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

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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 usable 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.
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 and Chong, 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, 1999a); up-flow anaerobic filtration (Borja and Banks, 1999b); 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 Pleanajit 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 deteriorate 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 fresh 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 world’s 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 ignored. 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.,
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, 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)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>80-90</td>
</tr>
<tr>
<td>pH</td>
<td>4.7</td>
</tr>
<tr>
<td>Biochemical Oxygen Demand BOD; 3days at 30 °C</td>
<td>25,000</td>
</tr>
<tr>
<td>Chemical Oxygen Demand</td>
<td>50,000</td>
</tr>
<tr>
<td>Total Solids (T.S)</td>
<td>40,500</td>
</tr>
<tr>
<td>Total Suspended Solids (T.S.S)</td>
<td>18,000</td>
</tr>
<tr>
<td>Total Volatile Solids (T.V.S )</td>
<td>34,000</td>
</tr>
<tr>
<td>Oil and Grease (O&amp;G)</td>
<td>4,000</td>
</tr>
<tr>
<td>Ammonia-Nitrate (NH$_3$-N)</td>
<td>35</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen (TKN)</td>
<td>750</td>
</tr>
</tbody>
</table>

*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)

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

* 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

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

www.ijsrp.org
Evaporation Solid concentrate from process can be utilized as feed material for fertilizer manufacturing.


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, (Ulrich 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 (Metcalfe 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 digesting 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 efficiency with 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 (García-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 (García-Calderon et al., 1998; Sowmeyan and Swaminathan, 2008); brewery wastewater (Alvarado-Lassman et al., 2008); ice-cream wastewater (Borja and Banks, 1995ab; 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 (García-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 apparent that 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 (Moham 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/m²day (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 (acidogenesis 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/m²day 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 Ganjipour (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 shown in Table 4.
### Table 4: Advantages and disadvantages of various types of anaerobic treatment methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantage</th>
<th>Disadvantages</th>
<th>References</th>
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<tr>
<td>Conventional anaerobic digestion (pond and digester)</td>
<td>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.</td>
<td>Large volume for digestion. Long retention times. No facilities to capture biogas. Lower methane emission.</td>
<td>Chan and Chooi (1984).</td>
</tr>
<tr>
<td>Fluidized bed</td>
<td>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.</td>
<td>High power requirements for bed fluidization. High cost of carrier media. Not suitable for high suspended solid wastewaters. Normally does not capture generated biogas.</td>
<td>Leslie Grady et al. (1999).</td>
</tr>
<tr>
<td>CSTR</td>
<td>Provides more contact of wastewater with biomass through mixing. Increased gas production compared to conventional Method.</td>
<td>Less efficient gas production at high treatment volume. Less biomass retention.</td>
<td></td>
</tr>
<tr>
<td>Anaerobic contact process</td>
<td>Reaches steady state quickly.</td>
<td></td>
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<tr>
<td></td>
<td>Short hydraulic retention time.</td>
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<tr>
<td></td>
<td>Produces relatively high effluent quality.</td>
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<td></td>
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<tr>
<td>Settleability of biomass is critical to successful performance.</td>
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VII. FACTORS AFFECTING ANAEROBIC DIGESTER PERFORMANCE

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; Patil 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 acetate 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 °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 °C was higher than operation at 37 °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

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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 Madanwar, 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 (Simappa, 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 (Simappa, 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 al., 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 H₂ 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 (Daums 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 et al., 1999) or differentiation between hydrogenotrophic and acetoclastic methanobacteria, and within this group between Methanoseta 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 al., 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

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<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
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| 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. | 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 are not quantitative. | 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.

<table>
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<tr>
<th>Fluorescent in situ hybridization (FISH)</th>
<th>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).</th>
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<tr>
<td>Easy and fast if required probes are available.</td>
<td>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).</td>
</tr>
<tr>
<td>Allows direct visualization of non-cultured microorganisms.</td>
<td>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).</td>
</tr>
<tr>
<td>Generally quantitative.</td>
<td>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.</td>
</tr>
<tr>
<td>Quantification of specific microbial groups is also possible, in contrast to conventional techniques (most probable number, plate counts) or other molecular techniques.</td>
<td>Quantification can be tedious and subjective (manual counting) or complex (image analysis).</td>
</tr>
<tr>
<td>Differential/preferential detection of active microorganisms.</td>
<td>Structural analysis of aggregates (granular sludge, biofilms) requires a confocal microscope and an image analysis environment (expensive, trained personnel necessary).</td>
</tr>
<tr>
<td>Apt for routine use, highly trained and specialized personnel is not necessary, only a basic knowledge of microscopy and laboratory experience are required.</td>
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**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 thermopallidum 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. thermopallidum 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°C. Gas production, organic acid consumption and methanogenic population were tracked in a maize silage reactor operating at 37°C (Blume et al., 2010). Hydrogenotrophic Methanobacterials dominated at OLRs equal or greater than 3.7 g-DOM/(L·d). In contrast, aceticlastic Methanosaetaeaceae 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 25-40°C 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 acetilastic Methanosarcinaceae and 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°C, while rods and coccoids dominated at 60°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). Lentisphaeraeae dominated the community with 98% of the clone library sequences.

Capacity of anaerobic wastewater treatment bioreactors to form biomass granules was tested at 15°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.

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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 *Methanoculleus* dominated a mesophilic reactor. Kim et al. (2010) characterized a mesophilic sludge used for thermal acidogenesis of swine wastewater at 51°C. The DGGE profiles indicated that *Pseudomonas mendocina*, *Bacillus halodurans*, *Clostridium hastiforme*, *Gracilbacter 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 biofluidized 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 10^5 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 *Methanosetaecaeae* 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 *Methanosaricinales* or methanogenesis (Hwang et al., 2010). The effect of nitrite and ammonium on two methanotrophic bacteria, *Methylococcus albus* and *Methylocystis* sp., was tested (Nyerges et al., 2010). *M. albus* 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 µM 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. Álvarez 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 Ca²⁺ leaching was observed in granular sludge samples in the presence of 20-g Na²/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 H₂ 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 celluloletic 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 been 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 thermophilic 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.

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