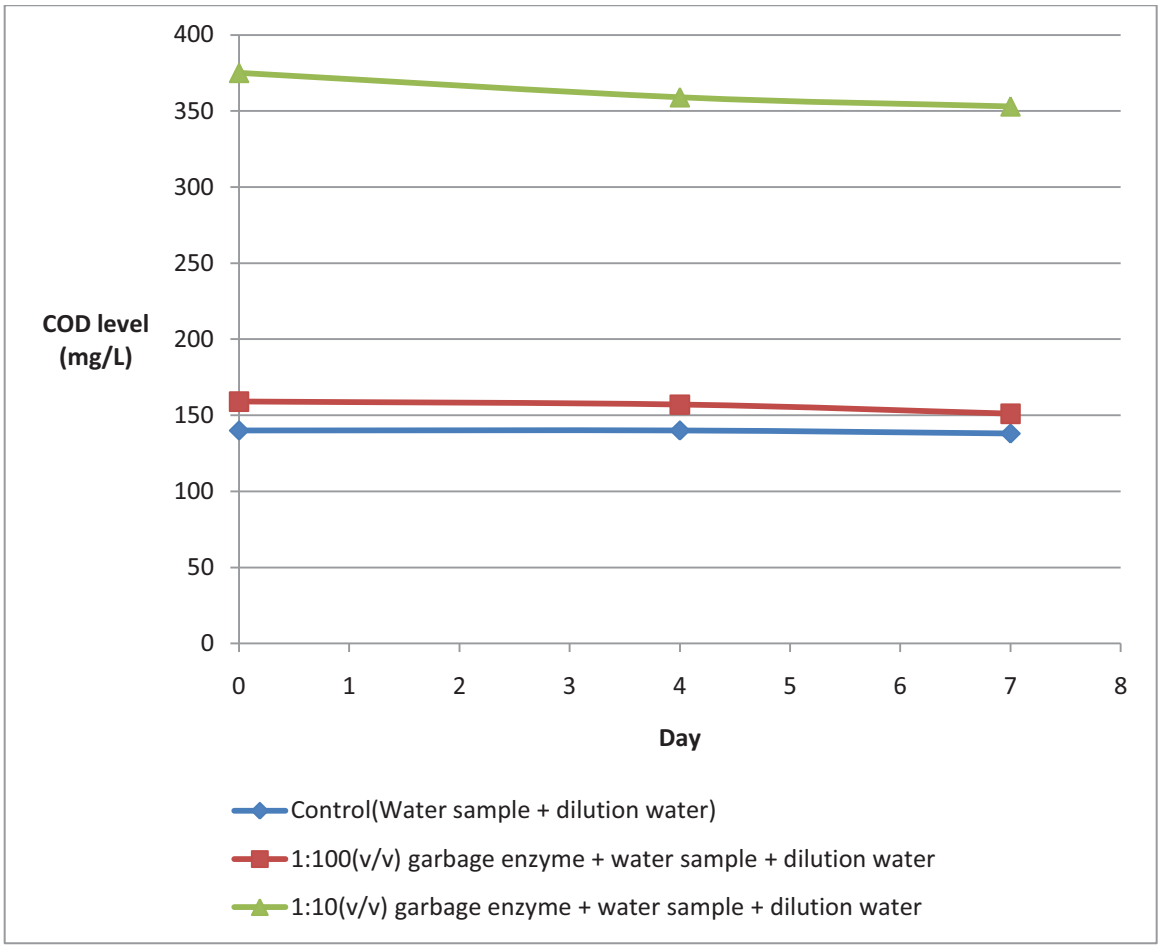

Figure 4.5: COD level versus Day (Effect of 1:10(v/v) and 1:00(v/v) concentration garbage enzyme).

Figure 4.5 shows that the sample treated with 1:10(v/v) concentration of garbage enzyme had higher COD value than sample treated with 1:100(v/v) concentration of garbage enzyme. This observation of higher COD value in more concentrated garbage enzyme usage could be due to the fact that higher concentration of garbage enzyme contained higher amount of organic matter. This COD value measure the organic matter in sample as the value of COD shows the oxygen equivalent of the organic content that can be oxidized by potassium dichromate ($K_2Cr_2O_7$) using silver sulfate (Ag_2SO_4) as a catalyst under acidic conditions (H_2SO_4) (Vyrides and Stuckey,2008). Such result proved that the garbage enzyme itself was organic matter as proposed by Dr. Rosukon.

Moreover, there was a proportional relationship between COD value (organic matter) and the concentration of garbage enzyme. However, in terms of treatment this higher value of COD in sample treated with more concentrated garbage enzyme of 1:100(v/v) gives rise to undesired situation in which the organic matter in sample increased when compared to the control sample whereby it was almost two times the amount. Thus, the purpose of treatment could not make sense by adding more organic matter that would be required further treatment if more concentrated garbage enzyme was used. However, this experiment also shown that the less concentrated garbage enzyme of 1:100(v/v) was more suitable to be used in treatment water sample whereby the COD value of addition garbage enzyme did not causes significant increased of COD value compared to control sample.

Meanwhile, there was no significant drop of COD value from initial day until day 7 for concentration of 1:10(v/v) and 1:100(v/v) garbage enzymes which reflect the rate of garbage enzyme activity was relatively the same. This observation could be due to the fact that the water sample contained low amount of organic matter. This organic matter which

was the substrates for degradation by microorganisms tend to be the limiting agent where its less availability causes rate of degradation of organic matter relatively constant and thus directly lead to no significant difference in COD values. In terms of water quality, the Interim National River Water Quality Standards for Malaysia suggest that under Class IIB, the COD of water for recreational use with body contact should be 25 mg/L. However, Figure 4.5 shows that COD value for control samples of the recreational lake water have exceeded the water quality standards by exhibiting 140 mg/L COD value. The results suggest that the water at Taman Bersatu Lake is not suitable to be used for recreational activities.

4.2.1 Percentage of COD removal

However, according to the calculated percentage of COD removal, sample with more concentrated garbage enzyme shows better removal result compared to less concentrated garbage enzyme in the same period of incubation or treatment. Despite 1:10 (v/v) garbage enzyme which contributes more organic matter to the sample but its ability to degrade organic matter was not suppressed owing to the higher amount of enzymatic reaction on degradation process. Despite according to Mak Oi Tong (2000), metal ions are vital and play roles in metal catalysts for hydrolytic reactions and redox reagents. However, as the result showed in Figure 4.6, percentage of COD removal was not that significant for both concentrations, suspected factors lead to this problem was probably due to the effect of oxygen saturated dilution water that suppress the enzyme reactivity as those dilution water consist of metal ions in nutrient supplements such as Mg^{2+} and Ca^{2+} (cofactors) which could causes saturation of binding site on enzyme and reducing the reactivity of

garbage enzyme. Therefore, to prove the effect nutrient supplement towards garbage enzyme activity, such oxygen saturated dilution water was replaced with solely oxygenated water.

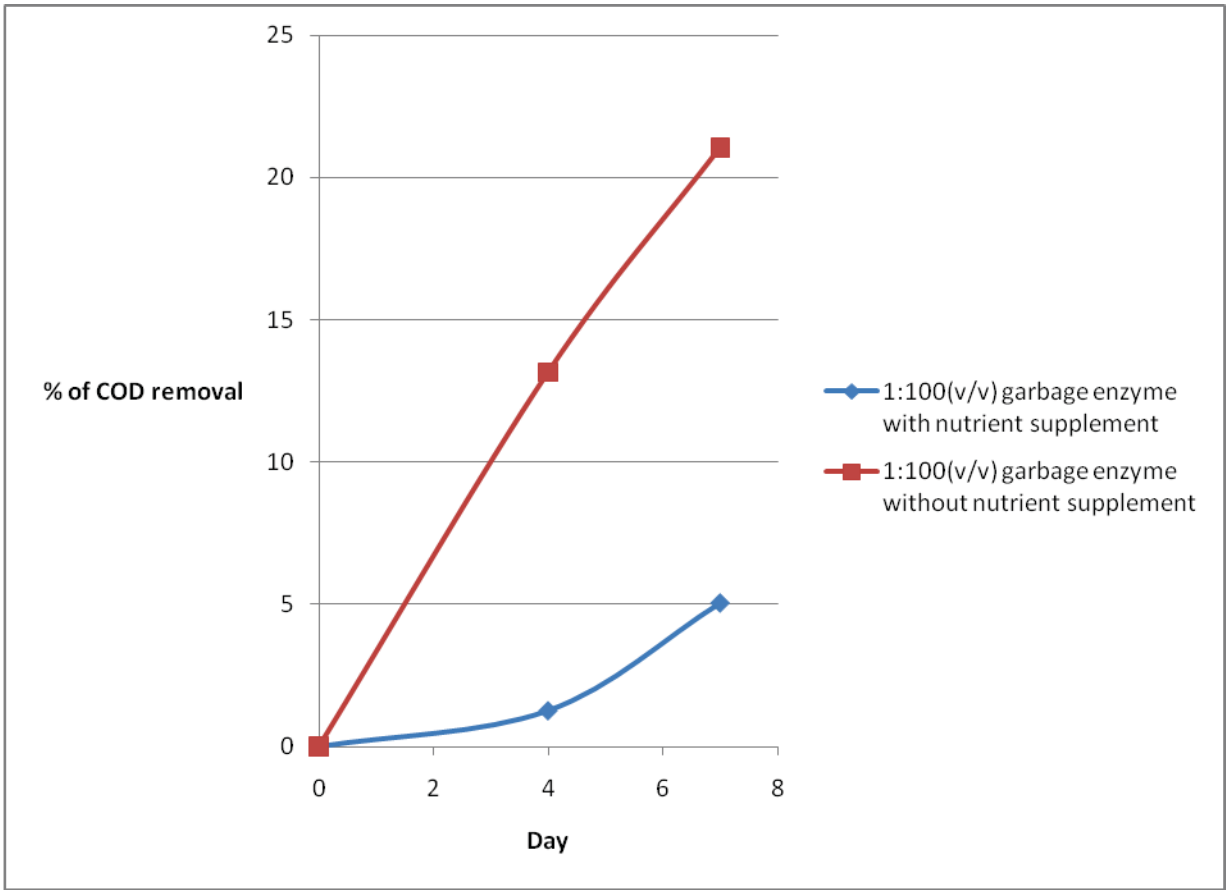

Figure 4.6: Percentage of COD removal versus Day.

Based on the COD removal percentage obtained, the sample treated with same amount of garbage enzyme concentration but without nutrient supplements shows percentage COD removal of almost 4 times than the sample treated with nutrient supplement conditions. Therefore, this result significantly proved that garbage enzyme works better in conditions without nutrient supplement.

4.3 Determination of Bacteria Growth in garbage enzyme treated lake water sample

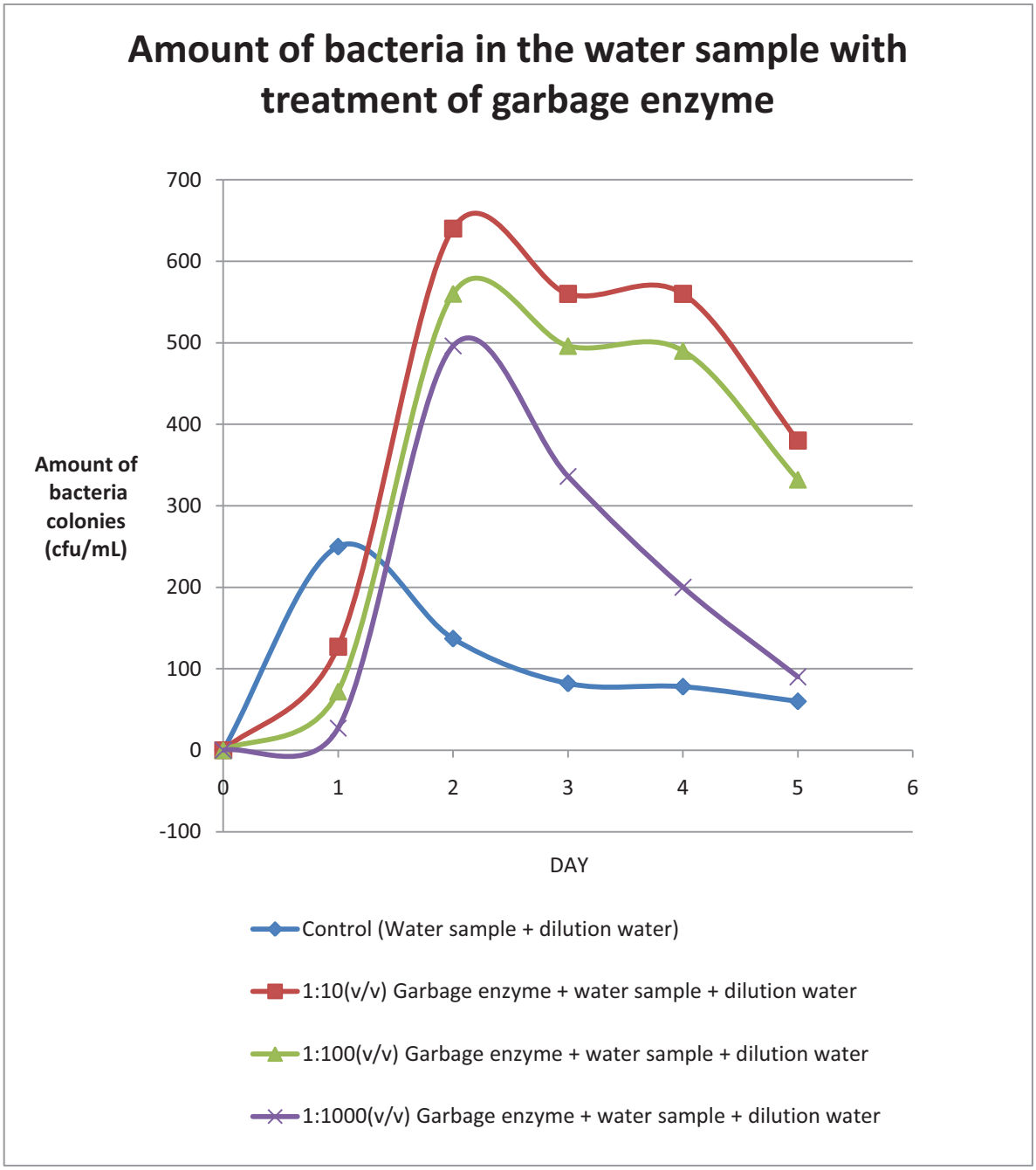

Figure 4.7: Amount of bacteria growth versus day.

From the Figure 4.7, it shows that the relationship of garbage enzyme concentration was proportional to the amount of bacterial growth in samples whereby more concentrated 1:10(v/v) garbage enzyme treated sample shown higher growth of bacterial compared to sample treated with 1:100(v/v) garbage enzyme and most diluted 1:1000(v/v) garbage enzyme. Therefore, this result proved that the garbage enzyme in the sample might possibly contained of bacteria which addition of more concentrated amount of garbage enzyme actually acclimating the number of bacteria number in the sample that in turn could enhancing the rate of degradation of organic matter through aerobic respiration. Moreover, this experiment also proved the fact that in previous Experiment which showed high COD value in 1:10(v/v) garbage enzyme treated sample could be due to the presence of high number of bacteria in garbage enzyme itself as shown in this study.

Moreover, the from bacteria growth curve as well as, it was observed that all conditions of garbage enzyme concentration actually showing similar trend whereby the highest growth of bacteria number in day 2 subsequently reduced in number on following days of observations. This observation of reduction trend in numbers of bacteria in sample can be explained by Shammas and coworkers (2006) who proposed such observation could be due to substrate inhibitory effects (Figure 4.8). There were two reasons owing to this inhibitory effects; firstly, it could be due to the concentrations of organic matter in the water sample were generally low. This is because the organic matter serve as the food or nutrient for the aerobic respiration and bacteria growth, so, once the organic matter (food) become depleted the bacteria growth were suppressed or die off. Second, the trend of bacteria reduction could also possibly due to when toxicity of organic matter to microbial activity is relatively

high. This reason reflects the bacteria were intolerant to the toxicity compound in the water sample such as those heavy metals which lead to die off or reduction of bacteria number.

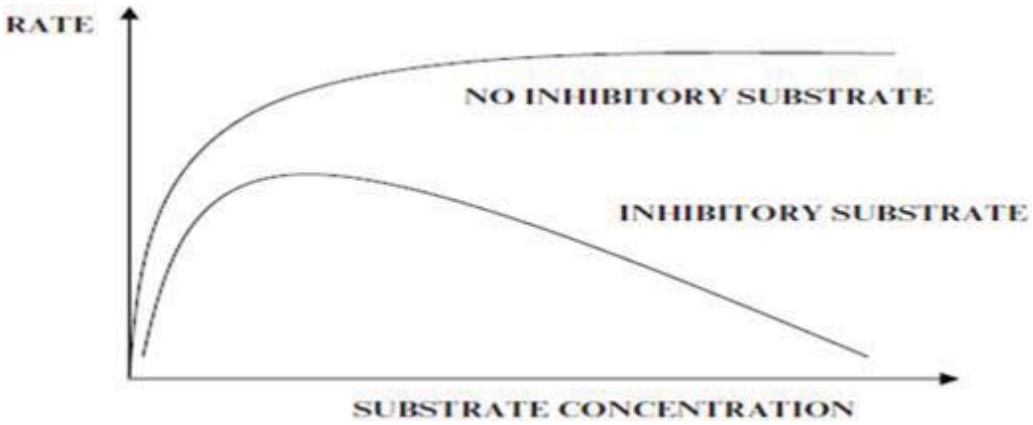

Figure 4.8: Rate of bacterial growth versus substrate concentration.

4.4 Microscopic view of microorganism found in recreational lake water sample

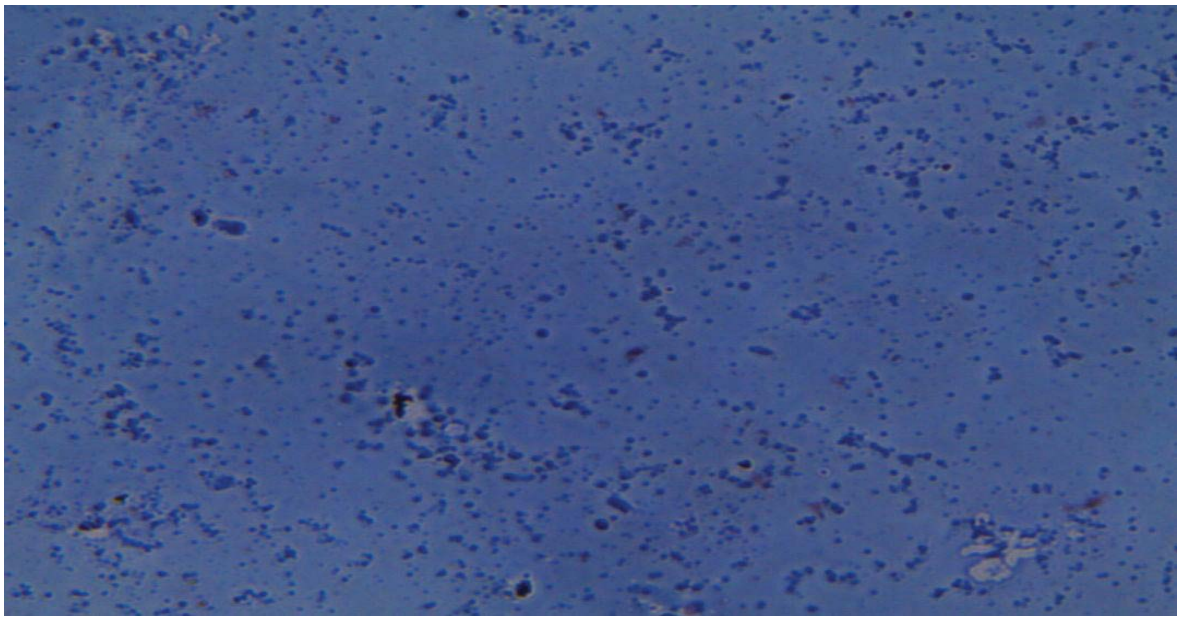

Figure 4.9: Manny types of microorganism in terms of shape were seen from the water sample which proved that possible reduction in dissolved oxygen is due to bacteria.

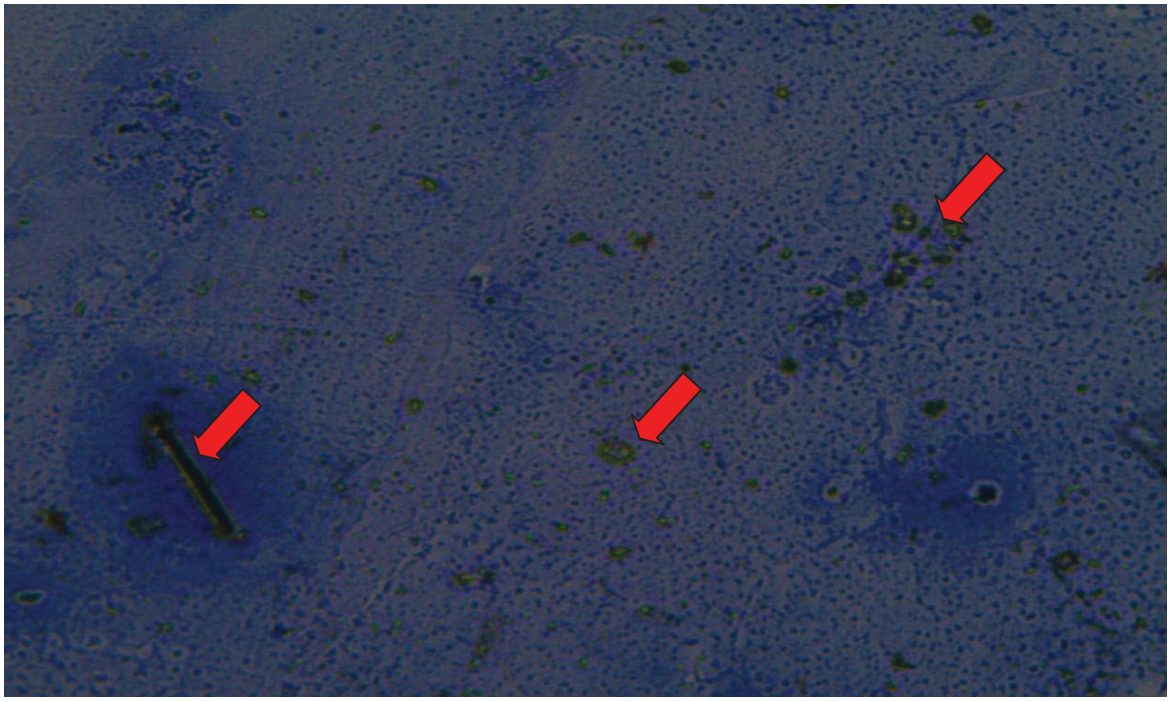

Figure 4.10: Algae were seen in water sample which could probably providing oxygen for bacteria respiration.

4.5 Effect of nutrient availability on the garbage enzyme activity

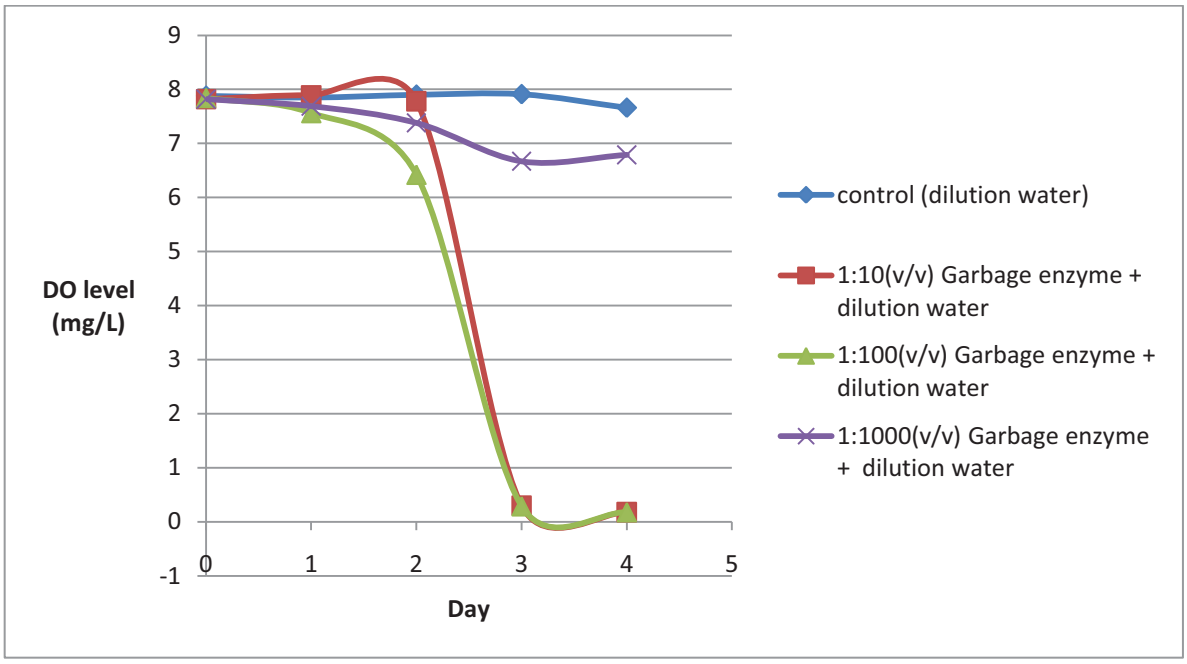

Figure 4.11: DO level versus Day (Effect of nutrient on the DO uptake by garbage enzyme bacteria (with oxygenated dilution water).

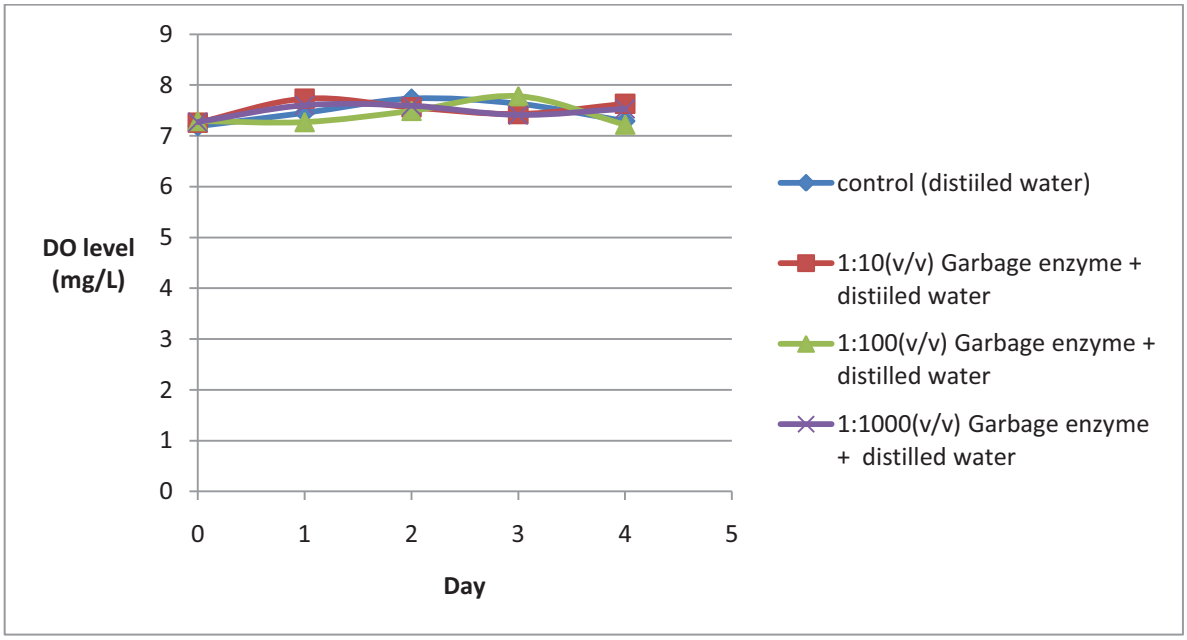

Figure 4.12: DO level versus Day (Effect of nutrient on the DO uptake by garbage enzyme bacteria (with distilled water).

From the above Figures 4.11 and 4.12 respectively, it clearly show that the nutrient availability was significantly affecting the DO uptake in the sample with treatment of garbage enzyme compared to those samples treated with distilled water (without nutrient). In this case, emphasis on two concentrations at 1:10(v/v) garbage enzyme and 1:100(v/v) garbage enzyme showing increase in DO uptake as DO level at the final day of measurement yielding a value of almost zero. However, this was in contrast to those treated with distilled water whereby at these two respective garbage enzyme concentrations, there was relatively constant measurement in terms of DO uptake. Therefore, such result proved that the garbage enzyme's bacteria works better in nutrient availability conditions based on the DO uptake as it enhance the aerobic respiration rate. The nutrients used in oxygenated dilution water such as Phosphate buffer, Magnesium sulfate, Calcium chloride and Ferric chloride were important for microorganism survival. According to Shammas and colleagues (2006), microbial metabolism requires these elements as nutrients for synthesis and energy generation. Therefore, we can expect that the nutrient added does increased the reproduction of bacteria number and directly increasing the dissolved oxygen consumption in the sample.

In addition, to further prove the validity of the effect of nutrient deficiency on bacterial growth, a bacteria culture from sample on agar plate was carried out. Based on the result obtained, there was no growth of bacteria colonies can be observed (Appendix: Figure 2). Therefore, since there was no bacteria colony which means that there was no bacteria growth and thus the DO uptake is relatively constant for the aerobic respiration.

4.6 Recovery percentage of acetic acid extraction using acid-base separation method

Based on the recovery test of acetic acid trough GC-MS, the calculated amount of recovery percentage of 2.80% shown that the method of acid-base separation was not the effective and efficient way to extract acetic acid from the solvent under acidic conditions. In other words, the difficulties in extracting acetic acid could probably due to the unsuitable organic solvent used. In this case, the dichloromethane which acts as organic solvent might not able to extract the acetic acid from solution as expected. For extraction of acetic acid, considerations towards factors such as distribution coefficient and miscibility with water should be taken into accounts whereby the desired properties of solvents should have high distribution coefficient, good selectivity towards solute and little or no miscibility with feed solution (Cheresources.com, 2010).

Solvent	Distribution Coefficient(K) air-water systems at 40°C	Miscibility with water
Ethyl Acetate	62.4	10
MIBK	139.5	2.0
Dichloromethane	5.65	1.3

Table 4.1: Common solvents for acetic acid extraction (Labhut,2010).

Apart from that, the low recovery percentage of acetic acid extraction might also caused by the instrumental setback especially GC-MS used to detect the amount of acetic acid extracted. In this case, GC-MS might not able to perform perfectly in terms of detection due to air leak that allowed contaminants to flow in. Hence, the properties such as boiling point, molecular weight, and etc of these contaminants could probably similar to acetic acid. Therefore, the similarity of properties between acetic acid and contaminants might lead to difficulties for detection due to overlapping of molecules. Hence, a lower peak area was observed than expected.

4.7 Determination of garbage enzyme property through acid-base separation of Acetic acid extraction

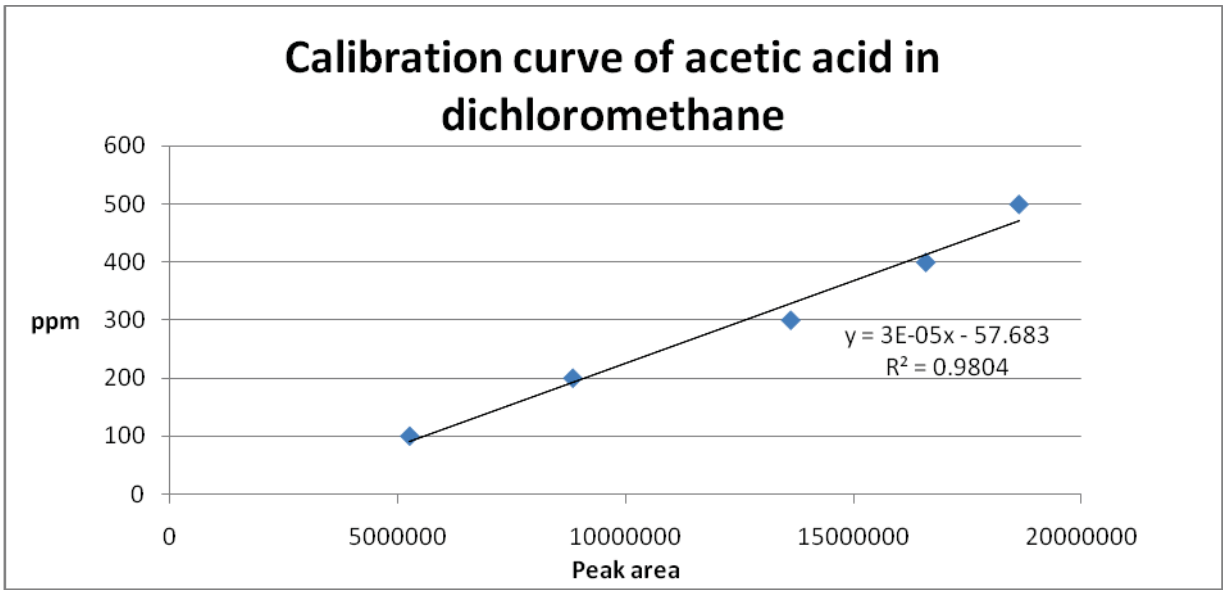

Figure 4.13: Calibration curve of acetic acid in dichloromethane.

Based on the calibration curve obtained, the amount of acetic acid extracted from the garbage enzyme was able to be calculated. The concentration of the acetic acid extracted from garbage enzyme was found out to be 102.21 ppm which reflects the recovery test of only 2.8% of acetic acid was able to be extracted. Hence, in order to find out the actual concentration of acetic acid in garbage enzyme, assumption of 100% recovery has to be made. Therefore, the actual amount of acetic acid inside the 50mL garbage enzyme was calculated to be 3650.36 ppm.

4.8 Effect of the DO uptake for the acidic/basic extracted garbage enzyme compared to control garbage enzyme (non-extracted)

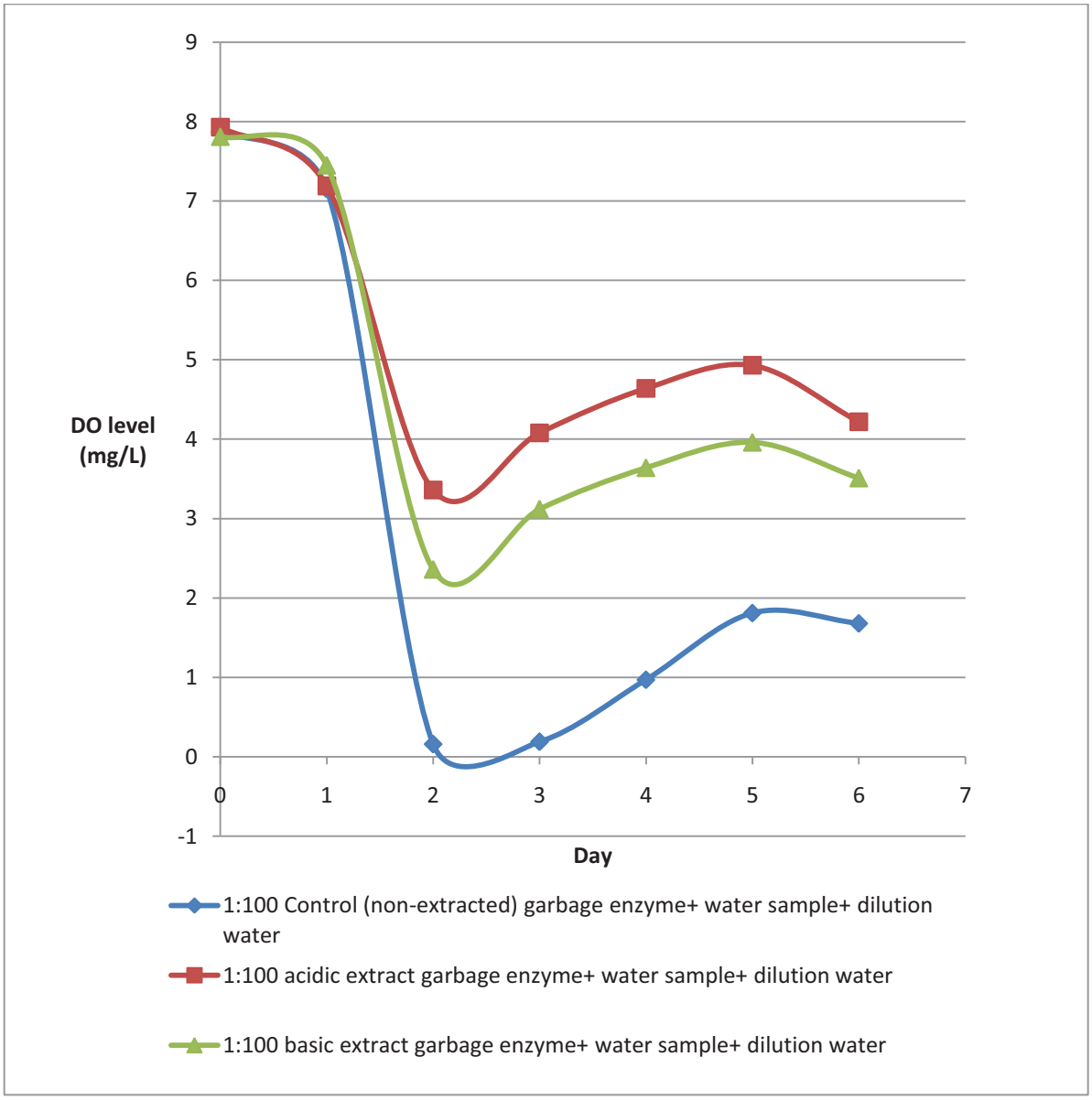

Figure 4.14: DO level versus Day (Effect of the DO uptake for the Basic/Acidic extracted garbage enzyme compared to control garbage enzyme (non-extracted)).

Based on Figure 4.14, DO uptakes were restricted for those samples with garbage enzyme that have undergone acid-base separation method. The control garbage enzyme (non-extracted) did show increment in DO uptake where its DO level manage to reach almost zero on second day of measurement which is compatible to the earlier optimization experiment conducted. However, the expected flat curves of DO uptake after Day 2 was not obtained mainly due to the instrumental error of DO meter measurement as the probe of DO meter probably have degraded after excessive usage. Meanwhile, Figure 4.14 also shows that the acidic extracted garbage enzyme was the one that highly affected in terms DO uptake as its DO uptake was the lowest. This result of DO uptake in fact was even lower than basic extracted garbage enzyme treated sample.

Therefore, the result of DO uptake of respective garbage enzyme proved that acid-base separation managed to remove the acid and basic compound from the garbage enzyme that might influence the efficiency in DO uptake. However, the main interest of this experiment was the fact that acidic extracted garbage enzyme was no longer efficient in catalyzing DO uptake. Hence, the main reason deduce for this observation was that the acetic acid effect was removed in the process of separation which lead to possibility that the garbage enzyme indeed was made up of acetic acid.

4.9 Simulation effect of acetic acid on DO uptake

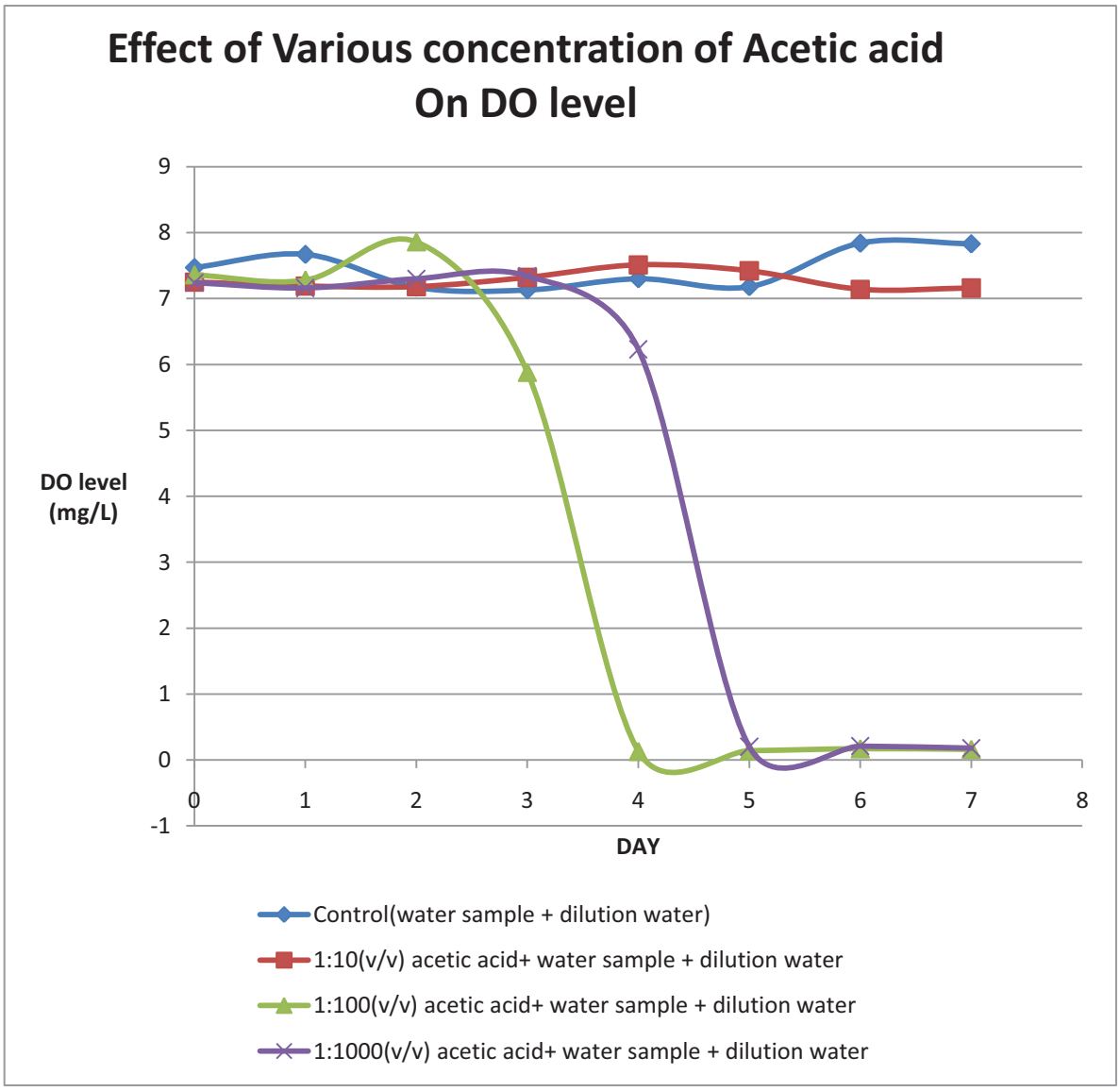

Figure 4.15: DO level versus Day (Effect of Various concentration of Acetic acid On DO level).

Based on the Figure 4.15, it shows that acetic acid basically does shown the same trend of DO uptake just like the effects of garbage enzyme in previous experiment. However, the acetic acid works better at lower concentration of acetic acid rather than garbage enzyme which have proportional effectiveness in terms of DO uptake with garbage enzyme concentrations. Meanwhile, based on the result obtained as well, it proved that at high concentration of acetic acid, 1:10(v/v) the effectiveness of acetic acid was completely disabled in which DO uptake remained constant throughout the period of incubation. Its ineffectiveness in terms of DO uptake was mainly due to the fact that at high concentration, it tends to kill off those microorganisms in the water sample and thus lead to constant DO level as the aerobic respiration did not occurred.

In addition, the acetic acid as well proved to be taking more time to reach the highest DO uptake at day 4 compared to garbage enzyme treated sample at day 2. In other words, the acetic acid at lower concentrations especially 1:100(v/v) can simulate the capability to act like garbage enzyme but at slower rate of DO uptake. For the rate of aerobic respiration or degradation which did not show the effectiveness as garbage enzyme could be owing to the fact that acetic acid does not possess the catalysis ability where garbage enzyme has to boost the aerobic respiration of microorganism in samples.

Therefore, through this experiment of acetic acid simulation, there was possibility that acetic acid could be used to treat water quality just like the garbage enzyme. However, in order to obtain the desired result of effectiveness and efficiency as garbage enzyme the actual amount of acetic acid inside the garbage enzyme must be found out so that a compatible comparison can be made.

5.0 Conclusion

The water quality is an important element to keep the ecosystem of water body in balance. Despite recreational lake does not receive much attention of pollution from the public, its actual status of pollution should not be ignored as it is part our environment. Therefore, the study emphasizes on the preparation of garbage enzyme from fruit peels to improve the water quality of recreational lake.

Based on the result and discussion of parameters such as DO, BOD, COD, nutrient availability, bacteria growth curve, and property of garbage enzyme this shows a significant evidence of the garbage enzyme effectiveness and efficiency. The findings of this study shows that garbage enzyme with 1:100(v/v) concentration proved to be the idea dosage for the treatment as its percentage of COD and BOD removal was more significant. Results of this study demonstrate the potential of garbage enzymes in water treatment purposes as well as a alternative treatment method that require less cost and expertise to operate. Besides, acetic acid existence is strongly related to garbage enzyme property which directly determining the functionality and effectiveness of garbage enzyme in treatment of water.

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Appendix:

Figure 1: Recreational Lake at Taman Bersatu, Rawang (location of water sample collection)

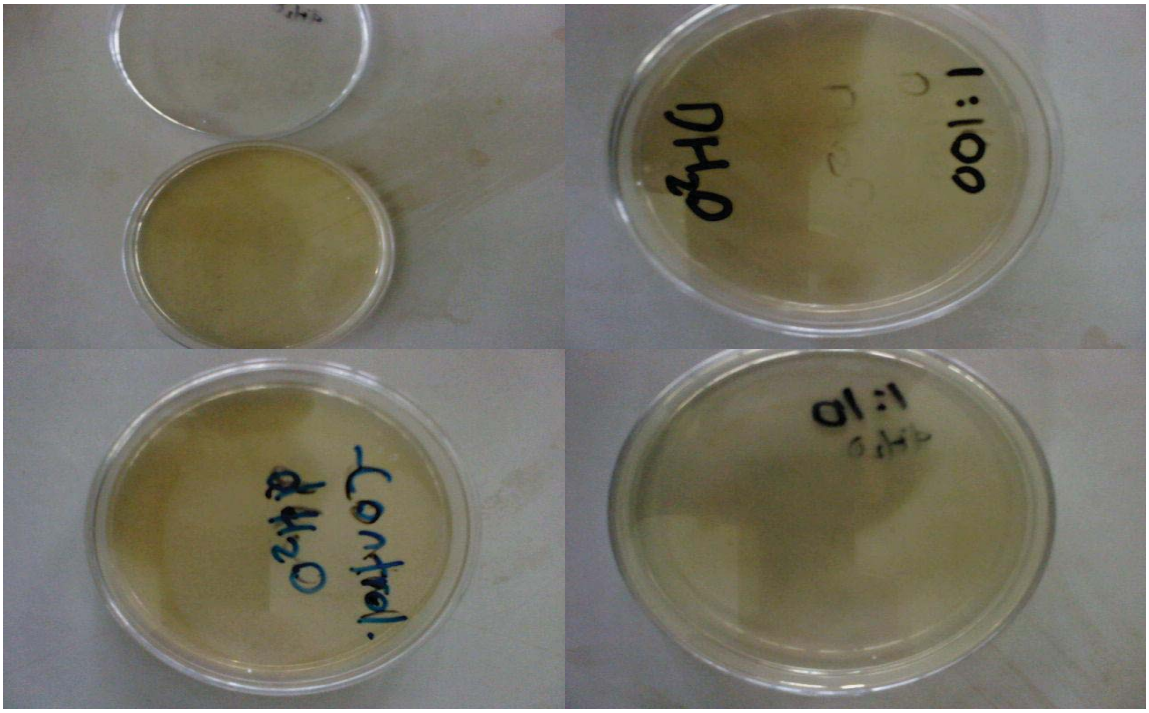

Figure 2: No growth of bacteria can be seen for those garbage enzymes in distilled water medium, therefore, the DO level is relatively constant.

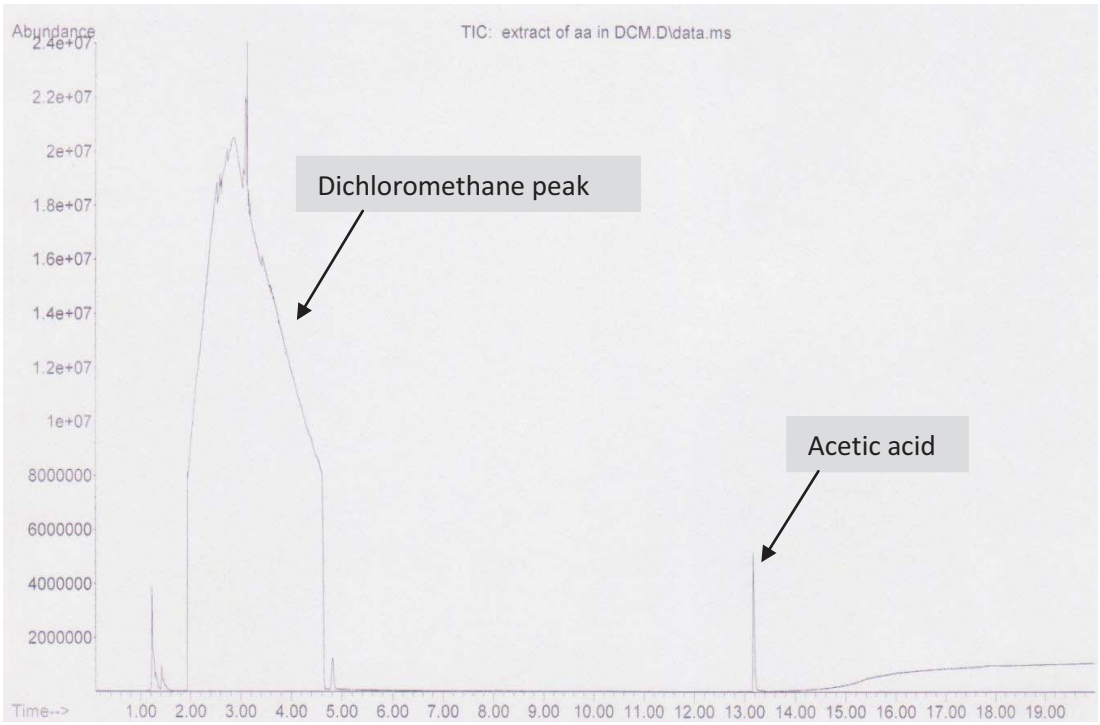

Figure 3: Mass spectrum of acetic acid extracted from dichloromethane.

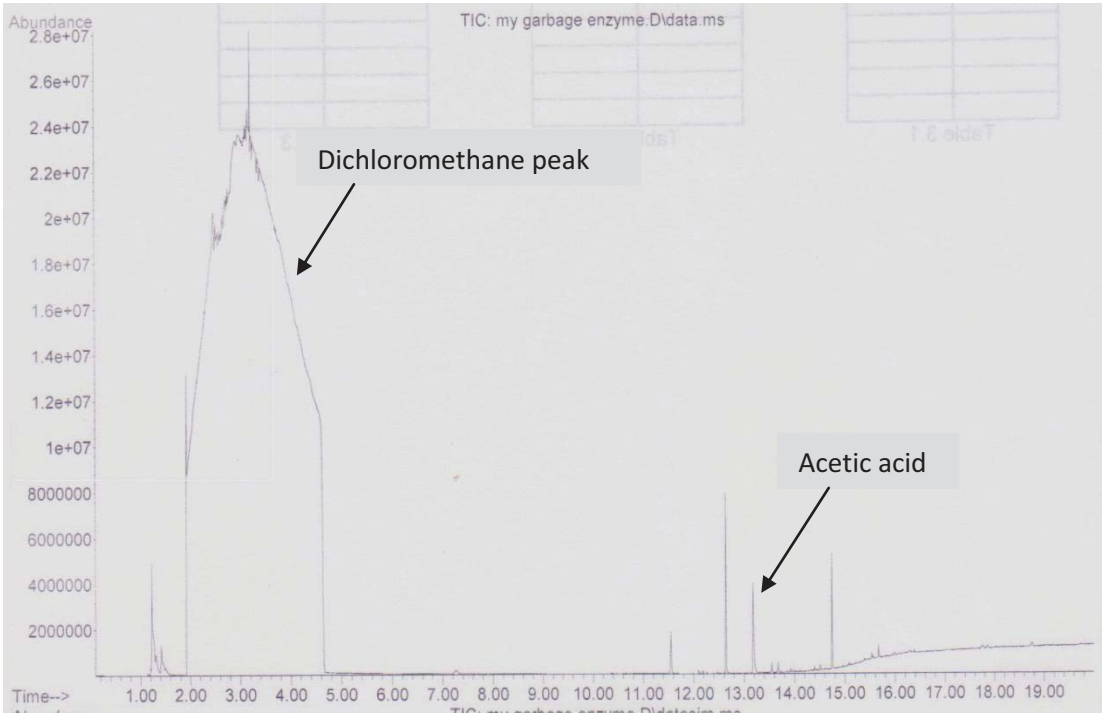

Figure 4: Mass spectrum of acetic acid extracted from garbage enzyme.

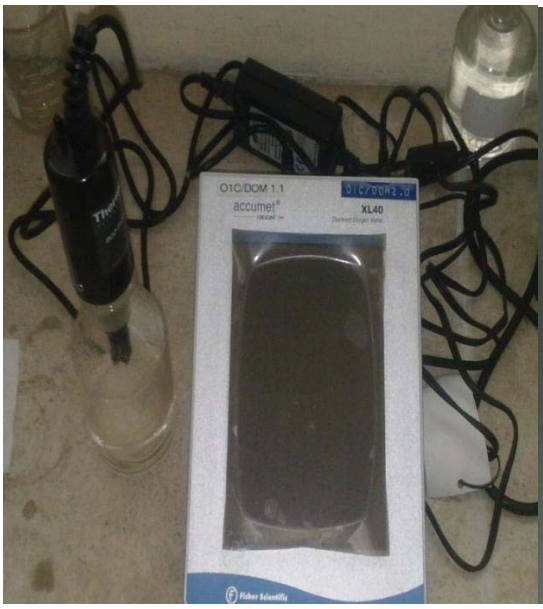

Figure 5: DO meter (Model: XL 40) used to measure DO level in samples

Figure 6: Merck Thermoreactor (Model: TR620) used to heat COD tube at148˚C for 2 hours

Figure 7: Metler Toledo pH meter (Model: 320) used to adjust the pH during acid-base separation.

Figure 8: GC-MS from Agilent Technologies with GC model: 7980A and MS model: 5975C inert XL MSD used in acetic acid analysis.

Figure 9: Merck UV Spectroquant (Model: Pharo 100) used to measure COD in samples.

Figure 10: BOD LE-519 incubator used to incubate samples at 20˚C

Figure 11: Hirayama Autoclave used during preparation of TSA agar medium at 121˚C for 2 hours.

Figure 12: Memmert incubator used to incubate agar plate cultured with bacteria at 37˚C.

Sample	Initial DO	DO level				
		Day 1	Day 2	Day 3	Day 4	Day 5
10mL of 1:10(v/v) Garbage enzyme + 50mL of lake water sample+ 240mL oxygenated dilution water.	7.32	7.25	0.14	0.24	0.16	0.17
10mL of 1:100(v/v) Garbage enzyme + 50mL of lake water sample+ 240mL oxygenated dilution water.	7.34	6.27	0.10	0.22	0.14	0.13
10mL of 1:1000(v/v) Garbage enzyme + 50mL of lake water sample+ 240mL oxygenated dilution water	7.26	7.20	7.10	7.09	7.17	7.14
50mL of lake water sample+ 240mL oxygenated dilution water (Control)	7.34	7.24	7.35	7.37	7.50	7.64

Table 1: Effect of various concentration garbage enzyme [1:10(v/v), 1:100(v/v), 1000(v/v)] and a control sample on the Dissolved Oxygen (DO) uptake for 5 days.

$$\textbf{\% of BOD removal} = \frac{\boldsymbol{Initial\ DO - Final\ DO}}{\boldsymbol{Initial\ DO}} \times \mathbf{100\%}$$

Sample / Day	1:10(v/v) Garbage enzyme			1:100(v/v) Garbage enzyme			1:1000 (v/v) Garbage enzyme		
	Initial DO	Final DO	**% of BOD removal**	Initial DO	Final DO	**% of BOD removal**	Initial DO	Final DO	**% of BOD removal**
0	7.26	7.26	**0**	7.34	7.34	**0**	7.32	7.32	**0**
1	7.26	7.25	**0.14**	7.34	6.27	**1.99**	7.32	7.20	**1.64**
2	7.26	0.14	**98.0**	7.34	0.10	**98.64**	7.32	7.10	**3.01**
3	7.26	0.24	**96.7**	7.34	0.22	**97.00**	7.32	7.09	**3.14**
4	7.26	0.16	**97.8**	7.34	0.14	**98.09**	7.32	7.17	**2.05**
5	7.26	0.17	**97.66**	7.34	0.13	**98.23**	7.32	7.14	**2.46**

Table 2: Percentage of BOD removal by several of garbage enzyme concentration.

Day	COD level (25-1500mg/L)		
	1:100(v/v) garbage enzyme + 50mL lake water sample + 240mL O_2 saturated dilution water	1:10(v/v) garbage enzyme + 50mL lake water sample + 240mL O_2 saturated dilution water	Control (50mL of Lake water sample + O_2 saturated dilution water)
0	159	375	140
4	157	359	140
7	151	353	138

Table 3: Organic contents of garbage enzyme at 1:10 and 1:100(v/v) on lake water sample on COD level (25-1500mg/L) for 7 days.

$$\% \text{ of COD removal} = \frac{Initial\ COD - Final\ COD}{Initial\ COD} \times 100\%$$

1:10(v/v) garbage enzyme + 50mL lake water sample + 240mL O_2 saturated dilution water				1:100(v/v) garbage enzyme + 50mL lake water sample + 240mL O_2 saturated dilution water			
Day	Initial COD	Final COD	% of COD removal	Day	Initial COD	Final COD	% of COD removal
0	375	375	0	0	159	159	0
4	375	359	4.27	4	159	157	1.27
7	375	353	5.87	7	159	151	5.06

Table 4: Percentage of COD removal by garbage enzyme with nutrient supplement.

1:100(v/v) garbage enzyme + 50mL lake water sample + 240mL Oxygenated water			
Day	Initial COD	Final COD	% of COD removal
0	190	190	0
4	190	165	13.16
7	190	150	21.05

Table 5: Percentage of COD removal by garbage enzyme without nutrient supplement.

Day	Amount of bacterial colonies (cfu/ml)			
	10mL of 1:10(v/v) Garbage enzyme + 50mL of lake water sample+ 240mL oxygenated dilution water	10mL of 1:100(v/v) Garbage enzyme + 50mL of lake water sample+ 240mL oxygenated dilution water	10mL of 1:1000(v/v) Garbage enzyme + 50mL of lake water sample+ 240mL oxygenated dilution water	50mL of lake water sample + 240mL of oxygen saturated dilution water (Control)
0	0	0	0	0
1	127	72	27	250
2	640	560	496	137
3	560	496	336	82
4	560	490	200	78
5	380	332	90	60

Table 6: Amount of bacterial colonies found in the water sample treated with garbage enzyme for 5 days.

Day	DO level			
	10mL of 1:10(v/v) Garbage enzyme + 290mL of oxygenated dilution water	10mL of 1:100(v/v) Garbage enzyme + 290mL of oxygenated dilution water	10mL of 1:1000(v/v) Garbage enzyme + 290mL of oxygenated dilution water	300mL of distilled water (Control)
0	7.82	7.84	7.82	7.88
1	7.89	7.56	7.69	7.85
2	7.78	6.42	7.38	7.90
3	0.30	0.29	6.67	7.91
4	0.18	0.18	6.79	7.66

Table 7: Effect of nutrient availability on the DO uptake by garbage enzyme bacteria (with oxygenated dilution water).

Day	DO level			
	10mL of 1:10(v/v) Garbage enzyme + 290mL of distilled water	10mL of 1:100(v/v) Garbage enzyme + 290mL of distilled water	10mL of 1:1000(v/v) Garbage enzyme + 290mL of distilled water	300mL of distilled water (Control)
0	7.19	7.29	7.27	7.19
1	7.45	7.27	7.60	7.45
2	7.73	7.49	7.59	7.73
3	7.43	7.78	7.41	7.63
4	7.63	7.22	7.53	7.29

Table 8: Effect of nutrient availability on the DO uptake by garbage enzyme bacteria (with distilled water).

Preparation of acetic acid concentration at 300 ppm:

$$\frac{\text{Weight of acetic acid (g)}}{Volume\ of\ Solvent\ (mL)} \times 10^6 = 300\ \text{ppm}$$

$$Weight\ of\ acetic\ acid = \frac{300\ ppm \times 50mL}{10^6}$$

$$= 0.015g$$

$$\rho_{(acetic\ acid)} = \frac{Weight\ of\ acetic\ acid\ (g)}{Volume\ of\ acetic\ acid\ (mL)}$$

$$Volume\ of\ acetic\ acid\ (mL) = \frac{0.015g}{1.049g/mL}$$

$$= 0.0143\ mL \times 1000$$

$$= 14.3\mu L$$

Recovery percentage of acetic acid extraction using acid-base separation method:

300 ppm acetic acid in dichloromethane	Peak Area
Initial(Without extracted with Dichloromethane)	15969493
Final(Extracted with dichloromethane)	447400

Table 9: Peak area of non-extracted acetic acid and extracted acetic acid at 300 ppm.

$$\text{Recovery percentage} = \frac{Final\ peak\ area}{Initial\ peak\ area} \times 100\%$$

$$= \frac{447400}{15969493} \times 100\%$$

$$= 2.80\ \%$$

Determination of garbage enzyme properties through Extraction of Acetic acid

Concentration of acetic acid prepared	Peak Area
100 ppm of acetic acid in 50mL dichloromethane	5256396
200 ppm of acetic acid in 50mL dichloromethane	8839304
300 ppm of acetic acid in 50mL dichloromethane	13622050
400 ppm of acetic acid in 50mL dichloromethane	16588765
500 ppm of acetic acid in 50mL dichloromethane	18633792
Extracted acetic acid from garbage enzyme	5627332

Table 10: Calibration curve of acetic acid in dichloromethane and amount of acetic acid extracted from garbage enzyme.

Calculation of acetic acid concentration in garbage enzyme:

From the trend function obtained through Microsoft Excel 2010, the concentration of acetic acid correspond to peak area of 5627332 was found out to be 102.21 ppm.

Since, the recovery of acetic acid through acid-base separation was only 2.80%. Assumption of 100% recovery would give rise to actual acetic acid concentration in 50mL garbage enzyme:

2.80 % = 102.21 ppm

100 % = actual amount of acetic acid

Cross multiplication:

$$\text{The actual amount of acetic acid in 50mL garbage enzyme} = \frac{100\% \times 102.21 \text{ ppm}}{2.80\%}$$

$$= 3650.36\ ppm$$

Day	DO level		
	10mL of 1:100(v/v) unextracted(control) garbage enzyme + 50mL of lake water sample + 240mL oxygenated dilution water	10mL of 1:100(v/v) acidic extracted garbage enzyme + 50mL of lake water sample + 240mL oxygenated dilution water	10mL of 1:100(v/v) basic extracted garbage enzyme + 50mL of lake water sample + 240mL oxygenated dilution water
0	7.87	7.93	7.81
1	7.15	7.19	7.45
2	0.16	3.36	2.36
3	0.19	4.08	3.12
4	0.97	4.64	3.64
5	1.81	4.93	3.96
6	1.68	4.22	3.51

Table 11: Effect of the DO uptake for the acidic/basic extract garbage enzyme compared to control garbage enzyme (non-extracted).

Day	DO level			
	10mL of 1:10(v/v) glacier acetic acid + 50mL of lake water sample+ 240mL oxygenated dilution water	10mL of 1:100(v/v) glacier acetic acid + 50mL of lake water sample+ 240mL oxygenated dilution water	10mL of 1:1000(v/v) glacier acetic acid + 50mL of lake water sample+ 240mL oxygenated dilution water	50mL of lake water sample + 240mL of oxygen saturated dilution water (Control)
0	7.25	7.36	7.24	7.47
1	7.19	7.28	7.16	7.67
2	7.18	7.86	7.30	7.17
3	7.32	5.88	7.34	7.13
4	7.51	0.13	6.23	7.30
5	7.42	0.14	0.20	7.18
6	7.14	0.17	0.21	7.84
7	7.16	0.16	0.18	7.83

Table 12: Effect of Various concentration of acetic acid On DO level.