STUDY OF COD REMOVAL BY ANAEROBIC DIGESTION USING MIXED CULTURE FROM SEWAGE

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ABSTRACT

The purpose of this study is to investigate the application of mixed culture which was taken from the sewage to remove the chemical oxygen demand (COD) in the wastewater. Synthetic wastewater, which is glucose solution, was used in this study. Four different concentration of glucose, 1g/l, 2g/L 3g/L and 4g/L were used. This study attempts to reduce the concentration of COD in the glucose solution using biological treatment. The selected colony of bacteria which been grew on the agar plate was employed to the glucose solution for the anaerobic digestion to occur. The organic matter that contributed to COD will be degraded by the bacteria under anaerobic conditions using batch process. The experiment was run in the closed Schott bottle, which act as the anaerobic digester rig. Rubber tube was connected from the top of the Schott bottle to allow migrating of the biogas produced towards the water displacement unit for biogas collection. The experiment was conducted for five days of treatment time. The analysis on COD reduction percentage, turbidity reduction and observation on biogas production were conduct throughout this study. From the result obtained the glucose with concentration of 1g/L show the most COD reduction percentage which is 61.4%. The reduction percentages were decrease as the glucose concentration increased. The COD reduction for 2g/L of glucose was 50.55%, 38.88% for 3g/L of glucose and 31.28% for 4g/L of glucose. The turbidity for all glucose solution used also shows reducing trends. There is production of biogas during the anaerobic digestion process and was observed through the water displacement unit. Bubble built up in the water displacement unit represents the biogas that produced.

ABSTRAK

Tujuan kajian ini dijalankan adalah untuk mengkaji penggunaan kultur campuran yang diambil dari air sisa buangan domestik bagi merawat permintaan oksigen kimia (COD) di dalam air sisa. Air sisa tiruan, iaitu larutan glukosa digunakan di dalam kajian ini. Empat kepekatan berbeza telah digunakan iaitu 1 g/L, 2 g/L, 3 g/L dan 4 g/L. Bakteria digunakan untuk merendahkan kepekakatan COD di dalam larutan glukosa tesebut. Koloni bakteria yang dihidupkan di atas piring agar dipilih untuk digunakan dalam proses pencernaan anaerobik. Kandungan organik yang menyumbang kepada COD dicerna oleh bakteria di dalam keadaan anaerobik. Eksperimen dijalankan didalam botol Schott tertutup, yang berfungsi sebagai ruang pencernaan anaerobik. Tiub getah disambungkan daripada penutup botol Schott bagi membolehkan pergerakan biogas yang terhasil ke arah unit pengumpulan gas. Eksperimen ini dijalankan selama lima hari. Analisis dilakukan terhadap penurunan nilai COD, penurunan kekeruhan dan pemerhatian terhadap penghasilan biogas. Berdasarkan keputusan yang terhasil, larutan glukosa 1 g/L menujukkan penurunan nilai COD yang paling tinggi iaitu 61.4%. Peratus penurunan nilai COD berkurang apabila nilai kepekatan larutan glukosa meningkat. Penurunan COD untuk glukosa berkepekatan 2 g/L ialah 50.55%, 38.88% untuk kepekatan 3 g/L dan 31.28% kepekatan 4 g/L glukosa. Kekeruhan untuk semua larutan glukosa yang digunakan juga menunjukkan trend penurunan. Biogas terhasil semasa pencernaan anaerobik berlaku dan ia dapat dilihat melalui unit perpindahan air. Gelembung-gelembung gas yang terbentuk menunjukkan terdapat biogas yang terhasil.

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BOD	-	Biological oxygen demand
COD	-	Chemical oxygen demand
EDC	-	Electron donating capacity
kPa	-	Kilopascal
L	-	Liter
mg	-	Milligram
NTU	-	Nephelometric turbidity unit
ppm	-	Part per million
rpm	-	Revolution per minute
°C	-	Degree celcius

CHAPTER 1

INTRODUCTION

1.1 Background of study

Wastewater originates predominantly from water usage by residences and commercial and industrial establishments, together with groundwater, surface water and storm water. Domestic and commercial water uses are the main source of wastewater. The wastewater typically contains organic pollutant, suspended solids, nutrients and pathogens. All of these pollutants need to be take care first before the wastewater can be passing out through the river or being recycled. For reducing the environmental and health hazards, all of these pollutants need to be brought down to allowable limits for the safe disposal of wastewater. The failure in treat the wastewater could cause various bad effects either towards environment or human. All commercial and industrial facilities have limits on the concentration of pollutants they can discharge to the sewer.

Waste-water quality may be defined by its physical, chemical, and biological characteristics. Chemical oxygen demand (COD) is one of the chemical water-quality parameter. The COD test is widely used in measuring the organic strength of domestic and industrial waste. COD test is used as measure of oxygen requirement of a sample that is susceptible to oxidation by strong chemical oxidant. The value of COD will show about how much organic pollutant exist in that wastewater.

The COD test are the most appropriate test for assessing the organic wastewater strength from a theoretical and practical point of view (Mara and Horan, 2003). The dichromate reflux method is preferred over procedures using other oxidants because its superior oxidizing ability, applicability to a wide range of sample and ease to manipulate (Williams, 2001).

Variety of methods can be applied in order to remove or reduce the chemical oxygen demand including physical, chemical and biological method. Anaerobic digestion, which is a part of biological method, is widely used in reducing the chemical oxygen demand. By the act of anaerobic bacteria, the biodegradable material will be break down to simpler substances which reduce the level of chemical oxygen demand in that wastewater and make it safe toward the human and environment.

1.2 Problem Statement

Wastewater usually contains high organic contents in it. The extent of organics content is shown by the COD value. So, the study was conducted on how the organics content can be degraded in order to remove the COD concentration in the wastewater. Anaerobic digestion was chose to be implement since it is a low cost treatment compare with the other alternative. It requires no oxygen supply of which reduces substantially to the cost of wastewater treatments. The anaerobic digestion can be practically applied at any place and any scale. Beside that, the final product which is methane gas derive from substrate breakdown contribute to the generation of green energy. The employing of mixed culture rather than single culture is because of its COD removing ability. Based on previous research, although pure culture effective, it is probable that mixed culture would be more effective. Mixed culture can adapt better to changing condition during growth. The anaerobic digestion is conducted in anaerobic digester which provides suitable operating condition for the process in order to promote the degradation. This leads to successful COD removal and the production of biogas.

1.3 Objective of Study

The main objective of this research is to reduce the chemical oxygen demand (COD) level of synthetic waste water using a biological method employing bacteria which was isolated from sewage wastewater.

1.4 Scope of Study

The scopes of this research are:

(a) To study the efficiency of digestion process by the percentage of chemical oxygen demand removal.

(b) Synthetic wastewater used in this study is glucose solution and the system is a mixed culture or consortium consisted of different bacteria.

(c) To reduce the turbidity through the anaerobic digestion.

(d) To observe the biogas production from the anaerobic digestion.

(e) To design the anaerobic digester rig.

CHAPTER 2

LITERATURE REVIEW

2.1 COD Removal

The organic matters in wastewaters have traditionally been characterizes by several parameters like chemical oxygen demand (COD) and biological oxygen demand (BOD). COD is becoming more common and more important to fractionate especially with respect to biological nutrient removal. The COD test allows measurement of waste in terms of the total quantity of oxygen required for oxidation to carbon dioxide and water. The level of COD is a critically important factor in evaluating the extent of organic pollution in waste water.

COD is expressed in milligrams per liter (mg/L), which indicates the mass of oxygen consumed per liter of solution. Older references may express the units as parts per million (ppm) (Sawyer *et al*., 2003). Therefore, COD measures the potential overall oxygen requirements of the waste water sample including oxidizable components not determined in the BOD analysis (Timothy *et al*., 1988).

Chemical oxygen demand test is the most appropriate test for assessing organic wastewater strength from a theoretical and practical point of view. It measure the electron donating capacity (EDC) which is directly related to the free energy changes in the oxidation of organics, does not require sophisticated analytical equipment, as such, is appropriate for practice in the field, gives result in a short time, allows electron balances to be made over the biological system, does not oxidize ammonia so it give only the electron donating capacity of organic compounds and also include the non-biodegradable organics (Mara and Horan, 2003).

2.2 COD and BOD

Bacteria require oxygen to breathe and oxidize organic substances; the total amount of which is called oxygen demand (Inoue, 2002). The biochemical oxygen demand (BOD) test measures oxygen uptake during the oxidation of organic matter. It has wide applications in measuring waste loading of treatment plants and in evaluating the efficiency of treatment processes. It is of limited use in industrial waste water containing heavy metal ions, cyanide, and other substances toxic to microorganisms (Nor, 2006).

Although COD is comparable to BOD, it actually measures chemically oxidizable matter. Generally COD is preferred to BOD in process control applications because results are more reproducible and are available in a few minutes or hours rather than five days. In many industrial samples, COD testing may be the only feasible course because of the presence of bacterial inhibitors or chemicals that will interfere with BOD determination (Sawyer *et al.*, 2003). COD measures the total concentration of organics matter, while BOD indicates the total amount of biodegradable organics matter. The various forms of COD in wastewater are illustrated as in Figure 2.1.

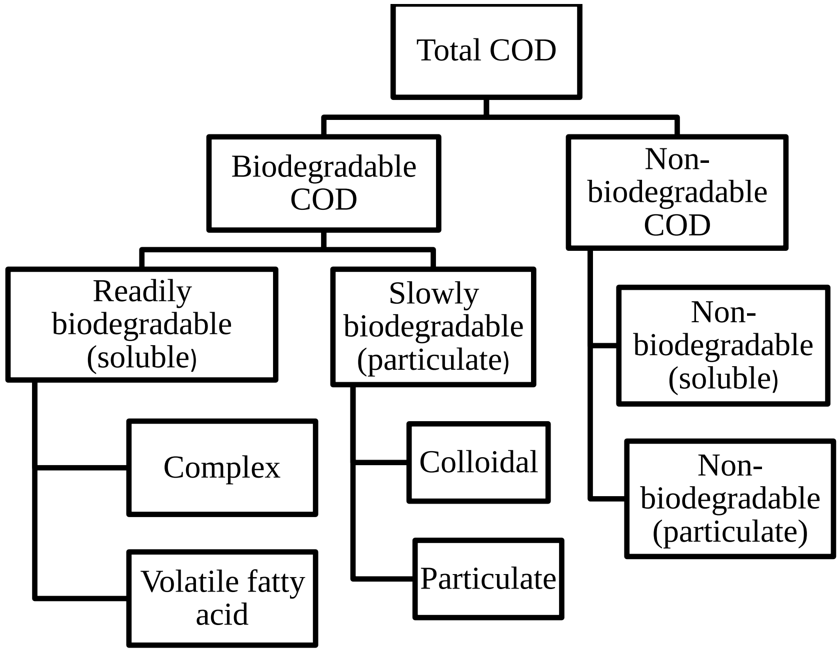

Figure 2.1: Fractionation of COD in wastewater (Metcalf and Eddy, 2003)

2.3 Methods of COD removal

The aims of wastewater treatment are to convert the waste materials present in wastewaters into stable oxidized end products which can be safely discharge to inland or coastal waters without any adverse ecological effects.

Wastewater treatment is essentially a mixture of settlement and biological or chemical unit processes. Unit treatment processes can be classified into five stages (Gray, 2004):

a) Preliminary treatment: the removal and disintegration of gross solid, the removal of grit and the separation of storm water. Oil and grease are also removed at this stage if present in large amount.

b) Primary treatment (sedimentation): the first major stage of treatment following preliminary treatment, which usually involves the removal of settle able solids which are separated as sludge.
c) Secondary (biological) treatment: the dissolve and colloidal organics are oxidized in the present of microorganisms.
d) Tertiary treatment: further treatment of a biologically treated effluent to remove BOD, COD, bacteria, suspended solid, specific toxic compounds or nutrients.
e) Sludge treatment: the dewatering, stabilization, and disposal of sludge

Many approaches have been reported for COD removal of waste water ranging from chemical, physical, biological method and combined method.

Treatment of the waste water via biological methods can be achieved by three approaches, involving fungi, algae, and bacteria. Although the biological treatment of waste water is important, it cannot completely remove soluble components, such as endocrine disrupters and pesticides (Ilyin *et al*., 2004). The most common treatment through the biological method was by employing the bacteria. The bacteria remove the dissolved organic load from the water by degrade it to simpler components. Biological treatment encompasses basically aerobic and anaerobic treatment. The organic matters will be degrades by microorganism during the treatment process.

2.3.1 Aerobic Digestion

Aerobic digestion is a bacterial process occurring in the presence of oxygen. Under aerobic conditions, bacteria rapidly consume organic matter and convert it into carbon dioxide. The operating costs used to be characteristically much greater for aerobic digestion because of the energy used by the blowers, pumps and motors needed to add oxygen to the process (Hammer and Hammer Jr., 2005).

Figure 2.2 below summarize the biochemical transformation occurring in anaerobic digester. Biodegradable particulate organic matter is hydrolyzed and converted into biodegradable soluble organic matter, releasing nutrients such as ammonia and phosphate. The biodegradable soluble organics matter is then converted into carbon dioxide, water and active biomass through the action of heterotrophic bacteria. The active biomass, in turn, undergoes decay, resulting in the generation of additional carbon dioxide and water, along with inactive biomass. Non-biodegradable particulate organic matter in the effluent is not affected by the digestion process and become a portion of the digested solids (Leslie *et al*., 1999).

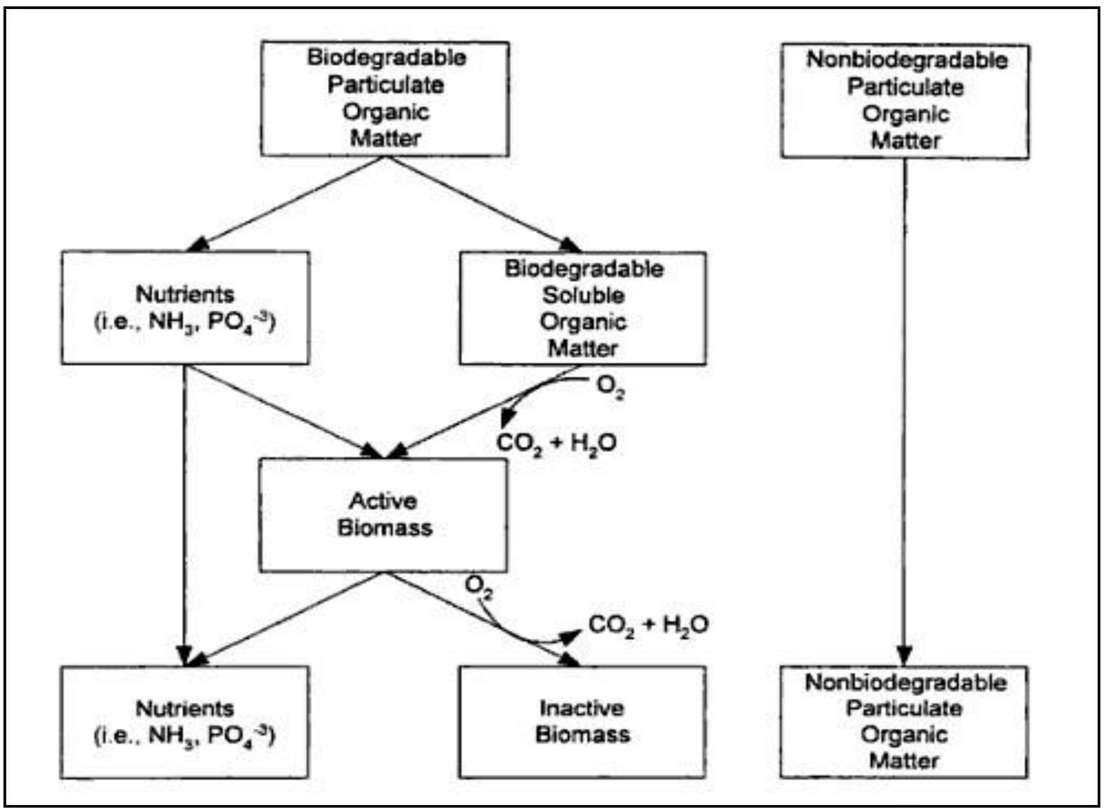

Figure 2.2: Schematic diagram of the events occurring during the aerobic digestion (Leslie *et al*., 1999).

In an aerobic operation, oxygen is essential for success operation of the systems. The most common aerobic processes are activated sludge and lagoon, active or trickling filters, and rotating contactors.

2.3.2 Anaerobic Digestion

Anaerobic digestion occurs as the result of a complicated set of chemical and biochemical reactions. Reaction occurs within the context of a complex ecosystem involving many types of bacteria, with each type providing a unique and indispensable biotransformation (Vesilind, 2003). Anaerobic digestion processes can employed for resource conservation, production of biogas and other useful end product from biomass and for environmental protection through waste and wastewater treatment (Johansson and Burnham, 1992). Organics pollutants from wastewater can be effectively removed through the modern high-rate anaerobic wastewater treatment. Its cost also low compared to the aerobic treatment because this method does not depend on the supply of electricity or other energy source. Meanwhile, the end product of the process is biogas, which is a useful energy carrier.

In the digestion process, consortiums of anaerobic bacteria participate in the decomposition of complex substrate. In the degradation of complex waste, the conversion of each group of organic compounds (protein, carbohydrate and lipid) require its own characteristic group of organism (Johansson and Burnham, 1992). The following main process can be distinguished.

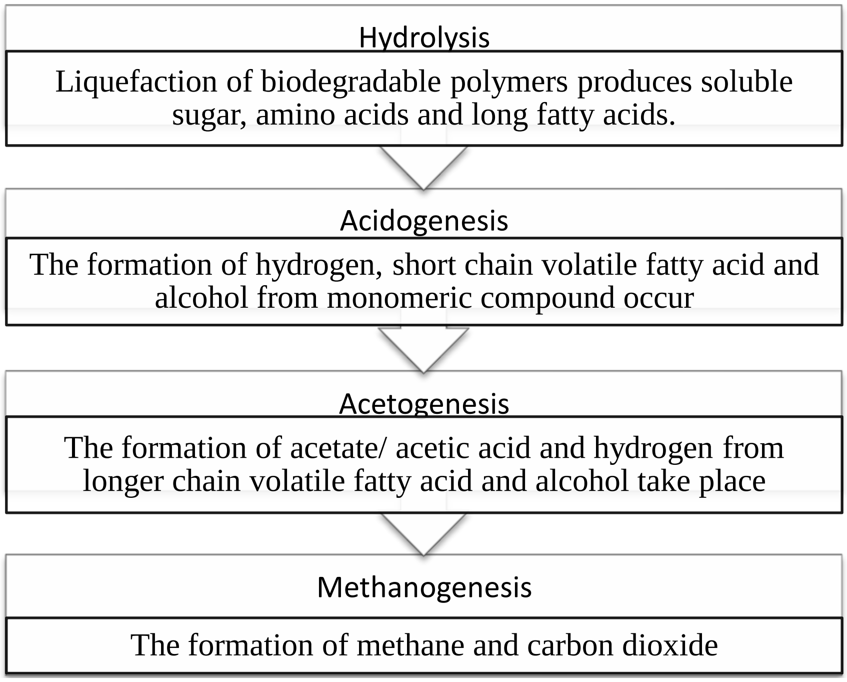

Figure 2.3: Main process in anaerobic digestion

Substantial removal in biodegradable compounds occurs only during the methanogenic step. This last stage only possible when the original organic compound have been converted effectively into the methanogenic substrates in the preceding steps. In step prior to methanogenesis, a decrease in chemical oxygen demand (COD), which is the measure of chemically oxidizable organic material in waste, occur only if hydrogen, H_2 is produced and release from the liquid phase where hydrogen has a stoichiometric chemical oxygen demand of 16 grams COD per gram of H_2 (Johansson and Burnham, 1992).

Anaerobic digestion has several advantages over aerobic processes (Bitton, 2005). Anaerobic digestion uses readily available CO_2 as an electron acceptor. It requires no oxygen, the supply of which adds substantially to the cost of wastewater treatment. Anaerobic digestion produces lower amounts of stabilized sludge since the energy yields of anaerobic microorganisms are relatively low. Most of the energy derived from substrate breakdown is found in the final product, CH_4. Anaerobic digestion produces a useful gas, methane. Anaerobic digestion is suitable for high-strength industrial waste.

There is preservation of the activity of anaerobic microorganism, even if the digester has not been fed for long period of time.

Anaerobic digestion is affected by temperature, pH, retention time, chemical composition of wastewater, competition of methanogens with sulfate-reducing bacteria, and the presence of toxicants.

2.4 Bacteria

2.4.1 Wastewater bacteria

Bacteria are single-cell plants. Bacteria metabolize the organics in wastewaters with the production of new microbial cell mass. The bacteria that can metabolize the maximum amount of the different organics predominate. While most bacteria in wastewater treatment systems utilize organics for their metabolism, there is an important group of bacteria that utilize inorganic compounds for their metabolism. As a net result, the two groups of bacteria in wastewater do not compete with each other for their nutrients and both grow in the same environment. Normal municipal wastewaters contain between 105 and 107 bacteria/ml (Runion, 2009).

Most of the bacteria that absorb the organic material in a wastewater treatment system are facultative in nature. This means they are adaptable to survive and multiply in either anaerobic or aerobic conditions. The nature of individual bacteria is dependent upon the environment in which they live. Usually, facultative bacteria will be anaerobic unless there is some type of mechanical or biochemical process used to add oxygen to the wastewater. When bacteria are in the process of being transferred from one environment to the other, the metamorphosis from anaerobic to aerobic state takes place within a couple of hours (Pearl, 2007).

Anaerobic bacteria are common in sewage and other wastewaters. They originate from the human intestine, although others sources include land drainage, storm water and biological process in industry. In sewage, the faecal bacteria outnumbered the other microorganism present, although no really satisfactory method of counting anaerobic bacteria have been developed. But as coliforms count are in excess of 10^6 - 10^7 ml^{-1} in sewage, and coliforms only represent a small portion of total anaerobic bacteria, the total count must be high (Gray, 2004).

2.4.2 Methane-Forming Bacteria

The anaerobic digestion is a complex biochemical process in which several group of anaerobic and facultative organism simultaneously absorb and break down organics matter. The anaerobic process is essentially controlled by methane-producing bacteria. Methane-forming bacteria or methanogens are microorganisms that produce methane as a metabolic byproduct in anoxic conditions. . It is specialized as group of Archaea that utilize a limited number of substrates, mostly acetate, carbon dioxide, and hydrogen for methane production or methanogenesis (Gerardi, 2006). Methanogens are oxygen-sensitive anaerobes and found in habitats that are rich in degradable organic compounds. Through the bacteria degradation, oxygen is rapidly removed in that habitat.

Bacteria grow at a relatively low rate and have generation times which range from slightly less than 2 days to about 22 days. Methane formers are very sensitive to pH, substrate composition and temperature. If the pH drops below, methane formation stops, and there is no decrease in organic content of sludge. The methane bacteria are highly active in the mesophilic and thermophilic ranges. The mesophilic ranges is 26°C - 43°C and thermophilic ranges is 45°C -65°C. Essentially, most of the digester operates within mesophilic range (Cheremisinoff, 1996).

Methanogenesis take place through three basic biochemical reactions that are mediates by three different groups of methane-forming bacteria which are acetoclastic methanogens, hydrotrophic methanogens, and methyltropic methanogens (Gerardi, 2006).

2.5 Biogas production

Biogas is the waste product of the bacteria feeding off the input biodegradable feedstock. Biogas produced in anaerobic digesters consists of 50%–80% methane, 20%–50% carbon dioxide, and trace levels of other gases such as hydrogen, carbon monoxide, nitrogen, oxygen, and hydrogen sulfide (Venkata *et al*.,2008). Most of the biogas is produced during the middle of the digestion, after the bacterial population has grown.

The quantity and quality of biogas produced during anaerobic digestion depends on the feed characteristics. Several methods are available to estimate methane generation from waste stream during anaerobic digestion. Methane production can be estimated from the chemical oxygen demand (COD) or ultimate biochemical oxygen demand (BOD) stabilization based on the fact that 1 kg COD destroyed produced $0.35m^3$ CH_4 at standard temperature and pressure (Kumar, 2008).

Gas production, especially methane increase with increasing organic loading to the digester until methane-forming bacteria are no longer capable of degrading volatile acids. The volume, rate and composition of the biogas produced are indicative of digester performance. A decrease in volume of biogas, rate of biogas production, or percent of methane composition is an early indicator of digester failure.

Treatability of waste or substrate by anaerobic digesters usually determined by monitoring the biogas production. The rate and volume of methane produced during anaerobic digestion of a waste can be used to determine its relative rate of conversion. The more rapid and larger quantity of biogas produced, the more easily the waste is treated.

When volatile acid production occurs more rapidly than volatile acid consumption, that is methane production, an upset condition occurs in an anaerobic digester. The digester becomes acidic. Because methane-forming bacteria are very sensitive to acidic condition, methane production decrease as volatile acid concentration increases. Methane production usually terminates with the digester pH drops below 6.0 (Gerardi, 2003).

CHAPTER 3

MATERIAL AND METHODS

3.1 Materials

All the glassware and other apparatus used in this study were sterilized by autoclaving at 121°C, 103 kPa for 15 minutes.

The synthetic wastewater used in this study is the glucose solution that was prepared with four different concentration; 1 g/L, 2 g/L, 3g/l, and 4 g/L.

The bacteria used in this study were mixed culture which was taken from the sewage treatment plant. The liquid sample of sewage was stored in chiller at 5°C.

Nutrient Broth was prepared by dissolving 8 g of Nutrient Broth (Merck®) in distilled deionized water (DDW) (1 L) and sterilized by autoclaving at 121°C, 103 kPa for 15 minutes. The sterilized culture medium was then kept at 5°C.

Nutrient agar (NA) plate was prepared by dissolving 20 g of NA powder (Merck®) in distilled deionized water (1 L) and sterilized by autoclaving at 121°C, 103 kPa for 15 minutes. The agar was then cooled down to about 50°C before being poured in sterile Petri dish.

3.2 Anaerobic Digester Preparation

Anaerobic digester is setup using 2 L Scott bottle which is equipped with stopper and rubber tube. The rubber tubes are used for sampling and migration of gas that produced during the process. Water displacement unit was constructed next to the digester and is connected through the tube for observation of gas generation. The schematic diagram of the anaerobic digester is as Figure 3.1.

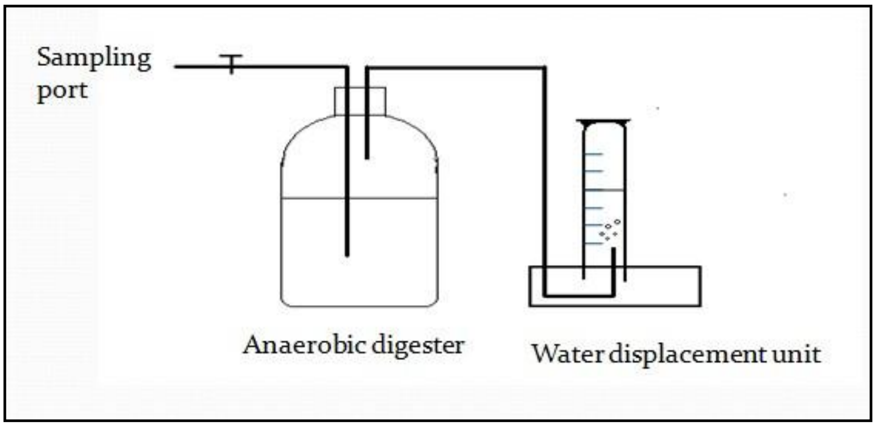

Figure 3.1: Schematic diagram of anaerobic digester.

3.3 Bacteria culture preparation

Source of bacteria culture was taken from the sewage treatment plant. 10 ml of sewage that contain consortium of bacteria in it was added into 100 ml nutrient broth. The mixture of the sewage and nutrient broth were incubated in incubator shaker for 24 hours at 30°C and 200rpm. After been incubate, that bacteria culture was diluted. Dilution was made starting from 10^{-1} until 10^{-7} dilution factor.

The bacteria culture was transferred onto agar plate for growth. The technique which been used was spread plate technique. With the pipette, 1 ml of each dilute solution was transferred to the middle of the agar which been labeled with aseptic technique. The suspended solution was spread properly at the surface of the agar using the sterile gas spreader. The bacteria culture which had been spread on the agar plate was incubate for 48 hours at 30°C.

After 48 hours incubation, the bacteria growth for each dilution factor was examined. The most suitable bacteria colony was selected and transferred into 150 ml glucose solution. The bacteria culture was acclimatized with the glucose solution for 24 hours at 30°C and 200 rpm.

3.4 COD reduction using mixed culture.

In this experiment, the factor which been study was the effect of glucose concentration on the COD removal. The mixed culture were used to reduce the COD of four different concentration of glucose, which were 1 g/L, 2 g/L, 3 g/L and 4 g/L.

The four different concentration of glucose was prepared in anaerobic digester which been setup earlier. 1500mL glucose solution for each concentration was prepared in each digester. Then, the acclimatized mixed culture was transferred into each digester with different concentration of glucose. The sample was analyzed to get the initial COD value.

The anaerobic digestion process for COD reduction was conducted for five days of treatment time. The COD analysis was carried out everyday for five days.

3.5 Turbidity reduction using mixed culture

In this experiment, the turbidity of the sample also had been analyzed during the 5 days of treatment. The initial turbidity value was determined at the beginning of process. Then, the turbidity value was determined for each day interval of time.

3.6 Observation of the biogas production

In this experiment, the biogas production was also been observed. The biogas that produced was collected from the anaerobic digester through the rubber tube into the water displacement unit. The production of biogas was observed through the air bubble in the water displacement unit.

3.7 Chemical Oxygen Demand analysis

Sample from the anaerobic digester was taken by sucking into syringe through the rubber tube at the digester. The sample was then diluted to 10^{-1} dilution factor. 2 ml of diluted sample was mixed with COD reagent vials (high range) and been held at 150°C for 2 hours in the COD reactor. After 2 hours, it was cooled and analyzed with the spectrophotometer.

3.8 Turbidity analysis

10mL of collected sample from the anaerobic digester was put in the turbidity tube. The sample was analyzed with the turbidity meter.