Palm Oil Mill Effluent (POME) Treatment "Microbial Communities in an Anaerobic Digester": A Review.

Jeremiah David Bala, Japareng Lalung and Norli Ismail

Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia (USM), 11800, Pulau, Penang, Malaysia

Abstract- Industrialization is vital to a nation's socio – economic development. It provides ready employment opportunities for a good percentage of the population. Although industrialization is inevitable, various devastating ecological and human disasters which have continuously occurred, implicate industries such as palm oil industry as major contributors to pollution problems and environmental degradation of various magnitude. As a result environmental problems have increased in geometric proportion over the last three decades with improper practices being largely responsible for the gross pollution of the aquatic environment with concomitant increase in waterborne diseases. Pollution of the environment with palm oil mill effluent (POME) is generated during palm oil processing which is carried out in mills where oil is extracted from the palm fruits. Large quantities of water are used during extraction of crude palm oil from the fresh fruits and about 50% of the water results in palm oil mill effluent. Palm oil mill effluent (POME) is an important source of inland water pollution when released into local rivers or lakes without treatment because it is a highly polluted wastewater that pollutes the environment if discharged directly due to its high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) concentration. Anaerobic digestion treatment of palm oil mill effluent has been considered to have a number of advantages over the conventional aerobic process. It saves the energy needed for aeration, converts organic pollutants into methane gas, a readily useable fuel, needs low nutrient requirement and produces low biomass. This technology in recent years has been applied for the treatment of many high-strength industrial wastewaters. This review discusses the various ongoing anaerobic digestion treatment of POME including their advantages and disadvantages, other related treatment technologies currently practice in palm oil mill industries, the potential of using the molecular biology techniques to provide detailed profile of the microbial community structure and establish the phylogenetics of microorganisms in bioreactors used for POME treatment and given insight into the microbial communities of wastewaters using the modern molecular biology techniques including their merits and demerits with emphasis on biological wastewater treatment processes that exploit an environment devoid of oxygen, inhibition of methanogenesis including anaerobic process and the potential uses and utilization of POME.

Index Terms- Anaerobic digestion, Effluent, Microbial communities, Molecular biology techniques, POME, Treatment, Wastewater.

I. INTRODUCTION

Oil palm (*Elaeis guineensis*) is one of the most versatile crops in the tropical world. The production of palm oil, however, results in the generation of large quantities of polluted wastewater commonly referred to as palm oil mill effluent (POME) (Najafpour *et al*., 2006). Typically, 1 t of crude palm oil production requires 5–7.5 t of water; over 50% of which ends up as POME. This wastewater is a viscous, brownish liquid containing about 95–96% water, 0.6–0.7% oil and 4–5% total solids (including 2–4% SS, mainly debris from the fruit). It is acidic (pH 4–5), hot (80–90 ^{0}C), nontoxic (no chemicals are added during oil extraction), has high organic content (COD 50,000 mg/L, BOD 25,000 mg/L) and contains appreciable amounts of plant nutrients (Singh *et al*., 1999 ; Borja *et al*.,1996). Palm oil mill effluent (POME) is an important source of inland water pollution when released into local rivers or lakes without treatment. POME contains lignocellulolic wastes with a mixture of carbohydrates and oil. Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) of POME are very high and COD values greater than 80,000 mg/L are frequently reported. Incomplete extraction of palm oil from the palm nut can increase COD values substantially (Oswal *et al*., 2002). POME has generally been treated by anaerobic digestion resulting in methane as a value added product (Sinnappa, 1978a; Borja *et al*., 1995; Zinatizadeh *et al*., 2006; Busu *et al*.,2010; Baharuddin *et al*., 2010; Chotwattanasak and Puetpaiboon, 2011).

Anaerobic treatment is the most suitable method for the treatment of effluents containing high concentration of organic carbon(Perez *et al*.,2001). Considering the high organic character of POME, anaerobic process is the most suitable approach for its treatment. Interest in anaerobic hybrid technology (combination of different anaerobic systems into a single bioreactor) has grown as it couples the recovery of usable energy with good process efficiency and stability (Zinatizadeh *et al.*, 2006). The up-flow anaerobic sludge fixed film (UASFF) bioreactor as an anaerobic hybrid reactor, is a combination of an up-flow anaerobic sludge blanket (UASB) reactor and an immobilized cell or fixed film (FF) reactor (Metcalf and Eddy,2003). The fixed film (FF) reactor or immobilized cell whose portion is positioned above the UASB section prevents sludge washout and helps in retaining a high biomass concentration in the reactor. Several researchers have successfully used the UASFF reactor to treat various kind of wastewaters such as starch, swine, slaughterhouse (Shaji, 2000; Suraruk *et al.*, 1998; Borja *et al.*, 1998).

Anaerobic treatment of wastewater has been considered to have a number of advantages over the conventional aerobic process. It saves the energy needed for aeration, converts organic

pollutants into methane gas, a readily useable fuel, needs low nutrient requirement and produces low biomass. The technology in recent years has been applied to the treatment of many high-strength industrial wastewaters (Herbert and Chan, 1997; Faisal and Unno, 2001).

Anaerobic digestion has been employed by most palm oil mills as their primary treatment of POME (Tay, 1991). More than 85% of palm oil mills in Malaysia have adopted the ponding system for POME treatment (Ma *et al.*, 1993) while the rest opted for open digesting tank (Yacob *et al.*, 2005). These methods are regarded as conventional POME treatment method whereby long retention times and large treatment areas are required (Poh andChong,2009). High-rate anaerobic bioreactors have also been applied in laboratory-scaled POME treatment such as up-flow anaerobic sludge blanket (UASB) reactor (Borja and Banks, 1994a); up-flow anaerobic filtration (Borja and Banks, 1994b); fluidized bed reactor (Borja and Banks, 1995a,b) and up-flow anaerobic sludge fixed-film (UASFF) reactor (Najafpour *et al.*, 2006). Anaerobic contact digester (Ibrahim *et al.*, 1984) and continuous stirred tank reactor (CSTR) have also been studied for treatment of POME (Chin, 1981).

Other than anaerobic digestion, POME has also been treated using membrane technology (Ahmad *et al.*, 2006, 2007), aerobic activated sludge reactor (Vijayaraghavan *et al.*, 2007), and evaporation method (Ma *et al.*, 1997).

The environment is becoming more polluted due to the various wastes discharged from wide range of industrial applications. The economic growth in developing and developed countries has resulted in significant increase in production which in turn generates huge amount of undesirable wastes (Yuliwati *et al.*, 2012). Palm oil mill effluent (POME) is undoubtedly the largest waste generated from the oil extraction process (Yacob *et al.*, 2006).

According to Prasertsan and Prasertsan (1996), during processing in the palm oil mill more than 70% (by weight) of the processed fresh fruit bunch (FFB) was left over as oil palm waste. According to Pleanjai *et al.* (2004), fiber, shell, decanter cake and empty fruit bunch (EFB) accounts for 30, 6, 3 and 28.5% of the FFB respectively. According to Yacob *et al.* (2006), 381 palm oil mills in Malaysia generated about 26.7 million tonnes of solid biomass and about 30 million tonnes of palm oil mill effluent (POME) in 2004. Discharging the effluents or by products on the lands may lead to pollution and might deteriote the surrounding environment. There is a need for a sound and efficient management system in the treatment of these by- products in a way that will help to conserve the environment and check the deterioration of air and river water quality (Rupani *et al.*, 2010). Treatment of POME is essential to avoid environmental pollution. Thus, there is an urgent need to find an efficient and practical approach to preserve the environment while maintaining the sustainability of the economy.

The present review discusses comprehensively the various ongoing aspects of anaerobic digestion methods for palm oil mill effluent (POME) treatment including their advantages and disadvantages, given insight into the microbial communities of wastewaters using the modern molecular biology techniques which include cloning of 16S rDNA, Denaturant gradient gel electrophoresis (DGGE) and Fluorescent in situ hybridization (FISH) which provides very precise taxonomical information, characteristic band patterns for different samples and make possible to identify microorganisms at any desired taxonomical level, depending on the specificity of the probe used respectively and other related treatment technologies currently practice in palm oil mill industries, the future promise and potential of using the molecular biology techniques to provide detailed profile of the taxonomical microbial community structure and establish the phylogenetics of microorganisms in bioreactors used for POME treatment with emphasis on biological wastewater treatment processes that exploit an environment devoid of oxygen, inhibition of methanogenesis including anaerobic process and the potential uses and utilization of POME.

II. PALM OIL MILL EFFLUENT (POME)

Palm oil is one of the two most important vegetable oils in the world's oil and fats market following Soya beans (Harley, 1988). Oil palm (*Elaeis guineensis*) is the most productive oil producing plant in the world, with one hectare of oil palm producing between 10 and 35 tonnes of fresh fruit bunch (FFB) per year (Harley, 1988; Ma *et al.*, 1996). The palm has a life of over 200 years, but the economic life is 20-25 years (nursery 11-15 months, first harvest is 32-38 months from planting and peak yield is 5-10 years from planting) (Igwe and Onyegbado, 2007). Usually, the harvested part is the fruit "fruit bunch"whereby oil is obtained from the fleshy mesocarp of the fruit. Oil extraction from flesh amounts to at least 45-46% while kernel accounts for at least 40-50%. The palm has a highly varied nutrient demand which depends mainly on the yield potential determined by the genetic make-up of the planting material and on yield limit set by climatic factors such as water, effective sunshine and temperature (Igwe and Onyegbado, 2007).

Crude palm oil contains fatty acid ester of glycerol commonly referred to as triglycerides, therefore, contributing to the worlds need of edible oil and fats. It is composed of approximately 50% saturated fats (primarily palmitic acid) and 40% unsaturated fats (principally linolenic and oleic acid); a unique composition if compared with other major fats (Usoro, 1974). The distinctive colour of the oil is due to the fat soluble carotenoids (pigment) which are also responsible for its vitamins E (tocopherols and tocotrienols) content (Igwe and Onyegbado, 2007). Despite the importance of the edible oil and fats extracted from the palm fruits, the POME contains residual oil which effect on the environment cannot be ignore. Treatment and disposal of oily wastewater, such as palm oil mill effluent is presently one of the serious environmental problems contributors. Palm oil mill wastes have existed for years but their effects on environment are at present more noticeable. The oily waste has to be removed to prevent interfaces in water treatment units, avoid problems in the biological treatment stages, and comply with water-discharge requirements (Ahmad *et al.*, 2005). Oily wastewater containing oil and grease are considered as hazardous pollutants particularly in the aquatic environments, because they are highly toxic to the aquatic organisms.

Characteristics of palm oil mill effluent depend on the quality of the raw material and palm oil production processes in palm oil mills. The extraction of crude palm oil from fresh fruit bunches (FFB) requires huge amounts of water (Rupani *et al.*,

2010). It has been estimated that 5- 7.5 tonnes of water is required for producing 1 tonne of crude palm oil and more than 50% of the water ends up as palm oil mill effluent (POME) (Ma,1999a, Ma.1999b, Ahmad *et al.*, 2003). Sethupathi (2004) has categorized three major processing operations responsible for producing the POME. Sterilization of FFB, clarification of the extracted crude palm oil (CPO), hydrocyclone separation of cracked mixture of kernel and shell hydrocyclone contributes about 36, 60 and 4% of POME respectively in the mills. Lorestani (2006) estimated that in Malaysia about 53 million m^3 POME is being produced every year based on palm oil production in 2005 (14.8 million tonnes). Yacob *et al.* (2005) estimated that about 0.5- 0.75 tonnes of POME will be discharged from mill for every tonne of fresh fruit bunch.

Wastewater composition depends mainly on the season, raw matter quality and the particular operations being conducted at any given time. Typically, palm oil mill wastewater is low in pH because of the organic acids produced in the fermentation process, ranging about 4-5. It also contains large amounts of total solids (40,500 mg/L), oil and grease (4000 mg/L) (Ma, 2000) Wastewater includes dissolved constituents such as high concentration of protein, carbohydrate, nitrogenous compounds, lipids and minerals, which may be converted into useful materials using microbial processes. The effluents from palm oil mill can cause considerable environmental problems, if discharged untreated (Singh *et al.*, 2010; Davis and Reilly.1980). Therefore, the challenge of converting POME into an environmental friendly waste requires an efficient treatment and effective disposal technique.

Table 1: Characteristics of Raw Palm Oil Mill Effluent (POME)

Parameters	Value
Temperature (°C)	80-90
pH	4.7
Biochemical Oxygen Demand BOD3; 3days at 30 °C	25,000
Chemical Oxygen Demand	50,000
Total Solids (T.S)	40,500
Total Suspended Solids (T.S.S)	18,000
Total Volatile Solids (T.V.S)	34,000
Oil and Grease (O&G)	4,000
Ammonia-Nitrate (NH_3-N)	35
Total Kjeldahl nitrogen (TKN)	750

*All values, except pH and temperature, are expressed in mg/L Source: Ma (2000).

Table 2: Effluent Discharge Standards for Crude Palm Oil Mills (Environmental Quality Act 1974, 2005)

Parameter	Unit	Parameter Units (second schedule)	Remarks
Biochemical Oxygen Demand BOD; 3days-30°C	mg/L	100	
Chemical Oxygen Demand (COD	mg/L	*	
Total Solids	mg/L	*	
Suspended Solids	mg/L	400	
Oil and Grease	mg/L	50	
Ammoniacal Nitrogen	mg/L	150	Value of filtered sample
Total Nitrogen	mg/L	200	Value of filtered sample
pH	-	5-9	
Temperature	°C	45	

* No discharge standard after 1984 Source: Pierzynski (2005).

III. CHARACTERISTICS OF PALM OIL MILL EFFLUENT (POME)

Huge quantities of waste are produced in the palm oil mill industry. The process of oil extraction results in generation of liquid waste commonly named as palm oil mill effluent (POME)(Rupani *et al.*, 2010). Palm oil mill effluent is generated mainly from oil extraction, washing and cleaning processes in the mill and these contains cellulosic material, fat, oil and grease etc (Agamuthu, 1995). Palm oil mill effluent also contains substantial quantities of solids, both suspended solids and total dissolved solids in the range of 18,000 mg/L and 40,500 mg/L respectively (Table 1). These solids are commonly named palm oil mill sludges (POMS). The solid waste that are produced in the process of extraction are the leaves, trunk, decanter cake, empty

fruit bunches, seed shells and fiber from the mesocarp (Rupani *et al.*, 2010).

Fresh POME is a hot, acidic (pH between 4 and 5), brownish colloidal suspension containing high concentrations of organic matter, high amounts of total solids (40,500 mg/L), oil and grease (4,000 mg/L) COD (50,000 mg/L) and BOD (25,000 mg/L) (Ma, 2000). The characteristics of typical POME is given in Table 1. According to Vairappan and Yen (2008), 66.8 million tonnes of POME was generated in year 2005. The raw or partially treated POME has an extremely high content of degradable organic matter. As no chemicals were added during the oil extraction process, POME is considered as non toxic, but it is identified as a major source of aquatic pollution by depleting dissolved oxygen when discharged untreated into the water bodies (Khalid and Wan Mustafa, 1992). However it also contains appreciable amounts of N, P, K, Mg and Ca (Habib *et al.*, 1997 and ,Muhrizal *et al.*, 2006), which are the vital nutrient elements for plant growth. Due to the non toxic nature and fertilizing properties, POME can be used as fertilizer or animal feed substitute, in terms of providing sufficient mineral requirements. Agamuthu *et al.* (1986) has also reported the increase of organic nitrogen leading to the production of a better fertilizer in POME.

Muhrizal, (2006) reported that POME contains high content of Al as compared to chicken manure and composted sawdust. According to Habib *et al.* (1997) toxic metals, such as Pb, can also be focused in POME, but their concentrations are usually below sub lethal levels (> 17.5 µg /g) (James *et al.*, 1996). According to James *et al.* (1996), Pb is found in POME as a result of contamination from plastic and metal pipes, tanks and containers where Pb is widely used in paints and glazing materials. The effluent discharge standards for crude palm oil mills (Environmental Quality Act 1974, 2005) are presented on Table 2.

IV. ANAEROBIC DIGESTION

Anaerobic digestion is the degradation of complex organic matters under the absence of oxygen. This process is time consuming as bacterial consortia responsible for the degradation process requires time to adapt to the new environment before they start to consume on organic matters to grow (Poh and Chong,2009).

In the process of degrading POME into methane, carbon dioxide and water, there is a sequence of reactions involved; hydrolysis, acidogenesis (including acetogenesis) and methanogenesis (Gerardi, 2003). Hydrolysis is where complex molecules (i.e., carbohydrates, lipids, proteins) are converted into sugar, amino acid and etc. In the step of acidogenesis, acidogenic bacteria will break down these sugar, fatty acids and amino acids into organic acids which mainly consist of acetic acid (from acetogenesis) together with hydrogen and carbon dioxide. Hydrogen and carbon dioxide will be utilized by hydrogenotropic methanogens while acetic acid and carbon dioxide will be utilized by acetoclastic methanogens to give methane as a final product(Gerardi,2003).

Methanogenesis is the rate limiting step in anaerobic digestion of POME (Ibrahim *et al.*, 1984). As such, conventional anaerobic digesters require large reactors and long retention time to ensure complete digestion of treated influent. Nonetheless, high-rate anaerobic bioreactors have been proposed (Borja and Banks,1994a,b, 1995a,b; Najafpour *et al.*, 2006; Ibrahim *et al.*, 1984) to reduce reactor volume, shorten retention time as well as capture methane gas for utilization.

Table 3: Advantages and Disadvantages between Anaerobic and Alternative Treatment Methods

Treatment Types	Advantages	Disadvantages	Reference
Anaerobic	Low energy requirements (no aeration), producing methane gas as a valuable end product, generated sludge from process could be used for land applications.	Long retention time, slow start-up (granulating reactors), large area required for conventional digesters.	Metcalf and Eddy (2003), Borja *et al.* (1996a).
Aerobic	Shorter retention time, more effective in handling toxic wastes.	High energy requirement (aeration), rate of pathogen inactivation is lower in aerobic sludge compared to anaerobic sludge, thus unsuitable for land applications.	Leslie Grady *et al.* (1999), Doble and Kumar (2005).
Membrane	Produce consistent and good water quality after treatment, smaller space required for membrane treatment plants, can disinfect treated water.	Short membrane life, membrane fouling, expensive compared to conventional treatment.	Ahmad *et al.* (2006), Metcalf and Eddy (2003.

Evaporation	Solid concentrate from process can be utilized as feed material for fertilizer manufacturing.	High energy consumption.	Ma *et al.* (1997).

Source**:** Poh and Chong (2009).

V. ANAEROBIC AND ALTERNATIVE POME TREATMENT METHODS.

Aerobic treatment, membrane treatment system and evaporation method are the currently available alternative methods for POME treatment (Poh and Chong,2009). The advantages and disadvantages for anaerobic and alternative treatment methods are shown in Table 3. In terms of energy requirement for POME treatment operation, anaerobic digestion has a stronger advantage over other alternative methods as it does not require energy for aeration. Furthermore, anaerobic POME treatment produces methane gas which is a value-added product of digestion that can be utilized in the mill to gain more revenue in terms of certified emission reduction (CER) (Poh and Chong, 2009;).For instance the open digesting tank for POME treatment without land application, capital cost quoted by Gopal and Ma (1986) for a palm oil mill processing 30 tons FFB/h is RM 750,000. Based on the Chemical Engineering Plant Cost Index in 2006, (Ullrich and Vasudevan 2004) the capital cost for this system is estimated to be RM 1,147,842 in 2006. Comparing this to the capital cost for a membrane system in POME treatment for a palm oil mill processing 36 tons FFB/h at RM 3,950,000 (Chong, 2007), it is obvious that the former anaerobic treatment has better advantage over other treatment methods in terms of capital cost. The only two significant drawbacks of anaerobic treatment are long retention times and long start-up period. However, the problem of long retention times can be rectified by using high-rate anaerobic bioreactors while the long start-up period can be shortened by using granulated seed sludge (McHugh *et al.*, 2003), utilizing seed sludge from same process (Yacob *et al.*, 2006b) or maintaining suitable

pH and temperature in the high-rate anaerobic bioreactor for growth of bacteria consortia (Liu *et al.*, 2002). Untreated wastewater with BOD/COD ratio of 0.5 and greater can be treated easily by biological means (Metcalf and Eddy, 2003). With reference to the published values of BOD and COD in Data for Engineers: POME (2004), aerobic and anaerobic treatment is suitable for POME treatment since the BOD/COD ratio is of 0.5. In comparison of these two treatment methods, the anaerobic treatment can be regarded to be more suitable for POME treatment due to its lower energy consumption while producing methane as a value-added product in the process (Poh and Chong, 2009).

VI. TYPES OF ANAEROBIC TREATMENT METHODS

A. Conventional treatment systems

Ponding system is the most common treatment system that is employed in palm oil mills for the treatment of POME with more than 85% of the mills having adopted this method (Poh and Chong, 2009). Ponding system comprises of de-oiling tank, acidification ponds, anaerobic ponds and facultative or aerobic ponds (Chan and Chooi, 1984). Number of ponds varies according to the capacity of the palm oil mill. Facultative or aerobic ponds are necessary to further reduce BOD concentration in order to produce effluent that complies with Federal Subsidiary Legislation, 1974 effluent discharge standards.

A typical size of an anaerobic pond in a palm oil mill which has a processing capacity of 54 tons per hour is 60.0 x 29.6 x 5.8 m (length x width x depth which is approximately equivalent to half the size of a soccer field. Size of pond depends on the capacity of the palm oil mill as well as the area available for ponds) (Yacob *et al.*, 2006a). Anaerobic ponds have the longest retention time in ponding system which is around 20–200 days (Chan and Chooi, 1984). Investigations by Yacob *et al.* (2006a) showed that anaerobic pond had a higher emission of methane with an average methane composition of 54.4% compared to open digester tank. In addition to that, the methane composition from anaerobic ponds was also found to be more consistent in the gaseous mixture. Methane emission in anaerobic ponds is influenced by mill activities and seasonal cropping of oil palm (Yacob *et al.*, 2006a). Open digesting tanks are used for POME treatment when limited land area is available for ponding system (Poh and Chong, 2009). Yacob *et al.* (2005) investigated on the methane emission from open digesting tanks where each tanks was half the capacity of anaerobic ponds (3600 m3) with retention time of 20 days. Emission of methane gas from open digesting tank was found to be less than anaerobic pond with an average methane composition of 36.0%. Lower methane composition is due to the transfer of oxygen into the tank when feed is induced into the tank. Mixing in digesting tanks improves the digestion process as bacteria consortia are brought into more contact with food (Leslie Grady *et al.*, 1999). Nevertheless, mixing in open digesting tank only depends on slow bubbling and eruption of biogas which causes low conversion of methane gas (Poh and Chong, 2009).

B. Anaerobic Filtration

Anaerobic filter has been applied to treat various types of wastewater including soybean processing wastewater (Yu *et al.*, 2002a), wine vinases (Nebot *et al.*, 1995; Pérez *et al.*, 1998), landfill leachate (Wang and Banks, 2007), municipal wastewater (Bodkhe, 2008), brewery wastewater (Leal *et al.*, 1998), slaughterhouse wastewater (Ruiz *et al.*, 1997), drug wastewater (Gangagni Rao *et al.*, 2005), distillery wastewater (Acharya *et al.*, 2008), beet sugar water (Farhadian *et al.*, 2007) and wastewater from ice-cream manufacture (Hawkes *et al.*, 1995; Monroy *et al.*, 1994). Borja and Banks (1994b, 1995b) have also utilized anaerobic filter for POME treatment. The packing allows biomass to attach on the surface when raw POME feed enters from the bottom of the bioreactor while treated effluent together with generated biogas will leave from the top of the bioreactor. Anaerobic filter is selected for wastewater treatment because (i) it requires a smaller reactor volume which operates on a shorter hydraulic retention times (HRTs) (ii) high substrate removal efficiency (Borja and Banks, 1994b), (iii) the ability to maintain high concentration of biomass in contact with the wastewater

without affecting treatment efficiency (Reyes *et al.*, 1999; Wang and Banks, 2007), and (iv) tolerance to shock loadings (Reyes *et al.*, 1999; Van Der Merwe and Britz, 1993). Besides, construction and operation of anaerobic filter is less expensive and small amount of suspended solids in the effluent eliminates the need for solid separation or recycle(Russo *et al.*, 1985).

However, filter clogging is a major problem in the continuous operation of anaerobic filters (Bodkhe, 2008; Jawed and Tare, 2000; Parawira *et al.*, 2006). So far, clogging of anaerobic filter has only been reported in the treatment of POME at an organic loading rate (OLR) of 20 g COD/l/day (Borja and Banks, 1995b) and also in the treatment of slaughterhouse wastewater at 6 g COD/l/day. This is due to the fact that other studies were conducted at lower OLRs which had lower suspended solid content compared to POME. In general, anaerobic filter is capable of treating wastewaters to give good effluent quality with at least 70% of COD removal efficiencywith methane composition of more than 50% (Poh and Chong,2009).

Investigations have been done to improve the efficiency of anaerobic filtration in wastewater treatment. For instance, Yu *et al.* (2002a) found that operating at an optimal recycle ratio which varies depending on OLR will enhance COD removal. However, methane percentage will be compromised with increase in optimal recycle ratio. Higher retention of biomass in the filter will also lead to a better COD removal efficiency. In order to optimize the retention of biomass on the filter media surface and trapped suspended biomass within the interstitial void spaces, Show and Tay (1999) suggested the use of support media with high porosity or open-pored surfaces. It was also suggested that continuously fed system gives better stability and greater degradation efficiency in anaerobic filters (Nebot *et al.*, 1995).

C. Anaerobic Fluidized Bed Reactor

Fluidized bed reactor exhibits several advantages that make it useful for treatment of high-strength wastewaters. It has very large surface areas for biomass attachment (Borja *et al.*, 2001; Toldrá *et al.*, 1987), enabling high OLR and short HRTs during operation (Garcia-Calderon *et al.*, 1998; Sowmeyan and Swaminathan,2008). Furthermore, fluidized bed has minimal problems of channeling, plugging or gas hold-up (Borja *et al.*, 2001; Toldrá *et al.*,1987). Higher up-flow velocity of raw POME is maintained for fluidized bed reactor to enable expansion of the support material bed. Biomass will then attach and grow on the support material. In this way, biomass can be retained in the reactor (Poh and Chong, 2009). Investigations have been done on the application of fluidized bed to treat cutting-oil wastewater (Perez *et al.*, 2007); real textile wastewater (Sen and Demirer, 2003); wine and distillery wastewater (Garcia-Calderon *et al.*, 1998; Sowmeyan and Swaminathan, 2008); brewery wastewater (Alvarado-Lassman *et al.*, 2008); ice-cream wastewater (Borja and Banks, 1995a; Hawkes *et al.*, 1995); slaughterhouse wastewater (Toldrá *et al.*, 1987); pharmaceutical effluent (Saravanane *et al.*, 2001) and POME (Borja and Banks, 1995b).
Inverse flow anaerobic fluidized bed is capable of tolerating higher OLRs compared to up-flow configuration. Alvarado-Lassman *et al.* (2008) showed that inverse flow fluidized bed shows excellent stability when overload is applied. It was found that in general, anaerobic fluidized bed is able to operate at higher OLRs, implying that less reactor volume will be required to operate at lower OLRs (Poh and Chong ,2009).

The type of support material in the fluidized bed plays an important role to determine the efficiency of the entire treatment system (Garcia-Calderon *et al.*, 1998; Sowmeyan and Swaminathan,2008) for both inverse flow and up-flow systems. Studies using fluidized bed to treat ice-cream wastewater showed different COD removal efficiencies when different support materials were used. Hawkes *et al.* (1995) found that fluidized bed using granular activated carbon (GAC) gave about 60% COD removal while Borja and Banks (1995a) obtained 94.4% of COD removal using ovoid saponite. Thus suitable support material needs to be selected to obtain high COD removal efficiency in the system.

In POME treatment, fluidized bed was found to be a better treatment method compared to anaerobic filter due to its ability to tolerate higher OLRs and its better methane gas production. Shorter HRT (6 h) also proved to be an advantage of fluidized bed over anaerobic filter (1.5–4.5 days) in POME treatment (Poh and Chong, 2009).

D. Anaerobic Contact Digestion

Contact process involves a digester and a sedimentation tank where sludge from digester effluent is left to settle and the effluent is recycled back into the digester. This process has been implemented in POME (Ibrahim *et al.*, 1984); ice-cream wastewater,alcohol distillery wastewater (Vlissidis and Zouboulis, 1993) and fermented olive mill wastewater treatment (Hamdi and Garcia, 1991).Concentrated wastewaters are suitable to be treated by anaerobic contact digestion since relatively high quality effluent can be achieved (Leslie Grady *et al.*, 1999). In the study of fermented olive mill wastewater treatment, anaerobic contact was capable of reaching steady state more quickly compared to anaerobic filter; however, more oxygen transfer in the digester (due to mixing) causes this process to be less stable (Poh and Chong. 2009 ; Hamdi and Gracia,1991)). While scum formation was reported in POME treatment pilot plant (Ibrahim *et al.*,1984), instability was not reported in other treatment systems. Despite the problems that might be encountered in anaerobic contact, this system has been able to remove COD efficiently, achieving up to 80% removal efficiency (Vlissidis and Zouboulis,1993).

E. Continuous Stirred Tank Reactor (CSTR)

CSTR is equivalent to a closed-tank digester with mixer. The mechanical agitator provides more area of contact with the biomass thus improving gas production. In POME treatment, CSTR has been applied by a mill under Keck Seng (Malaysia) Berhad in Masai, Johor and it is apparently the only one which has been operating continuously since early 1980s (Tong and Jaafar, 2006). Other applications of CSTR on wastewater treatment include dilute dairy wastewater (Chen and Shyu, 1996); jam wastewater (Mohan and Sunny, 2008) and coke wastewater (Vázquez *et al.*, 2006) where coke wastewater was treated in aerobic conditions.

The CSTR in Keck Seng's palm oil mill has COD removal efficiency of approximately 83% and CSTR treating dairy wastewater has COD removal efficiency of 60%. In terms of methane composition in generated biogas, it was found to be

62.5% for POME treatment and 22.5–76.9% for dairy wastewater treatment (Poh Chong. 2009). Another study on POME treatment using CSTR has been investigated by Ugoji (1997) where results indicated that COD removal efficiency is between 93.6–97.7%. The difference of COD removal efficiency between the two published results by Keck Seng and Ugoji is due to the different operating conditions where the latter study was done in laboratory scale. In the plant scale POME treatment at Keck Seng's palm oil mill, the treated wastewater could not be assumed to be well mixed due to the large volume of feed which might affect the overall efficiency of the COD removal.

Ramasamy and Abbasi (2000) attempted to upgrade the performance of CSTR by incorporating a biofilm support system (BSS) within the existing reactor. Low-density nylon mesh were rolled into cylinders and inserted into the CSTR. This BSS functions as a support media for growth of biomass. From this study, it was found that efficiency of CSTRs can be improved without biomass recycling.The implementation of BSS into CSTR can be useful to increase COD removal efficiency as well as biogas production in POME treatment.

F. Up- Flow Anaerobic Sludge Blanket (UASB) Reactor

UASB was developed by Lettinga *et al.* (1980) whereby this system has been successful in treating a wide range of industrial effluents including those with inhibitory compounds. The underlying principle of the UASB operation is to have an anaerobic sludge which exhibits good settling properties (Lettinga, 1995). So far, UASB has been applied for the treatment of potato wastewater (Kalyuzhnyi *et al.*, 1998; Lettinga *et al.*, 1980; Parawira *et al.*,2006); domestic wastewater (Barbosa and Sant'Anna, 1989; Behling *et al.*, 1997); slaughterhouse wastewater (Sayed *et al.*, 1984); ice-cream wastewater (Hawkes *et al.*, 1995); POME (Borja and Banks, 1994c); pharmaceutical wastewater (Stronach *et al.*,1987); instant coffee wastewater (Dinsdale *et al.*, 1997); sugar-beet wastewater (Lettinga *et al.*, 1980). UASB has a relatively simple design where sludge from organic matter degradation and biomass settles in the reactor. Organic matter from wastewater that comes in contact with sludge will be digested by the biomass granules.

In general, UASB is successful in COD removal of more than 60% for most wastewater types except for ice-cream wastewater. Hawkes *et al.* (1995) suggested that the lower COD removal percentage from ice-cream wastewater was due to design faults in the reactor's three phase separator and high contents of milk fat that were hard to degrade.

POME treatment has been successful with UASB reactor, achieving COD removal efficiency up to 98.4% with the highest operating OLR of 10.63 kg COD/m^3day (Borja and Banks, 1994c). However, reactor operated under overload conditions with high volatile fatty acid content became unstable after 15 days. Due to high amount of POME discharge daily from milling process, it is necessary to operate treatment system at higher OLR. Borja *et al.* (1996a) implemented a two-stage UASB system for POME treatment with the objective of preventing inhibition of granule formation at higher OLRs without having to remove solids from POME prior to treatment.This method is desirable since suspended solids in POME have high potential for gas production while extra costs from sludge disposal can be avoided. Results from this study showed the feasibility of separating anaerobic digestion into two-stages (acidogensis and methanogenesis) using a pair of UASB reactors.The methanogenic reactor was found to adapt quickly with the feed from the acidogenic reactor and also tolerate higher OLRs. It was suggested that OLR of 30 kg COD/m^3day could ensure an overall of 90% COD reduction and efficient methane conversion.

UASB reactor is advantageous for its ability to treat wastewater with high suspended solid content (Fang and Chui, 1994; Kalyuzhnyi *et al.*, 1998) that may clog reactors with packing material and also provide higher methane production (Kalyuzhnyi *et al.*,1996; Stronach *et al.*, 1987). However, this reactor might face long start-up periods if seeded sludge is not granulated. A study by Goodwin *et al.* (1992) has proved that reactors seeded with granulated sludge achieved high performance levels within a shorter start-up period. It could also adapt quickly to gradual increase of OLR (Kalyuzhnyi *et al.*, 1996).

G. Up-Flow Anaerobic Sludge Fixed-Film (UASFF) Reactor

UASB and anaerobic filter has been integrated to form a hybrid bioreactor – UASFF. This hybrid reactor combines the advantages of both reactors while eliminating their respective drawbacks. As such, UASFF is superior in terms of biomass retention, reactor stability at shock loadings and operation at high OLRs while eliminating the problems of clogging and biomass washout in anaerobic filter and UASB (Poh and Chong, 2009). Ayati and Ganjidoust (2006) has proven that UASFF is more efficient compared to UASB and anaerobic filter in the treatment of wood fiber wastewater.Other investigations of wastewater treatments using UASFF includes sugar wastewater (Guiot and van den Berg, 1985); dairy wastewater (Córdoba *et al.*, 1995); slaughterhouse wastewater (Borja *et al.*, 1995c, 1998; Lo *et al.*, 1994); wash waters from purification of virgin olive oil (Borja *et al.*, 1996b); coffee wastewater (Bello-Mendoza and Castillo-Rivera, 1998); brewery wastewater (Yu and Gu, 1996) and POME (Najafpour *et al.*, 2006). This hybrid reactor is generally capable of tolerating OLRs higher than UASB and anaerobic filter. Clogging is not reported in studies on the performance of hybrid reactor. UASFF is also able to achieve COD removal efficiency of at least 70% and above except for wood fiber wastewater as wood fiber is harder to degrade. Methane production for UASFF is also at a satisfactory level. In the treatment of POME, Najafpour *et al.* (2006) found that internal packing and high ratio of effluent recycle are both vital to control the stability of the UASFF reactor. Internal packing effectively retained biomass in the column while effluent recycle produced internal dilution to eliminate effects of high OLR. The advantages and disadvantages of each of the anaerobic treatment methods aforementioned are showed in Table 4.

Table 4: Advantages and disadvantages of various types of anaerobic treatment methods

	Advantage	Disadvantages	References
Conventional anaerobic digestion (pond and digester)	Low capital cost. Low operating and maintenance cost. Able to tolerate big range of OLR (pond) thus can easily cope POME discharge during high crop season. Recovered sludge cake from pond can be sold as fertilizer.	Large volume for digestion. Long retention times. No facilities to capture biogas. Lower methane emission.	Chan and Chooi (1984).
Anaerobic filtration	Small reactor volume. Producing high quality effluent. Short hydraulic retention times. Able to tolerate shock loadings. Retains high biomass concentration in the packing.	Clogging at high OLRs. High media and support cost. Unsuitable for high suspended solid Wastewater.	Borja and Banks (1994b, 1995b)
Fluidized bed	Most compact of all high-rate processes. Very well mixed conditions in the reactor. Large surface area for biomass attachment. No channeling, plugging or gas hold-up. Faster start-up.	High power requirements for bed Fluidization. High cost of carrier media. Not suitable for high suspended solid wastewaters. Normally does not capture generated biogas.	Leslie Grady *et al.* (1999).
UASB	Useful for treatment of high suspended solid wastewater. Producing high quality effluent. No media required (less cost). High concentration of biomass retained in the reactor. High methane production.	Performance dependant on sludge settleability. Foaming and sludge floatation at high OLRs. Long start-up period if granulated seed sludge is not used. Granulation inhibition at high volatile fatty acid concentration.	Lettinga (1995), Kalyuzhnyi *et al.* (1998), Goodwin *et al.* (1992).
UASFF	Higher OLR achievable compared to operating UASB or anaerobic filtration alone. Problems of clogging eliminated. Higher biomass retention. More stable operation. Ability to tolerate shock loadings. Suitable for diluted wastewater.	Lower OLR when treating suspended solid wastewaters.	Ayati and Ganjidoust (2006).
CSTR	Provides more contact of wastewater with biomass through mixing. Increased gas production compared to conventional Method.	Less efficient gas production at high treatment volume. Less biomass retention.	

Anaerobic contact process	Reaches steady state quickly. Short hydraulic retention time. Produces relatively high effluent quality.	Less stable due to oxygen transfer in digesting tank. Settleability of biomass is critical to successful performance.	Hamdi and Garcia (1991).

Source**:** Poh and Chong (2009).

VII. FACTORS AFFECTING ANAEROBIC DIGESTER PREFORMANCE

The few major factors that greatly influence digester performances in POME treatment are pH, mixing, operating temperature, and organic loading rates into the digester.

A. pH

The microbial community in anaerobic digesters are sensitive to pH changes and methanogens are affected to a greater extend (Leslie *et al.*, 1999). An investigation by Beccari *et al.* (1996) confirmed that methanogenesis is strongly affected by pH. As such, methanogenic activity will decrease when pH in the digester deviates from the optimum value (Poh and Chong, 2009). Optimum pH for most microbial growth is between 6.8 and 7.2 while pH lower than 4 and higher than 9.5 are not tolerable (Gerardi, 2006).Several cases of reactor failure reported in studies of wastewater treatment are due to accumulation of high volatile fatty acid concentration, causing a drop in pH which inhibited methanogenesis (Parawira *et al.*, 2006; Patel and Madamwar, 2002). Thus, volatile fatty acid concentration is an important parameter to monitor to guarantee reactor performance (Buyukkamaci and Filibeli,2004). It was found that digester could tolerate acetic acid concentrations up to 4000 mg/l without inhibition of gas production (Stafford, 1982). To control the level of volatile fatty acid in the system, alkalinity has to be maintained by recirculation of treated effluent (Najafpour *et al.*, 2006; Borja *et al.*, 1996a) to the digester or addition of lime and bicarbonate salt (Gerardi, 2003).

B. Mixing

Mixing provides good contact between microbes and substrates, reduces resistance to mass transfer, minimizes buildup of inhibitory intermediates and stabilizes environmental conditions (Leslie Grady *et al.*, 1999). When mixing is inefficient, overall rate of process will be impaired by pockets of material at different stages of digestion whereby every stage has a different pH and temperature (Stafford, 1982). Mixing can be accomplished through mechanical mixing, biogas recirculation or through slurry recirculation (Karim *et al.*, 2005a).Investigations have been done to observe the effects of mixing to the performance of anaerobic digesters. It was found that mixing improved the performance of digesters treating waste with higher concentration (Karim *et al.*, 2005b) while slurry recirculation showed better results compared to impeller and biogas recirculation mixing mode (Karim *et al.*, 2005c). Mixing also improved gas production as compared to unmixed digesters (Karim *et al.*,2005b). Intermittent mixing is advantageous over vigorous mixing (Kaparaju *et al.*, 2008; Stafford, 1982), where this has been adopted widely in large-scale municipal and farm waste digesters (Stafford, 1982). Rapid mixing is not encouraged as methanogens can be less efficient in this mode of operation (Gerardi, 2003). However, Karim *et al.* (2005b) mentioned that mixing during start-up is not beneficial due to the fact that digester pH will be lowered, resulting in performance instability as well as leading to a prolonged start-up period. Mixing in palm oil mills which depend on biogas produced (Ma and Ong, 1985) are less efficient compared to mechanical mixing as digesters are not perfectly mixed. Further investigation on effects of mixing on POME should be undertaken to obtain a suitable mode of mixing for the best digester performance.

C. Temperature

POME is discharged at temperatures around 80–90 ^{0}C (Zinatizadeh *et al.*, 2006) which actually makes treatment at both mesophilic and thermophilic temperatures feasible especially in tropical countries like Malaysia. Yet, anaerobic POME treatments in Malaysia are conducted only in the mesophilic temperature range. Various studies have been conducted to investigate the feasibility of operating wastewater treatment systems in the thermophilic temperature range such as sugar, high-strength wastewater (Wiegant *et al.*, 1985; Wiegant and Lettinga, 1985) and POME (Cail and Barford,1985; Choorit and Wisarnwan, 2007). These studies have reported successful system operation in the thermophilic temperature range, with POME treatment having treatment rates more than four times faster than operation in the mesophilic temperature range (Cail and Barford, 1985). Similarly, high production of methane was also observed from the treatment of sugar wastewater in this higher temperature range .

Effect of temperature on the performance of anaerobic digestion was investigated. Yu *et al.* (2002b) found that substrate degradation rate and biogas production rate at 55 ^{0}C was higher than operation at 37 ^{0}C. Studies have reported that thermophilic digesters are able to tolerate higher OLRs and operate at shorter HRT while producing more biogas (Ahn and Forster, 2002; Kim *et al.*, 2006; Yilmaz *et al.*, 2008). However, failure to control temperature increase can result in biomass washout (Lau and Fang, 1997) with accumulation of volatile fatty acid due to inhibition of methanogenesis. At high temperatures, production of volatile fatty acid is higher compared to mesophilic temperature range (Yu *et al.*, 2002b). Many operators prefer to have digesters operating in mesophilic temperature due to better process stability. Nevertheless, investigation on digester stability by Kim *et al.* (2002) proved that disadvantages of thermophilic digesters can be resolved by keeping microbial consortia in close proximity.

A cost benefit analysis done on anaerobic POME treatment system with biogas recovery for heat generation and digester effluent for land application indicated that operation in the thermophilic range provide the fastest payback to investment(Poh and Chong, 2009). The cost benefit analysis for POME treatment system that utilizes biogas for electricity generation and digester effluent for land application also showed a faster payback (Yeoh, 2004).Yeoh (2004) also stated that if all POME in Malaysia is to be treated at thermophilic temperature where recovered biogas is fully utilized for electricity energy

generation, it would generate 2250 million kWh which contributes approximately 4% of national electricity demand in 1999. This shows the potential of operating POME treatment systems in thermophilic temperature.

D. Organic Loading Rates

Various studies have proven that higher OLRs will reduce COD removal efficiency in wastewater treatment systems (Torkian *et al.*, 2003; Sánchez *et al.*, 2005; Patel and Madamwar, 2002) However, gas production will increase with OLR until a stage when methanogens could not work quick enough to convert acetic acid to methane. OLR is related to substrate concentration and HRT, thus a good balance between these two parameters has to be obtained for good digester operation. Short HRT will reduce the time of contact between substrate and biomass (Poh and Chong, 2009).

VIII. OTHER RELATED TREATMENT TECHNOLOGIES FOR POME

A. Tank Digestion and Facultative Ponds

In this system, raw effluent after oil trapping is pumped to a closed tank which has a retention time of about twenty days. The liquid is mixed by means of horizontal stirrers.

The methane gas (CH4) generated is flared off into the atmosphere, but the flaring of the CH4 is unacceptable and calls for improvement on this method. (Igwe and Onyegbado,2007). Digested liquid is discharged into a holding pond before it is disposed on land (Songehe, 1974). Tony and Bakar Jaafar, (2004) ; Hassan *et al.*(2009) have also investigated POME treatment using closed anaerobic digestion tanks.

B. Tank Digestion and Mechanical Aeration

This group consists of cooling/acidification ponds, an anaerobic digestion tank and an aeration pond. Raw effluent after oil trapping is pumped to the acidification pond through a cooling tower and retained for one to two days. It is then mixed with an equal volume of liquid from the anaerobic digester before it is fed back to the digester and the achievement recorded indicates that the effluent water has been treated (Igwe and Onyegbado,2007). The hydraulic retention time of the digester is about twenty days. The digested liquid is discharged to an aeration pond with two floating aerators. The liquid is aerated for twenty days before it is discharged (Karel *et al.*, 1974). Yacob et al .(2009) and Poh and Chong, (2009) have also reported the use of open digestion tanks for POME treatment.

C. Decanter and facultative ponds

In a few mills, decanters are used to separate the fruits juice after pressing into liquid and solid phase, the liquid which is mainly oil is fed to the conventional clarification process. The water resulting from the clarification station is recycled (Igwe and Onyegbado,2007). The solid is either disposed off on land or is dried in a rotary drier to about 10% moisture and then used as fuel. Thus, the effluent which consists of only the sterilizer condensate and waste from the hydrocyclone is greatly reduced in volume and is treated in a series of ponds (Wood, 1984). Chan and Chooi, (1984) elucidated that ponding systems also comprises of facultative or aerobic ponds used in the treatment of POME. Chin *et al.* (1996) have treated POME using a pond system.

D. Anaerobic and facultative ponds

This system consists of a series of ponds connected in series for different purposes. The effluent after oil trapping is retained in an acidification buffering pond for about two or three days, the resulting effluent is then treated in an anaerobic pond with a hydraulic retention time of thirty to eighty days depending on the mills (Igwe and Onyegbado,2007).This digested liquid is further treated in a series of facultative ponds before it is discharged. In some cases, part of the digested liquid is recycled to the acidification and buffering pond. The total hydraulic retention time of the system ranges from 75 to 120 days (Donne, 1981). Technologies currently undergoing intensive research and development include fluidized bed reactor (Idris *et al.*, 2003), up-flow anaerobic sludge blanket (UASB) reactor (Borja *et al.*, 1996; Chaisri *et al.*, 2007), up-flow anaerobic sludge fixed-film (UASFF) reactor (Zinatizadeh *et al.*, 2006a,b, 2007a,b) and membrane technology (Ahmad *et al.*, 2006a,b, 2009; Wu *et al.*, 2007). Other treatment system consists of a combination of mechanical chemical process and ponds (Sinnappa, 1978b). The raw effluent after oil trapping is separated into water and solid phases using a three- phase decanter. The oil is returned to the main line while the solid is dried in a rotary drier after the filter press. The water containing dissolved and suspended solids is treated with coagulants and flocculants to remove as much solids as possible before it is fed to an anaerobic digester which has a hydraulic retention time of about ten days. The digested liquid is further treated in an aeration tower and then oxidized (Sinnappa, 1978b).

IX. POTENTIAL USES AND UTILIZATION OF POME

Due to the huge quantities of POME generated by the oil palm industry, it is not a good practice to discharge the wastewater into the environment without utilizing it properly. Recently, the infiltration of POME into the groundwater tables and aquifer systems, which constitutes an accumulative, threatening and detrimental deterioration to the survival of aquatic life forms, the ecology and the food chains, is interpreted as one of the most intransigent paradoxes around the world (Yusoff and Hansen, 2007). In view of the aforementioned, the sustainability of the conversion of POME into useful substitutes for animal feed, fertilizers and carotene have attracted a huge energetic focus, mainly attributed to its abundant accessibility and low price (Hii *et al.*, 2012).

A. POME AS FEED FOR ANIMAL AND AQUACULTURAL ORGANISMS

Due to the rich content of organic matter, POME was used as a dietary substitute for pigs, poultry and small ruminants as well as aquacultural organisms (Wu *et al.*, 2009; Devendra, 2004). Generally, POME itself cannot be applied as food for animals. It always serves as a replacement of a regular diet constituent. In pig and poultry (i.e. chicken) farming, POME has proved to be an economical replacement for maize (regular diet constituent) and soybean meal, showing the same good feeding results (Devendra, 2004; Hutagalung *et al.*, 1977; Ho, 1976; Yeong *et al.*,1980). The Malaysian Agricultural Research Development (MARDI) even proved that POME can be used as the supplementary food for sheep and goats (Devendra and Muthurajah, 1976). Further researches using grass supplemented with dried POME or treated with POME also showed better

forage intake and better food digestion than with grass alone (Vadiveloo, 1988; Agamuthu *et al.*, 1996; Phang and Vadiveloo, 1991).Meanwhile, POME has also played a role in serving as food for fish (Babu *et al.*, 2001) and aquacultural organisms, such as chironomid larvae, also known as ‘‘bloodworms’’ (Habib *et al.*,1997). The reports showed that production of the chironomid larvae was significantly higher in POME than in algal cultures (Hii *et al.*, 2012). This described POME as a good source of nutritional supplement for aquacultural organisms. These chironomid larvae, in turn, can present valuable live food for fish or cultured invertebrates (Shaw and Mark 1980; Yusoff *et al* ., 1996).

X. MOLECULAR BIOLOGY TECHNIQUES AND THEIR USES IN WASTEWATER TREATMENT

Identification of microorganisms by conventional methods requires the isolation of pure cultures followed by laborious characterization experiments. These procedures are therefore inadequate for study of the biodiversity of a natural or engineered ecosystem. A new set of molecular techniques developed during the 1990s revolutionized microbial ecology research. The possibility of identifying specific populations of microorganisms in their native habitat/niche or environment without the need to isolate them is revolutionizing microbial ecology and giving rise to various new applications in numerous research fields.

In wastewater treatment, microbial molecular ecology techniques have been applied mainly to the study of flocs (activated sludge) and biofilms that grow in aerobic treatment systems (trickling filters) (Sanz and Kochling, 2007).These techniques include: Denaturant Gradient Gel Electrophoresis (DGGE), Fluorescent in situ Hybridization (FISH) and Cloning of 16S rDNA.

A. CLONING of 16S rDNA

Cloning and sequencing of the gene that codes for 16S rRNA is still the most widely used in the field of microbial ecology. This methodology implies the extraction of nucleic acids, amplification and cloning of the 16S rRNA genes, followed by sequencing and finally identification and affiliation of the isolated clone with the aid of phylogenetic software (Sanz and Kochling, 2007).

Several examples of cloning of 16S rDNA illustrate its potential in the wastewater treatment area. Cloning was employed to establish with precision the phylogenetic position of filamentous bacteria in granular sludge that were previously affiliated, by in situ hybridization, to the division of green non-sulfur bacteria (Sekiguchi *et al.*, 2001); or to determine the prevalent sulfate reducing bacteria in a biofilm (Ito *et al.*, 2002). The microbial communities residing in reactors for treating several types of industrial wastewater have also been determined by means of 16S rDNA cloning and sequencing (Sanz and Kochling, 2007). Egli *et al.* (2003) examined the microbial composition and structure of a rotating biological contactor biofilm for the treatment of ammonium-contaminated wastewaters. In their 16S rDNA clone libraries, they found the sequences of several previously undetected and uncommon microorganisms, as well as others that were confirmed to be associated with the process by FISH analysis. The study also confirmed the predicted functional structure of a mixed aerobic/anaerobic biofilm developed in the presence of high ammonium concentrations (Sanz and Kochling, 2007). A description of the microbial communities responsible for the anaerobic digestion of manure and manure/lipid mixtures in continuously stirred tank reactors (CSTR) was published in 2003 by Mladenovska *et al.* (2003). Phylogenetic analysis of the sequences obtained showed a narrow range of diversity, with most of the screened microorganisms belonging to the Methanosarcina genus (Sanz and Kochling, 2007).

Zhang *et al.* (2005) investigated the cloning approach in systems dedicated to the degradation of organic compounds. Working with a methanogenic reactor adapted to phenol degradation, the researchers used cloning in conjunction with in situ hybridization analysis to give a detailed picture of the population, as well as to identify the species responsible for phenol transformation (Sanz and Kochling, 2007). Using the cloning of 16S rDNA technique, several researchers (Hata *et al.*, 2004; Ferrera *et al.*,2004; Chen *et al.*, 2004) have investigated the microbial community structure and established the phylogenetics of microorganisms in various bioreactors for wastewater treatment.

In general, cloning and rRNA gene library construction have been applied in combination with other techniques in wastewater treatment. Cloning of the whole gene yields far more exact phylogenetic information than other molecular techniques such as FISH and DGGE (Sanz and Kochling, 2007).

B. DENATURANT GRADIENT GEL ELECTROPHORESIS (DGGE)

Denaturant gradient gel electrophoresis is based on the differing mobility on a gel of denatured DNA-fragments of the same size but with different nucleic acid sequences, thus generating band patterns that directly reflect the genetic biodiversity of the sample. The number of bands corresponds to the number of dominant species. Coupled with sequencing and phylogenetic analysis of the bands, this method can give a good overview of the composition of a given microbial community (Sanz and Kochling, 2007).

DGGE method has been employed in the characterization of a wide array of habitats, such as soil, bacterioplankton, hot springs, continental waters, etc (Sanz and Kochling, 2007).The technique is less widely used in anaerobic wastewater treatment, though in recent years DGGE seems to be increasingly popular as it has been used for the evaluation of the granular sludge’s microbial diversity from UASB reactors treating brewery (Chan *et al.*,2001), alcohol distillery (Akarsubasi *et al.*, 2006), and unbleached pulp plant wastewaters (Buzzini *et al.*,2006).

The technique is not used alone but rather as part of a combined approach with other methods, for example with in situ hybridization in the study of sulfate reducing bacteria (Santegoeds *et al.*, 1998) or phosphorous elimination (Onda *et al.*, 2002). Both these are good examples of the advantages of combining fingerprinting with in situ hybridization. The authors managed to trace the most probable protagonist in the process by evaluating DGGE band intensity and then designing a specific probe with the help of the predominant band sequence, in turn enabling quantification of the candidate and confirmation of the results obtained by DGGE (Sanz and Kochling, 2007).

The most important application of DGGE is monitoring dynamic changes in microbial communities, especially when many samples have to be processed. There are multiple applications of DGGE related to anaerobic digestion processes. These include: studies on differences between mesophilic and thermophilic reactors, demonstrating the lower biodiversity in thermophilic reactors used for the treatment of residual waters generated by the pharmaceutical industry (Lapara *et al.*,2000); analysis of the changes observed in the bacterial diversity of an anaerobic digester for treating urban solid waste (Silvay *et al.*,2000); studies on the changes in bacterial communities in a continuous stirred tank reactor (CSTR) in response to dilution rate (Ueno et ai., 2001). Nakagawa *et al.* (2002) monitored changes in an ethylbenzene-degrading acterial consortium in enrichment cultures under anaerobic, sulfatereducing conditions. By monitoring the predominant bacterial species over a period of 127 days, they identified a dominant bacterium that was present throughout the whole incubation period and most likely to be the microorganism responsible for ethylbenzene degradation. Both spatial and temporal changes in microbial community profiles were monitored by Pereira *et al.* (2002), in a study of expanded granular sludge bed (EGSB) reactors for the treatment of oleic acid. With this approach, the researchers were able to add another dimension to the analysis and compare the change in microbial communities in different layers of the sludge bed, as well as changes over the time (Sanz and Kochling, 2007).

Recently, Xing *et al.* (2005) used DGGE fingerprinting to monitor changes in the microbial community of a hydrogen producing bioreactor during the different phases of the process. The authors detected shifts in the population during start-up followed by stabilization once the process was running, and also found that cometabolism and mutual relationships played an important role in the microbial community involved in biological H_2 production(Sanz and Kochling, 2007). In another study, Roest *et al.* (2005) monitored microbial populations in a UASB reactor for treating paper mill wastewater over 3 years. With a combination of different molecular techniques and even conventional microbiological methodology, the authors were able to accurately describe the biological component of the process.

Several researchers have described changes in the microbial community taking place in different reactors (Connaughton *et al.*(2006); Liu *et al.* (2002); Park and Lee (2005).

C. FLUORESCENT IN SITU HYBRIDIZATION (FISH)

One of the ways to overcome some of the problems of studying microbial populations of a microcosm without resorting to traditional methodology is to use fluorescent probes. These are short sequences of DNA (16–20 nucleotides) labeled with a fluorescent dye. These sequences recognize 16S rRNA sequences in fixed cells and hybridize with them in situ (DNA–RNA matching). Microorganisms can be identified, localized and quantified in almost every ecosystem with hybridization (Amann *et al.*, 1990). The specificity of the probe enables detection/identification on any desired taxonomic level, from Domain down to a resolution suitable for differentiating between individual species. Previous knowledge of the expected microorganisms in the sample is often required to apply this method successfully. To target a particular species, a specific probe must be ready or its 16S rRNA sequence must be available (Sanz and Kochling, 2007). The use of oligonucleotide probes targeting 16S rRNA presents a revolution in microbial ecology, both for basic research and practical applications. Within the area of wastewater treatment, hybridization techniques are by far the most extensively used ones.

The applications of FISH in the wastewater treatment field have been directed towards study of the microorganisms taking part in the biological elimination of nitrogen and, to a lesser extent, phosphorous. Previous studies have dealt with the composition of nitrifying populations in bioreactors (Kim *et al.*, 2001;,Mosquera *et al.*,2005;,Okabe and Watanabe,2000), the predominant role of the ammonia- oxidizing Nitrosococcus and the nitrite-oxidizing Nitrospira in the nitrification process (Daims *et al.*,2001), or practical guidelines for developing highly efficient nitrifying biofilms (Tsuneda *et al.*,2000) FISH successfully identified anammox bacteria in different reactor types and wastewaters (Egli *et al* .,2001).

Studies that further illustrate the application of FISH in anaerobic digestion have dealt with the interaction and distribution of trophic groups, such as sulfate reducing bacteria and methanogenic archaea in methanogenic/sulfidogenic reactors (Santegoeds etal.,1999) or differentiation between hydrogenotrophic and acetoclastic methanobacteria, and within this group between Methanosaeta and Methanosarcina (Gonzalez *et al.*,2001;Rocheleau *et al.*,1999).

Researchers have combine complementary techniques in their studies which is evident in the work of Diaz *et al.* (2006) who have studied the microbial composition and structure of different types of granule in a UASB reactor that treated wastewater from a brewery. The authors used FISH, DGGE, cloning, and electron microscopy to gain insight into the structure, function and physical appearance of methanogenic granules. The use of multiple techniques was necessary to elucidate the structure-function relationship of the different granules (Sanz and Kochling, 2007). Roest *et al.* (2005) studied in depth the microbial community of granules from a reactor treating paper mill wastewater with a similar approach.

In situ hybridization has been also used as a molecular tool to describe microbial communities in other anaerobic wastewater treatment systems besides UASB reactors. A few studies include: analysis of the microbial composition of the biomass inside an anaerobic baffled reactor (Plumb *et a*l.,2001); various studies of membrane reactor systems [Luxmy., 2000;Rosenberger *et al.*, 2000]; the identification and characterization of anammox microorganisms in different systems by Jetten *et al.* [Jetten *et al.*, 2005] and the observation of anaerobic biofilm development (Araujo *et al.*, 2000).The advantages and disadvantages of the three (3) types of molecular biology techniques are presented in Table 5.

Table 5: Advantages and Disadvantages of the three (3) types of Molecular biology techniques

	Advantages	Disadvantages	References
Cloning of 16S rDNA	Complete 16S rRNA sequencing allows: *very precise taxonomic studies and phylogenetic trees of high resolution to be obtained; *design of primers (for PCR) and probes (for FISH). If time and effort is not a limiting factor, the approach covers most microorganisms, including minority groups, which would be hard to detect with genetic fingerprinting methods. Identification of microorganisms that have not been yet cultured or identified.	Very time consuming and laborious, making it unpractical for high sample throughput. Extraction of a DNA pool representative of the microbial community can be difficult when working with certain sample types (e.g. soil, sediments). Many clones have to be sequenced to ensure most of individual species in the sample are covered. It is not quantitative. The PCR step can favor certain species due to differences in DNA target site accessibility. This technology may be too complex, need specialized personnel and equipment.	Sanz and Kochling, (2007).
Denaturant gradient gel electrophoresis (DGGE)	Permits rapid and simple monitoring of the spatial-temporal variability of microbial populations if just band patterns are considered. It is relatively easy to obtain an overview of the dominant species of an ecosystem. It is adequate for analysis of a large number of samples (far more than cloning).	Depending on the nature of the sample, extraction and amplification of representative genomic DNA can be difficult (as in cloning). After the PCR amplification, the DNA copy number – which depends on abundance of a particular microorganism and the ease of amplification of the 16S rRNA – can be very different (as in cloning). The intensity the bands obtained on a DGGE gel may therefore vary (not quantitative). The number of detected bands is usually small, which implies: *the number of identified species is also small; *the bands correspond, although not necessarily, to the predominant species in the original sample. The sequences of the bands obtained from a gel correspond to short DNA fragments (200–600 bp), and so phylogenetic relations	Sanz and Kochling, (2007).

		are less reliably established than with cloning of the whole 16S rRNA gene. In addition, short sequences are less useful for designing new specific primers and probes.	
Fluorescent in situ hybridization (FISH)	Easy and fast if required probes are available. Allows direct visualization of non-cultured microorganisms. Generally quantitative. Quantification of specific microbial groups is also possible, in contrast to conventional techniques (most probable number, plate counts) or other molecular techniques. Differential/preferential detection of active microorganisms. Apt for routine use, highly trained and specialized personnel is not necessary, only a basic knowledge of microscopy and laboratory experience are required.	A prior knowledge of the ecosystem under study and the microorganisms most likely to be detected is necessary (combined use with other techniques may be necessary). If a particular microorganism has to be detected and quantified, its rRNA sequence must be known (if the corresponding probe has not yet been published). The design of a specific and unambiguously restrictive probe for a certain group of microorganisms is not always possible, especially if metabolic criteria are applied (e.g. nitrifying bacteria, halo-respiring bacteria). The design and optimization of hybridization conditions for a new probe is a difficult process that requires experience and dedication, and the results may not always be satisfactory. Quantification can be tedious and subjective (manual counting) or complex (image analysis). Structural analysis of aggregates (granular sludge, biofilms) requires a confocal microscope and an image analysis environment (expensive, trained personnel necessary).	Sanz and Kochling, (2007).

XI. MICROBIAL COMMUNITIES

Molecular biology tools are providing insight into the microbial community dynamics and structure during anaerobic

processes. This information can be used to improve treatment processes. The majority of tools used involve DNA extraction, 16S rRNA gene sequencing with polymerase chain reaction (PCR), quantitative PCR, clone libraries, fluorescence in-situ hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE).

A. Process dynamics linked to microbial community structure

Two-stage anaerobic digesters consisting of one acidogenic reactor and one methanogenic reactor were set-up to treat food waste-recycling wastewater (Shin, Han, *et al.*, 2010).Process performance in the reactors was stable with COD removal efficiencies of 73.0-85.9% even with microbial community shifts in both reactors. Similar findings by Wang *et al.* (2010) were found for two full-scale wastewater systems where bacterial community structure changed significantly while functionality remained stable. The wastewater treatment systems were anaerobic/anoxic/aerobic and anoxic/aerobic with nitrified water recirculation. The stability was measured using effluent BOD, total nitrogen and ammonia concentrations.

Clostridium thermopalmarium and *Clostridium novyi* were found to be key players in the hydrolysis of suspended organic matter in food waste-recycling wastewater (Kim, Song, *et al.*, 2010). *C. thermopalmarium* was the butyric acid producer, and C. novyi was the propionic acid producer. Maximum efficiency was found at a pH of 5.7 and temperature of 44.5^{o}C. Gas production, organic acid consumption and methanogenic population were tracked in a maize silage reactor operating at 37^{o}C (Blume *et al.*, 2010). Hydrogenotrophic *Methanobacterials* dominated at OLRs equal or greater than 3.7 g-DOM/(L·d), In contrast, aceticlastic *Methanosaetacaea* dominated at lower OLRs and disappeared at OLRs greater than 4.1 g- DOM/(L·d). A comparison of membrane-bioreactors and submerged-biofilter wastewater treatment plant (WWTP) showed significant differences in Archaea make-up (Gómez-Silván *et al.*, 2010). Treatment type and wastewater origin affected these results. Thirty-two different temperature-gradient gel electrophoresis (TGGE) bands were identified with five dominating the samples (Evans *et al.*, 2011).

B. Microbial characterization of isolates and communities

Methane production in anaerobic bioreactors can occur through syntrophic acetate-oxidizing bacteria. Westerholm *et al.* (2010) reported the isolation of one of these novel bacteria, *Syntrophaceticus schinkii*, from a mesophilic methanogenic digester. This bacterium is related to *Thermacetogenium phaeum* with 92% 16S rRNA sequence similarity. The isolate is capable of using ethanol, betaine and lactate as carbon and electron sources and grows in temperatures of 25-40oC and pH of 6-8. A different organism was isolated from a digester treating palm oil mill effluent (Zakaria *et al.*, 2010). The isolate is classified as a *Comamonas* sp. with the capacity to grow on acetic, propionic and n-butyric acids and is unique in its capacity to form polyhydroxyalkanoates.

Anaerobic digestion of cheese-processing wastewater showed dominance of aceticlastic *Methanosarcinaceae a*nd hydrogenotrophic *Methanomicrobiales* (Lee, Kim, *et al.*, 2010).

A thermophilic anaerobic digester for beet silage and beet juice was operated for seven years (Krakat *et al.*, 2010). Morphologically rods dominated at 55^{o}C, while rods and coccoids dominated at 60^{o}C. Hydrogenotrophic *Methanobacteriales* dominated the microbial community,which contrasts findings from Anaerobid Digestion Model 1 (ADM1), which attributes dominance to acetotrophic *Euryarchaeota* in these conditions. The microbial community structure was determined for a full-scale anaerobic digester treating industrial food waste and seeded with sludge from treated swine waste (Ike *et al.*, 2010). The microbial community structure deviated significantly from the seed sludge community, with *Actinomyces, Thermomonospora, Ralstonia* and *Shewanella* hydrolyzing and *Methanosarcina, Methanobrevibacter* and *Methanobacterium* producing methane.

Activated sludge was used to treat carbazole-containing wastewater in a 70**°**C ultrasound anaerobic reactor (Tan and Ji, 2010). *Pseudomonas* sp., *Comamonas* sp. and *Diaphorobacter* sp. were found to use carbazole as a carbon source. Anaerobic landfill leachate was analyzed with a 16S rRNA clone library (Limam *et al.*, 2010). *Lentisphaerae* dominated the community with 98% of the clone library sequences.

Capacity of anaerobic wastewater treatment bioreactors to form biomass granules was tested at 15^{o}C (O'Reilly *et al.*, 2010). *Methanocorpusculum* dominated, and only formed granules in the glucose fed bioreactor.

An anaerobic batch digester used for treating secondary sludge had an organic removal efficiency of 35% (Shin, Lee, *et al.*, 2010). *Fusibacter, Clostridium and Syntrophus* likely carried out acidogenesis.*Methanosarcinales and Methanomicrobiales* were present with the latter dominating.

Bergmann *et al.* (2010) also looked at methanogenic populations in a mesophilic biogas plant. Quantitative PCR determined that the methanogenic population was made of 84% *Methanomicrobiales*, 14% *Methanosarcinales* and 2% *Methanobacteriales.* In a study competed by Huang *et al.* (2010), hydrogen production was linked to the most dominant producer – *C. perfringens.*

XII. SWINE WASTEWATER

Li, *et al.* (2010) showed the close link between bacterial community makeup and treatment efficiency with a UASB reactor treating swine wastewater. Reactor acclimatization consisted of 3.5 g-COD/L influent, methane production of 9.5 L/d and a COD removal rate of 90%. At steady-state, the reactor had 3.0-6.0 g-COD/L influent, methane production of 9.5-13.2L/d and a COD removal rate of 90-95%. Microbial community diversity did not change significantly from start-up to steady-state operation. Contrasting findings were found in Kim *et al.* (2010) where two anaerobic batch digesters were seeded with anaerobic sludge from a WWTP to treat swine wastewater. Methane production differed in the two reactors from 4.5 L/L to 7.9 L/L. This difference was attributed to the abundance of *Methanomicrobiales* and propionate in the reactors. Abundance of *Methanobacteriales and Methanosarcinales* were found to be consistent in the two reactors.

Several researchers have elucidated methanogenic population composition in reactors treating swine wastewater.

Hydrogenotrophic methanogens such as *Methanobacteriales* dominated in a UASB reactor (Song *et al.*, 2010). Patil *et al.* (2010) found *Methanothermobacter* sp. and g-*Proteobacteria* dominated a thermophilic digester while *Firmicutes, Methanosarcina* and *Methanoculleu*s dominated a mesophilic reactor. Kim *et al.* (2010) characterized a mesophilic sludge used for thermal acidogenesis of swine wastewater at 51^{o}C. The DGGE profiles indicated that *Pseudomonas mendocina*, *Bacillus halodurans, Clostridium hastiforme, Gracilibacter thermotolerans and Thermomonas haemolytica* are present.

XIII. EFFECTIVENESS OF MOLECULAR BIOLOGY TECHNIQUES

Zhou *et al.* (2010) showed that the combined use of PCR-DGGE, gas chromatograph (GC) analysis and triphenyltetrazolium chloride (TTC) dehydrogenase activity test are effective in evaluating changes in microbial activity, structure and quantity. These microbiological tools were tested on a bio-fluidized bed with an anaerobic-oxic-oxic process for treating coking wastewater. Ramos *et al.* (2010) used a 16S rRNA clone library with restriction fragment length polymorphism (RFLP) analysis to determine microbial diversity in a UASB reactor. The use of *HaeIII* simplified the 162 clones down to 28 distinct organisms, providing a simple and fast method for identifying microbial diversity. The use of PCR-DGGE was applied to estimate microbial population sizes in a UASB reactor treating streptomycin (Liu, Yang, *et al.*, 2010). *E. coli* was inoculated and used as an internal standard, which allowed for good correlation between band intensity and population size. Microbial populations lower than 10^3 CFU/g were undetectable.

XIV. INHIBITION OF METHANOGENESIS

Methanogens are important in anaerobic sludge digestion. Chloroform and 2- bromoethanesulfonate are two known inhibitors of methanogenesis, but little is known of their impact on microbial communities (Evans *et al.*, 2011). Xu *et al.* (2010) completed a recent study that showed acetoclastic *Methanosaetaceae* were more sensitive to the inhibitors than hydrogenotrophic *Methanobacteriales* and *Methanomicrobiales*. This in turn affected methane production by the microbial community in the activated sludge.

In contrast, prolonged starvation of methanogens treating swine wastewater did not greatly affect cell numbers of *Methanosarcinales* or methanogenesis (Hwang *et al.*, 2010). The effect of nitrite and ammonium on two methanotrophic bacteria, *Methylomicrobium album* and *Methylocystis* sp., was tested (Nyerges *et al.*, 2010*). M. album* dominated in high nitrite levels, while *Methylocystis* sp. dominated in high ammonium levels

XV. INHIBITION OF THE ANAEROBIC PROCESS

Toxicants or inhibitors are mainly present from, but not necessarily limited to, differing compounds in the influent, excessive or limiting nutrients available for metabolism of the biomass, and waste products formed in the process (Evans *et al.*, 2011).

Martins *et al.* (2010) studied the use of Fenton's process for treating milk whey wastewater treatment effluent to produce a final effluent that could be discharged directly to the natural stream. They found that the hydrogen peroxide concentration and the ratio between H_2O_2:Fe^{2+} was important to total organic carbon (TOC) and COD removal. When the optimum of both was achieved a harmless effluent resulted.

Sabalowsky and Semprini (2010b) exposed two reductively dechlorinating anaerobic cultures (Evanite and Point Mugu) to high concentrations of chlorinated aliphatic hydrocarbons (CAH). Both cultures accumulated cis-1,2-dichloroethene (cDCE) in a batch-fed reactor to concentrations ranging from 9,000 – 12,000 uM before a loss in activity occurred. A concentration toxicity model was assembled incorporating CAH toxicity in terms of cell decay. A toxicity model that Sabalowsky and Semprini(2010a) assembled was extended to observations in continuous flow suspended and attached growth reactors.The model incorporating cDCE and trichloroethene (TCE) toxicity was predictive in determining that the cells in batch-fed growth are most sensitive to high concentrations of cDCE and TCE followed by the continuous flow stirred tank reactor and finally the attached growth being the least sensitive. Àlvarez *et al.* (2010) reviewed the inhibition caused by the antibiotics oxytetracycline (OTC) and Chlortetracycline (CTC) on pig manure anaerobic digestion (AD). The study found that varying concentrations of OTC and CTC combinations of 10, 50 and 100 mg/L fed to the reactor reduced methane production 56%, 60% and 62% respectively.

Dilute ethylene glycol aircraft deicing fluid was successfully treated using a four compartment anaerobic baffled reactor (ABR) (Marin *et al.*, 2010). The research team fed three dilute concentrations to the reactor and all achieved over 75% soluble COD removal. Acetoclastic activity changed throughout the study in each chamber suggesting that microbial differentiation was occurring in each chamber. Palatsi *et al.* (2010) fed manure and pulsed long- chain fatty acid (LCFA) into a thermophilic anaerobic digester to determine microbial toxicity. They found significant microbial community changes occurred during the inhibitory pulses. They used the IWA ADM1 model and changed the kinetics to account for the inhibition of the LCFA resulting in an improved fit. Organic overloading may have an inhibitory effect on the high-solids AD of municipal solid waste (MSW) (Schievano *et al.*,2010). The authors investigated a new approach by observing the putrescibility of organic mixtures. They found that measuring the organic loading calculated as OD_{20} (oxygen consumption in 20 h. biodegradation) was a very good indicator of inhibitory effects. Inhibition started at an OD_{20} > 17 – 18g-O_2/kg (Evans *et al.*, 2011).

Stone *et al.* (2010) studied the effects of Tylosin and Chlortetracycline (CTC) on swine manure digestion in the presence of sodium azide. CTC alone improved hydrolysis but inhibited methane and carbon dioxide production. Tylosin alone did not influence methane or carbon dioxide production but inhibited hydrogen and acetate-only microbial populations. Sodium azide alone enhanced biomass production and metabolic output.Sodium azide in the presence of Tylosin or CTC inhibited metabolism and methane and carbon dioxide production. Ismail *et al.* (2010) utilized four UASB reactors to evaluate EPS in a high saline environment. Reactor R1 was fed fully acidified

substrate while reactors R2 – R4 were fed partially acidified substrate. EPS was extracted by cation exchange. Bulk liquid Ca^{2}+ leaching was observed in granular sludge samples in the presence of 20-g Na^{+}/L.Extracted proteins were higher in reactors R2 – R4. An attempt to reduce recovery times by bioaugmentation after a transient toxic event in anaerobic digesters was studied (Schauer-Gimenez *et al.*, 2010). An H_2 utilizing culture was used as the bioaugmentation agent. It was found that recovery times do decrease after a transient toxic event and that propionate decreases and biogas production increases. Digesters that are adaptable will not benefit from this therapy but those with poorly adaptable microbes may benefit highly.

Addition of metal nutrient supplements to simulate acetoclastic methanogens was examined (Park,Bega, *et al.*, 2010). Two full-scale mesophilic digesters were examined using methane potential tests. Acetoclastic methanogens from a recently cleaned digester were not affected by low concentrations of trace metals including iron, cobalt and nickel. Another digester not having been cleaned for over 10 years was slightly affected with metal supplementation. Stressed acetoclastic methanogens are susceptible with trace metal supplements. Pirc *et al.* 2010) investigated cyanide influence on biogas production in AD of glucose. Cyanide was fed to the reactor at concentrations of 325 to 31,000 mg/L. Significant inhibition was found with cyanide concentrations greater than 2,600 mg/L.

XVI. CHALLENGES/RECOMMENDATION

The ponding system which is currently being practice by most mills to treat POME do not identify the individual microorganisms involve in degrading and utilizing the different components (oil and grease, total solids, total dissolve solids, total suspended solids, total volatile solids etc) in POME and hence discharge poor quality effluent into the environment. Knowledge of the biodiversity of the different composition of microbial consortium in the pond treating POME and bioreactors is crucial as this will establish the right compositions of individual microbial isolates or consortium to use at any particular given time in removing or reducing the components making up the overall COD and also to establish the substrates which the individual isolates utilize. In addition, the microorganisms are not established and hence the substrate they degrade and utilize is not ascertained. This lead to poor effluent discharge into the environment as the performance of the microorganisms with regards to the rate of reduction and removal of oily waste and cellulolytic material cannot be monitor since they are not known. This could pose challenges as the identities of the microbial isolates are not known and point to the limitation of this system.

It is worthy of note that the standard regulation governing the discharge of POME did not include COD and total solids(TS) in their schedule and excruciating as it may be, the standard has not being renew all these years. There is need for the government to look into the POME regulation standard with a view to fill in any missing gaps (inclusion of COD and TS) for better performance.

Since the identification of microorganisms by conventional methods requires the isolation of pure cultures followed by laborious characterization experiments, we therefore note here that the procedures are therefore inadequate for study of the biodiversity of a natural or engineered ecosystem like POME. A new set of molecular biology techniques developed during the 1990s has revolutionized microbial ecology research and hence we recommend the use of these techniques in monitoring the microbial population dynamic changes in microbial communities in POME.

These genetic fingerprinting techniques in molecular ecology will identify/detect, localize and quantify specific species of microorganisms utilizing and degrading the components' in POME both in the mesophlic and thermophlic stages in the treatment process. The predominant bacterial and fungal species will be identify and the most dominant species present in POME throughout the treatment process and responsible for the degradation and utilization will be establish and this is a step in the right direction as this will improve POME treatment since the organisms is establish and the substrate they utilize is ascertain.

We will like to state that the advantages of the molecular biology techniques in wastewater treatment are enormous as this will aid the identification of microorganisms that have not yet been culture or identify in POME treatment and when isolated, it could be the most suitable candidate organisms for bioremediation of polluted environment with POME.

To this end, the impact of POME on the environment calls for further studies in the areas of minimizing high COD and BOD load using other novel technologies or improve research technology for future advancement on the present status of POME treatment and continues utilization of POME as a suitable fermentation medium or substrates for the production of products such as organic acid, antibiotics, cellulase etc and for the production of fertilizer in order to reduce the burden caused by POME on the environment. Many palm oil mills are still unable to adhere to the wastewater discharge limits and thus resulting to a dramatic increase in the number of polluted rivers (Ahmad and Chan, 2009). The mills should routinely sample their pond in order to comply with government regulated standard for effluent discharge. The government on their own part should monitor the mills whether they comply with the said specifications and periodically make amendment and modifications in the regulation standard for POME discharge so as to better improve good quality effluent discharge into the environment.

XVII. FURTHER RESEARCH/STUDY

We will also like to reiterate and elucidate further that there is need to establish all the different composition of the microbial consortium in the anaerobic digester/bioreactor and pond use for POME treatment in mills as aforementioned in order to establish the most suitable microbial community or individual isolate utilizing and degrading the different components making up the overall COD in POME due to the inconsistency of POME. Secondly, for future improvement and advance research or improve technology in POME treatment, molecular biology techniques as earlier discussed should be use to provide more comprehensive study on the successional trend of microbial isolates utilizing and degrading POME in the anaerobic

bioreactor and the pond in mills as this can be use to improve treatment processes. Thirdly, the failure of the existing bioreactor/digester to achieve 100% removal of basic waste water parameters such as chemical oxygen demand (COD), therefore demand further research and development of novel bioreactor for effective treatment of POME(Jemeel *et al.*, 2011). This is a step in the right direction as this will improve POME treatment.

XVIII. CONCLUSION

Palm oil mill wastes have existed for years but their effects on environment are at present more noticeable. When discharged untreated, they may cause serious problem and deteriorates the environment. Due to the aforementioned, the palm oil industry faces the challenge of balancing the environmental protection, its economic viability and sustainable development. There is an urgent need to find an efficient and practical approach to preserve the environment while keeping the economy growing and maintaining the sustainability of the economy. Thus, while enjoying a most profitable commodity, the adverse environmental impact from the palm oil industry cannot be ignored. Hence, serious measures have to be taken in order to prevent the growing pollution and ecological degradation related to POME.

Considering the high organic concentration of POME, anaerobic process is the most suitable approach for its treatment. Hence, employing the biochemical abilities of microorganisms is the most popular strategy for the biological treatment of palm oil mill effluent. Microorganisms, than any other class of organisms, have a unique ability to interact both chemically and physically with a huge range of both man – made and naturally occurring compounds leading to a structural change to, or the complete degradation of, the target molecule. Anaerobic treatment of POME result in the production of methane as a value added product. Molecular biology tools is a veritable preferred and suggested technique which has the potential of providing insight into the microbial community dynamics and structure during anaerobic processes in wastewater treatment. In addition, the potential of using the molecular biology techniques to provide detailed profile of the microbial community structure and to establish the phylogenetics of microorganisms in bioreactors used for POME treatment will enhance wastewater treatment processes. This information can be used to improved POME treatment processes which will produce acceptable quality effluent before it can be discharged into the watercourse for land application with no harmful effect on the environment.

ACKNOWLEDGMENT

The authors would like to thank Universiti Sains Malaysia for the financial support under Research Grant, No.: 1001/PTEKIND/814147.

REFERENCES

[1] Acharya, B.K., Mohana, S. and Madamwar, D. (2008). Anaerobic treatment of distillery spent wash – a study on upflow anaerobic fixed film bioreactor. Bioresource Technology 99, 4621–4626.

[2] Agamuthu, P., Tan, E.L. and Shaifal, A.A. (1986). Effect of aeration and soil inoculum on the composition of palm oil effluent (POME). Agricultural Wastes 15, 121–132.

[3] Agamuthu, P., (1995). Palm oil mill effluent and utilization. In: C.A. Sastry, MA. Hashim and P. Agamuthu, (eds). Waste treatment plant. Narosa Publishing House, New Delhi, pp.338-360.

[4] Agamuthu, P., Sivaraj, S. and Mukherjee, T. K.(1996). Nutrition and in vitro digestion aspects of Napier grass (Pennisetum purpureum) grown with palm oil mill effluent (POME) as fertiliser for goat and sheep. Indian Journal of. Animal. Sciences. 66, 935–937.

[5] Ahmad, A., Ismail, S. and Bhatia. S. (2003). Water recycling from palm oil mill effluent pome) using membrane technology. Desalination, 157, 87-95.

[6] Ahmad, A.L., Sumathi, B.H. and Hameed, B.H. (2005).Residual oil and suspended solid removal using natural adsorbents chitosan, bentonite and activated carbon: A comparative study, Chemical Engineering Journal 108,179–185.

[7] Ahmad, A.L., Chong, M.F., Bhatia, S. and Ismail, S. (2006a). Drinking water reclamation from palm oil mill effluent (POME) using membrane technology. Desalination 191, 35–44.

[8] Ahmad ,A.L, Chong, M.F. and Bhatia, S. (2006b) Ultra filtration modeling of multiple solutes system for continuous cross-flow process. Chemical Engineeering Sciences 61, 5057–69.

[9] Ahmad, A.L., Chong, M..F. and Bhatia, S. (2007). Mathematical modeling of multiple solutes system for reverse osmosis process in palm oil mill effluent (POME) treatment. Chemical Engineering Journal 132, 183–193.

[10] Ahmad AL. and Chan CY. (2009). Sustainability of palm oil industries: an innovative treatment via membrane technology. Journal of Applied Sciences 9, 3074–3079.

[11] Ahn, J.-H. and Forster, C.F.. (2002). A comparison of mesophilic and thermophilic anaerobic upflow filters treating paper-pulp-liquors. Process Biochemistry 38, 257–262

[12] Akarsubasi, A.T, Ince, O., Oz. N.A., Kirdar, B.and Ince, B.K. (2006). Evaluation of performance, acetoclastic methanogenic activity and archaeal composition of full-scale UASB reactors treating alcohol distillery wastewaters. Process Biochemistry 41, 28–35.

[13] Alvarado-Lassman, A., Rustrián, E., García-Alvarado, M.A., Rodríguez-Jiménez, G.C.and Houbron, E. (2008). Brewery wastewater treatment using anaerobic inverse fluidized bed reactors. Bioresource Technology 99, 3009–3015.

[14] Álvarez, J. A.,Otero, L., Lema, J. M. and Omil, F. (2010). The effect and fate of antibiotics during the anaerobic digestion of pig manure. Bioresources. Technology 101, 8581-8586.

[15] Amann,R.I., Ludwig, W. and Schleifer, K.H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiololgy Review 59,143–69.

[16] Araujo, J.C., Brucha, G., Campos J.R. and Vazoller, R.F. (2000). Monitoring the development of anaerobic biofilms using fluorescent in situ hybridization and confocal laser scanning microscopy. Technology Water Sciences 41, 69–77.

[17] Ayati, B. and Ganjidoust, H. (2006). Comparing the efficiency of UAFF and UASB with hybrid reactor in treating wood fiber wastewater. Iranian Journal of Environmental Health Science Engineering 3, 39–44.

[18] Babu, M. K., Sukumaran, N., Kumar, M. G. V. and Murugesan, A. G. (2001). Fish culture in palm oil mill effluent. International. Journal of. Oil Palm Research., 2, 47–49.

[19] Baharuddin, A.S., Hock, L.S., Yusof., M.Z.M., Rahman., N.A.A., Shah., U.K.M., Hassan.,M.A., Wakisaka, M., Sakai, K. and Shirai, Y. (2010). Effects of palm oil mill effluent (POME) anaerobic sludge from 500m3 of closed anaerobic methane digested tank on pressed- shredded empty fruit bunch (EFB) composting process, African Journal of Biotechnology 9, 2427-2436.

[20] Barbosa, R.A. and Sant'Anna Jr., G.L. (1989). Treatment of raw domestic sewage in an UASB reactor. Water Research 23, 1483–1490.

[21] Beccari, M., Bonemazzi, F., Majone, M. and Riccardi, C., (1996). Interaction between acidogenesis and methanogenesis in the anaerobic treatment of olive oil mill effluents. Water Research 30, 183–189

[22] Behling, E., Diaz, A., Colina, G., Herrera, M., Gutierrez, E., Chacin, E., Fernandez, N. and Forster, C.F.(1997). Domestic wastewater treatment using UASB reactor.Bioresource Technology 61, 239–245.

[23] Bello-Mendoza, R. and Castillo-Rivera, M.F. (1998). Start-up of an anaerobic hybrid (UASB/filter) reactor treating wastewater from a coffee processing plant.Anaerobe 4, 219–225.

[24] Bergmann, I. Nettmann., E. Mundt, K. and Klocke, M. (2010). Determination of methanogenic Archaea abundance in a mesophilic biogas plant based on 16S rRNA gene sequence analysis. Canada Journal of Microbiology 56, 440-444.

[25] Blume, F., Bergmann, I., Nettmann, E., Schelle, H., Rehde, G., Mundt, K. and Klocke, M. (2010). Methanogenic population dynamics during semi-continuous biogas fermentation and acidification by overloading. Journal of Applied Microbiology 109, 441-450.

[26] Bodkhe, S. (2008). Development of an improved anaerobic filter for municipal wastewater treatment. Bioresources Technology 99, 222–226.

[27] Borja, R. and Banks, C.J. (1994a). Anaerobic digestion of palm oil mill effluent using an up-flow anaerobic sludge blanket reactor. Biomass and Bioenergy 6, 381–389.

[28] Borja, R. and Banks, C.J. (1994b). Treatment of palm oil mill effluent by upflow anaerobic filtration. Journal of Chemical Technology and Biotechnology 61, 103–109.

[29] Borja, R. and Banks, C.J.(1994c). Anaerobic digestion of palm oil mill effluent using an up-flow anaerobic sludge blanket reactor. Biomass and Bioenergy 6, 381–389.

[30] Borja, R. and Banks, C.J. (1995a). Response of an anaerobic fluidized bed reactor treating ice-cream wastewater to organic, hydraulic, temperature and pH shocks.Journal of Biotechnology 39, 251–259.

[31] Borja, R. and Banks, C.J. (1995b). Comparison of an anaerobic filter and an anaerobic fluidized bed reactor treating palm oil mill effluent. Process Biochemistry 30,511–521.

[32] Borja, R., Banks, C.J. and Wang, Z. (1995c). Performance of a hybrid anaerobic reactor,combining a sludge blanket and a filter, treating slaughterhouse wastewater.Applied Microbiology and Biotechnology 43, 351–357.

[33] Borja, R., Banks, C.J. and Sánchez, E. (1996a). Anaerobic treatment of palm oil mill effluent in a two-stage up-flow anaerobic sludge blanket (UASB) reactor. Journal of Biotechnology 45, 125–135.

[34] Borja, R., Alba, J. and Banks, C.J. (1996b). Anaerobic digestion of wash waters derived from the purification of virgin olive oil using a hybrid reactor combining a filter and a sludge blanket. Process Biochemistry 31, 219–224.

[35] Borja, R., Banks, C.J., Wang, Z. and Mancha, A.(1998). Anaerobic digestion of slaughterhouse wastewater using a combination sludge blanket and filter arrangement in a single reactor. Bioresource Technology 65, 125–133.

[36] Borja, R., González, E., Raposo, F., Millán, F. and Martín, A.(2001). Performance evaluation of a mesophilic anaerobic fluidized-bed reactor treating wastewater derived from the production of proteins from extracted sunflower flour. Bioresource Technology 76, 45–52.

[37] Busu, Z, Sulaiman, A, Hassan, MA, Shirai Y, Abdul-Aziz, S, Yacob, S and Wakisaka, M. (2010). Improved anaerobic treatment of palm oil mill effluent in a semi- commercial closed digester tank with sludge recycling and appropriate feeding strategy, Pertanika Journal of Tropical. Agricultural. Sciences 33, 27-37.

[38] Buyukkamaci, N. and Filibeli, A. (2004). Volatile fatty acid formation in anaerobic hybrid reactor. Process Biochemistry 39 1491–1494.

[39] Buzzini A.P., Sakamoto, I.K., Varesche, M B. and Pires, E.C. (2006). Evaluation of the microbial diversity in an UASB reactor treating wastewater from an unbleached pulp plant. Process Biochemistry 41,168–176.

[40] Cail, R.G. and Barford, J.P. (1985). Thermophilic semi-continuous anaerobic digestion of palm-oil mill effluent. Agricultural Wastes 13, 295–304.

[41] Chaisri, R., Boonsawang, P., Prasertsan, P. and Chaiprapat, S. (2007). Effect of organic loading rate on methane and volatile fatty acids productions from anaerobic treatment of palm oil mill effluent in UASB and UFAF reactors. Songklanakarin Journal of Science and Technology 29,311–23.

[42] Chan, K.S. and Chooi, C.F. (1984). Ponding System for palm oil mill effluent treatment. In: Proceedings of the Regional Workshop on Palm Oil Mill Technology & Effluent Treatment, pp. 185–192.

[43] Chan, O.C., Liu., W.T. and Fang, H.H.P.(2001). Study of microbial community of brewery-treating granular sludge by denaturing gradient gel electrophoresis of 16S rRNA gene. Water Science and Technology 43:77–82.

[44] Chen, T.H. and Shyu, W.H. (1996). Performance of four types of anaerobic reactors in treating very dilute dairy wastewater. Biomass and Bioenergy 11, 431–440

[45] Chen, C.L., Macarie, H., Ramirez, I., Olmos. A., Ong, S.L. and Monroy. O. (2004).Microbial community structure in a thermpohilic anaerobic hybrid reactor degrading terephthalate. Microbiology 150,3429–40.

[46] Chin, K.K. (1981). Anaerobic treatment kinetics of palm oil sludge. Water Research 15, 199–202.

[47] Chin, K.K., Lee, S.W. and Mohammad, H.H. (1996). A study of palm oil mill effluent treatment using a pond system. Water Science and Technology 34, 119–123.

[48] Chong, M.F. (2007). Modeling, simulation and design of membrane based palm oil mill effluent (POME) treatment plant from pilot plant studies. Ph.D. Thesis.Universiti Sains Malaysia.

[49] Choorit, W.and Wisarnwan, P. (2007). Effect of temperature on the anaerobic digestion of palm oil mill effluent. Electronic Journal of Biotechnology 10, 376–385.

[50] Chotwattanasak, J. and Puetpaiboon, U. (2001). Full scale anaerobic digester for treating palm oil mill wastewater. Journal of Substainable Energy and Environmental 2,133-136.

[51] Connaughton, S., Collins, G. and O'Flaherty, V. (2006). Development of microbial community structure and activity in a high-rate anaerobic bioreactor at 18 8C. Water Resources 40,1009–1017.

[52] Córdoba, P.R., Francese, A.P.and Sineriz, F.(1995). Improved performance of a hybrid design over an anaerobic filter for the treatment of dairy industry wastewater at laboratory scale. Journal of Fermentation and Bioengineering 79, 270–272.

[53] Daims H, Nielsen, J.L., Nielsen, P.H., Schleifer, K. and Wagner, M. (2001). In situ characterization of Nitrospira-like nitrite-oxidizing bacteria active in wastewater treatment plants. Applied Environmental Microbiology 67, 5273–5284.

[54] Data for Engineers: POME, (2004). Palm Oil Engineering Bulletin 71,34–35.

[55] Davis, J.B. and. Reilly, P.J.A .(1980). Palm oil mill effluent-a summary of treatment methods. Oleagineux 35, 323-330.

[56] Devendra, C. (2004) Integrated tree crops-ruminants systems: potential importance of the oil palm. Outlook Agriculture 33, 157–166.

[57] Devendra, C. and Muthurajah, R. N. (1976). The utilization of oil palm by-products by sheep. Processing and marketing, in: Proceeding. of the Malaysian International Symposium of Palm Oil, Kuala Lumpur.

[58] Dı´az, E.E., Stams, A.J.M., Amils, R and Sanz, J.L. (2006). Phenotypic properties and microbial diversity of methanogenic granules from a full-scale UASB reactor treating brewery wastewater. Applied Environmental Microbiology 72,4942–4949.

[59] Dinsdale, R.M., Hawkes, F.R. and Hawkes, D.L.(1997). Comparison of mesophilic and thermophilic upflow anaerobic sludge blanket reactors treating instant coffee production wastewater. Water Research 31, 163–169.

[60] Doble, M. and Kumar, A.(2005). Biotreatment of Industrial Effluents. Elsevier Butterworth-Heinemann, Oxford, United Kingdom. pp.19-38.

[61] Donne, T.P. (1981). Development of Palm oil Research and Report. Ministry of Sap an on Developments and Welfare, Sapan pp.110-120.

[62] Egli, K., Fanger, U., Alvarez, P.J., Siegrist, H., van der Meer, J.R. and Zehnder, A.J. (2001). Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate. Archives of Microbiology 175,198–207.

[63] Egli K., Bosshard, F., Werlen, C., Lais, P., Siegrist, H. and Zehnder, A.J. (2003). Microbial composition and structure of a rotating biological contactor biofilm treating ammonium-rich wastewater without organic carbon. Microbial Ecology 45,419–32.

[64] Evans, E.A., Evans, K.M.,Ulrich, A. and Ellsworth, S. (2011).Anaerobic Processes. Water Environment Research 83,1285-1332.

[65] Faisal, M.and Unno, H. (2001).Kinetic analysis of palm oil mill wastewater treatment by a modified anaerobic baffled reactor. Biochemical Engineering Journal 9, 25–31.

[66] Fang, H.H.P. and Chui, H.K. (1994). Comparison of startup performance of four anaerobic reactors for the treatment of high-strength wastewater. Resources,Conservation and Recycling 11, 123–138.

[67] Farhadian, M., Borghei, M. and Umrania, V.V.(2007). Treatment of beet sugar water by UAFB bioprocess. Bioresource Technology 98, 3080–3083.

[68] Federal Subsidiary Legislation – Environmental Quality Act 1974 [ACT 127]. Environmental Quality (Sewage and Industrial Effluents) Regulation 1979.[Online] Available: <http://www.doe.gov.my> (29/2/2008).

[69] Ferrera, I, Massana, R., Casamayor, E.O., Balague V., Sa´nchezm O. and Pedros-Alio, C. (2004). High-diversity biofilm for the oxidation of sulfide-containing effluents. Appl Microbiology Biotechnology 64,726–34.

[70] Gangagni, Rao, A., Venkata, Naidu, G., Krishna, Prasad, K., Chandrasekhar, Rao, N., Venkata. Mohan, N., Jetty, A. and Sarma, P.N.(2005). Anaerobic treatment of wastewater with high suspended solids from a bulk drug industry using fixed film reactor (AFFR). Bioresource Technology 96, 87–93.

[71] Garcia-Calderon, D., Buffiere, P., Moeltta, R. and Elmaleh, S. (1998). Anaerobic digestio of wine distillery wastewater in down-flow fluidized bed. Water Research 32, 3593–3600.

[72] Gerardi, M.H., (2003). The Microbiology of Anaerobic Digesters. Wiley-Interscience, New Jersey. pp. 51-57.

[73] Gerardi, M.H. (2006). Wastewater Bacteria. Wiley-Interscience, New Jersey. pp. 19– 31.

[74] Gómez-Silván, C., Molina-Muñoz, M., Poyatos, J. M., Ramos, A., Hontoria, E., Rodelas, B. and González-López, J. (2010). Structure of archaeal communities in membrane-bioreactor and submerged-biofilter wastewater treatment plants.Bioresource Technology 101, 2096-2105.

[75] Gonza´lez-Gil, G., Lens, P.N.L., Van Aelst, A, Van As, H., Versprille, A.I. and Lettinga, G. (2001). Cluster structure of anaerobic aggregates of an expanded granular sludge bed reactor. Applied Environmental Microbiology 67, 3683–92.

[76] Goodwin, J.A.S., Wase, D.A.J. and Forster, C.F. (1992). Pre-granulated seeds for UASB reactors: how necessary are they? Bioresource Technology 41, 71–79.

[77] Gopal, J. and Ma, A.N.(1986). The comparative economics of palm oil mill effluent treatment and resource recovery systems. National Workshop on Recent Developments in Palm Oil Milling Technology & Pollution Control.

[78] Guiot, S.R. and Van den Berg, L. (1985). Performance of an upflow anaerobic reactor combining a sludge blanket and a filter treating sugar waste. Biotechnology and Bioengineering 27, 800–806.

[79] Habib, M.A.B., Yusuf, F.M,. Phang, S.M. and Mohamed, S. (1997). Nutritional values of chironomid larvae grown in palm oil mill effluent and algal culture. Aquaculture, 158, 95-105.

[80] Hamdi, M. and Garcia, J.L. (1991). Comparison between anaerobic filter and anaerobic contact process for fermented olive mill wastewaters. Bioresource Technology 38, 23–29

[81] Hartley, C.N.S. (1988). The oil palm. 3rd Ed. Longman Scientific and Technical, UK., pp. 14-17.

[82] Hassan, MA, Sulaiman , A.,Shirai Y. and Abd-Aziz, S. (2009). Methane capture and clean development mechanism project for the sustainability of palm oil industry in Malaysia. Journal of Applied Science Research 5,1568–81.

[83] Hata, J., Miyata N., Kim, E.S., Takamizawa, K. and Iwahor,i K.(2004). Anaerobic degradation of cis-1,2-dichloroethylene and vinyl chloride by Clostridium sp. strain DC1 isolated from landfill leachate sediment. Journal of Bioscience and Bioengineering 97,196–201.

[84] Hawkes, F.R., Donnelly, T. and Anderson, G.K. (1995). Comparative performance of anaerobic digesters operating on ice-cream wastewater. Water Research 29,525–533.

[85] Herbert, H. and Chan, O.C. (1997).Toxicity of phenol towards anaerobic biogranules, Water Research. 31, 2229–2242.

[86] Hii, K.L., Yeap, S.P. and Mashitah, M.D. (2012). Cellulase production from palm oil mill effluent in Malaysia: Economical and technical perspectives, Engineering Life Sciences, 12, 7–28.

[87] Ho, K. P. (1976). Malaysia: sup the wastes. Planter 52, 296–297.

[88] Huang, Y., Zong, W., Yan, X., Wang, R., Hemme, C. L., Zhou, J. and Zhou, Z. (2010). Succession of the Bacterial Community and Dynamics of Hydrogen Producers in a Hydrogen-Producing Bioreactor. Applied Environmental Microbiology 76, 3387-3390.

[89] Hutagalung, R. I., Chang, C. C., Toh, K. M. and Chan, H. C. (1977). Potential of palm oil mill effluent as feed for growing-finishing pigs. Planter 53, 2–9.

[90] Hwang, K.,Song, M., Kim, W., Kim, N. and Hwang, S. (2010). Effects of prolonged starvation on methanogenic population dynamics in anaerobic digestion of swine wastewater. Bioresource. Technology 101, S2-S6

[91] Ibrahim, A., Yeoh, B.G., Cheah, S.C., Ma, A.N., Ahmad, S., Chew, T.Y., Raj, R. and Wahid, M.J.A., (1984). Thermophilic anaerobic contact digestion of palm oil mill effluent. Water Science and Technology 17, 155–165.

[92] Idris, A.B, Noor, M.J,M.M.and Al-Mamun, A. (2003). Modelling of anaerobic fluidized bed bioreactor in the treatment of palm oilmill effluent. Asian Journal of Microbiology, Biotechnology and Environmental Experimental Sciences 5,137–44.

[93] Igwe, J. C. and Onyegbado, C. C. (2007). A review of palm oil mill effluent (POME) water treatment. Global Journal of Environmental Research 1, 54–62.

[94] Ike, M., Inoue, D., Miyano, T., Liu, T. T., Sei, K., Soda, S. and Kadoshin, S. (2010). Microbial population dynamics during startup of a full-scale anaerobic digester treating industrial food waste in Kyoto eco-energy project. Bioresource. Technology 101, 3952-3957.

[95] Ismail, S. B., de La Parra, C. J., Temmink, H. and van Lier, J. B. (2010). Extracellular polymeric substances (EPS) in upflow anaerobic sludge blanket (UASB) reactors operated under high salinity conditions. Water Research 44, 1909-1917.

[96] Ito, T., Okabe, S., Satoh, H. and Watanabe, Y. (2002). Successional development of sulfate-reducing bacterial populations and their activities in a wastewater biofilm growing under microaerophilic conditions. Applied Environmental Microbiology 68, 1392–402.

[97] James, R., Sampath , K. and Alagurathinam, S. (1996). Effects of lead on respiratory enzyme activity, glycogen and blood sugar levels of the teleost Oreochromis mossambicus (Peters) during accumulation and depuration. Asian fisheries science Metro Manila 9, 87-100.

[98] Jameel, A.T., Muyibi, S.A. and Olanrewaju, A.A. (2011). Comparative study of bioreactors used for palm oil mill effluent treatment based on chemical oxygen removal efficiencies In: M.D.Z, Alam, A.T, Jameel and A, Amid, (eds). Current research and development in biotechnology engineering at International Islamic University Malaysia (IIUM) Vol. III. IIUM Press, Kuala Lumpur, pp.277-284. ISBN 9789674181444.

[99] Jawed, M. and Tare, V. (2000). Post-mortem examination and analysis of anaerobic filters. Bioresources Technology 72, 75–84.

[100] Jameel, A.T. and Olanrewaju, A.A. (2011). Aerobic biodegradation of oil and grease in palm oil mill effluent using consortium of microorganisms In: M.D.Z, Alam, A.T, Jameel and A, Amid, (eds). Current research and development in biotechnology engineering at International Islamic University Malaysia (IIUM) Vol. III. IIUM Press, Kuala Lumpur, pp. 43-51. ISBN 9789674181444.

[101] Jetten, M., Schmid, M., Van de Pas-Schoonen, K., Sinninghe Damste, J. and Strous, M. (2005). Anammox organisms: enrichment, cultivation, and environmental analysis. Methods in Enzymology 397:34–57.

[102] Kalyuzhnyi, S.V., Skylar, V.I., Davlyatshina, M.A., Parshina, S.N., Simankova,M.V.,Kostrikina, N.A. and Nozhevnikova, A.N. (1996). Organic removal and microbiological features of UASB-reactor under various organic loading rates.Bioresource Technology 55, 47–54.

[103] Kalyuzhnyi, S., de los Santos, L.E. and Martinez, J.R. (1998). Anaerobic treatment of raw and preclarified potato-maize wastewater in a UASB reactor. Bioresource Technology 66, 198–199.

[104] Kaparaju, P., Buendia, I., Ellegaard, L. and Angelidakia, I.(2008). Effects of mixing on methane production during thermophilic anaerobic digestion of manure:lab-scale and pilot-scale studies. Bioresource Technology 99, 4919– 4928.

[105] Karel, I.M., Sponge, Y. and Jujh, A. (1974). Disposal of sewage and other Borne waste. Butter Worth Press, London. pp: 50-61.

[106] Karim, K., Klasson, K.T., Hoffmann, R., Drescher, S.R., DePaoli, D.W. and Al-Dahhan, M.H.(2005a). Anaerobic digestion of animal waste: effect of mixing. Bioresource Technology 96, 1607–1612.

[107] Karim, K., Hoffmann, R., Klasson, K.T. and Al-Dahhan, M.H. (2005b). Anaerobic digestion of animal waste: effect of mode of mixing. Water Research 39, 3597–3606.

[108] Karim, K., Hoffmann, R., Klasson, T. and Al-Dahhan, M.H. (2005c). Anaerobic digestion of animal waste: waste strength versus impact of mixing. Bioresource Technology 96, 1771–1781

[109] Khalid, R. and. Wan Mustafa, W.A. (1992). External benefits of environmental regulation: Resource recovery and the utilisation of effluents. The Environmentalist 12, 277-285.

[110] Kim,I.S, Kim, S. and Jang, A. (2001). Activity monitoring for nitrifying bacteria by fluorescence in situ hybridization and respirometry. Environmental Monitoring Assessment 70, 223–31.

[111] Kim, M., Ahn, Y.-H. and Speece, R.E.,(2002). Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. Water Research 36, 4369– 4385.

[112] Kim, J.K., Oh, B.R., Chun, Y.N. and Kim, S.W. (2006). Effects of temperature and hydraulic retention time on anaerobic digestion of food waste. Journal of Bioscience and Bioengineering 102, 328–332.

[113] Kim, W., Hwang, K., Shin, S. G., Lee, S. and Hwang, S. (2010). Effect of high temperature on bacterial community dynamics in anaerobic acidogenesis using mesophilic sludge inoculum. Bioresource. Technology 101, S17-S22.

[114] Kim, M. D., Song, M., Jo, M., Shin, S. G., Khim, J. H. and Hwang, S. (2010). Growth condition and bacterial community for maximum hydrolysis of suspended organic materials in anaerobic digestion of food waste-recycling wastewater. Applied Microbiology and Biotechnology 85, 1611-1618.

[115] Kim, W., Lee, S., Shin, S. G., Lee, C., Hwang, K. and Hwang, S.(2010). Methanogenic community shift in anaerobic batch digesters treating swine wastewater. Water Research 44, 4900- 4907.

[116] Krakat, N., Westphal, A., Schmidt, S. and Scherer, P. (2010). Anaerobic digestion of renewable biomass: thermophilic temperature governs methanogen population dynamics. Applied Environmental Microbiology 76, 1842-1850.

[117] Lapara, T.M., Nakatsu, C.H., Pantea, L and Alleman, J.E. (2000). Phylogenetic analysis of bacterial communities in mesophilic and thermophilic bioreactors treating pharmaceutical wastewater. Applied Environmental Microbiology. 66:3951–3959.

[118] Lau, I.W.C. and Fang, H.H.P. (1997). Effect of temperature shock to thermophilic granules. Water Research 31, 2626–2632.

[119] Leal, K., Chachin, E., Gutierez, E., Fernandez, N. and Forster, C.F. (1998). A mesophilic digestion of brewery wastewater in an unheated anaerobic filter. Bioresource Technology 65, 51–55.

[120] Lee, C., Kim, J., Shin, S. G., O'Flaherty, V. and Hwang, S. (2010). Quantitative and qualitative transitions of methanogen community structure during the batch anaerobic digestion of cheese-processing wastewater. Applied. Microbiology and Biotechnology 87, 1963-1973.

[121] Leslie, G. Jr., C.P., Daigger, G.T. and Lim, H.C.(1999). Biological Wastewater Treatment, second ed. CRC Press. Revised & Expanded.

[122] Lettinga, G., van Velson, A.F.M., Hobma, S.W., de Zeeuw, W. and Klapwijk, A.(1980). Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. Biotechnology and Bioengineering 22, 699–734.

[123] Lettinga, G. (1995). Anaerobic digestion and wastewater treatment systems. Antonie van Leeuwenhoek 67, 3–28.

[124] Limam, R. D., Bouchez, T., Chouari, R., Li, T., Barkallah, I. Landoulsi, A. and Sghir, A. (2010). Detection of WWE2-related Lentisphaerae by 16S rRNA gene sequencing and fluorescence in situ hybridization in landfill leachate. Canada. Journal of. Microbiology 56, 846-852.

[125] Li, P., Wang, Y., Wang, Y., Liu, K. and Tong, L. (2010). Bacterial Community Structure and Diversity During Establishment of an Anaerobic Bioreactor to Treat Swine Wastewater. Water Science Technology 61, 243-252.

[126] Liu, W.T., Chan, O.C. and Fang, H.H.P.(2002). Microbial community dynamics during start-up of acidogenic anaerobic reactors. Water Research 36, 3203–3210.

[127] Liu, C.,Yang, J. L., Wu, G., Zhang, S., Li, Z. X. and Guo, J. B.(2010). Estimation of dominant microbial population sizes in the anaerobic granular sludge of a full-scale UASB treating streptomycin wastewater by PCR-DGGE. World Journal of Microbiology and Biotechnology 26, 375-379.

[128] Lo, K.V., Liao, P.H. and Gao, Y.C. (1994). Anaerobic treatment of swine wastewater using hybrid UASB reactor. Bioresource Technology 47, 153–157

[129] Lorestani, A.A.Z. (2006). Biological treatment of palm oil effluent (POME) using an up-flow anaerobic sludge fixed film (UASFF) bioreactor [TD899. P4 L8692006 f rb].

[130] Luxmy B.S, Nakajima, F. and Yamamoto, K. (2000). Predator grazing effect on bacterial size distribution and floc size variation in membrane-separation activated sludge. Water Science Technology 42,211–217.

[131] Ma, A.N. (1999a). Treatment of palm oil mill effluent. In: G. Singh, K.H. Lim, T. Leng and L.K. David, (eds) Oil palm and the environment: a Malaysian perspective. Malaysian Oil Palm Growers' Council Kuala Lampur, pp, 113-126.

[132] Ma, A.N. (1999b). Treatment of palm oil mill effluent. In: G. Singh, L.K. Huan, L. Teo and D.K. Lee, (eds) Oil palm and the environment. A Malaysian perspective. Malaysian Oil Palm Growers' Council, Malaysia, pp. 113-123.

[133] Ma, A.N. (2000). Environmental management for the oil palm industry. Palm Oil Development 30, 1-10.

[134] Ma, A.N. and Ong, A.S.H. (1985). Anaerobic digestion of palm oil mill. PORIM Bulletin 4, 35–45.

[135] Ma, A.N., Cheah, S.C. and Chow, M.C. (1993). Current status of palm oil processing wastes management. In: Waste Management in Malaysia: Current Status and Prospects for Bioremediation, pp. 111–136.

[136] Ma, A.N., Tajima, Y., Asahi, M. and Hannif, J. (1996). A novel treatment process for Palm oil Mill Effluent. Palm Oil Research Institute of Malaysia (PORIM) Technology, No.19, pp. 1-8.

[137] Ma, A.N., Tajima, Y., Asahi, M. and Hanif, J. (1997). Effluent treatment – evaporation method. PORIM Engineering News 44, 7–8.

[138] Mladenovska Z., Dabrowski, S. and Ahring, B.K. (2003). Anaerobic digestion of manure and mixture of manure with lipids: biogas reactor performance and microbial community analysis. Water Science Technology 48:271–278.

[139] Marin, J., Kennedy, K. J. and Eskicioglu, C. (2010). Characterization of an anaerobic baffled reactor treating dilute aircraft deicing fluid and long term effects of operation on granular biomass. Bioresource Technology 101, 2217-2223.

[140] Martins, R. C., Rossi, A. F., Castro-Silva, S. and Quinta-Ferreira, R. M. (2010). Fenton's process for post-biologically treated cheese production wastewaters final remediation. toxicity assessment. International Journal of Chemical Reactor Engineering 8, A142.

[141] McHugh, S., O'Reilly, C., Mahony, T., Colleran, E. and O'Flaherty, V.(2003). Anaerobic granular sludge bioreactor technology. Reviews in Environmental Science and Biotechnology 2, 225–245.

[142] Metcalf, E. (2003). Wastewater Engineering Treatment and Reuse, fourth ed.McGraw Hill, pp. 96–97.

[143] Mohan, S. and Sunny, N. (2008). Study of biomethanization of waste water from jam industries. Bioresource Technology 99, 210–213.

[144] Monroy, O., Johnson, K.A., Wheatley, A.D., Hawkes, F. and Caine, M. (1994). The anaerobic filtration of dairy waste: results of a pilot trial. Bioresource Technology 50, 243–251.

[145] Mosquera-Corral, A., Gonza´lez, F., Campos ,J. L. and Me´ndez, R. (2005). Partial nitrification in a SHARON reactor in the presence of salts and organic carbon compounds. Process Biochemistry 40, 3109–3118.

[146] Muhrizal, S., Shamshuddin, J., Fauziah, I . and. Husni, M.A.H.(2006). Changes in iron-poor acid sulfate soil upon submergence. Geoderma 131,110-122.

[147] Najafpour, G.D., Zinatizadeh, A.A.L., Mohamed, A.R., Hasnain Isa, M. and Nasrollahzadeh, H. (2006). High-rate anaerobic digestion of palm oil mill effluent in an upflow anaerobic sludge-fixed film bioreactor. Process Biochemistry 41, 370–379.

[148] Nakagawa, T., Sato, S., Yamamoto, Y. and Fukui, M. (2002). Successive changes in community structure of an ethylbenzene-degrading sulfate-reducing consortium. Water Research 36,2813–2823.

[149] Nebot, E., Romero, L.I., Quiroga, J.M. and Sales, D.(1995). Effect of the feed frequency on the performance of anaerobic filters. Anaerobe 1, 113–120.

[150] Nyerges, G., Han, S.K. and Stein, L. Y. (2010). Effects of ammonium and nitrite on growth and competitive fitness of cultivated methanotrophic bacteria. Applied Environmental Microbiology 76, 5648-5651.

[151] Okabe, S. and Watanabe, Y. (2000). Structure and function of nitrifying biofilms as determined by in situ hybridization and the use of microelectrodes.Water Science Technology 42, 21–32.

[152] Onda, S., Hiraishi, A., Matsuo, Y. and Takii, S. (2002). Polyphasic approaches to the identification of predominant polyphosphate-accumulating organisms in a laboratory-scale anaerobic/aerobic activated sludge system. Journal of General Applied Microbiology 48:43–54.

[153] O'Reilly, J., Lee, C., Chinalia, F., Collins, G., Mahony, T. and O'Flaherty, V. (2010). Microbial community dynamics associated with biomass granulation in low-temperature (15 °C) anaerobic wastewater treatment bioreactors. Bioresource Technology 101, 6336-6344.

[154] Oswal, N., Sarma, P.M., Zinjarde, S.S. and Pant, A.(2002). Palm oil mill effluent treatment by a tropical marine yeast. Bioresource Technology 85,35–37.

[155] Palatsi, J., Illa, J., Prenafeta-Boldú, F. X., Laureni, M., Fernandez, B., Angelidaki, I. and Flotats, X. (2010). Long-chain fatty acids inhibition and adaptation process in anaerobic thermophilic digestion: Batch tests, microbial community structure and mathematical modeling. Bioresource Technology 101, 2243-2251.

[156] Parawira, W., Murto, M., Zvauya, R. and Mattiasson, B. (2006). Comparative performance of a UASB reactor and an anaerobic packed-bed reactor when treating potato waste leachate. Renewable Energy 31, 893–903.

[157] Patel, H. and Madamwar, D. (2002). Effects of temperature and organic loading rates on biomethanation of acidic petrochemical wastewater using an anaerobic upflow fixed-film reactor. Bioresource Technology 82, 65–71.

[158] Patil, S. S., Kumar, M. S. and Ball, A. S. (2010). Microbial community dynamics in anaerobic bioreactors and algal tanks treating piggery wastewater. Applied Microbiology Biotechnology 87, 353-363.

[159] Park, J .S. and Lee, C.H. (2005). Removal of soluble COD by a biofilm formed on a membrane in a jet loop type membrane bioreactor. Water Research 39, 4609–4622.

[160] Park, C., Bega, A., Unlu, C., Chadderton, R. A., McKean, W. R., Kohl, P. M., Hunt, J. A., Keaney, J., Willis, J. L. and Duran, M. (2010). Acetoclastic methanogens in an anaerobic digester could be susceptible to trace metal supplementation. Water Science Technology 62, 2905-2911.

[161] Pereira, M.A., Roest, K., Stams, A.J.M., Mota, M., Alves, M. and Akkermans, A.D.L. (2002). Molecular monitoring of microbial diversity in expanded granular sludge bed (EGSB) reactors treating oleic acid. FEMS Microbiology and Ecology 41,95–103.

[162] Pérez, M., Romero, L.I. and Sales, D. (1998). Comparative performance of high rate anaerobic thermophilic technologies treating industrial wastewater. Water Research 32, 559–564.

[163] Perez, M., Romero, L.I. and Sales, D. (2001). Organic matter degradation kinetics in an anaerobic thermophilic fluidized bed bioreactor. Anaerobe 7, 25– 35.

[164] Perez, M., Rodriguez-Cano, R., Romero, L.I. and Sales, D. (2007). Performance of anaerobic thermophilic fluidized bed in the treatment of cutting-oil wastewater. Bioresource Technology 98, 3456–3463.

[165] Phang, O. C. and Vadiveloo, J. (1991). Effects of varieties, botanical fractions and supplements of palm oil byproducts on the feeding value of rice straw in goats. Small Ruminant Research 6, 295–301.

[166] Pirc, E. T., Levstek, M. and Bukovec, P. (2010). Influence of Cyanide on the Anaerobic Degradation of Glucose. Water Science Technology 62, 1799-1806.

[167] Pierzynski, G.M., Sims, J.T. and Vance, G.F, (2005). Soils and Environmental Quality, CRC Press

[168] Pleanjai, S. H., Gheewala, S. and Garivait, S. (2004). Environmental Evaluation of Biodiesel Production from Palm Oil in a Life Cycle Perspective.The Joint International Conference on Sustainable Energy and Environment (SEE)" Hua Hin, Thailand pp. 1-3.

[169] Plumb, J. J., Bell, J. and Stuckey, D.C. (2001). Populations associated with treatment of an industrial dye effluent in an anaerobic baffled reactor. Applied Environmental Microbiology 67, 3225–3236.

[170] Poh, P.E. and Chong, M.F.(2009).Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment, Bioresource Technology 100 ,1- 9.

[171] Prasertsan, S. and Prasertsan, P. (1996). Biomass residues from palm oil mills in Thailand: an overview on quantity and potential usage. Biomass Bioenergy 11, 87-395.

[172] Ramasamy, E.V. and Abbasi, S.A.(2000). Energy recovery from dairy waste- waters:impacts of biofilm support systems on anaerobic CST reactors. Applied Energy 65, 91–98.

[173] Ramos, C. G., Grilo, A. M., Sousa, S. A., Barbosa, M. L., Nadais,H. and Leitao, J. H. (2010). A new methodology combining PCR,cloning, and sequencing of clones discriminated by RFLP for the study of microbial populations: application to an UASB reactor sample. Applied Microbiology and Biotechnology 85, 801-806.

[174] Reyes, O., Sánchez, E., Rovirosa, N., Borja, R., Cruz, M., Colmenarejo, M.F., Escobedo,R., Ruiz, M., Rodríguez, X. and Correa, O. (1999). Low-strength wastewater treatment by a multistage anaerobic filter packed with waste tyre rubber. Bioresource Technology 70, 55–60.

[175] Rocheleau, S., Greer, C. W., Lawrence, J,R., Cantin, C., Laramee, L and Guiot, S.R. (1999). Differentiation of Methanosaeta concilii and Methanosarcina barkeri in anaerobic mesophilic granular sludge by fluorescent in situ hybridization and confocal scanning laser microscopy. Applied Environmental Microbiology 65,2222–2229.

[176] Roest, K., Heilig, H.G., Smidt, H., de Vos, W.M., Stams, A.J.M. and Akkermans, A.D.L. (2005). Community analysis of a full-scale anaerobic bioreactor treating paper mill wastewater. Systematic Applied Microbiology 28, 175–85.

[177] Rosenberger, S.,Witzig, R., Manz, W., Szewzyk, U. and Kraume, M. (2000). Operation of different membrane bioreactors: experimental results and physiological state of the microorganisms. Water Science Technology 41,269–277.

[178] Ruiz, I., Veiga, M.C., de Santiago, P. and Blázquez, R. (1997). Treatment of slaughterhouse wastewater in a UASB reactor and an anaerobic filter. Bioresource Technology 60, 251–258.

[179] Rupani, P.F., Singh, R.P., Ibrahim, M.H. and Esa, N.(2010). Review of Current Palm Oil Mill Effluent (POME) Treatment Methods:Vermicomposting as a Sustainable Practice, World Applied Sciences Journal 11, 70-81.

[180] Russo, C., Sant' Anna Jr., G.L. and de Carvalho Pereira, S.E. (1985). An anaerobic filter applied to the treatment of distillery wastewaters. Agricultural Wastes 14, 301– 313.

[181] Sabalowsky, A. R. and Semprini, L. (2010a). Trichloroethene and cis-1,2-dichloroethene concentration-dependent toxicity model simulates anaerobic dechlorination at high concentrations. II:Continuous flow and attached growth reactors. Biotechnology Bioengineering 107, 540-549.

[182] Sabalowsky, A. R. and Semprini, L. (2010b). Trichloroethene and cis-1,2-dichloroethene concentration-dependent toxicity model simulates anaerobic dechlorination at high concentrations: I.batch-fed reactors. Biotechnology Bioengineering 107, 529-539.

[183] Sánchez, E., Borja, R., Travieso, L., Martín, A. and Colmenarejo, M.F.(2005). Effect of organic loading rate on the stability, operational parameters and performance of a secondary upflow anaerobic sludge bed reactor treating piggery waste. Bioresource Technology 96, 335–344.

[184] Santegoeds, C.M., Ferdelman, T.G., Muyzer, G, and de Beer, D. (1998). Structural and functional dynamics of sulfate-reducing populations in bacterial biofilms. . Applied Environmental Microbiology 64,3731–3739.

[185] Santegoeds, C.M., Damgaard, L.R., Hesselink, G., Zopfi, J., Lens, P. and Muyzer, G. (1999). Distribution of sulfate-reducing and methanogenic bacteria in anaerobic aggregates determined by microsensor and molecular analyses. Applied Environmental Microbiology 65,4618–4629.

[186] Sanz, J.L. and Kochling, T. (2007). Molecular biology techniques used in wastewater treatment: An overview, Process Biochemistry 42 ,119–133.

[187] Saravanane, R., Murthy, D.V.S. and Krishnaiah, K. (2001). Treatment of anti-osmotic drug based pharmaceutical effluent in an upflow anaerobic fluidized bed system.Waste Management 21, 563–568.

[188] Sayed, S., de Zeeuw, W. and Lettinga, G. (1984). Anaerobic treatment of slaughterhouse waste using a flocculant sludge UASB reactor. Agricultural Wastes 11, 197–226.

[189] Schauer-Gimenez, A. E., Zitomer, D. H., Maki, J. S. and Struble, C.A. (2010). Bioaugmentation for improved recovery of anaerobic digesters after toxicant exposure. Water Research 44,3555-3564.

[190] Schievano, A., D'Imporzano, G., Malagutti, L., Fragali, E., Ruboni, G. and Adani, F. (2010). Evaluating inhibition conditions in high-solids anaerobic digestion of organic fraction of municipal solid waste. Bioresource Technology 101, 5728-5732.

[191] Sekiguchi, Y., Takahashi, H., Kamagata, Y., Ohashi, A. and Harada, H. (2001). In situ detection, isolation, and physiological properties of a thin filamentous microorganism abundant in methanogenic granular sludges: a novel isolate affiliated with a clone cluster, the green non-sulfur bacteria, subdivision I. . Applied Environmental Microbiology 67, 5740–5749.

[192] Sen, S. and Demirer, G.N. (2003). Anaerobic treatment of real textile wastewater with a fluidized bed reactor. Water Research 37, 1868–1878.

[193] Sethupathi, S. (2004). Removal of residue oil from palm, oil mill effluent (POME) using chitosan, Universiti.Sains Malaysia.

[194] Shaji, J.P. (2000). Development of a high rate anaerobic reactor for biomethanation of cassava starch factory effluent. Ph.D. Thesis, TNAU, Coimbatore:Department of Bioenergy, College of Agricultural Engineering, India;

[195] Shaw, P. C. and Mark, K. K. (1980). Chironomid farming: a means of recycling farm manure and potentially reducing water pollution in Hong Kong. Aquaculture 21, 155–163.

[196] Shin, S. G., Han, G., Lim, J., Lee, C. and Hwang, S. (2010). A comprehensive microbial insight into two-stage anaerobic digestion of food waste-recycling wastewater. Water Research 44, 4838-4849.

[197] Shin, S. G., Lee, S., Lee, C., Hwang, K. and Hwang, S. (2010). Qualitative and quantitative assessment of microbial community in batch anaerobic digestion of secondary sludge. Bioresource Technology 101, 9461-9470.

[198] Show, K.Y. and Tay, J.H. (1999). Influence of support media on biomass growth and retention in anaerobic filters. Water Research 33, 1471–1481.

[199] Silvey, P., Pullammanappallil, P.C., Blackall, L. and Nichols, P. (2000). Microbial ecology of the leach bed anaerobic digestion of unsorted municipal solid waste. Water Science Technology 41, 9–16.

[200] Singh, G., Huan, L.K., Leng, T. and Kow, D.L.(1999). Oil palm and the environment.SDN. Bhd, Kuala Lumpur: Sp-nuda Printing,

[201] Singh, R.P., Hakimi, M. I. and Esa, N. (2010).Composting of waste from palm oil mill: A sustainable waste management practice. Review in Environmental Science and Biotechnology DOI:10.1007/s11157-010-9199-2.

[202] Sinnappa, S. (1978a). Studies of palm oil mill waste effluent. Malaysian Agricultural Journal 51, 261–272.

[203] Sinnappa, S. (1978b). Treatment studies of palm oil mill wastes water pollution. University Press Bangkok, Thailand, pp, 21-25.

[204] Song, M., Shin, S. G. and Hwang, S. (2010). Methanogenic population dynamics assessed by real-time quantitative PCR in sludge granule in upflow anaerobic sludge blanket treating swine wastewater. Bioresource Technology 101, S23-S28.

[205] Songeha, S.U. (1974). National work shop on Biogas Technology. Proceedings in Kuala Lumpur, organized by the Malaysian working Group on food waste materials, SIRM Univ. of Malaysia, pp. 60-81.

[206] Sowmeyan, R. and Swaminathan, G. (2008). Evaluation of inverse anaerobic fluidized bed reactor for treating high strength organic wastewater. Bioresource Technology 99, 3877–3880.

[207] Stafford, D.A. (1982). The effects of mixing and volatile fatty acid concentrations on anaerobic digester performance. Biomass 2, 43–55.

[208] Stronach, S.M., Rudd, T. and Lester, J.N. (1987). Start-up of anaerobic bioreactors on high strength industrial wastes. Biomass 13, 173–197.

[209] Stone, J. J., Clay, S. A. and Spellman, G. M. (2010). Tylosin and chlortetracycline effects during swine manure digestion:Influence of sodium azide. Bioresource Technology 101, 9515- 9520.

[210] Sundram, K., Sambanthamurthi, R., and Tan, Y. A. (2003).Palm fruit chemistry and nutrition. Asia Pacific Journal of Clinical Nutrition 12, 355–362.

[211] Suraruk, B.,Wangnai, C., Chaiprasert, P. and Tanticharoen, M. (1998). Performance of an anaerobic hybrid reactor combining a filter and a sludge bed. In: Proceeding of the 10th Annual General Meeting of the Thai Society for Biotechnology for a Self-Sufficient Economy. pp.94–102.520.

[212] Tan, Y. and Ji, G. (2010). Bacterial community structure and dominant bacteria in activated sludge from a 70 °C ultrasound – enhanced anaerobic reactor for treating carbazole-containing wastewater. Bioresource Technology 101, 174-180.

[213] Tay, J.H. (1991). Complete reclamation of oil palm wastes. Resources, Conservation and Recycling 5, 383–392.

[214] Toldrá, F., Flors, A., Lequerica, J.L. and Vallés, S. (1987). Fluidized bed anaerobic biodegradation of food industry wastewaters. Biological Wastes 21, 55–61.

[215] Tong ,S.L., Bakar Jaafar, A. (2004). Waste to energy: methane recovery from anaerobic digestion of palm oil mill effluent. ENERGY SMART- 14 KON: pp.11456.

[216] Tong, S.L. and Jaafar, A.B.(2006). POME Biogas capture, upgrading and utilization. Palm Oil Engineering Bulletin 78, 11–17.

[217] Torkian, A., Eqbali, A. and Hashemian, S.J. (2003). The effect of organic loading rate on the performance of UASB reactor treating slaughterhouse effluent. Resources, Conservation and Recycling 40, 1–11.

[218] Tsuneda, S., Miyoshim, T., Aoi, Y. and Hirata, A. (2000). Tailoring of highly efficient nitrifying biofilms in fluidized bed for ammonia-rich industrial wastewater treatment. Water Science Technology 42,357–362.

[219] Ueno, Y., Haruta, S., Ishii, M. and Igarashi, Y. (2001). Changes in product formation and bacterial community by dilution rate on carbohydrate fermentation by methanogenic microflora in continuous flow stirred tank reactor. Applied Microbiology and Biotechnology 57, 65–73.

[220] Ugoji, E.O. (1997). Anaerobic digestion of palm oil mill effluent and its utilization as fertilizer for environmental protection. Renewable Energy 10, 291– 294.

[221] Ulrich, G.D. and Vasudevan, P.T. (2004). Chemical Engineering Process Design and Economics: A Practical Guide, second ed. Process Publishing Company.

[222] Usoro, E .J. (1974). The Nigerian oil palm Industry Ibadan University Press, pp. 1-3.

[223] Vadiveloo, J. (1988). Performance of young indigenous and crossbred goats fed forages supplemented with palm oil mill effluent. Small Ruminant Research 1, 369–379.

[224] Vairappan, C.S. and Yen, A.M. (2008). Palm oil mill effluent (POME) cultured marine microalgae as supplementary diet for rotifer culture. Journal of Applied Physics 20, 153-158.

[225] Van Der Merwe, M. and Britz, T.J. (1993). Anaerobic digestion of baker's yeast factory

[226] effluent using an anaerobic filter and hybrid digester. Bioresource Technology 43, 169–174.

[227] Vázquez, I., Rodríguez, J., Marañón, E., Castrillón, L. and Fernández, Y.(2006). Simultaneous removal of phenol, ammonium and thiocyanate from coke wastewater by aerobic degradation. Journal of Hazardous Materials B 137,1773–1780.

[228] Vijayaraghavan, K., Ahmad, D. and Abdul Aziz, M.E., (2007). Aerobic treatment of palm oil mill effluent. Journal of Environmental Management 82, 24–31.

[229] Vlissidis, A. and Zouboulis, A.I. (1993). Thermophilic anaerobic digestion of alcohol distillery wastewaters. Bioresource Technology 43, 131–140.

[230] Wang, Z. and Banks, C.J. (2007).Treatment of a high-strength sulphate-rich alkaline leachate using an anaerobic filter. Waste Management 27, 359–366.

[231] Wang, X., Wen, X., Criddle, C.,Yan, H., Zhang, Y. and Ding, K. (2010). Bacterial community dynamics in two full-scale wastewater treatment systems with functional stability. Journal of Applied Microbiology 109, 1218-1226.

[232] Westerholm, M., Roos, S. and Schnurer, A. (2010). Syntrophaceticus schinkii gen. nov., sp. nov., an anaerobic, syntrophic acetateoxidizing bacterium isolated from a mesophilic anaerobic filter. FEMS Microbiology Letter 309, 100-104.

[233] Wiegant, W.M. and Lettinga, G. (1985). Thermophilic anaerobic digestion of sugars in upflow anaerobic sludge blanket reactors. Biotechnology and Bioengineering 27, 1603–1607.

[234] Wiegant, W.M., Classen, J.A. and Lettinga, G. (1985). Thermophilic anaerobic digestion of high strength wastewaters. Biotechnology and Bioengineering 27, 1374–1381.

[235] Wood, B.J. (1984). A review on current methods of dealing with palm oil mill Effluent. Malaysian University Press, Kuala Lumpur, pp,19-32.

[236] Wu, T.Y., Mohammad, A.W., Md. Jahim J. and Anuar, N. (2007). Palm oil mill effluent (POME) treatment and bioresources recovery using ultrafiltration membrane: effect of pressure on membrane fouling. Biochemical Engineering Journal 35, 309–317.

[237] Wu, T. Y., Mohammad, A. W., Jahim, J. M. and Anuar, N. (2009). A holistic approach to managing palm oil mill effluent (POME): biotechnological advances in the sustainable reuse of POME. Biotechnology Advances 27, 40–52.

[238] Xing, D., Ren, N., Gong, M., Li, J. and Li, Q. (2005). Monitoring of microbial community structure and succession in the biohydrogen production reactor by denaturing gradient gel electrophoresis (DGGE). Science China C Life Science 48,155–162.

[239] Xu, K., Liu, H. and Chen, J. (2010). Effect of classic methanogenic inhibitors on the quantity and diversity of archaeal community and the reductive homoacetogenic activity during the process of anaerobic sludge digestion. BioresourceTechnology 101, 2600-2607.

[240] Yacob, S., Hassan, M.A., Shirai, Y., Wakisaka, M. and Subash, S. (2005). Baseline study of methane emission from open digesting tanks of palm oil mill effluent treatment. Chemosphere 59, 1575–1581.

[241] Yacob, S., Hassan, M.A., Shirai, Y., Wakisaka, M. and Subash, S. (2006a). Baseline study of methane emission from anaerobic ponds of palm oil mill effluent treatment. Science of the Total Environment 366, 187–196.

[242] Yacob, S., Shirai, Y., Hassan, M.A., Wakisaka, M. and Subash, S. (2006b). Start-up operation of semi-commercial closed anaerobic digester for palm oil mill effluent treatment. Process Biochemistry 41, 962–964.

[243] Yacob , S., Shirai, Y. and Ali, H. M. (2009). Treatment of palm oil wastewaters. Waste treatment in the food processing industry. CRC Press

[244] Yeoh, B.G. (2004). A technical and economic analysis of heat and power generation from biomethanation of palm oil mill effluent. Electricity Supply Industry in Transition: Issues and Prospect for Asia Conference, Thailand. Citeseer pp.14-16.

[245] Yeong, S. W., Syed Ali, A. B. and Faizah, M. (1980). the nutritive value of palm oil mill effluent product (PROLIMA) as a protein source in broiler diets. MARDI Res. Bulletin 8, 247–259.

[246] Yilmaz, T., Yuceer, A. and Basibuyuk, M. (2008). A comparison of the performance of mesophilic and thermophilic anaerobic filters treating papermill wastewater. Bioresource Technology 99, 156–163.

[247] Yu, H. and Gu, G. (1996). Biomethanation of brewery wastewater using an anaerobic upflow blanket filter. Journal of Cleaner Production 4, 219–223.

[248] Yu, H.Q., Hu, Z.H., Hong, T.Q., and Gu, G.W. (2002a). Performance of an anaerobic filter treating soybean processing wastewater with and without effluent recycle. Process Biochemistry 38, 507–513.

[249] Yu, H.Q., Fang, H.H.P. and Gu, G.W.(2002b). Comparative performance of mesophilic and thermophilic acidogenic upflow reactors. Process Biochemistry 38, 447– 454.

[250] Yuliwati, E., Ismail, A.F., Lau, W.I., Ng, B.C., Mataram, A. and Kassim , M.A. (2012). Effects of process conditions in submerged ultrafiltration for refinery wastewater treatment: Optimization of operating process by response surface methodology, Desalination 287, 350–361.

[251] Yusoff, M. F., Om, A. D. and Cheah, S. H. (1996). Use of agroindustrial effluent in augmenting microalgae production and fish fry growth in hatchery tanks. Journal of Aquaculture Tropics 11, 119–126.

[252] Yusoff, S. and Hansen, S. B.(2007). Feasibility study of performing a life cycle assessment on crude palm oil production in Malaysia. International Journal of Life Cycle Assessment 12, 50–58.

[253] Zakaria, M. R., Tabatabaei, M., Ghazali, F. M., Abd-Aziz, S., Shirai, Y. and Hassan, M. A. (2010). Polyhydroxyalkanoate production from anaerobically treated palm oil mill effluent by new bacterial strain Comamonas sp. EB172. World Journal of Microbiology and Biotechnology 26, 767-774.

[254] Zhang, T., Ke, S.Z., Liu, Y. and Fang, H.P. (2005). Microbial characteristics of a methanogenic phenol-degrading sludge. Water Science Technology 52, 73–78.

[255] Zhou, S., Wei, C., Liao, C. and Wu, H. (2010). Comprehensive study on dynamics of microbial community in Anaerobic-Oxic-Oxic process using PCR-DGGE, gas chromatography analysis, and dehydrogenase activity assays. World Journal of Microbiology and Biotechnology 26, 273-279.

[256] Zinatizadeh, A.A.L., Mohamed,A.R., Najafpour, G.D., Isa, M.H. and Nasrollahzadeh, H. (2006a). Kinetic evaluation of palm oil mill effluent digestion in a high rate up-flow anaerobic sludge fixed film bioreactor,Process Biochemistry 41,1038–1046.

[257] Zinatizadeh, A.A.L., Mohamed, A.R., Abdullah, A.Z., Mashitah, M.D., Hasnain Isa, M. Najafpour, G.D.(2006b). Process modeling and analysis of palm oil mill effluent treatment in an up-flow anaerobic sludge fixed film bioreactor using response surface methodology (RSM). Water Research 40, 3193–3208.

[258] Zinatizadeh, A.A.L., Mohamed, A.R., Mashitah, M.D., Abdullah, A.Z. and Isa, M.H. J. (2007a). Optimization of pre-treated palm oil mill effluent digestion in an up-flow anaerobic sludge fixed film bioreactor: a comparative study. Biochemical Engineering Journal 35,226–237.

[259] Zinatizadeh, A.A.L., Salamatinia, B., Zinatizadeh, S.L., Mohamed, A.R. and Hasnain Isa, M. (2007b). Palm oil mill effluent digestion in an up-flow anaerobic sludge fixed film bioreactor. International Journal of Environmental Research 1,264–271.

AUTHORS

First Author – Jeremiah David Bala, Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia (USM), 11800, Pulau, Penang, Malaysia. Jerrybrown316@yahoo.com

Second Author – Japareng Lalung, Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia (USM), 11800, Pulau, Penang, Malaysia. japareng@usm.my

Correspondence Author – Norli Ismail, Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia (USM), 11800, Pulau, Penang, Malaysia. norlii@usm.my; drnorliismail@gmail.com +6046532824