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Proper Treatment and Energy Recovery through Codigestion of Two Phase Olive Mill Waste

オリーブオイル残渣の混合メタン発酵による適正処理とエネルギー回収に関する研究

By

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ABSTRACT

Many agro-food industries produce a huge amount of waste in a form of biomass as a byproduct. Improper disposal of this waste can cause serious environmental problems. Olive milling is one of the main agro-food industries in the Mediterranean region. The short period of olive production leads to the uncontrolled discharge of the olive milling waste to the environment, causing serious environmental problems.

The two phase olive mill waste (2POMW) is the semisolid wet residue produced from the extraction of olive oil using two phase centrifugation. The doughy structure of this waste makes its storage and disposal difficult and can cause serious environmental problems because of its phytotoxicity and high organic content. Anaerobic digestion (AD) is an attractive process for the treatment of liquid and semisolid residues such as the 2POMW. In addition to the stabilization of the waste, energy can be recovered. In this study the main objective is to develop a process of anaerobic digestion in order to achieve both proper treatment and energy recovery for the 2POMW. Anaerobic digestion of the 2POMW is limited by the presence of lignocellulosic compounds; therefore, the first objective of this study is to enhance the hydrolysis of the 2POMW by a pretreatment step before AD. In addition, anaerobic digestion of the 2POMW as a sole source is limited by the presence of inhibitors, mainly long chain fatty acids (LCFA). This limitation can be overcome by digestion with another source of waste (food waste); codigestion dilutes the high concentration of inhibitors. Therefore the second objective of this study is to investigate the codigestion conditions, e.g., the mixing ratio which is required for the design of AD plants.

For the first objective, a series of batch experiments were performed to evaluate the effect of pretreatment. The pretreatment conditions performed were (1) mechanical pretreatment by size reduction, (2) alkaline pretreatment with different concentrations of NaOH (2.4, 6, 10, 20 and 30%) and (3) alkaline pretreatment with different concentrations of CaO (2.4, 6, 10, 20 and 30%). Following the pretreatment, anaerobic digestion was conducted in batch mode (using 200 mL vial with effective volume of 100 mL, 37 ºC) for 26 days. The effect of pretreatment on the amount of soluble organic compounds (represented as soluble chemical oxygen demand (sCOD)) and on methane production was determined. Since mechanical pretreatment had no effect on the sCOD, no improvement in methane production was observed. On the other hand, NaOH was able to solubilize part of the organic material. NaOH increased the concentration of sCOD, while the highest increase in the sCOD was for the 20% NaOH. Regarding methane production, when a loading rate of 0.88 ($gVS_{substrate}/gVS_{inoculum}$) of the NaOH pretreated 2POMW was applied without any pH control, the 6% NaOH pretreatment showed better performance than other treatments. The 20% NaOH pretreatment caused inhibition because of the high pH level inside the reactor (pH > 8.4). If pH is controlled, it is expected that methane production would increase. Degradation of the 2POMW by CaO was not sufficient to increase the sCOD, therefore, methane production from the CaO pretreated 2POMW was less compared with the NaOH pretreatments. Considering a full scale reactor system receiving food waste as a main substrate and the NaOH-treated 2POMW as a co-substrate, the NaOH concentration of 20% might be sufficient regarding the sCOD concentration. It was also expected that too large amount of sCOD in the loading rate might inhibit the AD process because of the production of volatile fatty acid (VFA).

Continuous reactor experiment (reactor with effective volume of 6 L, 37 ºC, hydraulic retention time of 30 days) for codigesting 2POMW with food waste was conducted. This study focused on investigating the mixing ratio of 2POMW to food waste in order to control the concentration of LCFA inside the reactor for a stable digestion process without inhibition. Mixing ratios of 3%, 4.3%, 5.7% and 8.3% were tested, considering the general total loading rate in COD. With increasing the mixing ratio, the organic loading rate as sCOD is also increasing, causing inhibition as expected before. To reduce the effect of sCOD on the AD process, the 2POMW used was with lower NaOH pretreatment for high mixing ratios, thus the organic loading rate as sCOD that was daily introduced into all reactors will be in the same level. There was no inhibition of methane gas production up to a mixing ratio of 4.3%; however, increasing the mixing ratio lead to higher oleic acid (the main LCFA in 2POMW) concentration and reduced methane gas production. Treatments of 10% NaOH-2POMW with 4.3% mixing ratio and 20% NaOH-2POMW with 3% mixing ratio were shown to be adequate concerning oleic acid concentration and methane gas production. Those treatments caused an increase in methane gas production by 548.5 mL/g-VS and 445.3 mL/g-VS respectively compared with the case of applying only food waste.

Our proposal of codigestion process of the 2POMW and food waste was applied to the existing biogas plant in Jordan which receives 60 t/d of food waste from different sources as a case study. This plant has the potential to produce 309 MWh of electricity per month. Applying 10% NaOH pretreated 2POMW in a mixing ratio of 4.3% (which showed the highest methane production in our study) during the five months of olive oil production can produce an additional 72.5 MWh per month, increasing the plant electrical production by 23.5%. This study showed that 2POMW, which has been illegally dumped, can be properly treated and that 2POMW can be a new renewable energy source for the existing biogas plant to recover additional energy significantly.

In conclusion, this study showed that NaOH pretreatment is an effective method to solubilize the lignocellulosic fraction of the 2POMW and to enhance methane production. Since the mono-digestion of 2POMW is limited by the high concentration of LCFA, this study proposed a codigestion system of 2POMW with Food waste. This study investigated the mixing ratio of 2POMW with food waste which is the main factor that controls the anaerobic digestion process. This study proposed a practical method for 2POMW to be successfully treated and converted to energy source by a combination of pretreatment and codigestion with food waste.

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INTRODUCTION

1.1 The motivation of the study

Many agro- food industries produce huge amount of waste in a form of biomass as a byproduct. Irregular disposal of this waste can cause a serious harm to the environment. However, such waste has received much attention as a renewable energy source. Recently, protecting the environment through a safe disposal of waste has gained much attention in order to guarantee a sustainable environment and support future needs. Moreover, the utilization of biowaste for energy production will decrease the dependency on the limited non-renewable energy sources.

Olive milling industry is one of the main industries in the Mediterranean region. The short period of olive oil production (November to March) leads to the uncontrolled discharge of the olive mill wastes to the environment. Mekki et al. (2007) reported the phytotoxic effect of olive mill wastewater (OMW) on soil microorganisms. Its negative impact on soil structure and composition has been shown as well. Moreover, the negative effect of OMW on aquatic ecosystem was studied (Karaouzas et al., 2011). Therefore, a safe disposal of this waste is needed.

There was a shift in olive oil production system from 3-phase extraction to 2-pahse extraction. This shift reduced the amount of olive mill waste water produced; however, it generates another kind of waste called two phase olive mill waste (2POMW). Since the shift to 2-phase extraction is continuing, this study attempted to find a proper treatment option for the 2POMW.

Anaerobic digestion (AD) is an environmentally sustainable process for the environment and its suitability for treating several wastes such as food waste (Kawai et al., 2014), manure (Rico et al., 2011) and other wastes has been proven. Anaerobic

digestion is a biological process in which complex organic materials are broken down into simpler compounds by microbes under anaerobic condition.
In order to achieve an optimum anaerobic digestion of 2POMW, several factors have to be considered. The anaerobic digestion of 2POMW can be inhibited by the presence of phenolic compounds and long chain fatty acids. Moreover, the lingocellulosic structure limits its biodegradability as it slows down the hydrolysis step. Therefore, this study tries to overcome these limitations and the energy recovery from the 2POMW.

In Jordan, Olive milling is one of the main industries. There are over 15 million olive tree producing around 105,000 t of olive. Pressing this olive generates huge amount of olive pomace and olive mill waste water. Up to date there is no proper disposal way which creates a serious environmental problem in the country. As well, regarding energy Jordan is considered as one of the poorest countries. Utilizing the 2POMW as an input for anaerobic digestion process has a dual advantage in protecting the environment and as an additional source of renewable energy. However the short period of oil production (5 months) is one of the main drawbacks of the process. Therefore, finding a proper way of utilizing the 2POMW in anaerobic digester, while keeping a whole year around reactor operation, will be addressed in this study.

1.2 Objectives of the study

Since hydrolysis of lignocellulosic material is a rate limiting step for anaerobic digestion, optimizing this step can improve the overall efficiency of the process and increase its economic profitability. The 2POMW contains significant amounts of lignocellulosic material. Several studies have shown that alkaline pretreatment facilitates the degradation of lignocellulosic materials such as wheat and corn and causes a reduction in the lignin and hemicellulose content (Monlau et al., 2012; Liang et al., 2013 and Zhu et al., 2010). Therefore, the objective of this study is to examine the effect of mechanical (size reduction) and alkaline (using NaOH and CaO) pretreatments on biogas production and to investigate the pretreatment conditions for a more effective biogas conversion from the 2POMW.

The anaerobic digestion of 2POMW is limited by the presence of toxic compounds such as oleic acid (a long chain fatty acids). This limits its application as a sole source for anaerobic digesters. Therefore, the second objective of this study is to propose a system in which food waste is treated in a digester that receives 2POMW and to investigate the codigestion conditions mainly the mixing ratio which is required for the design of AD plants.

Our proposal of codigestion process for the 2POMW and food waste was applied to the existing biogas plant in Jordan which receives food waste as case study to show that proper treatment and energy recovery can be achieved for the 2POMW.

1.3 Thesis overview

The following chapter (**chapter 2**) gives an insight about the olive oil production and the environmental problems associated with it. The major three processes of olive oil extraction were identified, that will help the reader to better understand the different kinds of waste produced from different olive oil processing techniques. The current disposal practices and the different treatment options were illustrated. An experiment was conducted to determine the effect of mechanical and alkaline pretreatment. That was discussed in **chapter 3** which reviewed some papers related to the anaerobic digestion and pretreatment of different lignocellulosic materials. The results of a series of batch experiments of different pretreatment conditions are shown and the effect of different pretreatment conditions on CH_4 production was clarified. In **chapter 4** the results from chapter 3 were used to investigate the codigestion conditions of food waste with the NaOH pretreated 2POMW. In **Chapter 5** the applicability of co-digesting 2POMW with food waste in the already existing biogas plant in Jordan was investigated. Finally **Chapter 6** shows the general conclusion of this research.

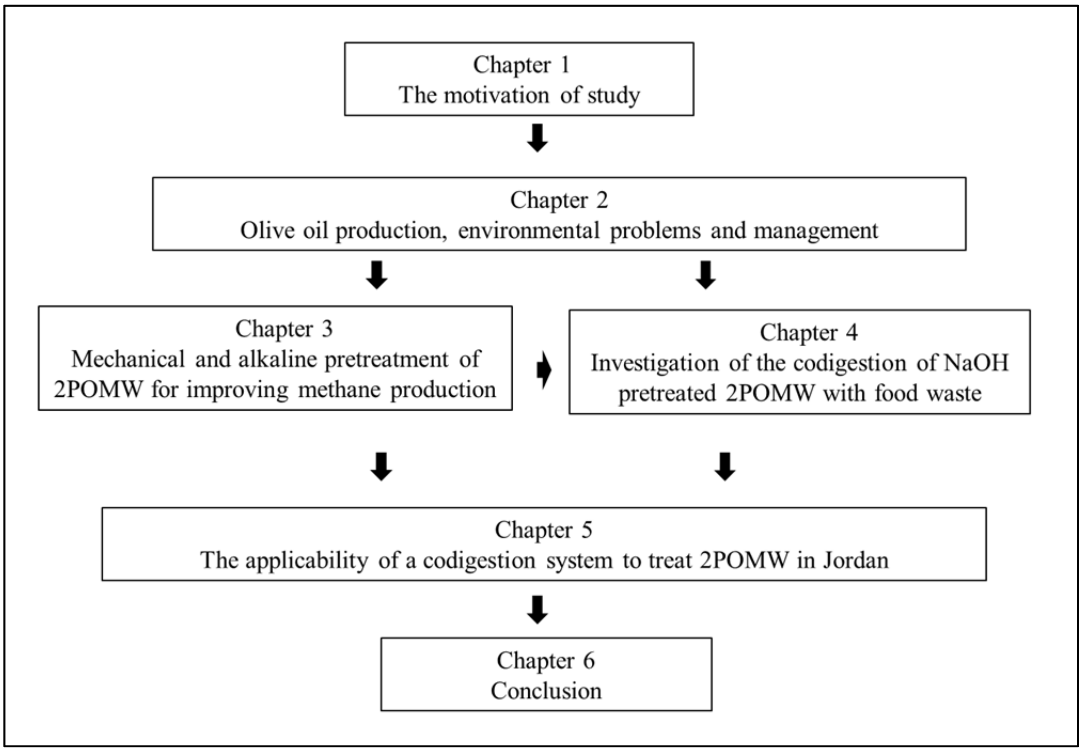

Fig. 1.1 The structure of the thesis

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CHAPTER 2

OLIVE OIL EXTRACTION WASTE: PRODUCTION, ENVIRONMENTAL RELATED ISSUES AND MANAGEMENT

2.1 Olive oil production and environmental consequences

Cultivation of olive trees is a common practice in all Mediterranean countries. The extraction of olive oil has a great economic and social importance in many countries. 99% of the olive oil production comes from the countries around the Mediterranean and the Middle East.

Spain is the first olive producing country with 2,400,000 ha is covered with trees, followed by Italy (1,140,685 ha) and Greece (765,000 ha). For the year 2004, olive oil production was 2,564,800 metric tons. The highest was for Spain with 978,700 tons, followed by Italy 633,700 ton, Greece 405,600 ton, Syria 125,800 ton, Tunisia 111,200 ton and Turkey 112,500 ton. (Niaounakis & Halvadakis 2006)

This industry is often associated with the generation of huge amount of byproducts that can induce adverse environmental problems on soil and water bodies due to their high phytotoxicity and organic carbon load. The effect of untreated olive mill wastewater on soil and water bodies is discussed elsewhere (Dermeche et al., 2013). Processing 100 kg of olives produces 35 kg of solid waste (olive pomace) and between 55 and 200 L of liquid waste (OMW) depending on the oil extraction process (Niaounakis el ta al., 2006). Therefore, finding an environmental friendly way of olive mill waste disposal is important.

Olive oil production is not a new process and has been practiced for a long time. However, in the last few decades disposal of olive mill wastes has been considered as a serious problem. This can be due to (1) The larger production size of olive oil, (2) the conversion from pressing to three- phase centrifugation which produces larger amounts of olive mill wastewater and (3) the exclusion of olive mill personnel from the decision making process (Kapellakis et al, 2006)

2.2 Olive oil extraction systems

2.2.1 Traditional pressing

This extraction process was used in the old days. In spite of its replacement with other methods, it is still used in some countries. This method involves crushing the wasted olives under a stone wheel until turning into paste. The paste is spread in mats and the mats are layered with alternative layers of metal disks. Then mats are put under pressure and squeezed to separate the solid residue (olive pomace) from the liquid (waste and oil). Finally the oil is separated by decanter. A small quantity of cold water is added to easily separate the oil from the other phases (Dermeche et al., 2013)

This process is characterized by its simplicity, technical feasibility and the small amount of olive mill waste water produced. Processing 1 ton of olive produces from 0.4 to 0.6 m^3 of OMW (Dermeche et al., 2013) and 400 kg olive pomace.

2.2.2 Three phase centrifugation

In order to increase olive oil yield, in the 1970s three phase centrifugation with higher efficiency and capacity was introduced. This process involves an initial decantation phase, which involves washing and grinding the olives to form pomace. The beaten olive paste is then made more fluidized by adding from 0.6 to 1.3 L of hot water per kilogram of olive (Alburquerque et al., 2004). Centrifugation is applied to separate the solid (olive pomace) from the liquid (waste water and oil), this is followed by a second decantation phase uses a vertical centrifuge to separate the olive oil from the vegetable water.

Three phase centrifugation has many advantages over traditional pressing: complete automation, smaller area needed, and higher efficiency. On the other hand, the major constraints of this extraction process are the use of large amount of fresh water and the production of large amount of waste water (Roig et al., 2006).

Extraction of one ton of olive generates 210 kg of olive cake, 1- 1.6 m^3 OMW (Alburquerque et al., 2004).

2.2.3 Two phase centrifugation

In order to reduce water consumption in the milling process and to reduce production of waste water, two phase centrifugation was introduced in the beginning of the 1990s. This process uses horizontal mounted centrifuge for primary separation of the olive oil from the vegetable solid and water. The process is the same as 3-phase, with the difference that instead of adding fresh water for horizontal centrifugation, the fruit water is recycled in a closed loop system. Recycling of fruit water increases the level of polyphenol, and so strengthens their biotic capacity as natural protectors against oxidation.

Since no water is used for olive oil processing, only small amount of waste water is produced; Extraction of one ton of olive generates 0.2 m^3 OMW. However, in this process liquid and solid residue come together to form a more humid pomace called two phase olive mill waste (2POMW) (IMPEL). Processing one ton of Olive produces 800 kg of 2POMW (Alburquerque et al., 2004).

The mass balance of the 3 processes is described in table 2.1:

Table 2.1. Approximate Input-Output Data for the Three Types of Olive Oil Production Processes (Azbar et al., 2004)

Production process	Input	Output	Amount of input	Amount of output
Traditional	Olive	Oil	1 ton	200 kg
	Washing water	Solid waste (25% water + 6% solid and oil)	0.1- 0.12 m^3	400 kg
	Energy	Wastewater (88% water + solid and oil)	40-63 kWh	400 m^3
Three-phase process	Olive	Oil	1 ton	200 kg
	Washing water	Solid waste (50%water + 4 % oil)	0.1- 0.12 m^3	500- 600 kg
	Fresh water for decanter	Wastewater (94% water + 4% oil)	0.5-1 m^3	1000-1200 m^3
Two phase process	Olives	Oil	1 ton	200 kg
	Washing water	Solid +water (60% water + 3% oil)	0.1-0.12 m^3	800-950 kg
	Energy		< 90- 117 kWh	

2.3 Types and characteristics of olive mill wastes

Three kinds of wastes generate from olive milling industry:

2.3.1 Olive mill waste water

OMW generates from the traditional pressing and the three phase centrifugation. The OMW comes from three phase centrifugation consists of the vegetable water in the olive fruit, the washing water before its crushing and the processing hot water added during oil extraction. In case of traditional pressing, only small amount of water is added, therefore, the pressing system results in a wastewater more concentrated in pollutants compared to the three-phase system (Azbar et al., 2004).

The chemical properties of the OMW depend on the extraction method, climate and olive variety. OMW is characterized by a violet dark brown color, low pH, and high electrical conductivity, and high phenolic content (Niaounakis & Halvadakis, 2006)

2.3.2 Olive pomace (olive husk)

Olive pomace is the solid residue produced after oil extraction using both the traditional pressing and the three phase centrifugation. Olive pomace contains crushed stones, skin, pulp and residual oil. Olive pomace from pressing has low moisture of about 25% (Niouakis & Halvadakis, 2006) while the three phase olive pomace is quiet richer in water 30%- 50% (Azbar et al., 2004)

2.3.3 Two phase olive mill waste

2POMW is the semi- solid wet residue from the extraction of olive oil using two-phase centrifugation, and it is a mixture of vegetable water from the olive fruit and solid waste.

The 2POMW is a thick sludge that in addition to the pulp, skin, crushed seed, it contains the vegetation water to increase the moisture content of the 2POMW to reach values between 55 and 70% (Niouakis & Halvadakis, 2006).

As mentioned before, this study is considering the treatment of 2POMW. The chemical composition of the 2POMW is presented in table 2.2. The 2POMW contains high amount of lignocellulosic compounds which limits its biological degradation. After extraction of olive oil some oil is still remained in the 2POMW. In addition the 2POMW contains high amount of toxic compounds such as phenolic compounds

Table 2.2. The main components of the organic fraction of 2POMW (dry bases) (Alburquerque et al., 2004)

Parameter	Range
Oil (%)	7.8-19.4
Cellulose (%)	14- 24
Hemicellulose (%)	27.3- 41.6
Lignin (%)	32.3- 55.7
Water soluble phenols (%)	0.6- 2.39
Total organic matter (g/kg)	848.9- 976

2.4 Current practices in olive mill wastes disposal in major olive oil producing countries

In Spain dumping olive mill wastewater in rivers has been forbidden since 1982. Since then, around 100 evaporation ponds were constructed to handle the produced OMW. After the switch to two phase centrifugation, the 2POMW is the major waste produced from the extraction process; about 4 million ton is produced every year. The produced 2POMW is usually treated by drying and second extraction with solvent. Polycyclic aromatic hydrocarbon was detected in the produced oil of the second extraction as a result of the drying process; this forced the producers to take some measures which increased the production cost. Finally the generated residue can be used for combined heat and power (CHP). The problem of 2POMW disposal has not been fully resolved and research is needed to enhance profitable use of it (Alburquerque et al., 2004).

Italy is the only country that has legislation for disposal and recycling of olive mill wastewater, Legislation (law No. 574/1996) for disposal of OMW in agricultural soil. For example, spreading of up to 80 m^3 ha^{-1} year $^{-1}$ OMW (generated by the continuous centrifugation system) is allowed in Italy (Ouzounidou et al, 2010). Therefore, the main application of OMW is spreading on lands. Olive cake is either sold, for example to mills as energy source or disposed (Niaounakis and Halvadakis, 2006). All the produced OMW is discharged to soil without treatment. Of the olive pomace 30% is used for composting while the remaining 70% undergoes second oil extraction (IMPEL 2003)

In Greece the management of the olive waste is not subjected to a specific regulation. Generally, waste water is neutralized with lime and disposed in evaporation ponds (Kavvadias et al 2010). Stamatakis (2010) mentioned that 58% of the waste is neutralized with lime before reaching natural water. Mostly, evaporation ponds are not in a proper design and so can result in soil and groundwater contamination. In addition, there are some cases of disposal in sea and rivers (Kavvadias et al., 2010). Of the three phase extraction 58% is treated and then disposed in creeks, of the pressing 58% is treated and disposed in creeks, 11.5% is discharged in rivers or sea and 19.5% is discharged to soil after treatment (IMPEL, 2003).

2.5 Treatment of olive milling wastes

The major constrains encountered when handling treatment of olive mill waste are: (1) The seasonal olive oil production (2) Olive production varies significantly from year to year, (3) olives are collected and extraction is conducted in the winter months (early November to March) and (4) Oil is extracted in a number of scattered mills (McNamara et al 2008).

Roig et al (2006) and McNamara et al (2008) reviewed several disposal and treatment options of olive mill wastes, such as evaporation, direct application to soil, animal feed, combustion, gasification, and biological treatment, such as composting, composting and anaerobic digestion.

The use of evaporation open ponds is the disposal method of OMW which is mostly adopted in many countries, but it has lots of limitations (1) bad odor (2) methane emission (3) infiltration into the soil (4) the need of large area (Roig et al., 2006).

The presence of proteins, minerals, and polysaccharides in OMW means that it has a potential to be used as a fertilizer or for irrigation. However, the abundance of phenolic compounds that are both antimicrobial and phytotoxic, causes phytotoxic and bio- toxic affect that limits the use of OMW as a fertilizer or irrigation water. (Mekki et al., 2007)

Exhausted olive cake (after drying and removal of residual oil) has a lower calorific value of 3922- 4445 kcal/kg (Azbar et al., 2004). Several studies have shown the potential of olive cake for energy generation through combustion (Abu-Qdais 1996; Alkhamis and Kablan 1996). However, high energy may be needed for the drying of the olive pomace before combustion

2.6 Alternative treatment option: Anaerobic digestion

Anaerobic digestion is a biological process in which organic matter is transformed to methane under anaerobic conditions. Methane can be used for energy to replace fossil fuels and thereby reducing carbon dioxide emissions. Anaerobic digestion is advisable because of its well-known advantages related to energy production and producing small amount of sludge. The high loading rate of olive mill waste makes anaerobic digestion a feasible alternative disposal method. In addition, the sludge remains after the codigestion of olive mill wastes is rich with mineral elements, which makes it useful for land application as a fertilizer (Fezzani and Cheikh 2007).

2.6.1 The process of anaerobic digestion

Anaerobic digestion (AD) is a series of biological processes in which microorganisms break down biodegradable material in the absence of oxygen. AD occurs in 4 steps (1) hydrolysis, where complex organic polymers such as proteins, fats and carbohydrates are broken down by extracellular enzymes in to Amino acids, LCFA and sugars (2) in acidogenesis the hydrolyzed products are fermented and converted into volatile fatty acids, alcohol and ketones. (3) acitogenesis is stage where the products of acidogenesis are converted to hydrogen, carbon dioxide and acetic acid. The hydrogen partial pressure must be low in this stage in order to be successful (4) the last stage is the methanogenesis where methane gas is formed. There are two main baths for methane formation; the conversion of acetate to methane by *Acetotrophic methanogens* and the conversion of hydrogen and carbon dioxide to methane by *Hydrogenotrophic methanogens*.

2.6.2 Technical problems

The chemical composition of 2POMW was represented in table 2.2. the high content of lignocellulosic compounds, phenolic compounds and oil induces some limitation for the anaerobic digestion process.

Inhibition related to the high oil content can be explained by the presence of long chain fatty acids (LCFA). LCFA can be adsorbed on bacterial surface which hinder the transportation of substrate (Pereira et al., 2005). The main LCFA contained in OMW were unsaturated LCFA, mainly oleic acid (Hamdi 1992)

Phenolic compounds exhibit antibacterial characteristic. Among the different phenolic compounds found in OMW, ortho-diphenolic compounds (caffic acid and protocatechuic) and mono-phenolic compounds (acid p-hydroxybenzoic acid and p-coumaric cid). Both types of phenols inhibit the acetate conversion for methane production, but ortho-phenols were shown to be more toxic (Borja et al., 1997). These compounds are recognized as simple phenolic compounds and together with LCFA inhibit the activity of methanogens. Another group is polyphenols, which contain darkly colored material and reduce the biodegradability of OMW (hamdi 1992).

Olive pomace and the 2POMW contain significant amounts of cellulose hemicellulose and lignin (table 2.2). This can inhibit the first step of hydrolysis of the anaerobic digestion process.

2.6.3 Measures to overcome technical problems

In order to overcome the previously mentioned constrains, one approach was to dilute with water; dilution reduces phenols and LCFA below the toxicity level for methanogens. Other approaches such as supplementing with nutrients to correct alkalinity and nitrogen content (Erguder et al 2000). In addition, to overcome this problem, several processes were applied such as: pretreatment, combined digestion with other waste has complementary properties, or two stage digestion. Those processes are discussed below.

a. *Pretreatment (to remove phenols and toxic compounds)*

As illustrated above, methanogenic activity is inhibited by the high concentration of phenolic compounds in OMW. A pretreatment step, using physico-chemical or biological processes, found to be effective and capable to decrease the toxicity of phenolic compounds.

Several physico- chemical methods were investigated for detoxifying OMW; 80% increase in biogas production was achieved after treating the OMW with acid cracking followed by coagulation- flocculation process using coagulating agents such as Al_2SO_4 (Azbar et al, 2008). Khoufi et al (2008) indicated high phenol removal efficiency when applying electro- coagulation pretreatment as well. Sabbah et al (2004) found that sand filtration and subsequent treatment with activated carbon is an effective pretreatment method to improve AD.

Fungi have been used effectively in treating OMW. Anaerobic digestion of OMW previously digested with Aspergillus niger resulted in 58% reduction of phenolic compounds (Hamdi and Garcia 1993) and hence a higher methane production than without previous pretreatment.

b. Codigestion

For a stable digestion process of olive mill wastewater, nitrogen content and alkalinity must be adjusted. Adding nutrients and chemicals increased the investment cost. Dilution with water resulted in an increase of the digester working volume. On the other hand, digesting with another complementary compound (high nitrogen, high alkalinity and high pH), can supplement olive mill waste with the required compounds. Codigestion dilutes the high concentration of phenols and LCFA as well.

As shown by Dareioti et al (2010) OMW has low pH (5), high COD (131 g O_2/l), low ammonium nitrogen (0.1 g N/L) and low alkalinity (1.5 g/L). It was found that liquid manure can work as a complementary substrate. Goberna et al (2010) showed that the high buffering capacity include in the cattle excreta, made it possible to treat the 2POMW without previous dilution, without addition of external base or nitrogen source. The 2POMW also supplied the excreta with fats, with their numerous biogas potential. Gelegenis et al (2007) indicated that codigestion of OMW with poultry manure was sufficient, but the optimal concentration of OMW in the mixture should be adjusted.

c. Single-phase and two-phase anaerobic digestion

In single phase all processes of anaerobic digestion from hydrolysis to methanogenesis take place in the same tank. Most of studies of anaerobic digestion of olive mill wastes were under single stage digestion. Some studies showed inhibition of anaerobic digestion as a result of LCFA and phenolic compounds accumulation (Hamdi, 1992).

Two-stage anaerobic digestion separates acidogenic bacteria and methnogenic bacteria in different tanks; different microorganisms have different requirement and growing conditions. Separation of phases allows controlling the operation conditions for each type of microorganism and allows the enrichment of different microorganisms in each process. According to Fezzani and cheikh (2010), by considering methane production, soluble COD and phenol removal, two-stage anaerobic digestion showed a better performance. About 94.3- 61.3 % removal of VFA in the methanogenic step was observed when two-stage anaerobic digestion was applied to treat olive mill wastewater (Rincon et al 2009). Furthermore, Rincon et al (2009) and Dareioti et al (2010) showed 40.7% and 18% reduction respectively of phenolic compounds before introducing the effluent to the methanogenic reactor.

2.7 Summary

Olive oil industry in one of the main industries in the Mediterranean region and the wastes come out from this process can represent a serious threat to the environment. There are three ways of olive oil extraction; traditional, three phase centrifugation and two phase centrifugation, according to which the generated waste will be different. In recent years the number of olive mills that uses the ecological two phase centrifugation is increasing. The waste generated form this waste is called the two phase olive mill waste (2POMW) and its doughy structure and high carbohydrate content makes management not an easy process. This study focuses on the anaerobic digestion (AD) of the 2POMW. There are many factors limit the AD of 2POMW; it's seasonal generation, high concentration of inhibitors and the high content of lignocellulosic material. Several studies have been done to optimize the organic loading rate of the 2POMW in the digester in order to control the concentration of inhibitors and increase methane production. However, no many studies have been done to improve the hydrolysis of the lignocellulosic portion of the 2POMW. Therefore, this study addressed the effect of pretreatment of 2POMW on improving its hydrolysis and methane gas production. In addition the codigestion of the 2POMW with food waste in order to facilitate its seasonal application and overcome inhibition by toxic compounds was discussed.

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CHAPTER 3

Mechanical and alkaline pretreatment of 2POMW for improving methane production

3.1. Introduction

Like other lignocellulosic biomass, the lignocellulose fraction of the 2POMW is hard to be enzymatically degraded. Cellulose, hemicellulose and lignin, the three main component of lignoccellulosic material, all together form lignocellulosic building blocks that prevents its attack and destruction by bacteria and enzymes. This is mainly attributed to (1) the degree of cellulose crystallinity (2) shield effect of lignin. Monlau et al. (2012) found a strong negative correlation between lignin content and the methane production.

Pretreatment can overcome this limitation by altering the physical or chemical properties, increasing the surface area, breaking the linkage between cellulose, hemicellulose and lignin, thus facilitating the accessibility of hydrolytic enzymes to the substrate. The pretreatment methods are classified to (1) physical; including thermal and mechanical pretreatments (2) chemical; alkali pretreatment, organosolvent and wet oxidation and (3) biological; enzymatic and fungal pretreatment (Mudhoo, 2012).

Monlau et al. (2012) compared 7 types of thermochemical pretreatment on sunflower stalks. Thermal pretreatment alone showed a slight increase in methane production; 3% and 14% increase of biochemical methane production (BMP) after pretreatment at 55 ºC and 170 ºC respectively. Farther increase in the BMP was obtained when acid pretreatment with FeCl2 and HCl was applied. Compared with acid pretreatment, alkaline pretreatment with NaOH, H2O2 and Ca(OH)2 was more effective in increasing the BMP efficiency; amongst, NaOH was the most effective with a 35% increase. As shown by Bruni et al. (2010) 66% higher methane production was obtained when digested manure biofibers were treated with CaO. This pretreatment gave the highest methane yield increase compared with the physical pretreatment by milling, biological treatment (enzyme and partial aerobic microbial conversion) and steam pretreatment with catalyst.

Alkaline pretreatment facilitates the degradation of lignocellulosic compounds, hence enhancing digestion and methane gas production. Pretreatment with NaOH caused a destruction of wheat plant structure and reduced its crystallinity (Taherdanak and Zilouei, 2014). A reduction in lignin and hemicellulose content after alkaline pretreatment was observed (Monlau et al., 2012; Liang et al., 2013 and Zhu et al., 2010).

Sonication and thermal pretreatment of olive pomace (the solid residue produced from 3- phase centrifugation) increased the soluble COD (sCOD) by 22% and 72%. The sonication increased methane production, but thermal pretreatment deteriorated anaerobic digestion. This was attributed to the release of some inhibitors with high temperature (Gianico et al., 2013). Rincon et al. (2013) reported only a slight (not significant) increase in methane yield after thermally pretreating the 2POMW. However, this treatment allowed a decrease in the lag period; therefore, the maximum methane yield was achieved faster.

Several studies have addressed the pretreatment of olive mill wastes, especially OMW, in order to overcome inhibition mainly by phenolic compounds (Borja et al., 1995; Sabbah et al., 2004); however, not much has been published about pretreatment in order to enhance the hydrolysis of the lignocellulosic component.

This study focuses on the pretreatment of 2POMW in order to facilitate hydrolysis and improve methane recovery. The 2POMW contains the pieces of stone and pulp plus the vegetation water. It is composed by water (60-70 %), lignin (13-15 %), cellulose and hemicellulose (18-20 %), fat (2.5-3 %) and mineral solids 2.5 % (Borja et al., 2002).

3.2 Materials and methods

3.2.1 Raw material

Two phase olive mill waste (2POMW) was used in this experiment. This waste was obtained from an olive oil factory located in Shyodo Island, Japan in sealed containers and transported to the laboratory. The material was stored at 4 ºC to maintain its original characteristics. The characteristics of the 2POMW are shown in table 3.1.

3.2.2 Microbial Inoculum

The inoculum was brought from a digester tank at a biogas plant processing kitchen waste in Takikawa city, northern Japan. The inoculum is characterized by pH of 8.39 ± 0.02, total solids (TS) 1.61 % ± 0.01 and volatile solids (VS) 0.71 % ± 0.007.

3.2.3 Mechanical pretreatment

Mechanical pretreatment by size reduction was tested. For size reduction, 328 g of 2POMW was ground in an open slotted homogenizer (Heidolph DIAX 900) with a rotation speed of 25000 rpm for 20 minutes. Sieve analysis was conducted to compare the pretreated and the untreated 2POMW. Sieve mesh sizes of 2 mm, 1 mm, 0.5 mm, 0.25 mm and 0.125 mm were used. Results of sieve analysis are shown in table 3.2. The mean weight diameter (MWD) was calculated as:

$$\mathrm{MWD} = \Sigma w_i \times x_i \qquad (1)$$

Where:

x_i: is the mean diameter of any particular size range of the 2POMW sample separated by sieving

w_i: is the weight of the sample in the size range as a fraction of the total weight

3.2.4 Alkaline pretreatment

The two bases (NaOH and CaO) were chosen in our experiment to conduct the alkaline pretreatment tests. The dose rate of NaOH and CaO and the pretreatment time were considered as the two important factors to determine the pretreatment condition. Five dosages of NaOH and CaO were chosen in our experiment; 2.4%, 6%, 10%, 20% and 30% (w/w TS). A specific amount of the 2POMW (depending on the loading rates described later) was added into digestion bottles. NaOH solution, that prepared to reach the above mentioned dosages, or CaO powder were added. The TOPMW was then mixed well under the condition of 75% moisture content. Pang et al. (2008) increased the moisture content to 80%. In our study it was found that 75% was enough to moist the 2POMW and to facilitate mixing with alkaline. Controls without alkaline addition were included. Finally the digestion bottles were closed with rubber septa and kept in the laboratory at 25 ºC for 6 days. This pretreatment time was determined by a preliminary test. The change of pH and the concentration of sCOD as a result of alkaline pretreatment were used as an indicator of 2POMW degradation. The pretreated 2POMW was used for conducting the biochemical methane potential tests without any additional treatment.

3.2.5 Biochemical methane potential (BMP) tests

A preliminary experiment was done to determine the appropriate loading rates to be used. Loading rates of 0.35, 0.88, 1.75 and 3.5 g-VS(substrate)/g-VS(inoculum) were tested. High loading rates of 1.75 and 3.5 g-VS(substrate)/g-VS(inoculum) caused inhibition with a pronounced lag phase. Therefore, loading rates of 0.35 and 0.88 g-VS(substrate)/g-VS(inoculum) were chosen to conduct the BMP test.

The BMP test was conducted in 200 mL digestion bottles with 100 mL effective working volume, the remaining 100 ml served as a head space. For NaOH pretreatment, two loading rates of 2POMW were tested; low loading rate of 0.35 g-VS(substrate)/g-VS(inoculum) and high loading rate of 0.88 g-VS(substrate)/g-VS(inoculum) as mentioned above. For CaO pretreatment only the high loading rate of 0.88 g-VS(substrate)/g-VS(inoculum) was studied. For the mechanical pretreatment experiment, the BMP of 0.88 g-VS(substrate)/g-VS(inoculum) for both pretreated and untreated 2POMW were compared. First, 100 mL of sludge (the inoculum) was supplemented to the digestion bottles that already contained the required amounts of pretreated or untreated 2POMW. At the beginning of the experiment, the head space was replaced with nitrogen gas to facilitate the anaerobic condition. The bottles were sealed with a butyl rubber septum- type stoppers and aluminum caps. Finally the bottles were placed in a water bath shaker with a shaking speed of 70 rpm. Mesophelic temperature of 37 ºC was maintained. Gas production was measured throughout the incubation period of 26 days. Test bottles were run in triplicate and mean value was used to represent our results.

Blank (without 2POMW) and control (with untreated 2POMW) were run in all experiments. For the high load NaOH pretreatment, at the end of the experiment pH and volatile fatty acid (VFA) were measured in order to evaluate their effect on inhibition.

3.2.6 Analytical methods

3.2.6.1 Biogas analysis

Biogas generation volume was measured by gas displacement using a 20 ml calibrated glass syringe; the needle of the syringe was inserted into the septum, the gas pressure inside the bottle pushes the syringe plunger according to the volume of biogas in the headspace. The methane content of the biogas was analyzed by injecting 0.15 ml in a gas chromatograph (model C-R8A Shimadzu, Japan) equipped with GW100- KA 1170 stainless steel column and thermal conductivity detector (TCD). Standard methane gas was used to draw the calibration curve.

3.2.6.2 Chemical composition analysis

TS and VS of the 2POMW and sludge were determined according to the standard methods of Japanese sewage association (1997). The pH value was tested by pH meter (D- 51, 9621C, Horiba, Japan).

Total phenolic content (TPC) of the 2POMW was determined as tyrosol equivalent (g tyrosol/Kg wet- 2POMW) using folin- Ciocalteau calorimetric method (Lafka et al., 2011); the 2POMW was extracted 3 times with ethyl acetate and the extract was evaporated under vacuum. The residue was then dissolved in methanol, then 0.2 mL of the extract (dissolved in methanol) was mixed with 20 mL water and 0.625 mL folin- Ciocalteau reagent, after 3 minutes 2.5 mL of (35%) Na_2CO_3 was added and the content was diluted to 25 mL with deionized water. One hour later, the absorbance was measured using (Hitatchi U- 1800) spectrophotometer.

The Chemical Oxygen Demand (COD) of the pretreated and not pretreated 2POMW was determined according to the dichromate open reflux method (Japanese sewage association 1997). The same procedure was used to determine the sCOD after passing the sample trough a 0.45 μm membrane filter paper.

Oil content was estimated gravimetrically according to the standard method for oil and grease determination (APHA standard methods 1999); the 2POMW was acidified to a low pH < 2 and extracted 3 times with hexane, the three extracts were combined and hexane was evaporated in a rotary evaporator and then overnight under vacuum and the residue weighted.

Oleic acid, which is well known as inhibitor in anaerobic digestion among LCFAs, was determined by injecting 1 μL of the oil extract (dissolved in hexane) into a gas chromatograph (model 14-B shimadzu, Japan) equipped with a flame ionization detector (FID) and a capillary column (BP21 30m× 0.25 mm).
In order to analyze the VFA of the digestate, a sample of 15 mL was centrifuged at 10,000 rpm for 10 minutes and then filtered through 0.45 μm membrane filter paper, and 1 μL was injected into a gas chromatograph (model 14-B shimadzu, Japan) equipped with a FID and a capillary column (GI005).

3.3 Results and discussion

3.3.1 Characterization of the 2POMW

The total phenolic content was found to be 9.3 g/ kg-TS (table 3.1). This value is in the low range of phenolic compounds of 6.2- 23.9 g/ kg TS as described in literature (Alburquerque et al., 2004). This concentration was found not to be inhibitive for the anaerobic digestion process. Fedorak and Hrudey (1984) showed a phenolic inhibition value of 1 g/L-reactor after adding different concentrations of phenol and alkylphenols to anaerobic bacteria and incubating at 37 ℃. However, since the TPC in our sample is low, the concentration of phenolic compounds for the two loading rates will be less than 0.1 g/L-reactor, which is 10 times less than the inhibiting concentration that was reported above. Oleic acid concentration above 30 mg/ L-reactor was reported by Lalman and Bagley (2001) to inhibit acetate degradation. Comparing with the value in table 3.1 and the loading rates were used in our experiment, for the high loading rate (0.88 g-VS(substrate)/g-VS(inoculum)) and the low loading rate (0.35g-VS(substrate)/g-VS(inoculum)) the oleic acid concentration inside the reactor will be 27 mg/ L-reactor and 11 mg/ L-reactor respectively. Therefore, no serious inhibition by oleic acid is expected.

Table 3.1. Characteristics of the 2POMW

Parameter	Value (Avg. +/- SD)
TCOD (g/Kg 2POMW)	547 ± 129
sCOD (g/Kg 2POMW)	111 ± 2
TS (%)	30.5 ± 0.3
VS (%)	24.9 ± 0.5
TPC (g/ Kg wet- 2POMW)	3.3 ± 0.6
(g/ Kg TS- 2POMW)	9.3 ± 0.7
Oil Content (%)	1.6 ± 0.5
Oleic acid (g/kg- 2POMW)	1.1 ± 0.6

*TPC: total phenolic compound

3.3.2 The effect of mechanical pretreatment

The effect of mechanical pretreatment on 2POMW is displayed in Table 3.2. The total phenolic content was not affected by mechanical pretreatment. After mechanical pretreatment the extracted oil content increased from 1.7% to 3.5%. Since mechanical pretreatment didn’t increase the sCOD, there was no change in methane production after pretreatment throughout the whole digestion period reaching values of 314 mL/ g-VS and 318 mL/ g-VS after 26 days for the untreated and mechanically treated TPMOW respectively.

Table 3.2. Effect of mechanical pretreatment on 2POMW and BMP

Parameters	Control (no pretreatment)	Mechanical size reduction
Mean weight Diameter (mm)	1.09 ± 0.04	0.56 ± 0.002
sCOD (g O_2/kg 2POMW)	111 ± 2	116 ± 2
Oil content (%)	1.7 ± 0.1	3.5 ± 0.2
TPC (g Tyrosol/kg 2POMW)	3.3 ± 0.6	3.2 ± 0.7
MP_{26} (ml/ g VS)	314 ± 91	318± 15

3.3.3 The effect of alkaline pretreatment

3.3.3.1 Optimizing the alkaline pretreatment process

An experiment was conducted to determine the adequate pretreatment time. The pretreatment time was determined according to the degradation degree of the organic matter in the 2POMW. The degree of alkaline consumption reflects the degree of degradation; as the hydroxide ion is consumed in hydrolyzing the ester bond between lignin and hemicellulose. This process produces carboxylic acid which reduces the pH. This consumption of OH^- was approximately estimated indirectly by measuring the pH. Figure 3.1 shows that just after the addition of the base, the pH was high; as a result of the high base concentration. However, as OH^- was consumed for degradation, an immediate reduction in pH occurred. This reduction was high in the first 3 days and started to level out afterwards. Therefore, in order to confirm sufficient degradation, a pretreatment period of 6 days was chosen in our study.

The amount of OH- consumed was estimated using the following formula:

$$|OH^-| = (10^{-pOHi} - 10^{-pOHf}) \times 0.1 \qquad (2)$$

Where,

$|OH^-|$: the amount of consumed OH^- (mole)

pOHi: the initial pOH

pOHf : the final pOH

pOH= -log (OH^-)

(OH^-): the concentration of OH- (Molar)

The working volume: 0.1 L

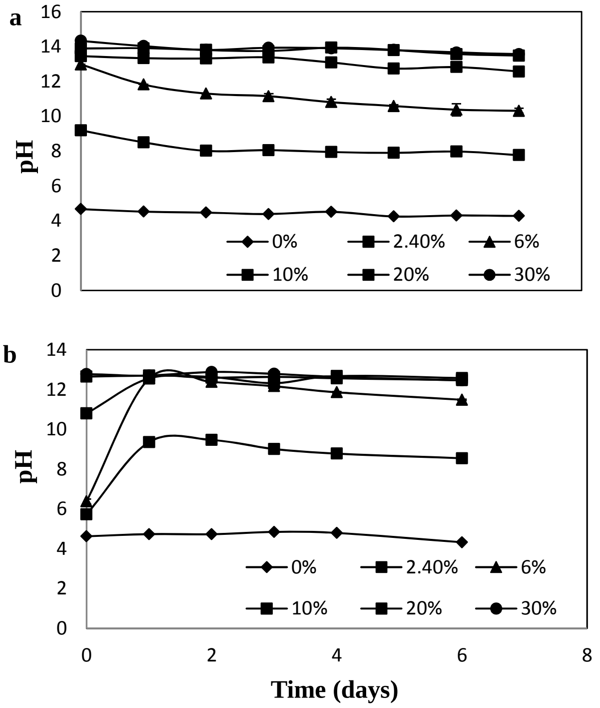

Fig. 3.1. The change of pH values over pretreatment time with (a) 2.4- 30% NaOH (b) 2.4- 30% CaO.

3.3.3.2 The effect of NaOH

Hendriks and Zeeman (2009) reviewed the effect of alkaline pretreatment on different ligonocellulosic biomass such as corn and wheat to enhance their digestibility. Alkaline pretreatment increases the surface area of the substrate, reduces crystallinity of cellulose, and weakens the bonds between lignin cellulose and hemicellulose. This facilitates the accessibility of hydrolytic enzymes to the substrate and increases the amount of organic matter available for microorganisms. In our study, the effect of alkaline pretreatment on degradation was estimated as a function of (1) sCOD and (2) alkaline consumption after 6 days of pretreatment (Table 3.3). Pretreatment with NaOH had a pronounced effect on dissolving part of the lignocellulosic material as indicated by the increase in sCOD. Except for the lowest concentration of 2.4%, an increase of NaOH concentration resulted in higher sCOD. These results were in consistent with the OH^- consumption which showed higher consumption with higher NaOH concentration. Sambusiti et al. (2012) showed higher NaOH concentration produced more sCOD from Sorghum plant.

Since NaOH pretreatment showed a high increase in the sCOD, two loading rates were selected for the anaerobic digestion tests. Figure 2b shows the produced methane within 26 days of incubation for the lower loading rate of 2POMW (0.35 g-VS$_{(substrate)}$/g-VS$_{(inoculum)}$). As can be seen, a higher cumulative methane yield in the range of 21.7% to 37% compared with the untreated 2POMW was obtained for all NaOH-pretreatments. This indicates that NaOH pretreated 2POMW samples were more easily available for microbial degradation. However, the 30% NaOH pretreated 2POMW initially inhibited methane production, most probably related to the high initial alkalinity inside the digester. Pretreating with 20% NaOH caused a rapid maximum daily methane production, which was 78.4% and 33.7% higher than the 6% and 10% treatments respectively. This was followed by a rapid decline in the digesting rate reaching a value similar to the control within 4 days (Figure 3.2a). This implies a faster digestion rate in the case of 20% NaOH compared with other pretreatments. This high initial methane production can be explained by the high sCOD of 272.5 g O_2/kg released upon treatment with 20% NaOH (Table 3.3).

A different trend was observed when the higher loading rate of 0.88g-VS$_{(substrate)}$/g-VS$_{(inoculum)}$ was applied into the reactor (Figure 3.3). The highest methane production was from the 6% NaOH treated sample (378.5 mL CH_4/g-VS), which was 20.3% higher compared with the untreated 2POMW. The daily methane production from the 2.4% pretreated 2POMW reached its maximum of 32.6 mL/ day on the second day, which was similar to the untreated 2POMW. A high methane production was shown for the 10% NaOH pretreated 2POMW as well. Pretreatment with 6% and 10% NaOH increased the sCOD by 51.9% and 65.4%, respectively compared with the untreated 2POMW, therefore, the improved anaerobic digestion of the 2POMW. On the contrary, higher concentrations of 20% and 30% showed inhibition throughout the incubation period. This is most probably attributed to the high initial pH for those treatments (Table 3.4). Zhu et al. (2010) observed that 7.5% NaOH concentration, which was the highest concentration used, inhibited the anaerobic digestion of corn stover, he attributed this to the high acidic condition (pH 6) caused by VFA accumulation. Butyric acid and Propionic acid inhibition concentration of >10,000 mg/L and 6000 mg/L respectively were reported (Khanal 2008). However, in our case pH and VFAs values measured at the end of the incubation confirmed that no such effect of acids build up occurred; the concentrations of VFA in all treatments were less than the inhibition level (Table 3.4). Rinzema et al. (1988) reported that high sodium ion concentration can impose toxicity for methanogens. Considering an anaerobic reactor system that receives food waste as a main substrate and the pretreated 2POMW as a co-substrate, the high pH of the pretreated 2POMW can neutralize the pH inside the reactor because the pH inside the reactor receiving mainly food waste is acidic. In this case, the NaOH concentration of 20% can be optimum regarding the sCOD concentration.

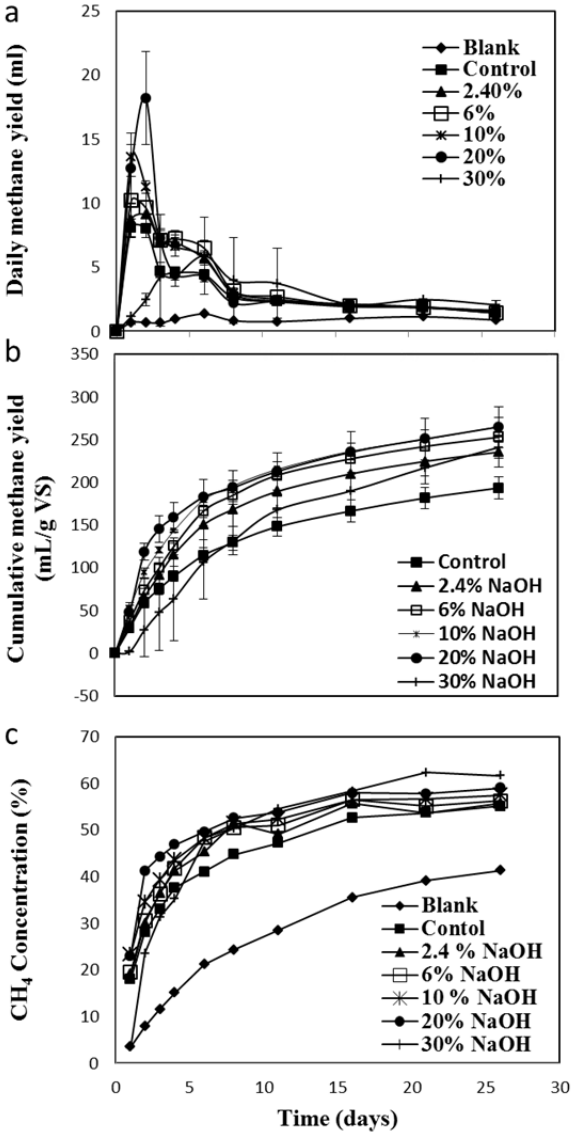

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Fig. 3.2 Methane production of low loading rate NaOH- pretreated 2POMW(0.35 g-VS$_{(substrate)}$/g-VS$_{(inoculum)}$) (a) Daily methane yield, absolute volume of methane gas produced from 100 mL reactor working volume (b) Cumulative methane yield, Values represent the methane production in all treatments minus the blank, values are represented as per volatile solid 2POMW (c) CH$_4$ concentration (%)

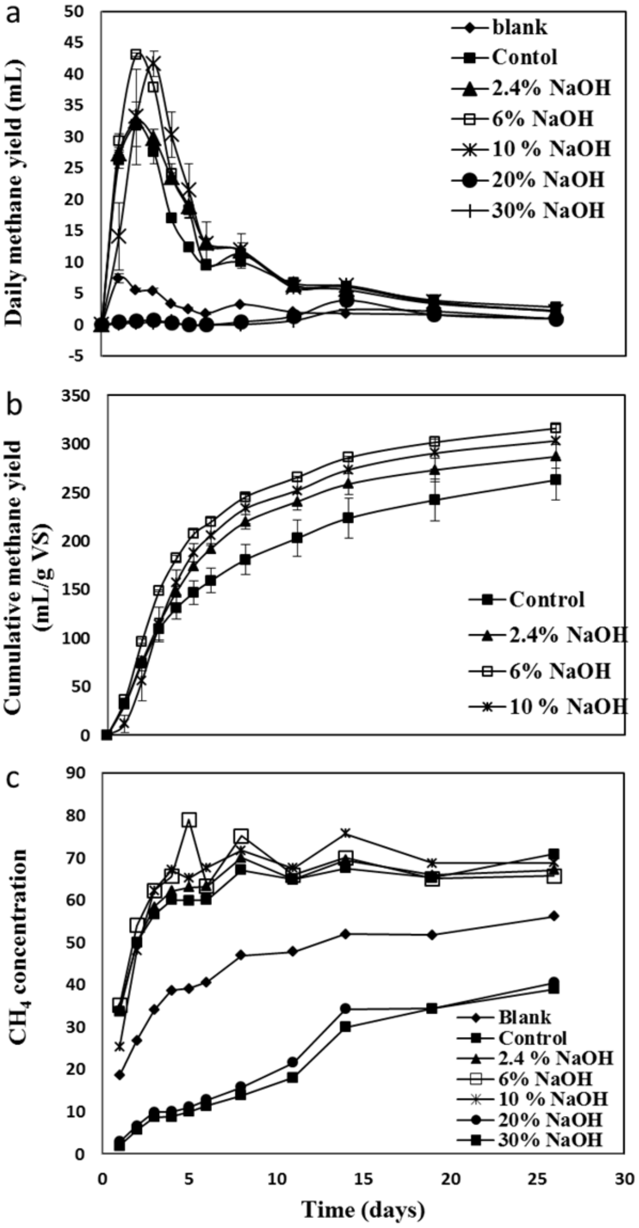

Fig. 3.3 Methane production of high loading rate NaOH- pretreated 2POMW(0.88 g-VS(substrate)/g-VS(inoculum)) (a) Daily methane yield, absolute volume of methane gas produced from 100 mL reactor working volume (b) Cumulative methane yield, Values represent the methane production in all treatments minus the blank, values are represented as per volatile solid 2POMW (c) CH_4 concentration (%)

Table. 3.3 Characteristics of the Two phase olive mill waste after pretreatment with different concentrations of NaOH and CaO

Pretreatment condition	pH_i	pH_f	OH^- consumed (mole)	sCOD ($g\ O_2/kg$)
No pretreatment	4.62 ± 0.20	4.32 ± 0.18	-	74.1 ± 10.6
NaOH				
2.4 %	9.19 ± 0	7.98 ± 0.03	1.5×10^{-6}	60.0 ± 7.1
6 %	12.97 ± 0.14	10.38 ± 0.35	9.3×10^{-3}	112.5 ± 24.8
10%	13.45 ± 0	12.82 ± 0.03	2.7×10^{-2}	122.5 ± 10.6
20%	13.89 ± 0	13.57 ± 0.01	4.1×10^{-2}	272.5 ± 17.7
30%	14.33 ± 0.36	13.65 ± 0.04	1.7×10^{-1}	265.0 ± 49.5
CaO				
2.4 %	9.36 ± 0.36	8.54 ± 0.04	1.9×10^{-6}	82.7 ± 8.5
6 %	12.55 ± 0.06	11.48 ± 0.03	3.2×10^{-3}	87.0 ± 8.5
10%	12.56 ± 0.11	12.47 ± 0.02	7.0×10^{-4}	85.0 ± 5.7
20%	12.69 ± 0.01	12.57 ± 0	1.2×10^{-3}	82.0 ± 4.2
30%	12.77 ± 0.01	12.45 ± 0.24	3.1×10^{-3}	68.0 ± 7.1

3.3.3.3 The effect of CaO

CaO didn't facilitate solubilization of lignocellulosic material; there was no significant difference in sCOD between CaO treated and untreated 2POMW (Table 3.3). However, a specific amount of the OH^- was consumed to degrade the material and bring the organic matter more available for hydrolyzing microorganisms. Taherdanak and Ziolouei (2014) showed that NaOH pretreatment modified the structure of wheat plant and reduced its crystallinity. In our study, the lowest OH^- consumption was for the lowest CaO concentration of 2.4% while the other treatments showed a higher OH^- consumption. Unexpectedly, the 10% CaO treatment showed less consumption of OH^- compared with 20% and 30% CaO this may be related to some error in measuring pH.

Figure 3.4 shows the result of anaerobic digestion of CaO pretreated 2POMW with the higher loading rate of 0.88 $g\text{-}VS_{(substrate)}/g\text{-}VS_{(inoculum)}$. A concentration of 30% showed less methane production compared with the untreated 2POMW, most probably due to the high initial pH value; initial pH was found to be 8.64. However, for all other CaO treatments, higher CaO concentration induced higher methane production. Pretreatment with 2.4%, 6%, 10% and 20% increased cumulative methane yield by 7.2%, 10.5%, 14.9% and 26.9% respectively compared with the untreated 2POMW (Figure 3.4). Although no increase in sCOD was observed, it was clear from the higher methane production rates (Figure 3.4a) that easily biodegradable materials were available at early stages of digestion. Results of OH^- consumption confirm that part of the 2POMW was degraded, even though it was not enough for increasing sCOD (Table 3.3). Liang et al. (2013) reported a reduction in pH after lime pretreatment. He attributed that to the lime consumption in disturbing the ester bonds and neutralizing the structural carboxylic acids that formed from the deacetylation of hemicellulose during pretreatment.

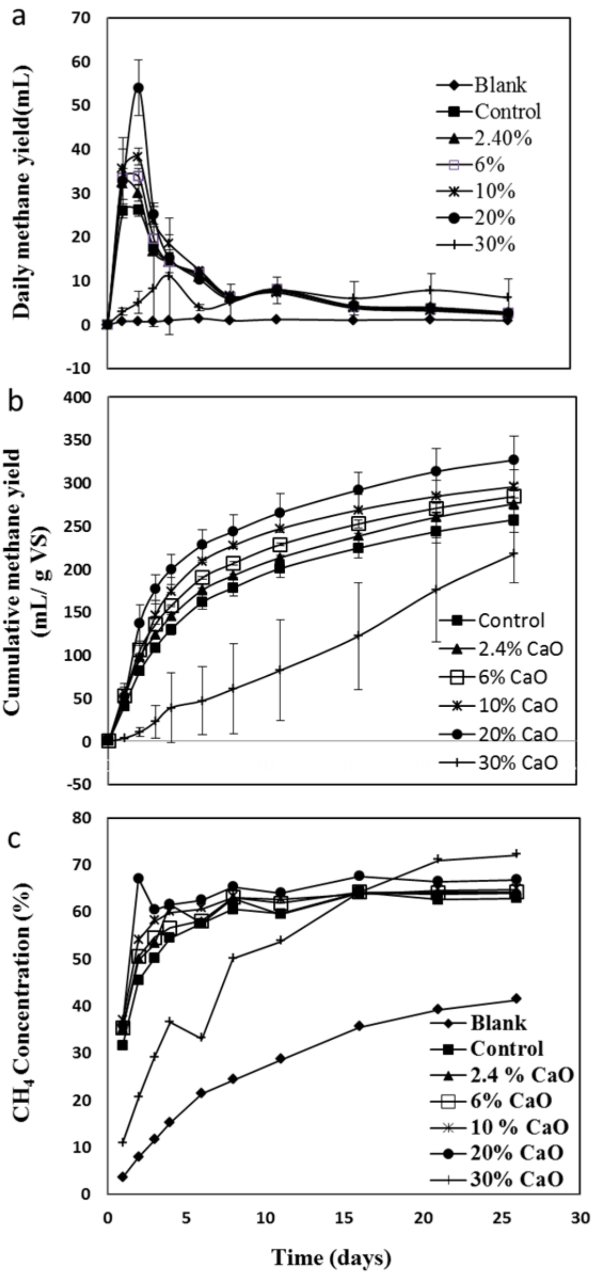

Fig. 3.4. Methane production of high loading rate CaO- pretreated 2POMW(0.88 g-VS$_{(substrate)}$/g-VS$_{(inoculum)}$) (a) Daily methane yield, absolute volume of methane gas produced from 100 mL reactor working volume (b) Cumulative methane yield, Values represent the methane production in all treatments minus the blank, values are represented as per volatile solid 2POMW (c) CH_4 concentration (%)

Table 3.4. Changes of pH and VFA after anaerobic digestion of NaOH pretreated 2POMW with loading rate of 0.88 g-VS TPMOW/ g-VS inoculum

Treatment	pH_i	pH_f	Acetic acid (mg/L-reactor)	Propionic acid (mg/L-reactor)	Butyric acid (mg/L-reactor)
Blank	8.39 ± 0.02	8.07 ± 0.01	4494 ± 828	2690 ± 125	4645 ± 571
Control	8.23 ± 0	7.75 ± 0	4974 ± 63	277 ±43	4895 ± 125
2.4%	8.30 ± 0.01	7.76 ± 0.01	5465	2507	3939
6%	8.36 ± 0	7.77 ± 0	4488 ± 1205	2638 ± 163	4381 ± 667
10%	8.49 ± 0.05	7.82 ± 0.02	5718 ± 695	2556 ± 78	4143 ± 349
20%	8.77 ± 0	7.61 ± 0.02	5144 ± 259	2605 ± 2	4415 ± 6
30%	8.83 ± 0.02	7.73 ±0.08	5974 ± 334	2602 ± 12	4268 ± 173

3.3.3.4 Comparison of pretreatment with NaOH and CaO

Comparing both bases used, NaOH had a stronger effect in improving the methane production; for the high loading rate used (0.88 $g\text{-}VS_{(substrate)}/g\text{-}VS_{(inoculum)}$), pretreating with 6% CaO achieved a methane production improvement by 10.5%. The same concentration of NaOH improved methane production by 20.3%. The NaOH pretreatment increased the sCOD and consumed three times the amount of OH^- that was consumed with the CaO pretreated sample. Bruni et al. (2010) observation was opposite to our results as he reported higher methane production for the CaO pretreated biofibers compared with NaOH. Our results showed that applying high concentration of CaO (20%) revealed 26.9% improvement in methane production (Figure 3.4). However, this has two disadvantages for full scale application; (1) the cost will be increased because of higher amount of chemical demand (2) increasing the amount of CaO makes the mixing process during pretreatment harder because of the viscous nature of CaO.

For both NaOH and CaO pretreatments, alkaline concentration of 2.4% resulted in the lowest OH^- consumption of 1.5×10^{-6} and 1.9×10^{-6} mole, respectively. While for higher concentrations up to 30%, NaOH had a higher degradation power than CaO; as indicated by higher sCOD and higher OH^- consumption.

The higher methane production after alkaline pretreatment can be explained by (1) modifying the structure of the 2POMW by reducing the crystallinity of cellulose and breaking the linkage between cellulose, hemicellulose and lignin, which makes cellulose more available to anaerobic microorganisms, and (2) solubilization of part of the lignocellulosic material as indicated by the increase in sCOD upon pretreatment.

3.4 Summary

Two phase olive mill waste contains specific amounts of cellulose, hemicellulose and lignin, which are not easily biodegradable and may limit the anaerobic digestion process.

1. This study showed that mechanical pretreatment had no effect on the sCOD and didn't improve methane gas production.
2. Alkaline pretreatment with NaOH and CaO facilitated the anaerobic digestion process by making the substrate more available to anaerobic microorganisms
3. NaOH showed higher methane yield than CaO; NaOH was stronger as it solubilized part of the lignocellulosic organic matter.
4. High alkalinity of alkaline treated 2POMW can inhibit the anaerobic digestion process under the condition of without pH neutralization. In this case, NaOH concentration of 6% has been shown to be reasonable for improving methane production.
5. Considering a full scale reactor system receiving food waste as main a substrate and the NaOH-treated 2POMW, the NaOH concentration of 20% might be sufficient regarding the soluble COD concentration.

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CHAPTER 4

Investigating the Codigestion Condition of Pretreated Two Phase Olive Mill Waste Mixed with Food Waste

4.1. Introduction

The treatment of olive mill wastes in a reactor as a sole source is limited by its high concentration of long chain fatty acids (LCFA) and phenolic compounds (Hamdi et al. 1992). Another drawback is the seasonal generation of olive milling wastes which is limited to the winter season (November to March), making the full scale application of olive milling wastes only for a short period economically unfeasible.

Research has been done in order to overcome the above mentioned problems. Several measures have been studied and proposed such as dilution with water to reduce the effect of inhibitors, pretreatment to remove inhibitors. However, dilution increases the effluent volume and pretreatment makes the process more complicated and increases the initial cost of the process.

Codigestion can cause a dilution effect and reduce the concentration of inhibitors inside the reactor. Another advantage is that another source of input can be available to the reactor throughout the whole year, while olive milling waste can be introduced in the olive oil production season. The codigestion of olive milling wastes has been proposed in many studies.

Codigestion of olive mill wastewater (OMW) with manure is the most widely studied mixture. The high alkalinity and nitrogen content in manure balance the low alkalinity and nitrogen content of the OMW. Co-digesting OMW with swine manure was investigated (Azaizeh and Jadoun 2010). Mixing ratios of 33% to 67% of OMW to swine manure resulted 85- 95% COD removal and a biogas production of 0.55 L/g COD. When treating OMW with poultry manure, a mixing ratio of 40% OMW to Poultry waste was critical as it showed highest gas production after which methane production started to decrease (Gelegenis et al 2007). Margarita et al (2010) found that treating OMW with liquid cow manure in a two stage continuous stirred tank reactor an attractive method to treat both wastes. Co-digesting 2POMW with cattle excreta in a mixing ratio of 3:1 and loading rate of 5.5 g COD L^{-1} d^{-1} produced 1096 mL biogas L^{-1} reactor d^{-1}, which was 337% higher compared with the cattle excreta alone.

Compared with the OMW, no much study has been done concerning the codigestion of 2POMW. Therefore, the objective of this study is to adjust the mixing ratio for the codigestion of 2POMW (previously pretreated with NaOH) with food waste for stable operation conditions and methane recovery without inhibition.

4.2. Materials and methods

4.2.1. Two phase olive mill waste

The two phase olive mill waste (2POMW) was obtained from an olive oil factory located in Shyodo Island, Japan in sealed containers and transported to the laboratory. The material was stored at 4 ºC to maintain its original characteristics. The characteristics of the 2POMW are shown above in table 3.1.

4.2.2. Food waste

The food waste was brought form a biogas plant processing kitchen waste in Takikawa city, northern Japan. The kitchen waste was treated in the biogas plant by 50% dilution with water and then shredding. Therefore, the sample received was a homogenous sample. The obtained food waste is characterized by chemical oxygen demand (COD) of 115 ± 79, soluble chemical oxygen demand (sCOD) of 52.5 ± 0.7, TS 4.86 % ± 0.06, VS 3.78 % ± 0.03 and pH of 4.66.

4.2.3. The anaerobic sludge

The anaerobic sludge used as inoculum was obtained from the biogas plant in Takikawa city. The main characteristics of the inoculum are: pH: 8.39 ± 0.02, TS: 1.61 % ± 0.01 and VS: 0.71 % ± 0.007.

4.2.4. Pretreatment with NaOH

Alkaline pretreatment with NaOH was chosen to carry out the continuous reactor experiment because it showed better improvement in the sCOD and methane production based on the previous experimental work (chapter 3). Pretreatments with 6%, 10% and 20% NaOH (w/w TS) were done. Pretreatments were conducted daily to allow a six day period of pretreatment before being introduced into the reactors. The TOPMW was mixed well with NaOH solution to reach the above mentioned concentrations under the condition of 75% moisture content.

4.2.5. Experimental setup

In order to determine the mixing ratio of 2POMW to food waste that will not cause inhibition because of high concentration of long chain fatty acids (LCFA), four treatments were conducted in a 6 L effective working volume continuous stirred tank reactors. Reactors were charged with 6 L of the anaerobic sludge and the then flushed with N_2 gas in order to insure anaerobic condition. The temperature of the reactors was kept 37 °C by putting the reactors in water baths. Continuous stirring was applied by placing a mechanical stirrer on the top of each reactor. Each reactor had a port from the upper part for feeding and withdrawing the digested sample. Initially, the reactors were daily fed with 200 mL of food waste and same amount of digestate was withdrawn. After stable state is achieved in all reactors, they started to receive, in addition to the food waste, pretreated 2POMW in mixing ratios of 3%, 4.3%, 5.7% and 8.3%. A control treatment with only food waste was conducted as well. With increasing the mixing ratio the 2POMW applied was with less NaOH pretreatment. Therefore, in the case of 3%, 4.3%, 5.7% and 8.3% the food waste was co-digested with 20%, 10%, 6% and 6% NaOH pretreated 2POMW. This experimental design was done to keep the sCOD coming from the 2POMW around similar levels for all reactors (table 4.1), thus, the effect of LCFA on methane gas production can be clarified. The operation conditions for all reactors and the experimental apparatus are presented in table 4.1 and figure 4.1 respectively.

Gas production, methane concentration and the pH of the effluent were measured daily throughout the whole experimental period. In addition, the LCFA concentration in the effluent was measured twice after a period of 15 days and 30 days. In order to insure that there was no inhibition of the high phenolic content, total phenolic content (TPC) as tyrosol was measured for reactor 5, which had the highest mixing ratio.

Table 4.1. The operating parameters of the continuous stirred tank reactors

Parameter	R1	R2	R3	R4	R5
HRT (days)	30	27.5	28.7	29.1	28.3
Temperature (℃)	37	37	37	37	37
NaOH pretreated 2POMW	-	6%	10%	20%	6%
Mixing ratio (2POMW/food waste)	0%	8.3%	4.3%	3%	5.7%
Food waste volume (mL/day)	200	200	200	200	200
2POMW volume(mL/day)	0	18	9	6	12
Loading rate as COD (g/L-sludge)	4	5.3	4.6	4.4	4.92
Loading rate as sCOD (g/L-sludge)	1.74	2.02	1.97	2.08	1.92

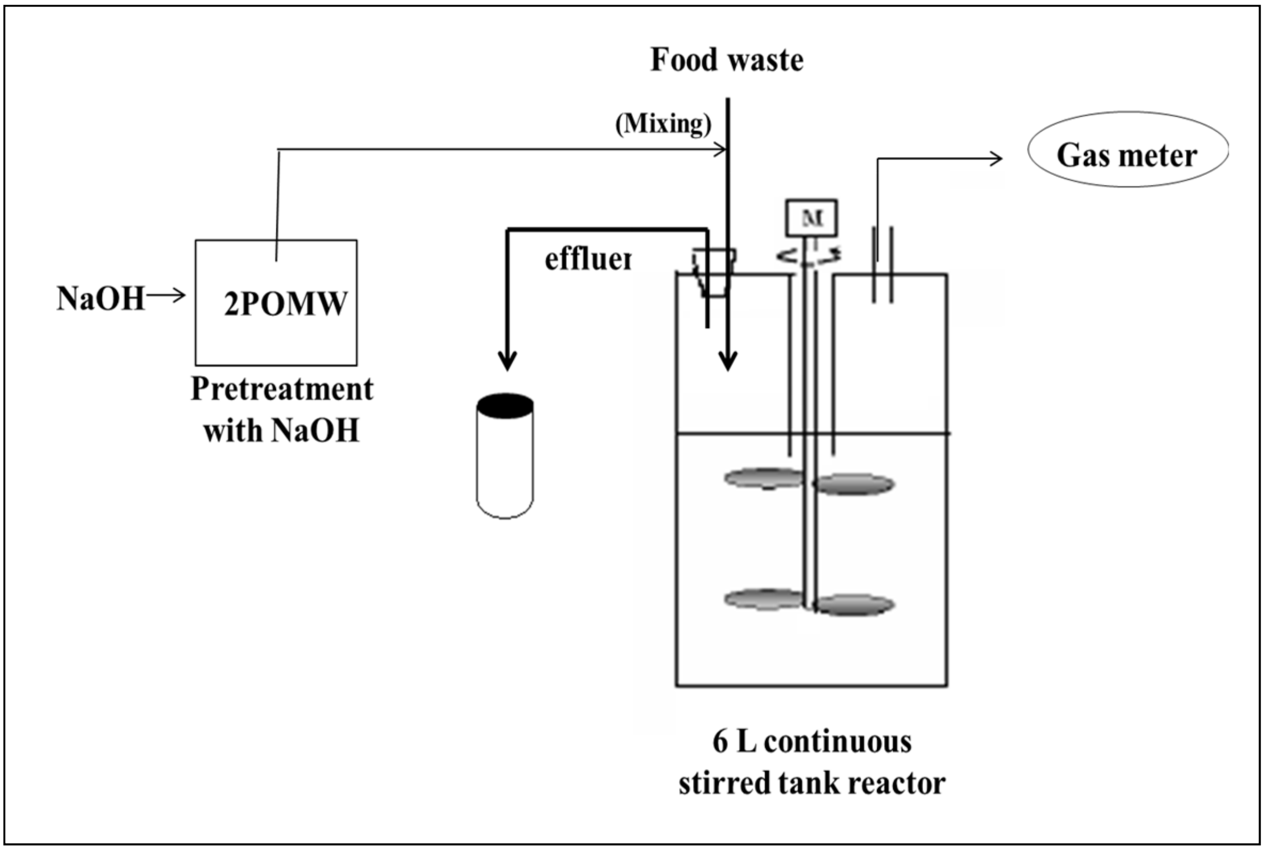

Fig.4.1. The experimental apparatus of the continuous stirred tank reactor experiment

4.2.6 Analytical methods

4.2.6.1. Biogas analysis

The volume of biogas produced was quantified by a gas generation counter placed on the top of each reactor. The methane content of the biogas was analyzed by injecting 0.15 ml in a gas chromatograph (model C-R8A Shimadzu, Japan) equipped with GW100- KA 1170 stainless steel column and thermal conductivity detector (TCD). Standard methane gas was used to draw the calibration curve.

4.2.5.2. Chemical composition analysis

Total solid contend (TS) and Volatile solid content (VS) of the food waste were determined according to the standard methods of Japanese sewage association (1997). The pH was tested by pH meter (D- 51, 9621C, Horiba, Japan).

Oil extraction of the sludge was done according to the standard method for oil and grease determination (APHA standard methods 1999); first the sludge was mixed with hexane (after reducing the pH to 2), after centrifugation the hexane (containing the lipid) was separated from the aqueous part, which was kept for the phenolic content measurement. Hexane was evaporated in a rotary evaporator and then overnight under vacuum and the residue weighted. Oleic acid, which is well known as inhibitor in anaerobic digestion among the LCFAs, was determined by injecting 1 μL of the oil extract (dissolved in hexane) into a gas chromatograph (model 14-B shimadzu, Japan) equipped with a flame ionization detector (FID) and a capillary column (BP21 30m× 0.25 mm).

Total phenolic content (TPC) in the sludge was determined as tyrosol equivalent (g tyrosol/Kg wet- 2POMW) using folin- Ciocalteau calorimetric method (Lafka et al., 2011); the aqueous part which was separated previously was extracted 3 times with ethyl acetate and the extract was evaporated under vacuum. The residue was then dissolved in methanol, then 0.2 mL of the extract (dissolved in methanol) was mixed with 20 mL water and 0.625 mL folin- Ciocalteau reagent, after 3 minutes 2.5 mL of (35%) Na2CO3 was added and the content was diluted to 25 mL with deionized water. One hour later, the absorbance was measured using (Hitatchi U- 1800) spectrophotometer.

The soluble chemical oxygen demand (sCOD) of the food waste was determined according to the dichromate open reflux method (Japanese sewage association 1997) after passing the sample trough a 0.45 μm membrane filter paper.

In order to analyze the VFA of the digestate, a sample of 15 mL was centrifuged at 10,000 rpm for 10 minutes and then filtered through 0.45 μm membrane filter paper, and 1 µL was injected into a gas chromatograph (model 14-B shimadzu, Japan) equipped with a FID and a capillary column (GI005).

4.3. Results and discussion

4.3.1. Substrates characterization

There are big differences in the composition of the pretreated 2POMW compared with food waste; the pretreated 2POMW had a high pH of 10.37, 12.82 and 13.57 for the 6%, 10% and 20% pretreated 2POMW compared with food waste which had pH of 4.66. The pretreated 2POMWs had high sCOD compared with food waste. The pretreated 2POMWs were high in their LCFA content, which is known to cause inhibition if being introduced as a sole source of substrate without any pretreatment or dilution.

Table 4.2. The characteristics of the food waste and the pretreated 2POMW

Properties	**6%NaOH pretreated 2POMW**	**10%NaOH pretreated 2POMW**	**20%NaOH pretreated 2POMW**	**Food waste**
pH	10.37 ∓ 0.34	12.82 ∓ 0.03	13.57 ∓ 0.01	4.66 ∓ 0
sCOD (g/kg)	112.5 ∓ 24.8	122.5 ∓ 10.6	272.5 ∓ 10.6	52.5 ∓ 0.7
Oleic acid (g/kg)	1.69 ∓ 0.29	7.57 ∓ 1.03	7.28 ∓ 1.83	-

4.3.2. The effect of mixing ratio on total phenolic content

The values in table 4.3 represent the TPC represented as (g Tyrosol/L digestate) for the treatment with the highest mixing ratio (6% NaOH-2POMW and 18% mixing ratio). After applying 2POMW, TPC increased compared with the blank (table 4.3), however, values were lower than the inhibition level which is 1g/L-sludge (Fedorak and Hrudey, 1984).

Table 4.3. The TPC in the digestate from reactor 5 (6% pretreated POMW and mixing ratio of 8.3%)

Time (days)	Blank (g-Tyrosol/L-sludge)	6% NaOH pretreated 2POMW (g-Tyrosol/L-sludge)
9	-	0.03 ± 0.02
19	0.02	0.04 ± 0.02
28	0.02	0.04 ± 0.02
30	0.02	0.04 ± 0.02

4.3.3. The effect of mixing ratio on LCFA

As shown in table 4.4, with increasing the mixing ratio, there was an increase in the amount of Oleic acid (the main LCFA in the 2POMW) which was measured after 15 days after starting the addition of 2POMW. These values decreased at the end of the experiment in all treatments, except the 8.3%, which kept at high level of 61.79 mg/L. The high oleic acid concentration in this treatment can contribute the inhibition of methane gas productions.

Table 4.4. The concentration of Oleic acid in all treatments after 15 days and 30 days of codigestion

Treatments	**Oleic acid concentration after 15 days (mg/L)**	**Oleic acid concentration after 30 days (mg/L)**
R1 (Blank)	4	9.5
R2 (8.3% mixing ratio× 6% NaOH)	41.53 ∓ 1.54	61.79 ∓ 7.08
R3 (4.3% mixing ratio× 10% NaOH)	6.11	5.02
R4 (3% mixing ratio× 20% NaOH)	9.8 ∓ 9.3	7.96
R5 (5.7% mixing ratio× 6% NaOH)	20.96 ∓ 14.6	10.4

4.3.4. The effect of mixing ratio on methane gas production

Mixing ratio is an important controlling factor that will determine the contribution of 2POMW in increasing the LCFA concentration (the main inhibitor of 2POMW) inside the reactor.

In the blank treatment (R1), methane production was constant throughout the whole period of the experiment. In the case of codigestion (figure 4.3- 4.6), there was increase in methane gas production just after starting to add 2POMW. For the 8.3% mixing ratio with 6% NaOH pretreatment (R2), there was an initial increase in methane gas production of about 300 mL CH_4/ g-$VS_{(2POMW)}$/day just after starting the addition of 2POMW (figure 4.3). This increase in methane production was calculated as the difference in methane gas production between after and before the start of the addition of 2POMW. This value was an average of two reactors under same condition. However, this increase was not sustained and the process was inhibited most probably related to the high oleic acid concentration as presented in table 4.4. When 10% NaOH pretreated 2POMW was added in a mixing ratio of 4.3% (R3), the highest increase in methane production of about 548.5 CH_4/g-$VS_{(2POMW)}$/day was achieved (figure 4.4). Applying 3% of the 20% NaOH pretreated 2POMW (R4) caused an increase in methane gas produciton of 445.3 CH_4/g-$VS_{(2POMW)}$/day (figure 4.5). The 6% NaOH pretreatment which had higher concentration of oleic acid (20.9 mg/L) showed the lowest increase in methane gas production of about 270 CH_4/g-$VS_{(2POMW)}$ when it was applied in a mixing ratio of 5.7% (R5). For R3, R4 and R5 the increase in methane produciton was not ihibited and was sustained until the end of the experiment. The values of R2, R4 and R5 was an average of two reactors, while the treatments of R1 and R3 represent one reactor. pH in all reactors was monitored and it was found to be constant and in the range 8-8.2.

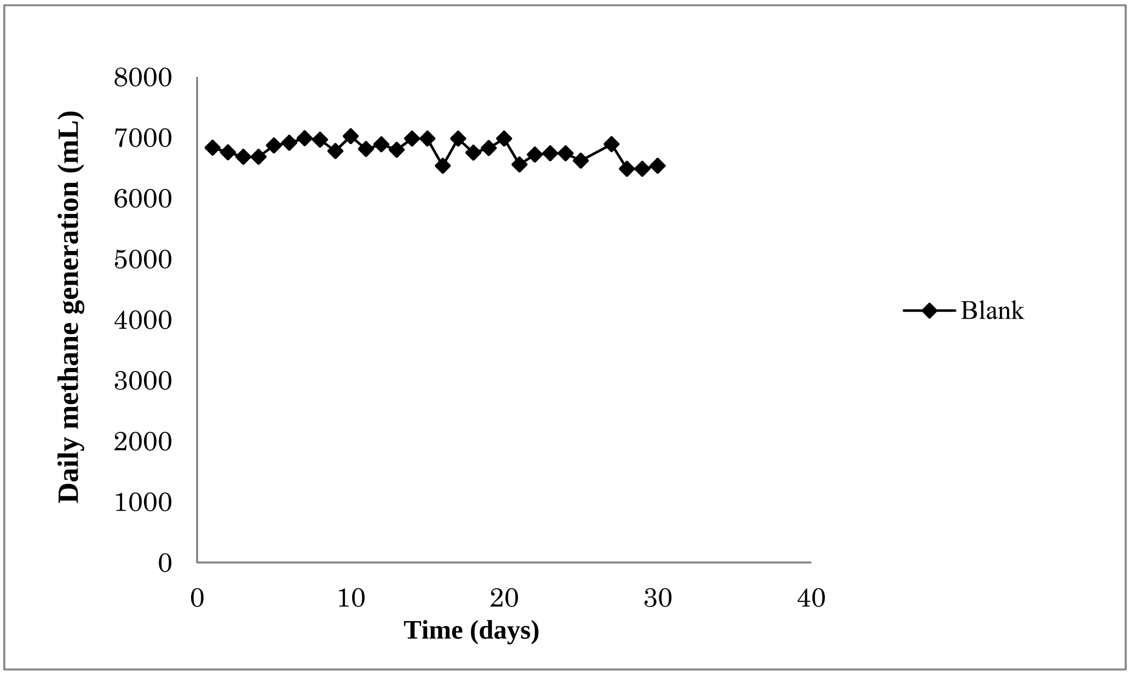

Fig. 4.2. Daily methane production from the blank reactor (R1)

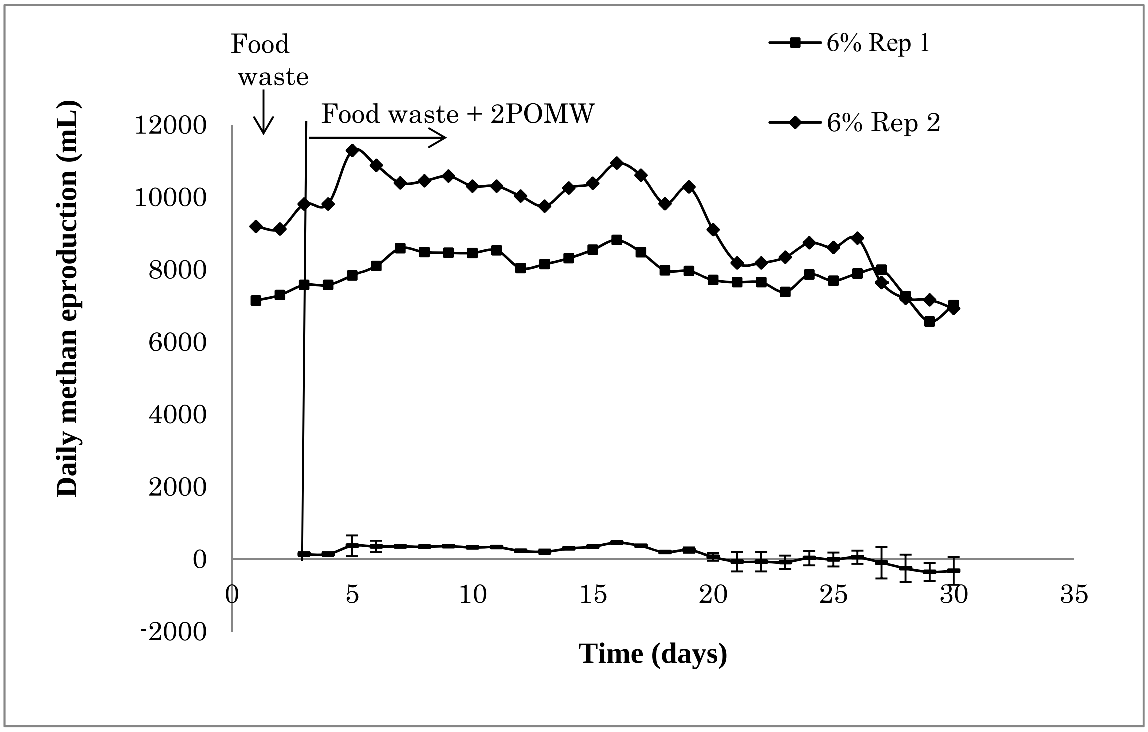

Fig. 4.3. Daily methane production from the codigestion of food waste with 6% NaOH-pretreated 2POMW with mixing ratio of 8.3% (R2)

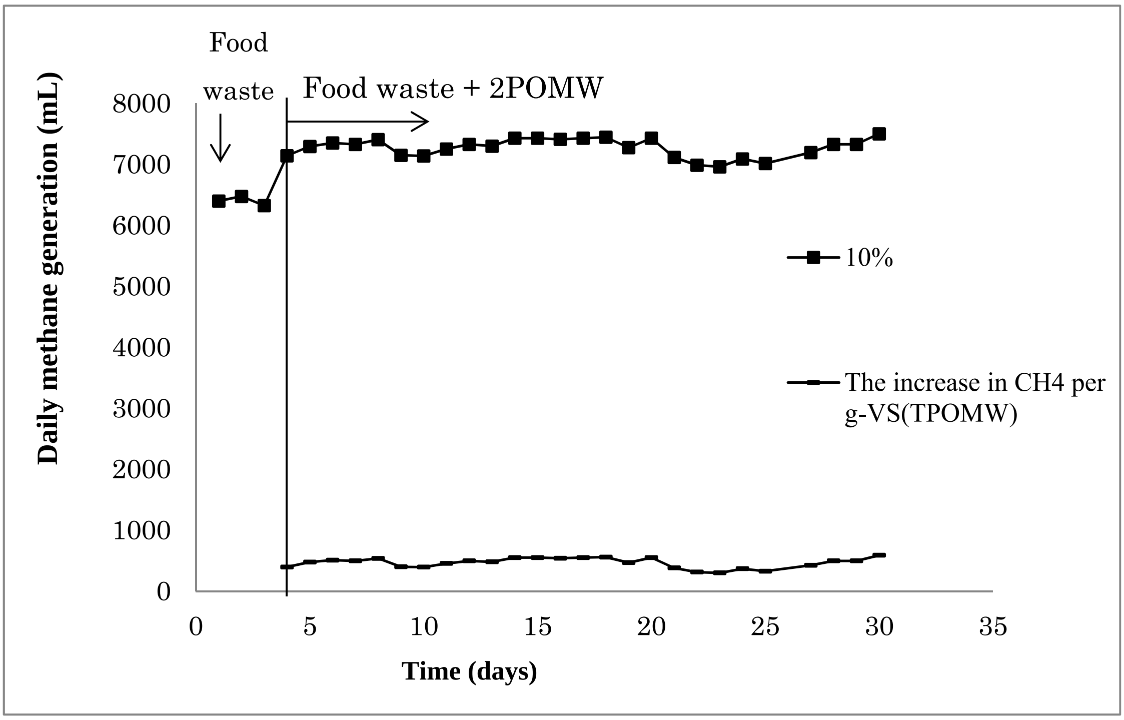

Fig. 4..4. Daily methane production from the codigestion of food waste with 10% NaOH- pretreated 2POMW with mixing ratio of 4.3% (R3)

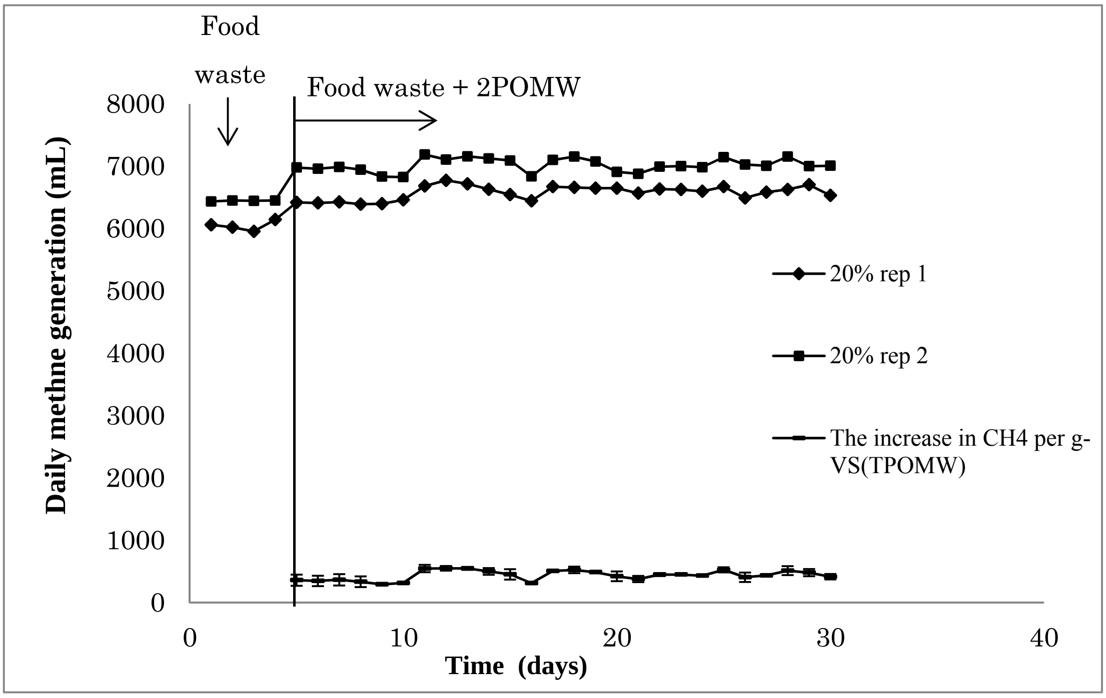

Fig. 4.5 Daily methane production from the codigestion of food waste with 20% NaOH-pretreated 2POMW with mixing ratio of 3% (R4)

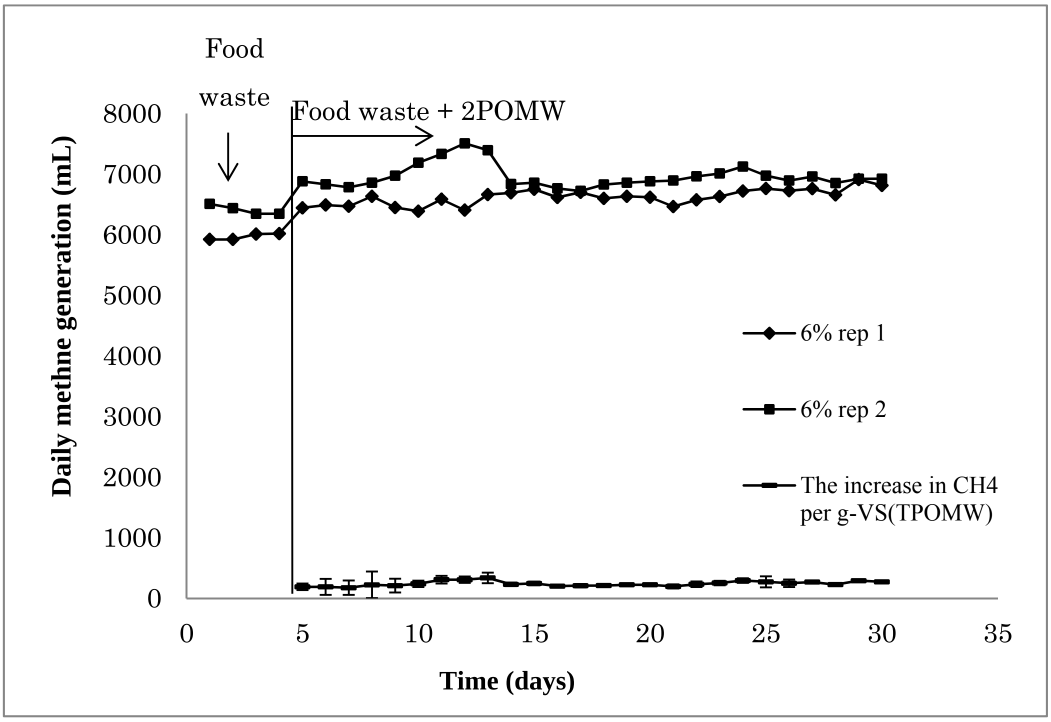

Fig. 4.6. Daily methane production from the codigestion of food waste with 6% NaOH- pretreated 2POMW with mixing ratio of 5.7% (R5)

Figure 4.7 shows the relation between oleic acid concentration and methane gas production. For low mixing ratios of 3% and 4.3% oleic acid concentration were low, 9.8 mg/L and 6.11 mg/L respectively. This was accompanied with high methane gas production from the 2POMW. With increasing the mixing ratio to 5.7%, oleic acid concentration increased and methane production decreased. A further increase in mixing ratio to 8.3% increased oleic acid concentration to 41.53 mg/L which was above the inhibition level 30 mg/L (Lalman and Bagley, 2001).

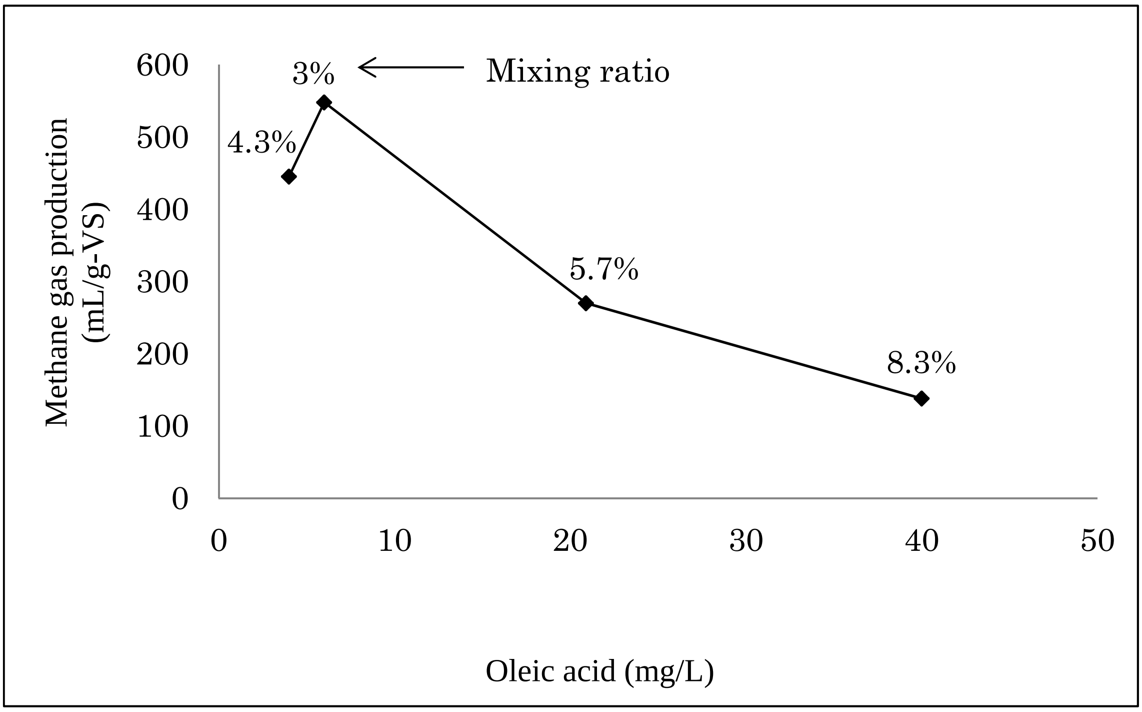

Fig. 4.7. The relationship between methane production and LCFA (measured after 15 days of codigestion)

4.4 Summary

Introducing the 2POMW as a sole source of input into anaerobic digesters is limited by the presence of inhibitors, as well as, its seasonal production. In this study the codigestion of NaOH pretreated 2POMW with food waste was investigated. Codigestion plays a dilution role and reduces the toxicity of inhibitors.

1. This study showed that the mixing ratio is an important factor for designing an anaerobic reactor for codigesting 2POMW because it affects the amount of LCFA inside the reactor.
2. The high LCFA concentration with high mixing ratio inhibited methane gas production.
3. There was no inhibition of methane gas production up to a mixing ratio of 4.3%.
4. Treatments of 10% NaOH pretreatment with 4.3% mixing ratio and 20% NaOH pretreatment with 3% mixing ratio are considered to be adequate regarding methane gas production.

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CHAPTER 5
THE APPLICABILITY OF A CODIGESTION SYSTEM TO TREAT 2POMW IN JORDAN

5.1 Olive oil industry in Jordan

As other Mediterranean countries, Jordan is rich with olive trees plantation. The number of olive trees was reported to be more than 116,000 thousand trees planted over an area of 150,000 acre (Department of statistics, 2012). About 83% of the olive fruits are converted to oil. There are 112 olive mills distributed in the country, mostly concentrated in the northern part. 71% of the olive mills are using three phase centrifugation, 22% are using the two phase centrifugation, while 7% are still applying the tradition pressing (final report).

Three main byproducts come out from the mills; olive oil, olive pomace and waste water. Abu Ashour et al., (2010) reported that in 2005 processing 106,750 of olive fruits generated 26,688 tons of olive pomace. Hamatteh et al (2010) reported that 80,000 ton of olive pomace is generated annually from olive mills. Until now, olive pomace does not have a proper way of management or economic value. Some of the pomace is used as a feeding source for animals, some is burnt in a small scale to produce heat; however, most of the waste is just dumped in the environment.

5.2 Jordan’s policy for renewable energy

The Government of Jordan faces challenges in the energy sector, rising demand due to population growth, increased per capita consumption and a reduction in the availability of market priced fuel. Jordan imports 96 percent of its oil and gas. Electricity generation in Jordan has been mainly dependent on natural gas, imported from Egypt.

The government is seeking an investment of US$18 billion in the sector of renewable energy, oil shale and nuclear power by 2020. Therefore, recently the Jordan`s parliament adopted the renewable energy and energy efficiency law (REEL) in April 2012. The plans aim to increase the renewable energy share in the energy mix from 2% to 7% by 2015 and to 10% by 2020 (Electricity regulation commission)

- ✓ 600 - 1000 MW Wind Energy.
- ✓ 300 - 600 MW Solar Energy.
- ✓ 30 - 50 MW Waste to Energy

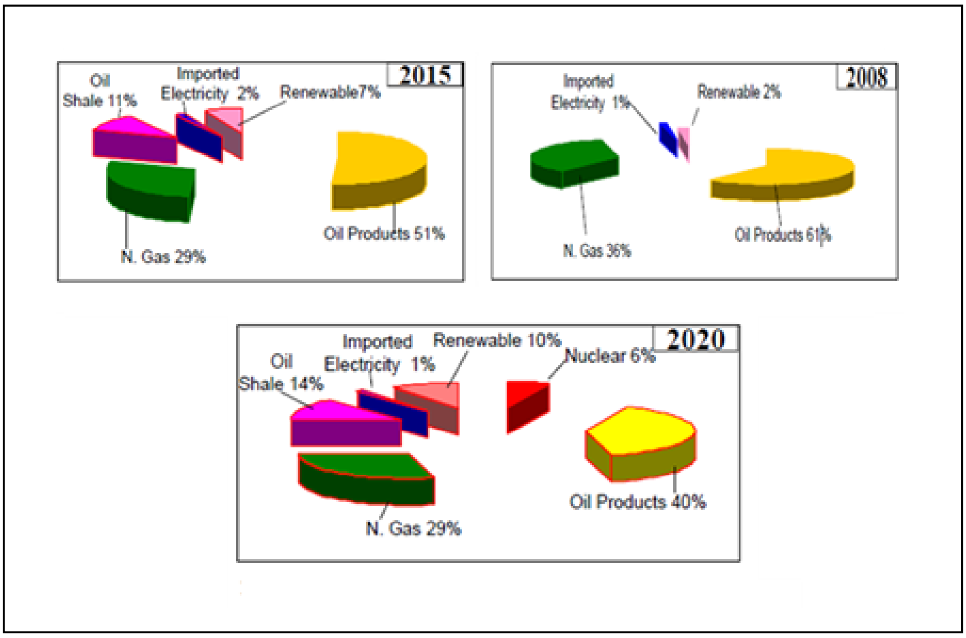

Fig. 5.1. The energy mix in Jordan (Electricity Regulatory Commission)

5.3 Biogas as a renewable energy

There is only one biogas plant in Jordan that receives food waste. This biogas plant is called *The Jordan Biogas Company (Russaifa biogas plant)* and it was established in 1998 and started operating in 2000 as a 1 MWh pilot project. This plant consists of two parts; (1) a system of 12 landfill gas wells (in the landfill) and (2) a Biogas plant (digester) receiving organic waste at a rate of 60 tons per day from various generators like hotels, restaurants and slaughterhouses in Amman (the capital city of Jordan). Unfortunately, the biogas digester stopped working in 2005 because of improper separation of the waste in Amman

The Jordan biogas company (JBC) is owned by (1) greater Amman municipality and (2) the central electricity generating company. Between the start-up in May 2000 and 2002, the JBC has sold electricity to the grid at an average 0.05 US $/kWh for a profit of nearly 225,700 US $.
Considering the daily application of 60 ton food waste, the plant has the potential to produce 3756 MWh of electricity every year.

The plant consists of:

1. Solid waste receiving inception
2. Liquid waste receiving
3. Mixing tank: solid and liquid wastes are mixed to reach 10% total solid.
4. Digester: 2000 m^3 reactor operates under mesophiloic condition (37 ℃) and hydraulic retention time of 25 days.
5. Separator: separates the digestate in to liquid and solid compost
6. Storage tank for the liquid compost
7. Gas cleaning unit to remove H_2S
9. Gas storage
10. Electricity production unit: JENBACHER engine works on biogas to turn on a generator which produces electricity with the capacity of 1 MWh.

5.4 The applicability of treating 2POMW in an already existing anaerobic digester

The applicability of 2POMW is limited by its short period of generation which is limited to the winter season (November to March). Therefore, treating 2POMW as a sole source for anaerobic digester is believed to be economically and technically unfeasible.

This study considers the application of 2POMW in the already existing digester of the

Jordan biogas company to be a supplementary input only introduced into the reactor in the period of olive oil production. This will increase the energy production in the winter season, when energy requirement is more because of the cold weather.

The digester of the Jordan biogas company is designed to receive 60 t/d food wastes. Considering the methane production capacity of 50 m^3 CH_4 per ton of food waste, 90000 m^3 CH_4 can be produced monthly. This amount of gas if converted to electricity gives 309 MWh, as one cubic meter of methane can be converted to 9.8 kWh (Van Erten –Jansen et al, 2012). In our study co-digesting 10% NaOH pretreated 2POMW in a mixing ratio of 4.3% with food waste showed the highest increase in methane production. In order to sustain the mixing ratio of 4.3%, 5.16 tons of 10% NaOH pretreated 2POMW must be applied, considering that the food waste is diluted with 50% water. Our study showed a methane production potential of 548.5 mL per one gram volatile solid of the 10% NaOH pretreated 2POMW. Therefore, applying 5.16 tons per day of the NaOH pretreated 2POMW renders 21142 m^3/month of methane, which if converted to electricity gives 72.5 MWh/ month of electricity. This increases the electricity production of the reactor by 23.2% (table 5.1).

Table 5.1. The calculation showing the increase in electricity production from the addition of 2POMW to the already existing plant receiving food waste in Jordan

Monthly electricity produced by the plant from the 60t/d food waste = 309 MWh	
Mixing ratio of 2POMW 4.3%	
Methane production potential from the 10% NaOH is 548.5 mL/g-VS	
Daily application of 2POMW	5.16 t/d
Daily application as volatile solid	VS = 24.9% (from table 3.1) 5.16× (24.9/100) = 1.28 t- $VS_{(2POMW)}$
Additional daily methane production	=1.28 t- $VS_{(2POMW)} \times 10^6$ g/t×548.5mL CH_4/ g-VS $\times 10^{-6}$ m^3/mL= 704 m^3
Additional monthly methane production	704 m^3/d× 30 d/month = 21142.04 m^3
Additional monthly electricity production	21142.04 m^3 CH_4× 9.8 KWh/m^3× 10^3MWh/KWh × 0.35 (efficiency) = 72.5 MWh/month
The increase in electricity production from the additional 2POMW	=72.5/309×100=23.5%

5.5 Summary

Olive milling waste is produced in Jordan in Large quantities. The treatment of 2POMW in anaerobic digestion for energy production is limited by its seasonal production.

- In this study the applicability of 2POMW in the already existing biogas plant in Jordan has been studied.

- Considering the results of our experiment, Applying 10% NaOH pretreated 2POMW to the already existing plant in Jordan can increase the electricity production efficiency by 23.5%.

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CHAPTER 6

Conclusion

Olive milling waste is one of the most problematic agro- food industrial wastes in the Mediterranean countries. Improper management and disposal of this waste can lead to serious environmental problems because of its high organic load and phytotoxic properties. The doughy structure of the 2POMW, which is produced by two phase centrifugation, makes its storage and disposal even more difficult. This study proposed a practical method for designing a system for anaerobic treatment of 2POMW coupled with energy recovery.

Chapter 1 shows the motivation of this study regarding the environmental problems caused by the uncontrolled discharge of olive milling waste to the environment. This led us to set several objectives which are discussed in this chapter as well. Finally, this chapter shows the structure of the thesis with a brief description of each chapter.

Chapter 2 presents some figures related to the olive oil production in the major olive oil producing countries. This chapter gives a description of the three methods of olive oil extraction which is important to understand and distinguish the different wastes that come out form different extraction process. Current practices of olive wastes disposal were discussed. The anaerobic digestion which was applied in our study was discussed; especially the limitations of the process and potential solutions to overcome inhibition.

Since the digestion of 2POMW is limited by its lignocellulosic composition, in **Chapter 3** the effect of pretreatment of 2POMW on facilitating its solubilization and degradation is presented. Among the pretreatments tested, alkaline pretreatment with NaOH showed the best performance in terms of both organic compounds solubilization and methane production. CaO could degrade part of the organic material; however, it was not sufficient to solubilize the organic compounds. Mechanical pretreatment by size reduction was not effective to enhance degradation. Choosing the proper concentration of NaOH for pretreatment is critical for the anaerobic digestion process; high NaOH concentration of 20% showed the highest solubilization, but introducing a large amount of 20% NaOH pretreated 2POMW without pH control can cause inhibition because of the high pH level. Therefore, in this batch experiment, 6% NaOH pretreatment showed the highest increase of methane production.

Chapter 4 proposed a codigestion system of 2POMW with food waste as a potential solution to overcome inhibition and to increase the electricity production of a biogas plant that regularly receives food waste. Introducing the 2POMW as a sole source of input into an anaerobic digester is limited by the presence of inhibitors, as well as, its seasonal production. In this chapter the codigestion of NaOH pretreated 2POMW with food waste was studied. Codigestion plays a dilution role and reduces the toxicity of inhibitors. In this study, the effect of mixing ratio was investigated; increasing mixing ratio caused an increase in the concentration of oleic acid, which inhibited the anaerobic digestion process. Up to mixing ratio of 4.3% there was no inhibition of methane gas production. Applying 10% NaOH pretreated 2POMW in a mixing ratio of 4.3% increased methane gas production by 548.5 mL CH_4/g-VS/d.

Chapter 5 proposed a system for treating 2POMW in Jordan. This chapter states some figures related to the olive oil production in Jordan and the effect of the generated waste on the environment. The Jordanian government intends to increase the renewable energy share in the energy mix in Jordan. Therefore, applying 2POMW in anaerobic digester can be an attractive option. The applicability of applying NaOH pretreated 2POMW in the already existing anaerobic digester was discussed and evaluated.

In conclusion this study proposed a system to facilitate the anaerobic digestion of 2POMW. Our proposed system showed that 2POMW can be successfully treated and converted to a source of energy through a step of pretreatment and codigestion with food waste; for example, introducing 10% NaOH pretreated 2POMW in a mixing ration of 4.3% into the already existing reactor in Jordan can increase the monthly electricity production of the reactor by 23.5%.