An-Najah National University

Faculty of Engineering and Information Technology

Chemical Engineering Department

Graduation Project II

Toward Biogas Production from Olive Mill Wastewater (OMWW) and Constructing Anaerobic Lab Scale Bioreactor

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Abstract

Olive oil production is a traditional industry in Palestine; about 20,754 tons of olive oil are produced annually in Palestine. However, Olive Mill Wastewater (OMWW) -which is the black liquid byproduct of the olive oil extraction process- management is a crucial environmental challenge facing the Mediterranean countries including Palestine. Based on previous studies, Co-digestion of OMWW with sludge from the digester in a designed bioreactor under specific conditions provides the possibility to eliminate the OMWW toxicity and produce biogas at the same time.

The main goal of this study is to treat the OMWW with sludge at different ratios under anaerobic digestion process at a mesophilic condition with an optimum temperature equal to 37°C using a laboratory-scale bioreactor.

The project started by constructing and build a laboratory-scale bioreactor. Depending on the economical and qualitative aspects 5 liters in size pressure cooker (four identical pressure cookers) was chosen to be used as a lab-scale bioreactor. Due to the sensitivity of the bacteria and sustainability, stainless steel was considered as a material of construction.

The choice of a pressure cooker as a constructed lab-scale bioreactor was basically to perform the digestion process to treat the OMWW samples. Thus, the four pressure cookers were modified in a way to reach the desired construction of the bioreactor. The modifications were done in manner to transform the pressure cooker into a lab-scale bioreactor as follows;¾" hole with a PVC pipe was inserted in the led as the feed inlet, a washing nozzle (stainless steel water tap), 2 PVC pipes (¾") with 3 elbows were also inserted; lastly, a gas tube was connected with the bike tire for gas collection purpose. The produced biogas will be utilized as the stirring system to prevent bacteria scum.

The project work was mostly involved around the construction part as explained above due to the current situations (the spread of Covid-19) but some tests were made to assure that the system is working properly and to check some parameters like leaking and stability. Another test was the cow manure test which was done to confirm the design stability.

This project is a continues work for the previous work that was accomplished in the first semester were fresh OMWW samples were collected from Al- Kafryat pressing mill, near Tulkarem and the Wastewater Treatment Plant (WWTP), near Nablus. Characterization of OMWW samples were done based on number of physical and chemical parameters such as chemical oxygen demand (COD), ammonia, total nitrogen, pH and total dissolved solids (TDS). The results that obtained were respectively 60,000 mg/L, 280 mg/L, 630 mg/L, 7.4 and 20,746 mg/L.

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Nomenclatures

BOD	Biochemical Oxygen Demand
CH_4	Methane
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank
HRT	Hydraulic Retention Time
LCM	Liquid Cow Manure
OLR	Organic Load Rate
OMWW	Olive Mill Wastewater
SRT	Solid Retention Time
TCOD	Total Chemical Oxygen Demand
TDS	Total Dissolved Solid
TP	Total Phenolic
TPOMWW	Two Phase Olive Mill Wastewater
TPOMWW	Two Phase Olive Mill Wastewater
TS	Total Solid
VFAs	Volatile Fatty Acids
TSS	Total Suspended Solid
WWTP	Wastewater Treatment Plant

Chapter One

1. Introduction

Wastewater is defined as a complex matrix that contains several concentrations such as solids (total solids 350–1200 mg/l), dissolved and particulate matter (chemical oxygen demand (COD) 250– 1000 mg/l), microorganisms (up to 109 number/ml), nutrients and heavy metals and micro-pollutants (Warwick *et al.*, 2013). Wastewater is classified mainly into four groups; domestic wastewater, industrial wastewater, storm-water, and infiltrations. These four groups can be present in a combined system or separate sewage systems (Henze *et al.*, 2008).

According to the studies, industrial wastewater is considered as one of the most pollutants elements for the water environment. Especially during the last decades, the discharge of industrial wastewater into rivers, lakes and coastal areas has significantly increased. Therefore, a specific treatment system is truly needed. Developing a treatment system is depended on the type of industrial wastewater as there are many types of industrial wastewater, each type requires special treatment technique (Hanchang, 2009).

The anaerobic digestion process is one of the most common treatments that are widely used to treat different industrial effluents. To maintain the anaerobic process, an appropriate bioreactor design is required. The selection of a specific bioreactor design includes a series of choices ranging from basic microbiology and biochemistry to process engineering and marketing which must contribute in a symmetric way to make a proper determination of the basic type of bioreactor system and its correct dimensions to provide an optimal environment for the biological system, operating at minimal cost in order to maximize the benefits and the productivity of the process (Asenjo, 1994).

Typically, the design of biological reactors involves determining operating conditions, sizing the reactor, controlling temperature and sterility, determining the means of feed introduction and product removal, designing for mixing and mass transfer and controlling operating variables, such as pH and oxygen concentrations (McDuffie, 2013). The importance of selecting the suitable construction of bioreactor is highly needed to achieve the desired results such as the amount of the biogas compositions and the optimum ratios to produce biogas (Mandenius, 2016)

The anaerobic digestion treatment with other types of waste (e.g. cow manure, chicken manure, and sludge from the wastewater treatment plant), in other terms anaerobic co-digestion treatment is the presuming solution for the reduction of the OMWW Chemical Oxygen Demand (COD), and generating the methane gas (Battista *et al.*, 2013).

1.1. Problem Statement

In the current Palestinian situation, the enormous produced amounts of OMWW composes a real threat to the environment. The annual OMWW production in the Mediterranean countries is estimated to amounts ranging from 7 to over 30 million m^3. In the West Bank, the annual production is up to 120,000 m^3 besides thousands of tons of solid olive residues. This huge amount of OMWW has no pre-treatment system before discharging it directly into Wadi or sewage networks (Nazzal, 2017).

The OMWW has a very high organic load, as the load is 100-150 times greater than the organic load of domestic wastewater (1080 mg/L) (Al-Khatib *et al.*, 2009). The process of discharging the OMWW directly into Wadi or the public sewage networks has a huge effect on the efficiency of the Wastewater Treatment Plant (WWTP) and the activity of microorganisms and the effluent quality.

The most problem of OMWW is the high organic content, acidity pH and high phenols content which is toxic to many microorganisms (Nazzal, 2017).

Despite the importance of the olive oil production industry for the Palestinian economy, the problem of produced OMWW is becoming a real and serious environmental problem. Thus, it's required to solve the problem and apply a reasonable and practical treatment technique. The proposed treatment in the project is an anaerobic co-digestion process taking into consideration the design of an anaerobic bioreactor to perform the target process.

1.2. Objectives

The main objectives of this project are:

1. Construct a laboratory-scale bioreactor with the required specifications (such as the size, the material of construction and temperature controlling).
2. To produce biogas from the anaerobic digestion of OMWW locally named as “Zibar” mixed with sludge (from digester) obtained from the domestic wastewater treatment plant.
3. To measure the volume of produced biogas and determine the methane (CH_4) content.
4. To examine the optimum ratio between OMWW and the sludge for maximum biogas production.

1.3. Challenges

There are many challenges that were encountered during the graduation project’s journey, here are some of these challenges:

- The spread of pandemic Covid-19, which threatened the entire world and because of it, part of the graduation project was suspended and thus the possibility to complete the planned experimental work.
- Time delay through the construction of lab-scale bioreactors due to the facilities to accomplish the desired design.

1.4 Constraints and Engineering Standards

Constraints:

- Operating the digestion process under mesophilic conditions at 37 ℃ (Stable, less energy and wide use in practice). Olive Mill Wastewater samples are only available seasonally (September - November), so that it was mandatory to store the samples in glass bottles inside the refrigerator at 4 ℃.
- Transfer the sludge for wastewater treatment plant to the laboratory immediately

Standards:

1. Chemical Oxygen Demand Test (COD), Ammonia Test, Total Nitrogen Test and Total Dissolved Solid Test must be done according to the standards.
2. The recommended bioreactor working is 3.5 L (70% of 5 L) (Kurt Eyer, 2020).
3. Retention time should be about (15–30) days to treat waste under mesophilic conditions.

1.4. Report Structure

The report consists of six chapters, the first one includes the introduction in general, also the problem statement, the objectives and the challenges of this project. The second chapter includes the literature review part that contains eleven sections: OMWW treatment, OMWW characteristics, potential studies for biogas production, olive pressing techniques (traditional and modern methods), olive oil production in Palestine, the environmental impact of OMWW, biogas from OMWW, anaerobic digestion stages, also factors affecting anaerobic digestion process through biogas and anaerobic digestion stages. Chapter three is about methodology and analytical methods. The fourth and fifth chapters contain lab-scale bioreactor construction and recommendations respectively. Finally, chapter six is about the conclusion

Chapter Two

2. Literature Review

2.1. Olive Mill Wastewater Treatment

There are several processes that could be used to treat the OMWW which includes the physical method, the physio-chemical methods (flocculation, filtration, incineration, open evaporating ponds, and coagulation), and biological treatment methods that reduce the organic load of OMWW.

2.1.1. Physical treatment

Physical processes are applied to separate the various phases (solids, liquids, and gases) of OMWW. However, physical processes are not able to achieve acceptable limits for toxicity and organic load alone when used in OMWW treatment. physical processes include: dilution, sedimentation, filtration, centrifugation, evaporation filtration, and dissolved-air flotation (Abushattal *et al.*, 2017).

2.1.2. Forced Evaporation

For two phase oil extraction process, the appropriate management and treatment option for OMWW in is the "natural evaporation method". It can be considered a low-cost solution to incorporation of wastewater pollutants into dried sludge. However, due to the long evaporation periods this technique is suitable only for low to medium wastewater flows. This alternative treatment is characterized by the generation of dried sludge that can be disposed of or reused as fertilizer (Shaheen and Abdel Karim, 2007).

2.1.3. Physio-chemical treatment

This method is mainly used to remove organic matter from the liquid phase by adding chemicals. Many techniques used in this method such as, neutralization technique, flocculation technique, precipitation, adsorption, chemical oxidation, and Ion exchange. However, it has many disadvantages such as; sludge-disposal problems, low efficiency, and high cost (Abushattal *et al.*, 2017).

2.1.4. Electrocoagulation

Electrocoagulation is a process that consists of creating a floc of metallic hydroxides within the effluent to be cleaned, by electro dissolution of soluble anodes. Compared with traditional flocculation and coagulation, electrocoagulation has, in theory, the advantage of removing the smallest colloidal particles; the smallest charged particles have a greater probability of being coagulated because of the electric field that sets them in motion. It is used to reduce the phenolic content of OMWW effluent. Electrocoagulation is an effective pre-treatment technique which aims to reduce the organic load of OMWW prior to an upcoming biological treatment which resulted in a higher volume of biogas compared to crude or diluted OMWW (Sounni *et al.*, 2018).

2.1.5. Biological treatment

Biological treatment is the most appropriate and modern cheapest way to treat OMWW. The biodegradable chemical species present in OMWW can be degraded by this method using microorganisms that break down these species with environmental consideration. Biological therapy can be achieved by using aerobic, anaerobic treatment (Abushattal *et al.*, 2017).

Detoxification processes run by fungi is more effective than that run by bacteria that applied to degrade the phenolic compound. The removal rate of fungi for COD as 40 – 88 %, for phenols as 60 – 100 %.

2.1.5.1. Aerobic processes

Aerobic processes are applied as a pre-treatment to reduce COD, TSS, and phenolic compounds which enhancing the anaerobic treatment. The aerobic digestion conditions include the availability of oxygen and nutrient where the aerobic microorganism thrives in these conditions. However, the aerobic treatment is not highly recommended due to the number of reasons: a. It is an expensive treatment. b. Needs dilution. c. High residence time is necessary. d. Requires a pH adjustment.

2.1.5.2. Anaerobic process

The anaerobic treatment is a cost-effective alternative, when it is compared to the aerobic treatment, especially for high organic industrial wastewater. Anaerobic wastewater treatment processes for effluent treatment of olive presses were tested in experimental measures (Shaheen and Abdel Karim, 2007).

Anaerobic treatments include the degradation of organic matter and the production of biogas such via a microorganism in the condition of absence of oxygen. Many parameters affect the anaerobic digestion process which is Solids Retention Time (SRT), Temperature, pH and Alkalinity. Anaerobic treatment is widely used especially for the production of biogases which has big capability to recover energy, saving energy, and reduce operational cost (Abushattal *et al.*, 2017).

2.2. OMWW Characteristics

Serious environmental problems arise when OMWW is discharged into the environment without applying any treatment or controls because OMWW contains a high amount of chemical species that resist degradation such as phenolic compound and high COD content.

In general, OMWW contains water with 83 wt %, 15 wt % organic compounds and the rest inorganic compounds as illustrated in Figure 2.9.2

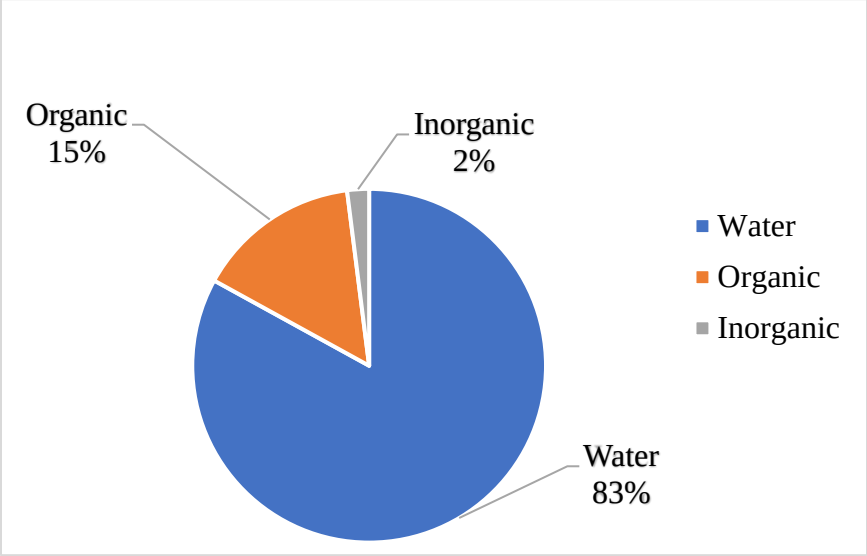

Figure 2.9.2.: The main composition of OMWW

Also, it is characterized by the high concentration of cations and anions, COD, BOD, Polyphenolic compounds, Fat and Nutrient.

Usually, OMWW can be described in dark violet to black colour, and its organic compound load is 100-150 times higher than the organic load of domestic wastewater, which is the highest percentage of all ingredients and has a specific oil odor (Yosef and Abdallah, 2016).

OMWW pH between 4.9 and 5.1, high electrical conductivity, high concentration of total suspends solid (TSS) and total dissolved solids (TDS), COD (up to 247 g/L) and high polyphenol concentration (up to 21 g/L).

Table 2 below shows the general characteristic of OMWW (Yosef and Abdallah, 2016).

Table 2.5.1 1: General Characteristic of OMWW from (2004 – 2016)

Parameter	Unit	Yosef (2016)	Aladham (2012)	Khtib *et al.*, (2009)	Basheer *et al.*, (2004)
BOD5	mg/L	33532	11375	45624.67	27500
COD	mg/L	246652	137525	98999.67	163500
TS	mg/L	75328	67478		
TSS	mg/L	62117	52014	16963.6	86840
TDS	mg/L	13211	15464	35212.67	
Total polyphenols	mg/L	21000	4592	3149.33	6800
pH		5.1	4.9	4.99	5

All of the above parameters must be taken into consideration in the design of a well-integrated treatment process of OMWW.

1.1. Potential Studies for Biogas Production from OMWW

Several studies were inducted all over the world to study the anaerobic treatability of OMWW. A group of these studies from different regions are illustrated below.

A study in Turkey was inducted to evaluate the anaerobic digestion process of a group of OMWW samples with a collected digester sludge from Ankara wastewater treatment plant. The substrate was injected in 125 mL serum bottles for 44 days, the gas production rate was monitored and measured using a gas displacement device. Additionally, the CH_4 content was measured using a 20 g/L KOH solution in other serum bottles. It was found that the CH_4 content of the produced biogas from olive-oil mill wastewater was 77 ± 6%. **Error! Reference source not found.** illustrates the cumulative gas production for the OMWW samples (Demirer *et al.*, 2000).

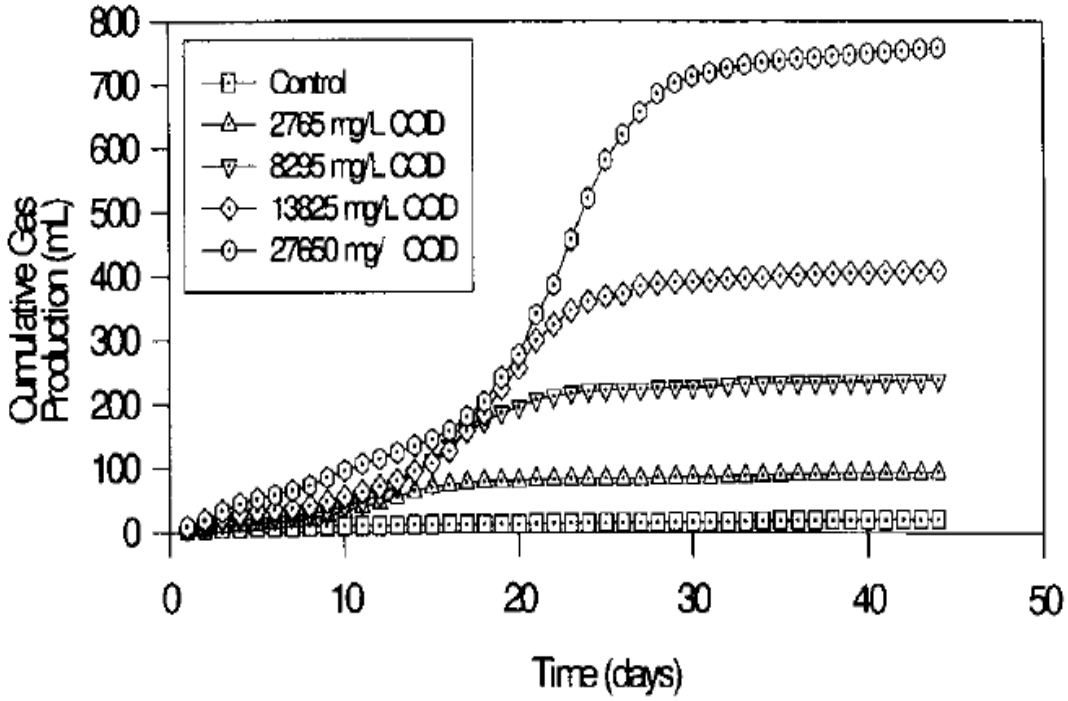

Figure 2.3. 1:Cumulative gas production for different OMWW samples (Demirer *et al.*, 2000).

According to a different study in Vegas Altas Spain, it was confirmed to perform an aerobic pretreatment for the collected OMWW samples. The aerobic pretreatment was followed up by the anaerobic digestion process. The anaerobic digestion process was incited and activated via injecting the sludge inoculum to the OMWW samples. The experiments were performed for a period of time that ensured that reliable results would be obtained. The results for the anaerobic digestion process (methane yield, pH, reduction of total chemical oxygen demand) are illustrated in Figure 2.3. 2. A reduction in polyphenols concentration of 78% (5 days aeration time), achieves a methane production of 0.39 m^3/kg removed TCOD and 65.17% TCOD degradation (González-González and Cuadros, 2015).

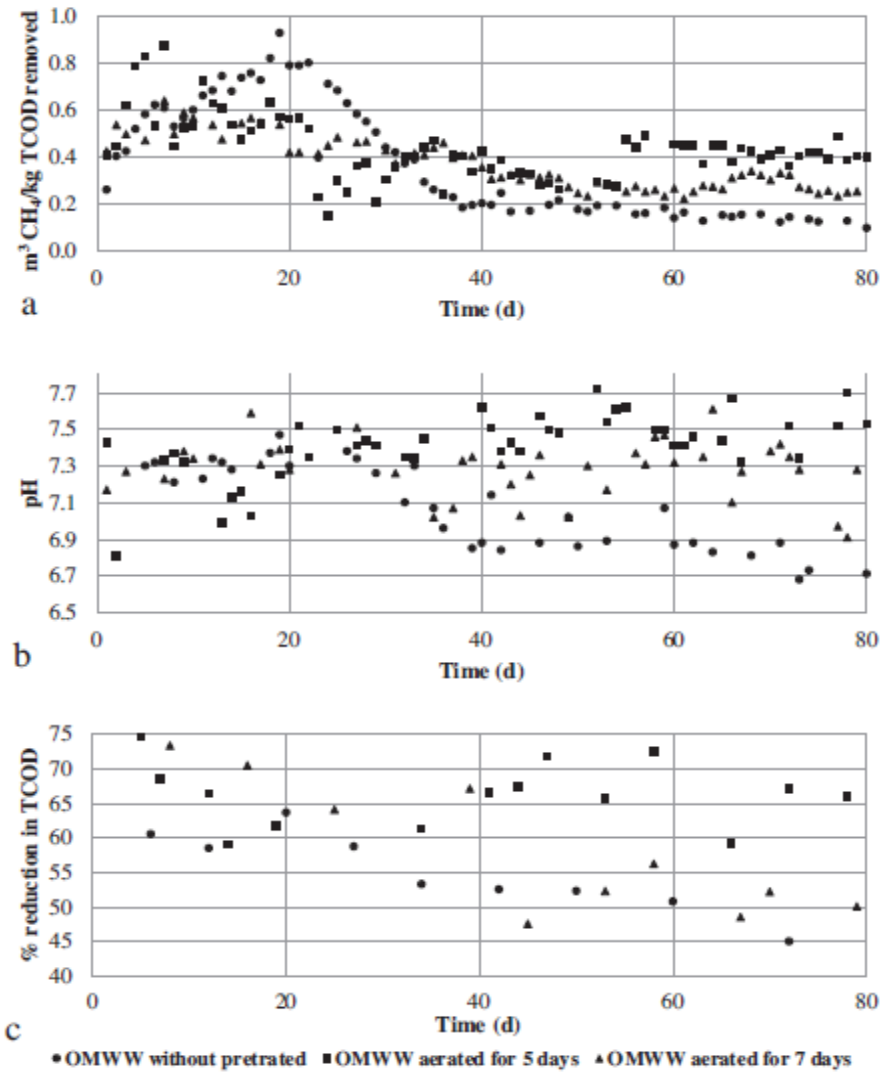

Figure 2.3. 2: Results of anaerobic digestion of OMWW: no pretreatment stage and 5- and 7-days aeration process. (a)Methane yield, (b) pH, (c) reduction of Total Chemical Oxygen Demand (González-González and Cuadros, 2015).

1.2. Anaerobic Bioreactor Design

The biochemical reactor is defined as a system that provides a biologically active environment, where the microorganisms and enzymes are immobilized, and biochemical reactions are performed (Somiya, 2013).

Generally, the anaerobic bioreactor design is dependent on the experimental basis. Each anaerobic system has its requirement and specifications based on several conditions and factors. There are different types of bioreactors are represented below in details (Khanal *et al.*, 2017):

1. **Continuous-Stirred Tank Reactor (CSTR):**

The CSTR is the most commonly used reactor configuration employed for anaerobic treatment of industrial effluent containing medium to high suspended solids with total solids (TS) content of 0.5% or higher. The contents in the reactor are maintained under completely-mixed conditions by mixing continuously or intermittently using the mechanical mixture, biogas sparging, or liquid recirculation. A schematic diagram for the CSTR is illustrated in Figure 2.4.1.

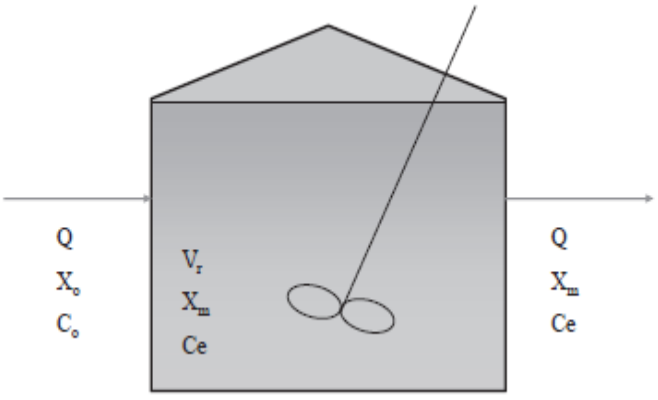

Figure 2.4. 1: Schematic diagram of continuous-stirred tank reactor (Khanal et al., 2017)

2. Covered Anaerobic Lagoon (CAL):

The CAL is an inexpensive option for treating the industrial effluents with TS content of 0.5- 3%. The CALs are designed as earthen pits constructed with impermeable liners (e.g., clay) at the bottom and sides. A typical CAL uses neither mechanical mixing nor external heating. It is operated at ambient conditions; thus, the treatment efficiency is tied to the geographical location and climate. Figure 2.4.2 shows the typical CAL.

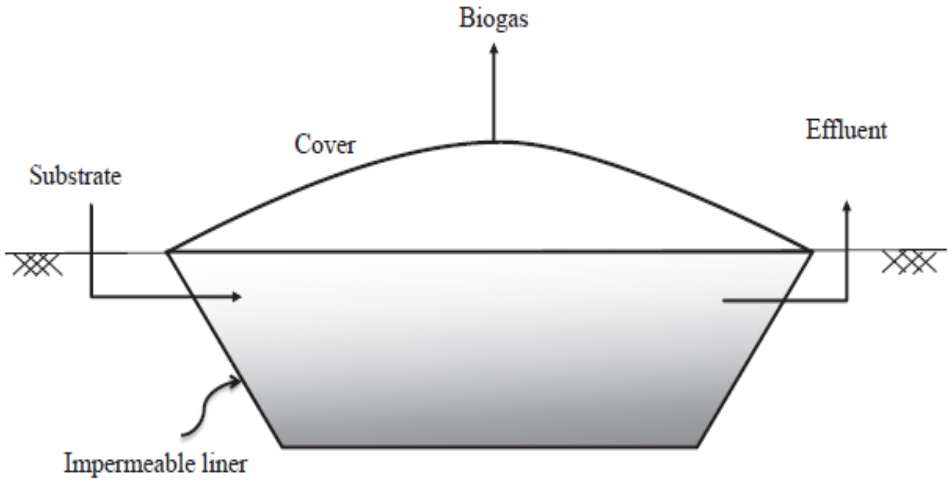

Figure 2.4. 2: Schematic diagram of a typical covered anaerobic lagoon (Khanal et al., 2017).

3. Anaerobic Contact Reactor (ACR)

The ACR is mainly used for treating the industrial wastewater with high suspended solids (e.g., wastewater from a meat packing plant). It mainly includes a CSTR and a downstream settling tank. The settled microbial biomass is recycled back to the reactor, due to the ACR configuration it maintains a high biomass concentration. Also, the produced biogas (CH4 and CO2) are removed from the liquid phase via degassifier to prevent biomass floating. Figure 2.4.3 below shows the ACR design.

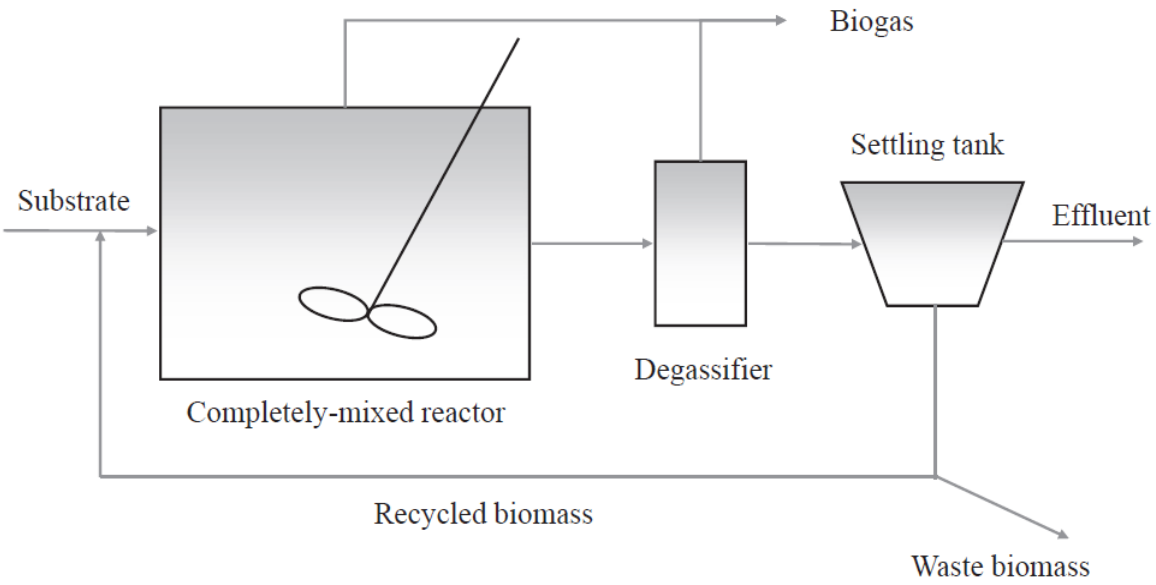

Figure 2.4. 3 :Schematic diagram of anaerobic contact reactor (Khanal et al., 2017).

4. Upflow Anaerobic Sludge Blanket Reactor (UASB):

The UASB reactor is a suspended growth reactor mainly utilized for the very high concentration of microbial biomass (e.g. food processing, agro-based industries, and other carbohydrate-rich industries) by promoting granulation. The anaerobic granules are 1-3 mm in diameter and dense enough to settle down in the reactor. The substrate is uniformly distributed at the bottom of the reactor, where the anaerobic granules come in contact with the organic matter and degrade it. UASB design based on two approaches, the first one the maximum allowable volumetric OLR to obtain the desired organic removal efficiency. And the other approach based on the specific sludge activity or the specific substrate utilization rate.

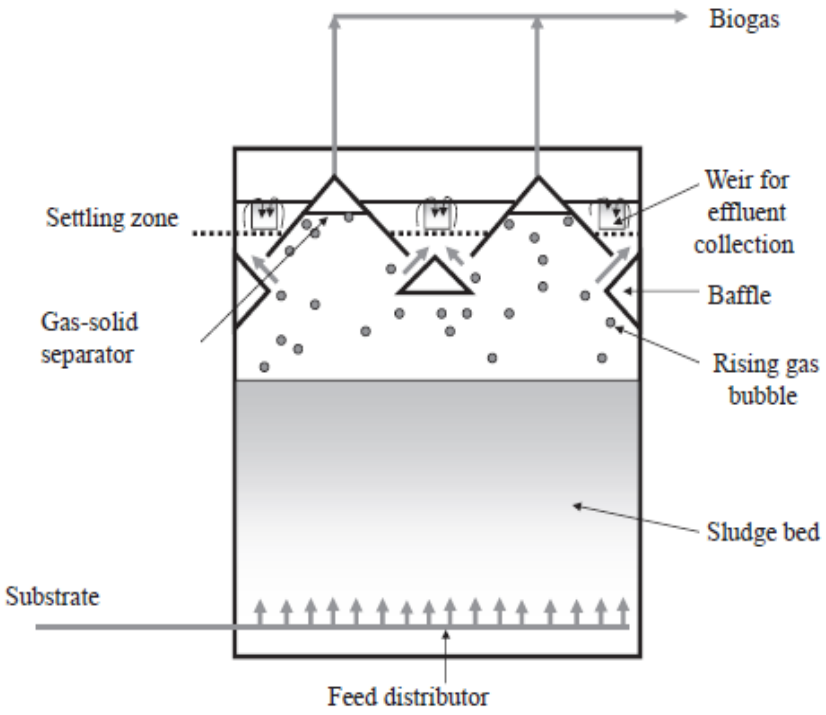

Figure 2.4. 4: Schematic diagram of a UASB reactor (Khanal et al., 2017).

5. Anaerobic Baffled Reactor (ABR)

The ABR consist of a group of baffles that are arranged in a certain way to force the wastewater to flow over and under the baffles. Microbial biomass accumulates between the baffles forming granular biomass with time. It has the advantage of promoting contact between the wastewater and the sludge blanket. However, it's not suitable to treat the industrial effluents with high suspended solid content. Figure 2.4.5 shows the ABR design.

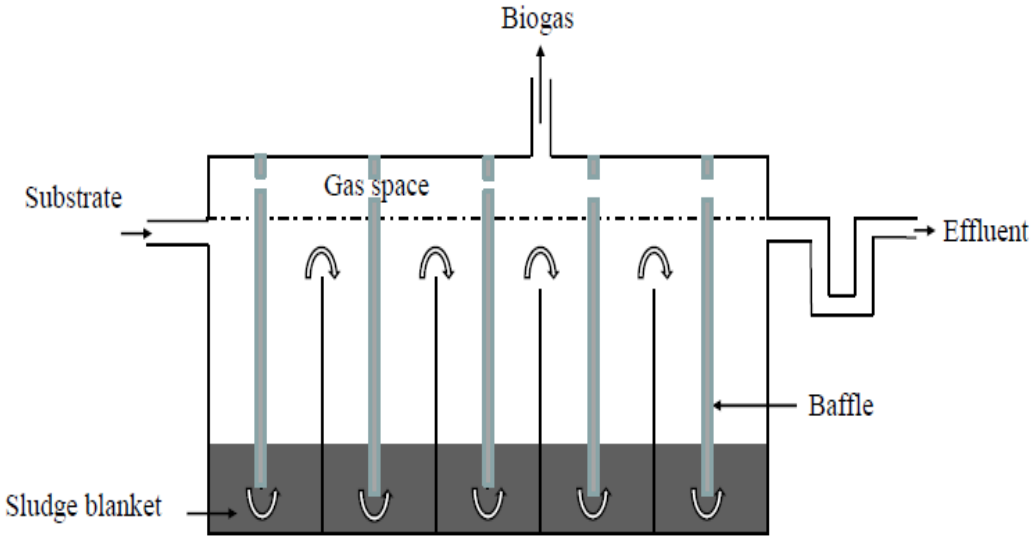

Figure 2.4. 5: Schematics of anaerobic baffled reactor (Khanal et al., 2017).

6. Anaerobic Sequencing Batch Reactor (ASBR)

The ASBR offers the ability to treat high-strength industrial effluents with medium solid content TS (0.5-4%). Since the ASBR is operating based on a sequence of cycles. The substrate is fed into the reactor during the "feed" cycle. The biomass is degraded in the react cycle, mechanical mixture or the produced biogas could be used as an agitation system. After a sufficient period of time (settle phase) all the microbial biomass will be completely degraded and the degree of the anaerobic treatment is achieved. Figure 2.4.6 illustrates the schematic diagram for ASBR.

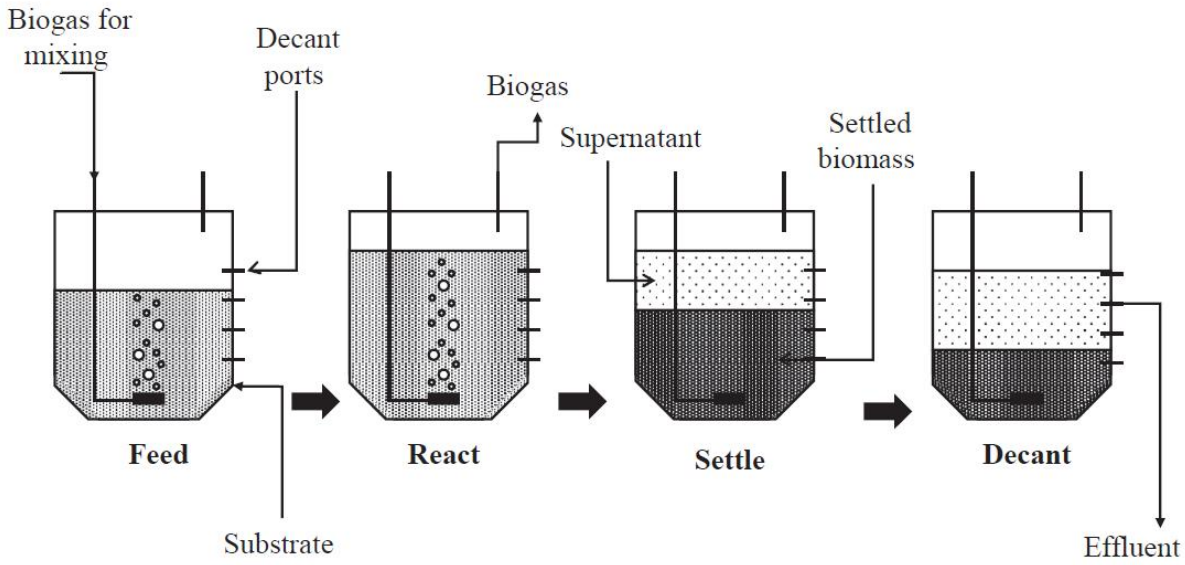

Figure 2.4. 6:Schematics of anaerobic sequencing batch reactor (Khanal et al., 2017).

7. Anaerobic Filter (AF):

The (AF) is a packed-bed attached-growth reactor primarily developed to treat highly soluble wastewater. The two common types of AR are the upflow and the downflow aerobic filter reactors. A large amount of microbial biomass is retained in an anaerobic filter. Thus, extremely long SRT can be achieved irrespective of HRT. The excess microbial biomass may require a periodic removal from the bottom of the reactor to prevent the filter blocking. Figure 2.4.7 demonstrates the schematic diagram of the AF reactor.

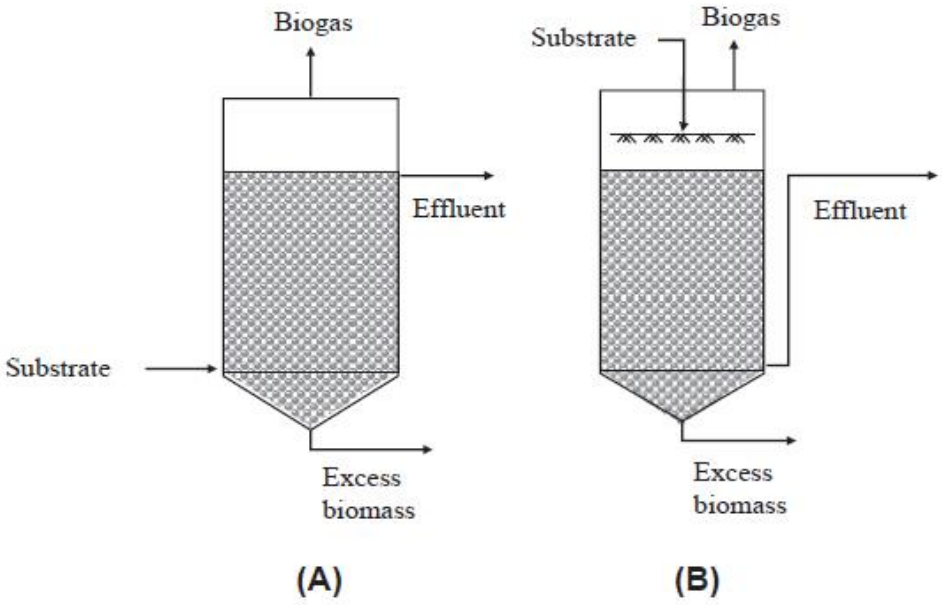

Figure 2.4. 7: Schematic diagram of an anaerobic filter (A) upflow anaerobic filter and (B) downflow anaerobic filter (Khanal *et al.*, 2017).

1.3. Biogas

Biogas originates from bacteria in the process of biodegradation of organic material under anaerobic (without air) conditions. The natural generation of biogas is an important part of the biogeochemical carbon cycle. Methanogens (methane-producing bacteria) are the last link in a chain of microorganisms, which degrade organic material and return the decomposition products to the environment. In this process biogas is generated, a source of renewable energy (Kossmann and Pönitz, 2011)

Biogas is a mixture of gases that is composed chiefly of composition as shown in Table 2.5.1 below.

Table 2.5. 1: Composition of Biogas (Kossmann and Pönitz, 2011)

Composition of Biogas	Vol %
Methane (CH_4)	40 - 70
Carbon dioxide (CO_2)	30 – 60
Other gases	5-1
Hydrogen (H_2)	0 – 1
Hydrogen Sulfide (H_2S)	0 – 3

1.3.1. Biogas Substrate

Biogas substrate, the production of biogas has started from biomass energy sources. Biomass has several different forms such as food crops (e.g. corn or sugar cane, sugar beets, canola), cellulosic plant material (e.g. switch grass or miscanthus), complex biomass (e.g. animal waste) and plants (e.g. Jatropha, soybeans, or sunflowers) (Rittmann, 2008). The major obstacle against using food crops as the energy source that the competition with food or feed production as long as all food crops require to be grown on a high-grade arable land. (Mussgnug *et al.*, 2010).

Combination of different biomass forms also considered a reliable source for biogas production. A considerable combination is pretreated OMWW with chicken manure (a rich source of nitrogen) and sludge (Sounni *et al.*, 2018).

1.4. Environmental Impact of OMWW

OMWW is a problematic waste that has become a major cause of health and environmental concerns. OMWW is frequently dumped, untreated, either in soil or into water sources causing:

1. Phytotoxicity,
2. The proliferation of insects,
3. Increasing salinity,
4. Reducing the permeability of the soil,
5. Decreasing the degree of aeration
6. Colouring of natural waters,
7. Changing plant growth and,
8. Pungent odors

According to different studies, the environmental impact of OMWW was investigated. It has long term effects on soil and crops as it contains phenolic compounds (Antimicrobial effects), fats, and salts. Therefore, it should not be used directly in agricultural areas. OMWW contains different concentrations of ionic species (K^{+1}, Na^{+1} and HCO3) that cause soil salinity if it is directly applied in irrigation without pre-treatment. Improper discharge of OMWW into domestic wastewater basins disrupts biological activities due to a large load of toxic organic compounds. Aerobic digestion in an open system causes strong odor and problems of surface and groundwater (Al-Khatib *et al.*, 2009).

Therefore, a precautionary measure should be taken before discharging non-dry water into the environment (soil and water) and disposing of untreated water directly without treatment is unacceptable.

The number of oil presses and methods of waste disposal of Zibar in Palestine in 2018 was shown in Table A- 5.

1.5. Factors affecting the anaerobic digestion process through biogas scope:

1. Temperature

   Temperature is considered as the most crucial parameter that directly affects the anaerobic process through two scopes; the first one is affecting the enzymes and co-enzymes. The other scope, it affects the methane yield and digestate (effluent) quality.

   Generally, anaerobic digestion bacteria are thermophilic (55-70℃) and mesophilic bacteria (25-50℃). It is observed that higher growth rates, higher metabolic rates, and higher productivity are all obtained via thermophilic digestion. However, it is more sensitive to environmental changes. In contrast, mesophilic digestion exhibits better process stability and richer in bacteria although lower methane yields and poor biodegradability (Mao *et al.*, 2015, Zhang *et al.*, 2014).

2. pH

   The pH value has a dominant role in both methanogenesis and acidogenesis processes since both methanogenic and acidogenic bacteria have optimal pH operating levels. The methanogenesis is most efficient at pH (6.5–8.2) and the optimal pH is 7.0. On the other hand, the optimum pH level for acidogenesis at (5.5-6.5). Otherwise, any change in pH levels will reduce the growth rate and bacteria activity (Mao *et al.*, 2015).

3. Carbon to nitrogen (C/N) ratio

   Carbon to nitrogen ratio is a sensitive parameter as it affects the process stability and gas production rate (Sievers and Brune, 1978). Low C/N ratio indicates a low protein solubilization rate and this led to lower total ammonia nitrogen rates in other words inhibition of ammonia. Whereas, a high C/N ratio means insufficient nitrogen to maintain cell biomass. Therefore, fast nitrogen degradation and lower gas production. The optimal C/N ratio is (20-30) and 25 is mostly used (Mao *et al.*, 2015).

4. Organic load rate (OLR)

   OLR represents the number of volatile solids fed into a digester per day under continuous feeding. Volatile solids represent the amount of solids that can be digested in the digestion process. The OLR depends on the waste type that is fed to the digester, as the types of waste determine the biochemical activity that will occur in the digester is directly associated with gas production, studies showed increasing OLR increases the production although extremely high OLR values cause bacterial inhibition (Mao *et al.*, 2015, Babaee and Shayegan, (2011)).

5. Retention time

Retention time is defined as the time required to complete the degradation process. There are mainly two types of retention time: solid retention time (SRT) and hydraulic retention time (HRT). The retention time is related to the microbial growth rate and depends on the process temperature, OLR and substrate composition. An average retention time of (15–30) days is required to treat waste under mesophilic conditions (Mao *et al.*, 2015). Thus, the retention time has a major effect on the production of bigas and digestion process in general. The Hydraulic Retention Time (HRT) has a critical effect on the production of methane, the longer HRT more likely, the substrate will be broken down and stabilized also, have proper interactions with bacteria within the digester. Hence, a longer HRT leads to increase the methane production. Although, some studies looked at the HRT, many set it as a constant ranging from 1 to 20 days. A few studies maintained the digestion until the production of methane fell or levelled off (Nelson, 2010).

Based on the previous studies, the higher amount of biogas will produce with longer HRT since the microorganisms have been accorded much more time to actively degrade materials. An increase in HRT might provide sufficient time for methanogens to mineralize the organic matter to methane and carbon dioxide. For example, after 12 h, volumes of 0.11, 0.16, 0.21 and 0.24 L/L leachate were achieved for HRT 12, HRT 24, HRT 36 and HRT 48, respectively (Baati *et al.*, 2018) as shown in Figure 4.6.1

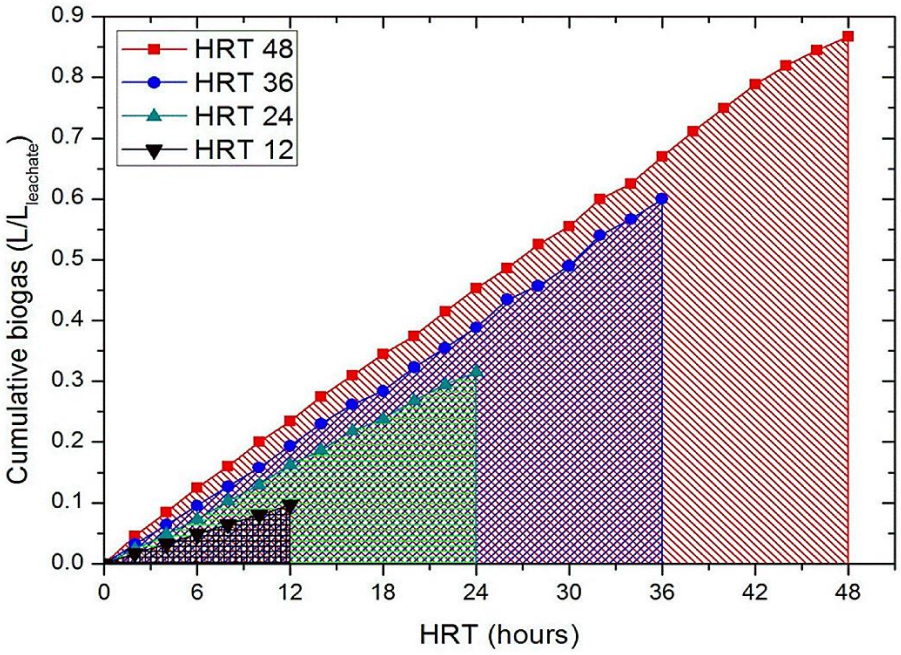

Figure 4.6. 1: Cumulative of Biogas with HRT (Baati et al., 2018)

1.6. Anaerobic Digestion Stages

Anaerobic digestion is a complex process that is capable to degrade any organic waste through a bacterial population mixture in the absence of oxygen. This degradation process produces a valuable gas mixture (mainly methane CH_4 and CO_2) under specific environmental conditions that are needed for bacteria growth. In respect of bacteria's enzymes, organic waste undergoes four main reactions as shown in Figure 2.8. 1 (Lastella *et al.*, 2002, Manchala *et al.*, 2017):

1. Hydrolysis

   Organic macromolecules, i.e. carbohydrates and fats, are converted to monomers in a de-polymerization process by enzymes. Produced monomers undergo degrading reactions and are converted to acetic acid, long-chain fatty acids and CO_2.

2. Acid Formation **(Acidogenesis)**

   It is generally defined as an anaerobic acid producing microbial process without an additional electron acceptor or donor. In this process, the long chain of fatty acids serves as both the electron donors and acceptors. The main products of the hydrolysis process are converted volatile fatty acids (VFAs) (e.g., acetate, propionate, butyrate, etc.), hydrogen and CO_2.

3. Acetic Acid Formation (**Acetogenesis)**

   Acetogens bacteria are responsible for producing enzymes that are used for conversion reactions of long-chain fatty acids to acetic acid, molecular hydrogen and CO_2. In other words, it's a further fermentation of VFAs to acetate, CO_2 and hydrogen, which are the precursors of methane formation.

4. Production of Methane (**Methanogenesis)**

   Acetic Acid is degraded with methane production using methanogenic bacteria. This bacterium is highly sensitive for O_2 concentration and pH value (7-8 for a well-balanced system).

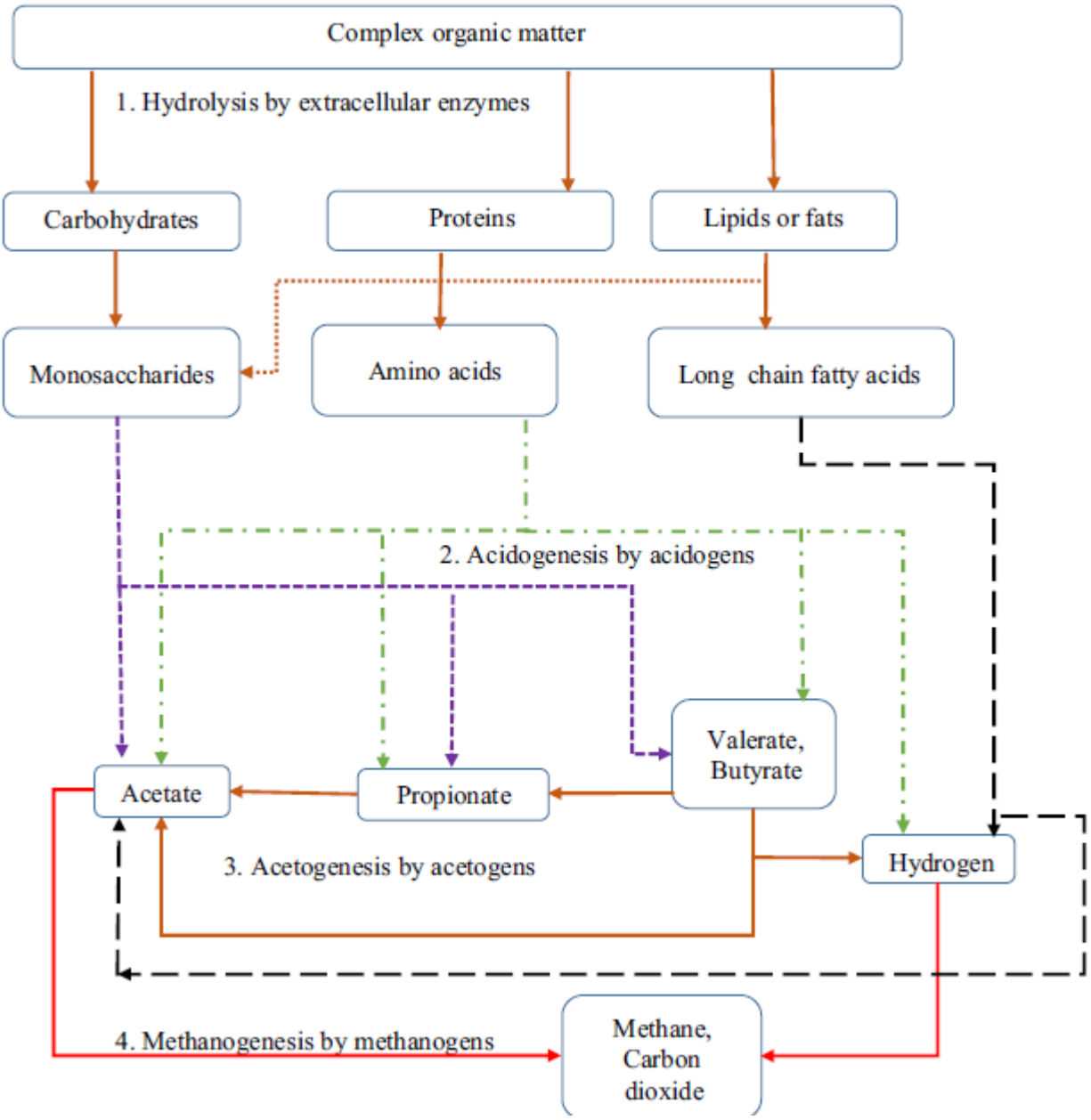

Figure 2.8. 1: Anaerobic digestion stages (Manchala et al., 2017)

1.7. Olive Pressing Techniques

1.7.1. Traditional Pressing Method.

The traditional pressing method, Pressure method (Stone mill). The oldest method for pressing olives to extract oil. The extraction of oil in this method is done by spreading the olive paste after crushing and kneading olives on filter mats stacked together and pressurized. This process doesn't require the addition of water to the olive paste unless it is difficult to separate the oily phase from other phases. If so, addition small quantities of water (3-5 L/100 kg of olives) during crunching and kneading occurs. The pressing mills are operated manually so that require more labor. However, the oil is extracted is high-quality oil with high polyphenol content (Di Giovacchino *et al.*, (1994), Khdair *et al.*, 2015).

1.7.2. Modern Method (Integral Centrifuge Systems)

a. **Three-phase centrifuge decanters.** By **the** 1970's, three-phase method was introduced. It's based on the industrial centrifuge for phase separation as shown in Figure 1. As a first step, olives are crashed to get a fine paste, then paste is pumped to a horizontal centrifuge, in this stage addition of warm water occurs to dilute the olive paste before it goes to the vertical centrifuge decanter and then components are separated to three streams; olive oil, aqueous waste (black water) and a wet solid (paste) (Angerosa and di Giovacchino, 1996). Three-phase decanters method is less bulky than the traditional method, requires a smaller number of labors so that the construction and working cost are much lower but it requires a lot of electric energy. However, the obtained oil yields in this method are lower than that from the traditional one and the paste is almost twice higher of moisture percentage. For the quality of produced oil, it is slightly less acidic, richer in coloring pigments and poorer in natural antioxidants that directly affect the oil flavor (Ranalli and Martinelli, 1995).

b. **Two-phase centrifuge decanters** were introduced by 1990. This pressing method is based on horizontal centrifuge so that needs a minimum amount of water to separate the olive oil from two-phase olive mill wastewater (TPOMWW) (ElMekawy *et al.*, (2014)). As long

as, the minimum amount of water is used the energy consumption is reduced. The quantity of processed olives is enhanced. Furthermore, the produced oil is higher in quality since it is richer in natural antioxidants (Ranalli and Angerosa, 1996). However, the produced oil requires a multi-stage process treatment to remove high acidity and organoleptic defects since the produced TPOMWW is considered as complex aqueous residue, this residue is highly concentrated of organic matter and toxic compounds such as polyphenols, polyalcohol's and volatile fatty acids (Borja *et al.*, (2006)).

Figure 2.9.2.1 shows three olive oil extraction techniques.

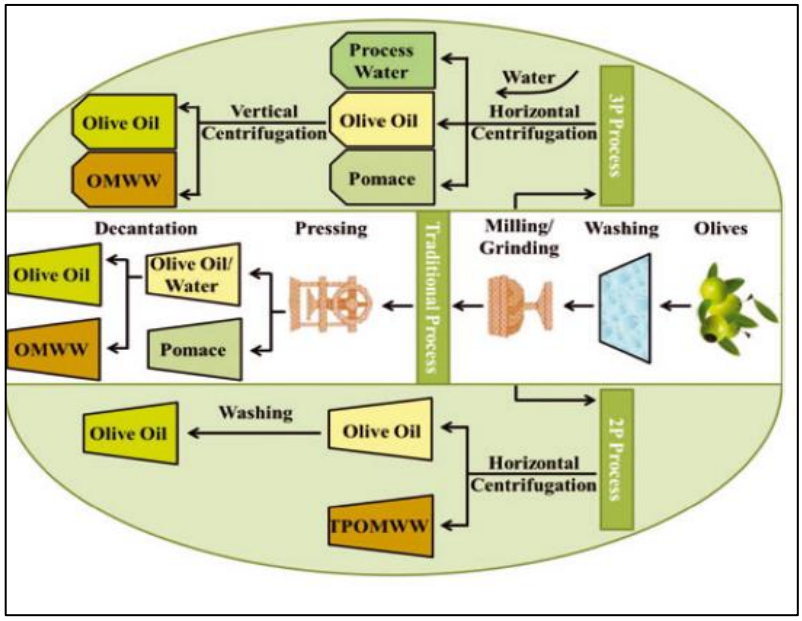

Figure 2.9.2. 1: Schematic flowchart of industrial olive oil extraction techniques; traditional, three-phase and two-phase centrifuge process (ElMekawy et al., 2014).

Chapter Three

3. Methodology and Analytical Methods

3.1. Analytical Methods

3.1.1. Measurement of Chemical Parameters

a. Chemical Oxygen Demand (COD)

COD was determined based on the Standard Methods. Details of the method are shown in **Appendix A**.

COD is the amount of consumed oxygen to chemically oxidize organic waste. It is a measure of water and wastewater quality and used to monitor wastewater treatment plant efficiency (Merk Mili Pore, 2019).

Two samples of fresh and stored OMWW were tested. Before the test is performed the samples were diluted 5 times (1:5) and 10 times (1:10) for stored and fresh samples respectively. Then the samples were digested at 150℃ using HI 839800 COD REACTOR, HANNA instruments for two hours. The test is held based on the EPA (Environmental Protection Agency) approved method 410.4 for COD medium range (0-1500) mg/L determination that uses mercuric sulfate ($HgSO_4$) as the oxidizing reagent. Then allowed to cool to room temperature. Spectrophotometer (HANNA) Colorimeter was used to read COD value.

b. Ammonia

Ammonia is a critical nutrient in biological wastewater treatment. It's a requirement for bacteria to make proteins, including enzymes needed to break down food. It's a reflective measure for nutrient deficiencies (Plaintest Water Analysis Technologies, 2019).

A fresh OMWW sample was filtered for two times using cotton filter paper, Whatman (41 grade, 90 mm diameter), to obtain a clear sample before performing the test. The

sample was diluted 5 times and the test performed according to the ASTM (American Society for Testing and Materials) Manual of Water and Environmental Technology, D1426-92, Nessler method. Four drops of the Nessler reagent were added to the filtrate sample to measure the concentration. The ammonia concentration was determined using HI 82314 Multiparameter Bench Photometer for Wastewater Treatment Application, HANNA. Details of method procedure in **Appendix B.**

c. Total Nitrogen

Total nitrogen is an essential nutrient in biological wastewater treatment. It reflects the nutrient efficiency (Enviroenmental Protection Agency, 2013).

A fresh OMWW sample was diluted by a factor (1:5) and digested at 150℃ using HI 839800 COD REACTOR, HANNA instruments for two hours, the test is performed based on chromotropic acid method Sodium metabisulfite was added to the sample to remove potential interferences. The concentration of total nitrogen was determined using HI 82314 Multiparameter Bench Photometer for Wastewater Treatment Application, HANNA. Details of method procedure in **Appendix C.**

3.1.2. Measurement of Physical Parameters

a. pH Value

The pH value of the solution was measured at ambient temperature using pH-Meter (Jenway 3310). The obtained pH value of OMWW samples was 7.4.

b. Total Dissolved Solids (TDS)

TDS is the measure of dissolved nutrients in the water, it reflects the water quality as it an indicator for the presence of contaminates (Water Research Center, 2014).

Three representative samples were used to conduct the test. Only one sample was filtered using glass microfiber filter paper, Whatman (934-AH, 47 mm

diameter) with vacuum filtration and the other two samples were centrifuged (KUBOTA 5100) at 3500 rpm for 15 min. All samples were placed in the oven at 180 ℃ for 15 min. After the samples were cooled down, they were weighed to perform the calculations. TDS was determined based on the standard method (HANNA). Details are shown in **Appendix D.**

Chapter Four

4. Lab-Scale Bioreactor

4.1. Overview

The main function of the bioreactor is providing a controlled environment to manage optimal growth and/or product formation in particular cell system employed.

The criteria for constructing a bioreactor didn't consist of a group of steps to follow, nor does it have a single correct solution to the problems that were faced. The consideration in the construction is many and varied, however, the accepted construction was based upon the available approaches and economic consideration.

At the earlier stages, it was intended to use the constructed bioreactor by the mechanical engineering department, which was a pilot-scale (8 litres) steel bioreactor (continuous stirred tank reactor (CSTR)) as shown in Figure 4.1.1 and Figure 4.1.2 respectively.

Figure 4.1. 1: Reactor tank with motor (Fares Khrashi, 2019)

Figure 4.1. 2: Bioreactor/digester schematic diagram (Fares Khrashi, 2019).

However, when the constructed bioreactor was examined number of defects were noticed as follows:

1. The digester cover was made of steel instead of stainless steel (as mentioned in the design report) which was completely covered with rust.
2. The digester cover was insulated with a piece of rubber which was completely raptured as shown in Figure 4.1.4.
3. Impeller flow pattern was chosen to be radial flow which is characterized with high shear and turbulence, however, for the anaerobic digestion (liquid materials) the axial flow should be used as it known for lower shear (Caframo Lab Solution, 2019).

   From an economical point of view, the maintenance of the previous defects would cost both money and time. Moreover, the expectations for maintenance success is low. So, it was decided to construct a sustainable lab-scaled bioreactor to perform the experimental part

Figure 4.1. 3: Bioreactor made of Steel and covered with rust and corrosion.

Figure 4.1. 4: Rubber for the cover was raptured

Figure 4.1. 5: Radial flow Impeller

New construction has been studied based on the available approaches and the economic aspects. the bioreactor construction steps all fully clarified in later sections.

4.2. Bioreactor Material of Construction

As mentioned before the anaerobic digestion process is a sensitive biological process. Due to the sensitivity of the bacteria that is responsible for breaking down the biodigester complex feed. For building up this liable bacterial society a long time is required (from 3-4 weeks) (Meegoda *et al.*, 2018). Thus, to manage a reliable and successful anaerobic digestion process with optimum biogas production, it's essential to select the proper type of construction material of bioreactor. Two scenarios were considered and Table (4.2.1) illustrates the reasons for choosing stainless steel over plastic.

Table 4.2. 1: Material of construction type and advantages, disadvantages.

Material of construction	Advantages	Disadvantages	Reference
Stainless Steel (selected)	• **Excellent corrosion resistance.** • **Long service (sustainable and durable)** • **No need for protective coatings.** • **Low maintenance.**	• High cost.	(Institute, 2016)
Plastic	• Low cost. • Easily portable.	• Short life span. • Plastic causes reduction in the digestion. • Plastics are nonbiodegradable.	(Rajendran *et al.*, 2012, Muthuswamy and Nemerow, 1990)

The challenges of the material of construction:

•Deciding whether to use plastic or stainless steel, it was decided (based on the researches) to use stainless steel since it is more suitable and sustainable for long term use even though plastic is cheaper and easier to work with.

•Searching the local market to find good stainless steel "pressure cooker" with a suitable price and shape as it is available in the local markets with reasonable price.

4.3. Construction Implementation

Further, overcoming the obstacles that were experienced in the selection of the material of construction. The bioreactor construction criteria were executed through studying different scenarios, each scenario has its requirement and specifications. In the first scenario, the construction of bioreactor could be accomplished by a specified design company with minimum time. However, from an economical point of view, this option would cost much. Another scenario was suggested to repair all malfunctions in the old construction and reuse it. Although, this scenario would cost both time and money and the possibility of failure is high.

The promising scenario involving all the economical and qualitative aspects is to use a modified stainless steel "pressure cooker" (5 litres), with dimensions 38.1 x 20.3 x 30.5 cm and 4.14 kg as the weight with silver color (Amazon, 2017). It is worth mentioning that using a pressure cooker as a bioreactor is a new unique option in this aspect. On the other hand, the available pressure cookers in the Palestinian local stores (Nablus) have some problems that are listed below:

1. The pressure cooker has a solid body (requires a lathe to create change).
2. The high cost of each pressure cooker (project execution requires four pressure cookers).

To cope with all pervious problems and obtain the desired construction certain modifications are needed. A deal for pressure cooker price was done to be within the project's budget. Also, some modifications were done in a lathe workshop (Al-Kurdi workshop/ Nablus city). Each modification was tested to make sure the progress was going properly. All modifications that were done to convert the "pressure cooker" to the required bioreactor construction for performing the digestion of olive mill wastewater and sludge are illustrated in Table 4.3.1 below**.**

Table 4.3. 1: Bioreactor construction specifications

Bioreactor Construction Requirement	Specifications	The Used Materials
Feed inlet	A hole ¾" in the pressure cooker lid.	¾" of PVC pipe was inserted in the hole to deliver the feed.
Effluent (outlet)	A hole ¾" is made in the bottom of pressure cooker body (2 cm above)	2 PVC pipes (¾") with 3 elbows for conjunction.
Gas collection system	A hole ½" in the pressure cooker lid.	Gas tube (double-check) connected with bike tire (26" diameter) to store the gas inside.
Washing nozzle	A hole ¾" in the bottom of the pressure cooker body.	¾" Stainless steel tank tap.
Agitation system	The same hole for the gas collection system.	Using a valve to block moving the produced biogas and keep it inside the reactor.

The studied scenarios are summed below in Figure 4.3.1.

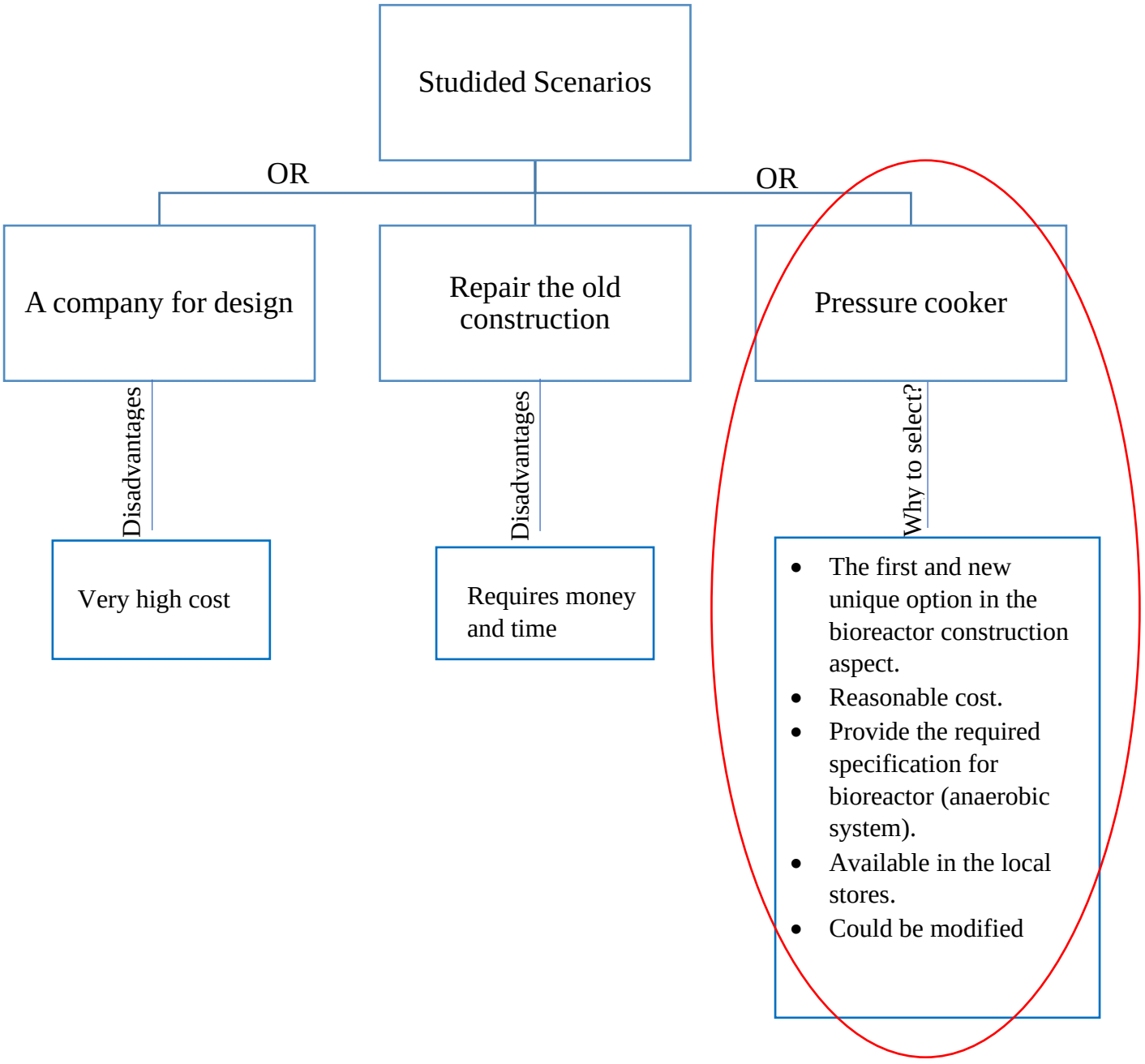

Figure 4.3. 1: Scenarios Summary

4.3.1. Modifications Implementation.

Many routes were applied to prevent leaking, first coating the bioreactor's cover with silicone adhesive and rubber (waiting 24 hours to dry and stick together) then applying the test, leaking was noticed. Another type of silicone adhesive (super 7, stronger than the previous one) was suggested as an alternative approach to solving the problem and the leak test was re-conducted, but this suggestion also failed. After many trials of using different kinds of adhesives, it was intended to clean and remove these adhesives, and use gasket maker (strong adhesive, outstanding oil resistance, excellent torque retention and suitable for high temperatures) to cover the leaking spots under the original rubber then applying the test, and no leak was noticed as shown in Figure 4.3.1. 2. The gasket maker was a good solution to the first part of the problem (leaking in different spots in the cover of pressure cooker). However, for the leakage around the drilled holes in both pressure cooker body and cover, the holes were covered with gasket maker fortified with rubbers (both sides) to ensure that there is no leak as illustrated in Figure 4.3.1. 3. Since the implemented material (as mentioned in Table 4.3.1) which is PVC was a different material than stainless steel material. As a conclusion, no adhesive can stand up with stainless steel material (based on the previous trials and tests), but in our project, the best solution was to use gasket maker fortified with rubbers to overcome this problem. Figure 4.3.1. 1summaries all the routes.

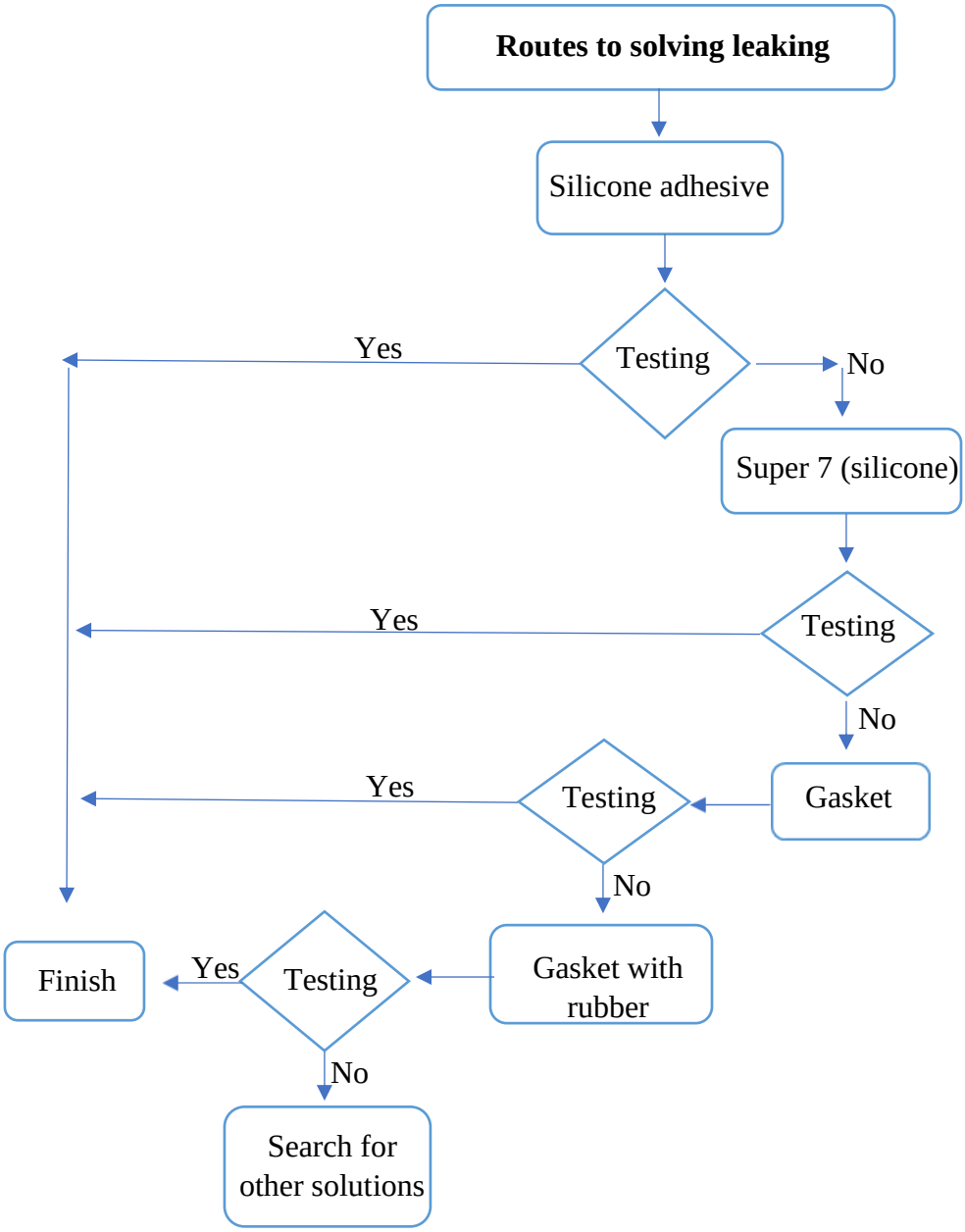

Figure 4.3.1. 1: Routes to solve leaking.

Figure 4.3.1. 2: Gasket maker with the original rubber.

Figure 4.3.1. 3: Drilled holes with rubbers.

4.3.2. Mixing Options (Stirring System)

Mixing in an anaerobic digester keeps the solids in suspension and homogenizes the incoming feed with the active microbial community of the digester content. The following options are all valid as a mixing system in the bioreactor:

1. Usage of an old pump by inserting it to the system.
2. Stirring by the produced biogas using a valve.
3. Inserting a mixer with certain dimensions to fit the bioreactor dimensions.

4.4.Schematic Diagrams for the Bioreactor Construction

The following scheme Figure 4.4. 1 illustrates the constructed bioreactor dimensions in cm and the bioreactor working volume. Typically, the working volume is 70-80% of the total bioreactor volume (Kurt Eyer, 2020). As the pressure cooker volume is 5 L, the working volume is 3.5 L (70% of 5 L).

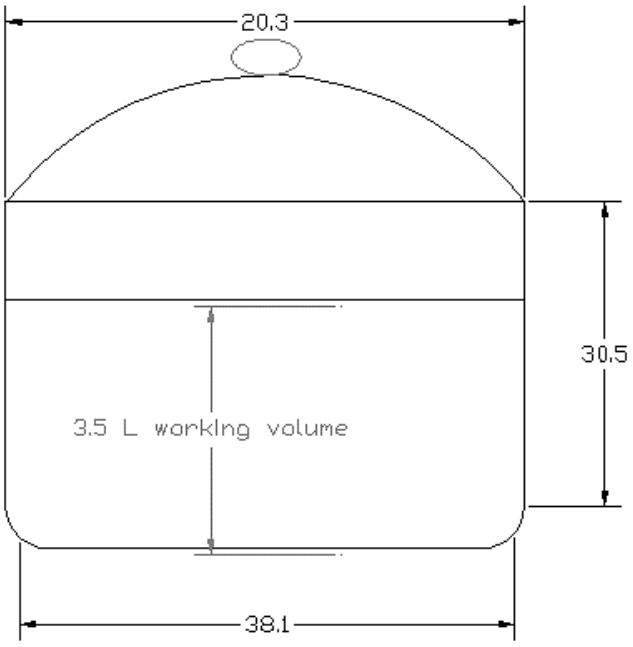

Figure 4.4. 1: The constructed bioreactor dimensions and working volume.

Whereas, the Figures below (Figure 4.4.2-Figure 4.4.6) show the bioreactor constructions stages:

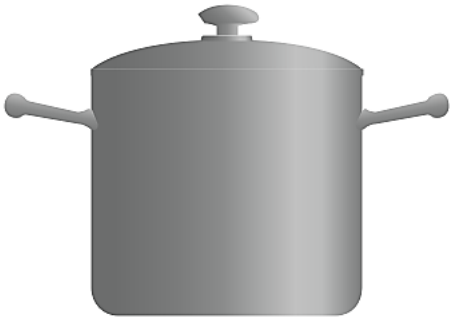

Figure 4.4. 2: A solid pressure cooker

Figure 4.4. 3: Effluent (Outlet hole.

Figure 4.4. 4: Washing nozzle (stainless steel water tap)

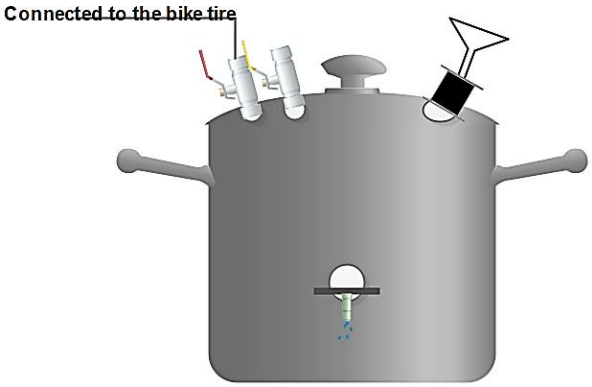

Figure 4.4. 5: Final schematic for bioreactor construction

Figure 4.4. 6: Feed inlet and Agitation system holes

4.5.Tests Methodology

4.5.1. Testing bioreactor for gas and fluid leakage

First, a leak test must be performed to make sure that there is no gases and fluids escape from the bioreactor. A leak test method is a quality control step to assure device integrity and solidity, it is

considered as a non-destructive test with no impact on the environment or operators. Several leak testing methods are available, but the most commonly used methods are bubble test, pressure and vacuum decay, tracer gas detectors (halogen, helium and hydrogen) and acoustic leak detection (Bergoglio and Mari, 2012). In our case, the bubble test method was selected.

Bubble Test Method:

This method is cheap and easy to apply, it allows detection of leaks up to 10-5 Pa.m³/s and is suitable for very large systems. Pressurizing technique was used in this experiment. The cover of the bioreactor was moistened with a foam-forming soap solution, then the pressure was applied to ensure the visibility of tiny leaks, it was observed that the cover was leaking and bubbles were formed as shown in Figure 4.4.1. 1. To observe if there were any leaks in the body, fluid leaking test was done by filling the bioreactor with water (liquid leak was noticed). Figure 4.3.1. 4 above illustrates all the work.

Figure 4.5.1. 1: Gas leaking test

4.5.2. Cow Manure Test

It was intended to conduct a test to check the constructed reactor and confirm construction stability. The cow manure was chosen as the feeding material (before injecting the olive mill wastewater and sludge that will be studied in this project.)

The cow manure samples were collected from Al-Afouri farms (Zawata, near Nablus). The samples were consisted of fresh and old cow manure and stored in glass bottles.

The methodology for performing the test as following:

1. The cow manure samples were mixed with a ratio of (2 L fresh: 1 L old) and diluted with tap water as shown in Figure 4.5.2. 1.
2. The feed was injected in the reactor through the feed inlet using a plastic funnel as shown in Figure 4.5.2. 2.
3. The reactor was incubated in the incubator at temperature (35-40)℃ as shown in Figure 4.5.2. 3.
4. The incubator was placed near the gas detector (to observe the gas leakage).
5. A thermometer was injected inside the incubator to check the temperature.
6. The produced gas from the digestion is collected in the bike tire as illustrated in Figure 4.5.2. 4.
7. The pH of the system was checked continuously (ensuring that the system has remained neutral 7) as shown in Figure 4.5.2. 5.
8. Sodium acetate (1.2 M) was added as feeding material for the bacteria.
9. The test was held for 4 days to ensure system stability.

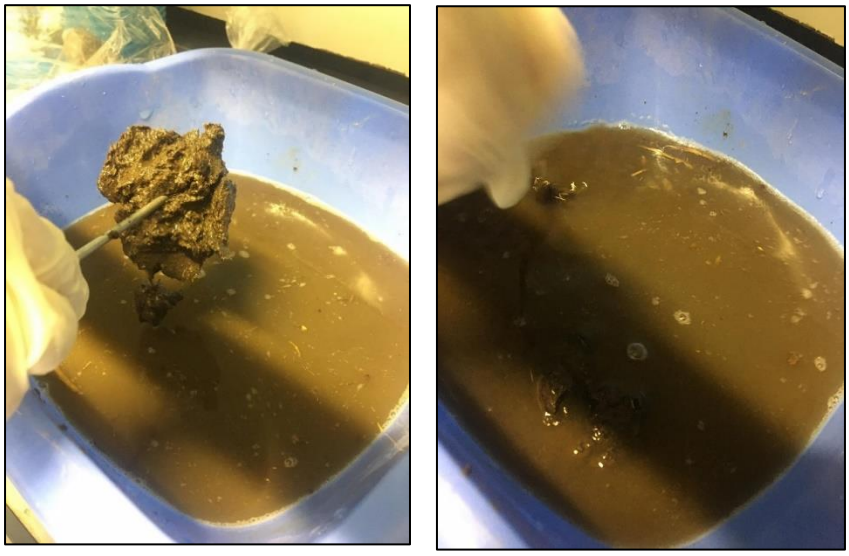

Figure 4.5.2. 1: Mixing fresh and old cow manure and diluted by tap water.

Figure 4.5.2. 2: Injecting the mixture to the reactor using a plastic funnel.

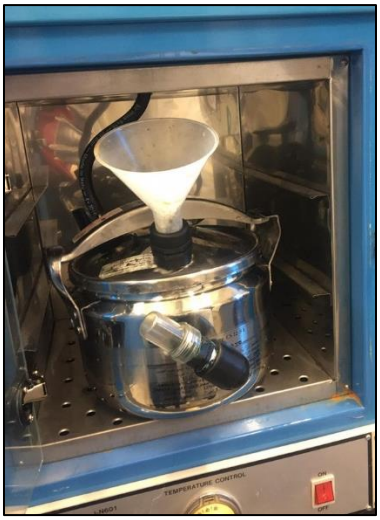

Figure 4.5.2. 3: Injecting the reactor inside the incubator.

Figure 4.5.2. 4: Produced gas is collected in the bike tire.

Figure 4.5.2. 5: Testing the pH for the sample from the cow manure mixture.

Chapter Five

5.1. Recommendations and Future work

For future work, it is proposed to preview the project's plan deeply in detail from an experimental point of view. Our role in the project is situated between operating the bioreactor and examine the quantity and quality of the produced biogas with other evolutionary techniques. All the sections below define the project plan.

a. Preparing the substrate materials

OMWW samples were collected from two different sources through the olive collecting season (October 2019). The majority of samples were collected from the wastewater treatment plant located near Nablus city. The samples were stored using glass bottles in the refrigerator at -4 ℃. However, the OMWW lacks efficient bacteria to initiate and activate the anaerobic digestion process. It was decided to use the sludge as the inoculum to activate the bio digestion process, the sludge (as Eng. Mohammed, the wastewater treatment Operator recommends) will be collected from the digester (a rich active bacterial society). After the collection, the sludge should be transported to the laboratory and injected immediately to the reactor.

b. Characterization of OMWW samples

The collected samples must be characterized again to determine their biological oxygen demand (BOD), chemical oxygen demand (COD), pH and total nitrogen before performing the experiments (the samples were characterized immediately after the collection).

c. Operating the designed bioreactors

As it is previously mentioned in the design chapter, four bioreactors were constructed with the same specifications. One of the bioreactors will be operated as a control (reference) bioreactor, thus it will be filled only with OMWW samples without addition of sludge. The three remained bioreactors will be filled with OMWW and sludge which are mixed with different ratios. Then the bioreactors will be incubated in the incubators to keep the temperature at 37 ℃ (mesophilic range) All bioreactors will be completely closed for nearly 40 days.

d. Analytical Methods

To quantify the anaerobic digestion process and the gas productivity, many of measurements are utilized, including pH, alkalinity, Chemical Oxygen Demand (COD), polyphenols concentration and Total Solids (TS). All tests will be conducted according to the standard methods (HANNA). The samples will be taken from the bioreactor sampler tap to monitor the previously mentioned parameters.

e. OMWW and Sludge Ratios

As already stated, the bioreactors substrate will consist of a mixture of OMWW and sludge at different ratios. The intended OMWW/Sludge ratios as follows (70:30, 80:20, 90:10). Referring to the previous studies, the presumed ratio has achieved the highest gas production levels and the highest COD removals is (70:30) of OMMW to sludge (Azbar *et al.*, 2004). Additionally, this ratio provides a pH level near 6.4 which is a perfect medium for the bacterial society activities and growth.

As illustrated in Figure *5.1. 1*the COD removal using (70:30) ratio is nearly 69.5%.

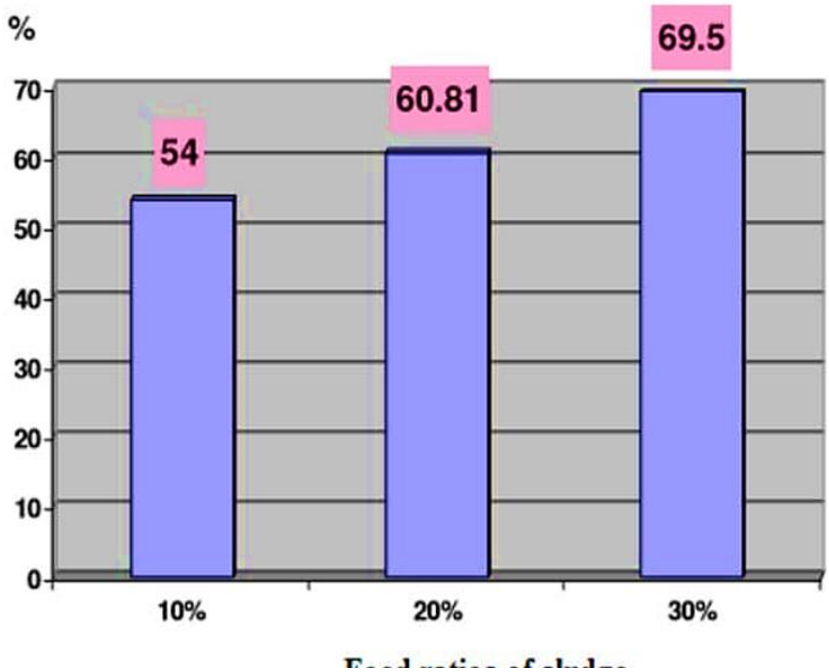

Figure 5.1. 2: COD removal percentage using (70:30) ratio (Azbar et al., 2004).

For total Nitrogen, the highest rate of transformation was almost 81.8% in the ratio (70:30) and the highest percentage was among other ratios as shown in Figure 5.1. 3.

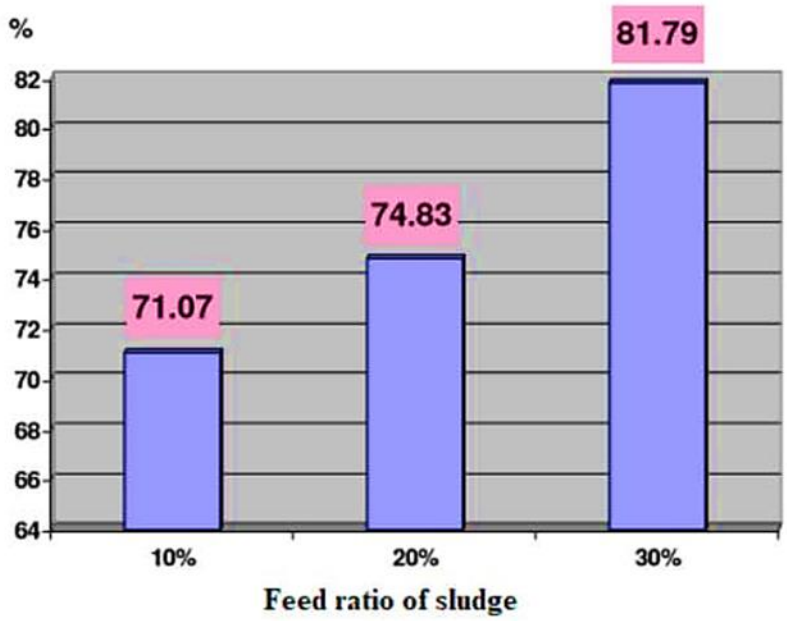

Figure 5.1. 4: Total Nitrogen percentage in using (70:30) ratio (Azbar et al., 2004).

Furthermore, the total polyphenols removal percentage was found the highest in (70:30) ratio and almost is 22.2% as shown in Figure 5.1. 5

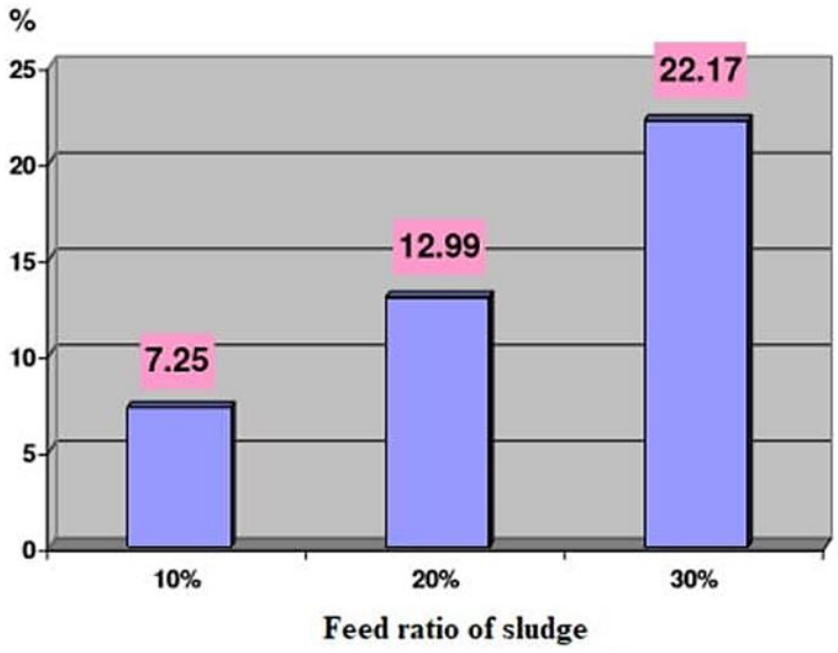

Figure 5.1. 6 : Polyphenols removal percentage in (70:30) ratio (Azbar et al., 2004).

f. Methods for Measuring Produced Biogas Volume.

The proposed method to measure the produced biogas volume is the Liquid Displacement Method. As long as, the biogas will be collected using a plastic tube and the tube will be connected to the other liquid basin which contains the graduated cylinder (1 L) and caustic soda solution (1 M). Biogas is first purified from CO_2 and H_2S by simultaneous reaction and absorption in a bath containing caustic soda solution (1M). Dissolved methane gas is released from caustic soda solution and assembled into an inverted 1-liter cylinder for quantitative measurement. The biogas will be collected from each bottle separately in an inverted graduated cylinder (Abushattal *et al.*, 2017).

The liquid displacement scheme is illustrated in *Figure 5.1. 7:*

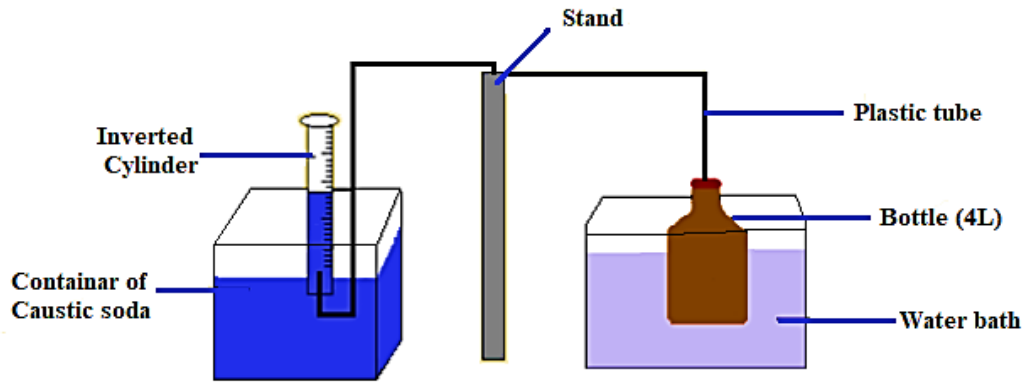

Figure 5.1. 8: Anaerobic digestion system including the biogas measurement (Abushattal et al., 2017)

g. Challenges could be faced:

- The digester sludge is a sensitive bacterial medium, thus it is required to collect the sample and inject them directly without any time delay, despite the distance between the WWTP and the university lab.
- Delay in time for the sludge samples collection due to the Nablus municipality protocols to have permission for samples collection.

Chapter Six

6. Conclusion

The main objective of this study was to evaluate the capability to treat the OMWW by the anaerobic co-digestion process (with the sludge from digester) and produce biogas, taking into account the construction of a new bioreactor to perform the experimental work using it. The bioreactor construction part and part of experimental testing techniques were accomplished before the quarantine. Based on the previously accomplished work the following conclusions were drawn:

1. The most appropriate material to construct the bioreactor is stainless steel compared to plastic (sustainable and corrosion resuscitated). A local modified “pressure cooker” was chosen.
2. No adhesive can stand up with stainless steel material based on the performed trials, for the project case, the best solution was to use gasket maker fortified with rubbers.
3. The constructed bioreactor suites well the anaerobic digestion process conditions and specifications as an experimental test using the cow manure was implemented for four days and no leakage observed from the produced gas.

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Appendices

Appendix A: Chemical Oxygen Demand Test

- **Method of testing**

Adaptation of the USEPA 410.4 approved method for the COD determination on surface waters and wastewaters. Oxidizable organic compounds reduce the dichromate ion (orange) to the chromic ion (green). The amount of remaining dichromate is determined

- **Required reagent**

Table A- 1: COD required reagent.

Code	Description	Q. ty/test	Q. ty/set
*	Reagent	1 vial	25 vials
-----	Deionized Water	0.2 mL	optional

*Reagent Vial identification: No letter, red cap.

Note: Store the unused vials in their container in a cool and dark place.

Dichromate could be replaced with mercuric sulphate (same procedure, method and ranges).

Interference may be caused by:

Chlorides (Cl-): above 20000 mg/L. Samples with higher chloride concentration should be diluted.

- **Measurement procedure**

Before starting to use the reagent kit it is recommended to read carefully all the instructions and the Health & Safety Data Sheet (HSDS). Pay particular attention to all warnings, cautions and notes. Failure to do so may result in serious injury to the operator.

Reagent Blank Correction: This method needs a reagent blank correction. A single blank vial may be used more than once. The blank vial is stable for several months (room temperature). For most accurate measurement, run a blank for each set of measurements and always use the same lot of reagents for blank and samples.

1. Choose a homogeneous sample. Samples containing settleable solids need to be homogenized with a blender.
2. Preheat the Hanna Reactor C 9800 to 150 °C (302°F). For correct use of the reactor follow Reactor Instruction Manual. Use of the optional HI 740217 safety shield is strongly recommended. Do not use an oven or microwave because leaking samples can generate a corrosive and possibly explosive atmosphere.
3. Remove the cap from two Reagent Vials.
4. Add exactly 0.2 mL of sample to one vial (sample vial), and 0.2 mL of deionized water to the other vial (blank vial), while keeping the vials at a 45-degree angle.
5. Replace the cap tightly and mix by inverting each vial a couple of times.

Warning: as the vials become very hot during mixing, be careful in handling them.

6. Insert the vials into the reactor and heat them for 2 hours at 150°C.
7. At the end of the digestion period switch off the reactor. Wait for twenty minutes to allow the vials to cool to about 120°C.
8. Invert each vial several times while still warm, then place them in the test tube rack. Warning: as the vials are still hot, be careful in handling them.
9. Leave the vials in the tube rack to cool to room temperature. Do not shake or invert them anymore otherwise the samples may become turbid.

10. Select the program number corresponding to Oxygen Demand, Chemical HR (COD) on the secondary LCD by pressing PROGRAM t and s.
11. Place the blank vial into the holder and push it completely down.
12. Press ZERO and "SIP" will blink on the display.
13. Wait for a few seconds and the display will show “-0.0-”. Now the meter is zeroed and ready for measurement.
14. Remove the blank vial.
15. Place the sample vial into the holder and push it completely down.
16. Press READ DIRECT and "SIP" will blink during measurement.
17. Multiply the reading on the Liquid Crystal Display by 10 to obtain the concentration in mg/L of oxygen demand.

Appendix B: Ammonia Test

Form HANNA'S catalog, standard methods were used (HANNA).

Ammonia Test

- **Method of testing**

Adaptation of the ASTM Manual of Water and Environmental Technology, D1426-92, Nessler method. The reaction between ammonia and reagents causes a yellow tint in the sample.

- **Required reagent**

Table A- 2: Ammonia required reagents.

Code	Description	Q.ty/test	Q.ty/set
*	Reagent	1 vial	25 vial
HI 93764-0	Nessler Reagent	4 drops	1 bottle

* Reagent Vial identification: A, green cap.

Note: Store the unused vials in their container in a cool and dark place.

Interference may be caused by:

• Organic compounds like: chloramines, various aliphatic and aromatic amines, glycine or urea above 100 ppm N (positive error), to eliminate these interferences distillation is required.

• Organic compounds like: aldehydes, alcohols (e.g. ethanol) or acetone above 1 % (negative error), to eliminate these interferences distillation is required.

• Sulfide: may cause turbidity.

- **Measurement procedure**

1. Select the program number corresponding to Ammonia HR on the secondary LCD by pressing PROGRAM t and s.
2. Remove the cap from a Reagent Vial.
3. Add exactly 1.0 mL of sample to the vial, while keeping the vial at a 45-degree angle.
4. Replace the cap and mix by inverting the vial a couples of times. This is the blank.
5. Mark the vial with a pencil on the white band to place an orientation sign. Use this sign to insert the test vial always in the same position into the holder.
6. Place the vial into the holder and push it completely down.
7. Press ZERO and “SIP” will blink on the display.
8. Wait for a few seconds and the display will show “-0.0-”. Now the meter is zeroed and ready for measurement.
9. Remove the vial.
10. Remove the cap and add 4 drops of HI 93764-0 Nessler Reagent.
11. Replace the cap tightly and mix by inverting the vial a couples of times. This is the sample.
12. Place the vial into the holder and push it completely down. Ensure that the vial orientation respect to the holder is the same as the blank.
13. Press TIMER and the display will show the countdown prior to the measurement. Alternatively, wait for 3 minutes and 30 seconds and press READ DIRECT. In both cases "SIP" will blink during measurement.

Appendix C: Total Nitrogen Test

- **Method of testing**

Chromotropic acid method. A Persulfate digestion converts all forms of nitrogen to nitrate. Then the reaction between nitrate and the reagents causes a yellow tint in the sample.

- **Required reagent**

Table A- 3: Total nitrogen required reagent.

Code	Description	Q.ty/test	Q.ty/set
*	Digestion Vial	1 vial	50 vial
-----	Deionized Water	0.5 ml	1 bottle
-----	Potassium Persulfate	1 packet	50 packets
-----	Sodium Metabisulfite	1 packet	50 packets
HI 93767-0	Total Nitrogen Reagent	1 packet	50 packets
**	Reagent Vial	1 vial	50 vial

* Digestion Vial identification: N, red cap.

**Reagent Vial identification: N, white cap.

Note: Store the unused vials in their container in a cool and dark place.

Interference may be caused by:

- Bromide (Br-): above 240 mg/L (positive error) .
- Chloride (Cl-): above 3000 mg/L (positive error).
- Chromium (Cr3+): above 0.5 mg/L.

- **Measurement procedure**

Before starting to use the reagent kit it is recommended to read carefully all the instructions and the Health & Safety Data Sheet (HSDS). Pay particular attention to all warnings, cautions and notes. Failure to do so may result in serious injury to the operator. Reagent Blank Correction: This method needs a reagent blank correction. A single blank vial may

be used more than once; the blank vial is stable up to one week if stored in a dark place at room temperature. Always use the same lot of reagents for blank and samples. For most accurate measurement, run a blank for each set of measurements.

1. Preheat the Hanna Reactor C 9800 to 105 °C (221°F). For correct use of the reactor follow Reactor Instruction Manual. Use of the optional HI 740217 safety shield is strongly recommended. Do not use an oven or microwave because leaking samples can generate a corrosive and possibly explosive atmosphere.
2. Remove the cap from two Digestion Vials (red cap vials).
3. Add the content of one packet of Potassium Persulfate for Total Nitrogen analysis to each vial.
4. Add exactly 0.5 mL of sample to one vial (sample vial), and 0.5 mL of deionized water to the other vial (blank vial), while keeping the vials at a 45-degree angle.
5. Replace the cap tightly and shake vigorously the vials for about 30 seconds until all the powder is completely dissolved.
6. Insert the vials into the reactor and heat them for 30 minutes at 105°C. Note: to obtain most accurate results, it is strongly recommended to remove the vials from the reactor after exactly 30 minutes.
7. At the end of the digestion place the vials carefully in the test tube rack and allow to cool to room temperature. Warning: as the vials are still hot, be careful in handling them.
8. Select the program number corresponding to Total Nitrogen HR on the secondary LCD by pressing PROGRAM t and s.
9. Remove the cap from the vials and add the content of one packet of Sodium Metabisulfite for Total Nitrogen analysis to each vial. Replace the cap tightly and shake gently the vials for 15 seconds.
10. Wait for 3 minutes (without shaking the vials) to allow the reaction to complete.
11. Remove the cap from the vials and add the content of one packet of HI 93767-0 Total Nitrogen Reagent to each vial. Replace the cap tightly and shake gently the vials for 15 seconds.
12. Wait for 2 minutes (without shaking the vials) to allow the reaction to complete.

13. Remove the cap from two Reagent Vials (white cap vials).
14. Add exactly 2.0 mL of digested sample (from the digested red cap sample vial) to one Reagent Vial (sample vial), and 2.0 mL of digested blank (from the digested red cap blank vial) to the other vial (blank vial), while keeping the vials at a 45-degree angle.
15. Replace the cap tightly and invert the vials 10 times. Warning: as the vials become hot during mixing, be careful in handling them.
16. Place the blank vial into the holder and push it completely down.
17. Press TIMER and the display will show the countdown prior to the measurement. Alternatively, wait for 5 minutes and press ZERO. In both cases "SIP" will blink during measurement.
18. Wait for a few seconds and the display will show “-0.0-”. Now the meter is zeroed and ready for measurement.
19. Remove the blank vial.
20. Place the sample vial into the holder and push it completely down.
21. Press READ DIRECT and "SIP" will blink on the display.
22. The instrument directly displays concentration in mg/L of total nitrogen (N) on the Liquid Crystal Display. The method detects all organic and inorganic forms of nitrogen present in the sample.
23. To convert the reading to mg/L of ammonia (NH3), multiply by a factor of 1.22.
24. To convert the reading to mg/L of nitrate NO3-, multiply by a factor of 4.43.

Appendix D: Total Dissolved Solid Test

- **Measurement procedure**

1- First, clean the crucibles (3 crucibles) and glass watches with deionized water and soap.
2- Put the crucibles and Whatmen filter paper (41, ashless, circles 90mm Dia, Cat No 1441 090), which is placed in the center of the glass watch in the oven at 105 ℃ for 15 minutes.
3- Mark the crucibles and the filter papers, weigh them.
4- Put 30 mL samples in 50 mL graduated cylinder and 50 mL centrifuge tubes.
5- Do vacuum filtration for the sample in 50 mL graduated cylinder.
6- Put the tubes in centrifuge, KUBOTA 5100, with 3500 rpm for 15 minutes in order to separate the solid and the liquid into two layers, which is easier while doing vacuum filtration.
7- Do vacuum filtration for the centrifuged tubes.
8- Put the filter papers with the precipitate in the glass watches and the filtrate in the crucibles.
9- Put them in the ovens in order to dry.
10- Weigh the crucibles and the filter papers, do calculations.

Figure A- 1: Hl 83214 Multi-parameter Bench photometer for wastewater treatment application.

Figure A- 2: Hl 839800 COD Reactor.

Figure A- 3: Hl93764-0, Nessler Reagent.

Figure A- 4: Ammonia test vial.

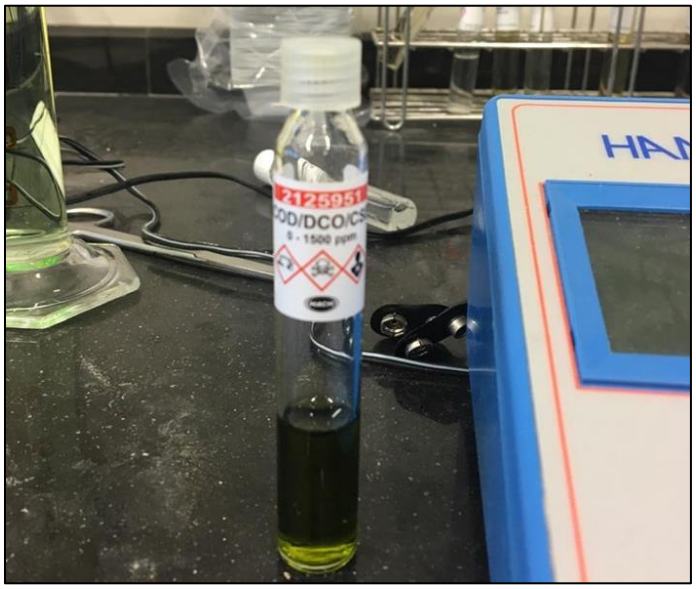
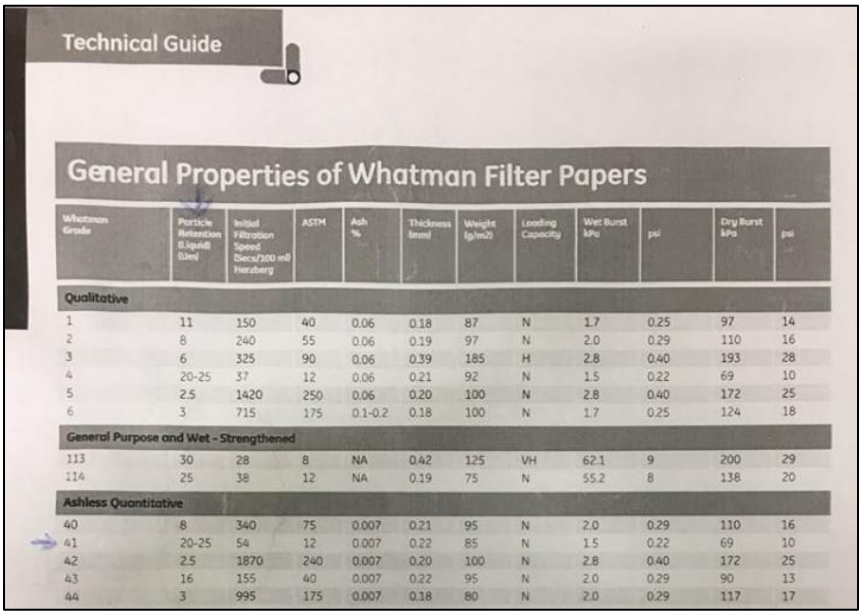


Figure A- 5: COD test vial after digestion.

Figure A- 6: KUBOTA 5100 centrifuge.

Figure A- 7: Vacuum flask.

Figure A- 8: Whatman filter paper.



Technical Guide

General Properties of Whatman Filter Papers

Whatman Grade	Particle Retention (Liquid) (Um)	Initial Filtration Speed (Secs/100 ml) Herzberg	ASTM	Ash %	Thickness (mm)	Weight (g/m2)	Loading Capacity	Wet Burst kPa	psi	Dry Burst kPa	psi
Qualitative											
1	11	150	40	0.06	0.18	87	N	1.7	0.25	97	14
2	8	240	55	0.06	0.19	97	N	2.0	0.29	110	16
3	6	325	90	0.06	0.39	185	H	2.8	0.40	193	28
4	20-25	37	12	0.06	0.21	92	N	1.5	0.22	69	10
5	2.5	1420	250	0.06	0.20	100	N	2.8	0.40	172	25
6	3	715	175	0.1-0.2	0.18	100	N	1.7	0.25	124	18
General Purpose and Wet - Strengthened											
113	30	28	8	NA	0.42	125	VH	62.1	9	200	29
114	25	38	12	NA	0.19	75	N	55.2	8	138	20
Ashless Quantitative											
40	8	340	75	0.007	0.21	95	N	2.0	0.29	110	16
41	20-25	54	12	0.007	0.22	85	N	1.5	0.22	69	10
42	2.5	1870	240	0.007	0.20	100	N	2.8	0.40	172	25
43	16	155	40	0.007	0.22	95	N	2.0	0.29	90	13
44	3	995	175	0.007	0.18	80	N	2.0	0.29	117	17

Figure A- 9: General properties of Whatman filter paper.

Appendix E: Olive Oil Production in Palestine.

Olive production is the backbone of Palestinian agriculture. It takes part in the social and economic of the Palestinian households especially in rural areas.

In 2018, there were 292 olive presses in Palestine, in which 260 we're operating and 32 were temporarily closed as shown in Table 1. In the operating presses, 248 were fully automatic, 12 half automatic and traditional presses (PCBS, 2018). Kknowing that the number of presses is affected by the seasonal production of olive (PCBS, 2016). The total quantity of olives pressed in 2018 was 59,345 tons, while in 2016 was 484,581 tons.

Table A- 4: Number of oil presses and methods of waste disposal of olive cake and waste water.

Government/ Automation Level	Method of waste disposal							No. of operating presses
	Olive cake			Waste water				
	Other	Sell	Farmer	Others	Tight cesspit	Sewage network	Porous cesspit	
Palestine	7	24	229	10	128	37	85	260
West bank	6	6	221	10	124	18	81	233
Jenin, Tubas and Northern Valleys	1	3	54	3	26	1	28	58
Tulkarm	0	1	30	1	24	3	3	31
Nablus	0	0	38	3	20	5	10	38
Qalqiliya	0	0	16	0	1	0	15	16
Salfit	0	0	24	0	24	0	0	24
Ramallah and Al- Bireh	0	2	29	2	19	2	8	31
Jerusalem	2	0	1	1	2	0	0	3
Bethlehem	3	0	3	0	0	3	3	6
Hebron	0	0	26	0	8	4	14	26
Gaza strip	1	18	8	0	4	19	4	27
Gaza and North Gaza	0	9	0	0	0	6	3	9
Deir AL-Balah	1	8	0	0	0	9	0	9
Khan Younis and Rafah	0	1	8	0	4	4	1	9
Traditional and half automatic presses	2	5	5	1	4	4	3	12
Automatic presses	5	19	224	9	124	33	82	248

Table A- 5: Number of oil presses and methods of waste disposal of Zibar in Palestine in 2018 (PCBS, 2018).

Government/ Automation Level	Method of waste disposal				No. of operating presses
	Zibar				
	Other	Tight cesspit	Sewage network	Porous cesspit	
Palestine	15	125	36	84	260
West bank	5	21	17	80	233
Jenin, Tubas and Northern Valleys	4	26	1	27	58
Tulkarm	2	23	3	3	31
Nablus	5	21	4	8	38
Qalqiliya	0	1	0	5	16
Salfit	0	24	0	0	24
Ramallah and Al- Bireh	3	17	2	9	31
Jerusalem	0	1	1	1	3
Bethlehem	1	0	2	3	6
Hebron	0	8	4	14	26
Gaza strip	0	4	19		27
Gaza and North Gaza	0	0	6	3	9
Deir AL-Balah	0	0	9	0	9
Khan Younis and Rafah	0	4	4	1	9
Traditional and half automatic presses	0	4	4	4	12
Automatic presses	15	121	32	80	248

Three types of oil extraction are utilized in West Bank (Shaheen, 2004):

1- Semi-automatic oil extraction process (it uses vertical hydraulic presses).

2- Fully automatic oil extraction process (it's done by using three phase decanters).

3- Traditional oil extraction process.