Anaerobic Co-Digestion of Algal Biomass and a Supplemental Carbon Source Material to Produce Methane

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Utah State University

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ANAEROBIC CO-DIGESTION OF ALGAL BIOMASS AND A SUPPLEMENTAL CARBON SOURCE MATERIAL TO PRODUCE METHANE

by

Yousef Soboh

A dissertation submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Biological Engineering

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ABSTRACT

Anaerobic Co-Digestion of Algal Biomass and a Supplemental Carbon Source Material to Produce Methane

by

Yousef Soboh, Doctor of Philosophy

Utah State University, 2015

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Department: Biological Engineering

Algae that are grown in wastewater treatment lagoons could be an important substrate for biofuel production; however, the low C/N ratio of algae is not conducive to anaerobic digestion of algae with economically attractive methane production rates. Increasing the C/N ratio in anaerobic, laboratory scale, batch reactors by blending algal biomass with sodium acetate resulted in increased methane production rates as the C/N ratio increased. The highest amount of methane was produced when the C/N was 21/1. When the C/N was 24/1, the biogas production rate decreased. Batch experiments were done to evaluate the effect of optimizing the C/N ratio on methane production from algae and to identify the most essential information needed to conduct research on co-digestion of algal biomass using the continuous, high-rate, up-flow anaerobic sludge blanket (UASB) reactor system. Based on the results obtained from batch reactor experiments,
anaerobic co-digestion of algal biomass, obtained by continuous centrifugation from the Logan City, Utah, 5th stage wastewater treatment lagoon, and sodium acetate was conducted using laboratory scale UASB reactors with the C/N ratio in the feedstock adjusted to 21/1. Duplicate, 34 L UASB reactor systems were built of poly(methyl methacrylate). Both reactors were seeded with 11 L of anaerobic sediment from the 3rd stage lagoon. The pH of the feedstock was adjusted to the neutral range. The feedstock was initially introduced at a low organic loading rate of 0.9 g/L·d with a hydraulic retention time (HRT) of 7.2 days and then increased up to 5.4 g/L·d and a HRT of 5.5 days. These organic loading rates corresponded to an initial influent chemical oxygen demand (COD) of 6.25 g/L and increased to 27.2 g/L. Methane production increased from 270 mL/g to 349 mL/g COD biodegraded. COD removal efficiency was 80% and biogas methane composition was 90% at steady state. Algal biomass contributed 33-50% of the COD in the feedstock depending on the COD of the algae paste from centrifugation. The shortest HRT at which steady state was not affected was 5.5 days. At lower HRT all monitored parameters showed a slight decrease after the 75th day of operation.
The demand on alternative energy is rapidly increasing because the reserves of conventional fuel are decreasing and their impacts on the environment are increasing. Developing renewable energy sources should receive utmost attention. The production of biofuels like methanol, biodiesel, and methane from various kinds of abundant biomass represents a very attractive energy source that can reduce the dependence on conventional fossil fuels energy.

Since algae contains high amounts of nitrogen and low carbon content, this research focused on anaerobic fermentation of algae to produce methane by blending algae with a supplemental carbon source material, sodium acetate. This was done to increase the carbon content of the material fed to the anaerobic process to improve its fermentation and thus increase the amount of methane produced. Laboratory experiments were conducted to determine the favorable proportionality of carbon and nitrogen content. These experiments showed that the best carbon to nitrogen ratio should be 21/1 by weight. A follow-on experiment was conducted for approximately 81 days with the carbon to nitrogen ratio adjusted to 21/1 in the mixture of algae and sodium acetate to be...
fermented. This experiment used a bioreactor called an up-flow anaerobic sludge blanket reactor with continuous flow. The results from this experiment showed that 80% of the organic matter decomposed and the methane content was approximately 90% of the total biogas produced. It was estimated that 349 mL of methane was produced by each gram of organic matter decomposed.
DEDICATION

To my beloved wife Abeer Ismail Tarayra for her love, support and patience
ACKNOWLEDGMENTS

I would like to thank my major advisors, Dr. Ronald Sims and Dr. Darwin Sorensen. I appreciate your camaraderie, enthusiasm, and support throughout the years. You both have been exceptional advisors and I thank you both very much for the opportunity. I would like to thank the other members my committee, Dr. Mac McKee, Dr. Charles Miller, Mr. Issa Hamud, for their support and encouragement.

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<td>A</td>
<td>Surface area</td>
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<tr>
<td>AAFED</td>
<td>Anaerobic Attached Film Expanded Bed</td>
</tr>
<tr>
<td>APB</td>
<td>Anaerobic Packed Bed</td>
</tr>
<tr>
<td>APHA</td>
<td>American Public Health Association</td>
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<tr>
<td>ATP</td>
<td>Adenosine Tri-Phosphate</td>
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<tr>
<td>BOD</td>
<td>Biochemical Oxygen Demand</td>
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<tr>
<td>C/N</td>
<td>Carbon to Nitrogen ratio</td>
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<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
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<td>COD&lt;sub&gt;in&lt;/sub&gt;</td>
<td>COD influent</td>
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<tr>
<td>COD&lt;sub&gt;out&lt;/sub&gt;</td>
<td>COD effluent</td>
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<tr>
<td>FB</td>
<td>Fluidized Bed</td>
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<tr>
<td>HRT</td>
<td>Hydraulic Retention Time</td>
</tr>
<tr>
<td>OLR</td>
<td>Organic Loading Rate</td>
</tr>
<tr>
<td>Q</td>
<td>Water flow rate</td>
</tr>
<tr>
<td>SCCM</td>
<td>Standard cubic centimeter</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SRT</td>
<td>Sludge Retention Time</td>
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<tr>
<td>TS</td>
<td>Total Solids</td>
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<tr>
<td>TSS</td>
<td>Total Suspected Solids</td>
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<td>UASB</td>
<td>Up-Flow Anaerobic Sludge Blanket reactor</td>
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<tr>
<td>V</td>
<td>Reactor volume</td>
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<tr>
<td>VFAs</td>
<td>Volatile Fatty Acids</td>
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<tr>
<td>VSS</td>
<td>Volatile Suspended Solids</td>
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<tr>
<td>ΔG°</td>
<td>Gibbs Free Energy</td>
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<tr>
<td>CVWRF</td>
<td>Central Valley Water Reclamation Facility</td>
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<td>WEF</td>
<td>Water Environmental Federation</td>
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CHAPTER 1

INTRODUCTION

Background

Anaerobic digestion of waste and wastewater is a proven technology (van Lier et al., 2001). For almost three decades it has been recognized that the successful application of anaerobic digestion not only provides methane as a renewable energy source and the mitigation of environmental impacts from wastes, but it provides other ecological benefits like sanitation, reduction in deforestation and offsets the need to import conventional fuels (BORDA, 1989). Recently the application of anaerobic digestion has increased particularly in the digestion of wastewater originating from industrial and agricultural activities to produce biogas (Borja et al., 1994; Landine et al., 1982). Anaerobic digestion technology could be considered as the heart of treatment and recovery technology (Figure 1) (DELFT, 1995) but it has not been practiced at its full potential. The success of anaerobic digestion is attributed to the development of high-rate reactor systems such as the Up-Flow Anaerobic Sludge Blanket (UASB) reactor, the Up-Flow Anaerobic Filter (UAF), the Anaerobic Attached Film Expanded Bed (AAFEB) reactor, the Fluidized Bed (FB) reactor, and the expanded granular sludge bed (EGSB) reactor that are all characterized by high sludge retention time (Lettinga et al., 1997). High-rate reactors are characterized by their ability to accommodate high organic loading rates because they contain high concentrations of biomass and provide sufficient sludge-water contact (Lettinga et al., 1997; Rajeshwari et al., 2000). The biomass is present in
Among the above mentioned reactor systems, the UASB reactor was reported to be the most efficient, especially as a pretreatment system for a wide range of different types of industrial wastewater including those containing some types of toxic and/or inhibitory substances for microorganisms (Lettinga, 1995).

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There are many reactor types used in anaerobic digestion and treatment of different types of wastes and wastewater. The most commonly used types are the completely mixed anaerobic reactor, the fluidized bed reactor, anaerobic filters, and
UASB reactors. Among these reactors, the UASB reactor is the most well demonstrated (Ersahin et al., 2011) and represents a proven, sustainable reactor (Lettinga, 1995).

The problems associated with anaerobic filters and FB reactors has led to development of unpacked reactors that still incorporate an immobilized form of particulate biomass (Anderson et al., 2003; Ersahin et al., 2011). In the 1970s, in the Netherlands, Lettinga et al. (1980) developed an unpacked, high-rate reactor called a UASB reactor. It is by far the most transformatory development in anaerobic treatment process technology in recent times. It has wide applications in treating relatively low strength wastewater as well as a wide range of industrial wastewater such as food, paper, brewery, yeast, chemical and other industrial wastewaters. (George et al., 2004). Influent is distributed at the base of the UASB reactor, travels upward through the sludge bed and passes around the inclined wall of the three phase separator and deflectors that provides a greater area for the effluent which in turn slows down the up-flow velocity, enhance solids detention in the reactor, and efficiency in solids separation from the effluent (Ersahin et al., 2011). The UASB reactor is shown in (Figure 2).

Sludge granules, formed after a few months of reactor operation, composed mainly of a dense, microbial community is responsible for decomposition of organic matter and the respiration of methanogens (Hulshoff Pol et al., 2004; Shah et al., 2014).

Good settling properties of sludge, low hydraulic retention times (less reactor volume), no costs for packing material, high biomass concentrations (30-80 g/L), effective solids/liquid separation, and accommodation of high organic loading rates can be obtained by UASB reactors (Speece, 1996). The only limitation of the UASB reactor is related to treating wastewaters containing high solid concentrations which inhibits the
formation of granulated sludge (George et al., 2004). This limitation is true for wastewater containing particulate wastes (Lettinga and Hulshoff Pol, 1991; Parawira, 2004). Important characteristics of suspended solids to be considered include biodegradability or the rate of degradation, SS size and surface area, biomass attachment to the material, SS density, and the tendency of SS to be adsorbed to the sludge (Lettinga and Hulshoff Pol, 1991).

The biogas produced in the sludge blanket becomes partially entrapped in the sludge and the free gas bubbles and particles with the attached gas tend to rise to the top of the reactor. Particles float to the surface of the degassing baffles on their way upward
and attached gas bubbles may be released. The degassed sludge particles then drop back to the digestion zone. The gas released from the sludge is usually collected in a container known as the gas collection dome located on the top of the reactor. Liquid containing some solids and biomass granules passes into the settling zone, where part of the residual solids are separated from the liquid and occasionally drop back through the buffer system to the settling zone. This helps achieve sufficient contact between the biomass and wastewater. The UASB system relies on the mixing brought about by the biogas generated and on an even feed inlet distribution, therefore, there is no mechanical mixing in a UASB reactor (Hulshoff Pol et al., 1983; van Haandle and Lettinga, 1994).

The UASB reactor technology is the most demonstrated and is the most frequently applied high-rate reactor system. Although anaerobic reactors were originally developed for mainly soluble and medium strength wastewater, it would be a serious mistake to exclude their applicability to more complex high strength and partially soluble wastewaters or low strength wastewater (<1000 mg COD/L) (Lettinga et al., 1984; Lettinga and Hulshoff Pol, 1991; Mrowiec and Suschka, 2010). The feasibility of grey water treatment in a UASB reactor was studied. The batch recirculation experiments showed that a total-COD removal of 79% can be achieved in grey-water treatment in the UASB reactor. Whereas continuous flow process showed a removal efficiency of 31-41% at HRTs of 20, 12, and 8 hours. The COD removal efficiencies were three times those obtained by septic tanks (Elmitwalli et al., 2007).

Previous studies showed that laboratory scale, two-stage anaerobic conversion of food waste to methane was efficient using a laboratory scale leaching bed reactor for acidification and a UASB reactor for methane production (Demirel and Yenigun, 2002;
UASB reactors have been used for anaerobic treatment of wastes generated from the sugar industry (Hampannavar and Shivayogimath, 2010). It showed 89.4% COD removal efficiency at an OLR of 16 g/L·d and 6 hrs retention time. Studies on a laboratory scale UASB and anaerobic packed bed (APB) treating potato leachate at increasing OLR (Parawira et al., 2006), showed better performance of UASB in terms of methane production rate (0.231 L CH$_4$/g COD biodegraded and 0.161 L CH$_4$/g COD biodegraded, respectively) at OLR of 6.1 and 4.7g COD/L·d, respectively. Both reactors showed over 90% COD removal efficiencies.

Olive mills wastewater that was a very oily substrate with a relatively high content of poly-phenols (Khatib et al., 2009) has been studied using pilot scale UASB reactor seeded with anaerobic digested sludge obtained from an anaerobic digester in a brewery industry. The removal efficiency, in terms of COD, reached 84% at a HRT of ≤ 3.5 days (Khatib et al., 2009). A pilot scale study was set up to investigate the principle design parameters for a UASB reactor for treating wastewater of small communities with low strength wastewater in Iran (Azimi and Zamanzadeh, 2004). The UASB showed a removal efficiency of BOD, COD, and TSS of 71, 63 and 65%, respectively. The temperature was 22-26 °C with a HRT of 6 hours whereas in colder periods the removal efficiencies dropped down to 54, 46, and 53%, respectively, with a HRT of 8 hrs (Azimi and Zamanzadeh, 2004).

The UASB reactors digest and treat a large variety of wastes and wastewaters of industrial and domestic sources to reduce environmental impacts and to produce valuable bio-energy (Lettinga and Hulshoff Pol, 1991). The main disadvantage of this type of reactor is the start-up process, i.e. the time needed to acclimatize the biomass to the
feedstock until the process is consistently operating under stable conditions without the accumulation of intermediates, such as VFA, hydrogen gas and carbon monoxide in addition to the long time the digester takes for granulation of sludge (Borja et al., 1994; Pullammanappallil et al., 1998; Rintala, 1991). According to Franco et al (2007), OLR must initially be about 1 g/L.d to avoid the accumulation of intermediates.

**Mass Balance Equation**

Within the UASB reactor (control volume), the mass balance for any given constituent takes the form:

Accumulation = input – output ± generation.

(Net rate of accumulation in the control volume) = (rate of flow into the control volume) - (rate of flow out of the control volume) + (net rate of generation in the control volume).

Each term in the mass balance equation has units of mass/time. The biodegradable fraction of organic material (COD) present in the influent, after exposure to anaerobic digestion in the UASB reactor will be converted to biomass COD; methane COD; and COD oxidized to CO₂ and other gases. At steady-state, when organic matter does not accumulate in the digestion system, the daily mass of influent COD is equal to the sum of the daily mass of (van Haandel and Lettinga, 1994):

(i) COD leaving the system as methane;

(ii) the excess sludge (biomass) COD produced;

(iii) the COD of effluent; and

(iv) COD oxidized.
Figure 3—Global mass balance applied to anaerobic reactor. Adapted from Franco et al. (2007).

The mass balance applied to anaerobic bioreactors allows the estimation of the amount of methane produced as shown in (Figure 3).

Where:
- $Q$: Wastewater flow rate (m$^3$/day)
- $Q_g$: gas flow rate (m$^3$/day)
- $COD_{inf}$: COD concentration of influent (kg COD/m$^3$).
- $COD_{eff}$: COD effluent concentration (kg/m$^3$).

**Research Motivation**

Algal biomass represents an important substrate for the production of renewable energy and reduction of greenhouse gas emission to the atmosphere (Dębowski et al.,
One type of algal biomass that represents a potentially viable source for biofuel production is waste grown algae (Salerno et al., 2009).

The tremendous amounts of algal biomass, particularly in wastewater lagoons and ponds, represent a potential resource for bio-energy and recovery of fertilizers containing nitrogen and phosphorous (Mulbry et al., 2005).

By anaerobic digestion, the algal waste to be handled can be reduced and a viable bioenergy source, methane, can be produced to offset the needs of fossil fuels and, as a result, protect the environment (Yen and Brune, 2007). The chemical energy stored in the algal biomass as a result of photosynthesis could be released as methane via anaerobic digestion. This concept was originally proposed over half a century ago by Oswald and Golueke (1960) when they proposed algae cultivation using wastewater in a raceway with subsequent anaerobic digestion of the biomass to methane. Chen and Oswald (1998) found that treating algal biomass by heating at 100 ºC for 8 hours was effective as a pretreatment process to decrease the recalcitrance of algal biomass to hydrolysis, resulting in a 33% improvement in the rate of methane production. Yen and Brune (2007) concluded that the resulting improvement in methane energy production would not be economically feasible because of the energy consumed in the pretreatment step.

The resistance of the cell walls to biodegradation and the low C/N ratio of algal biomass are the main obstacles encountered in anaerobic digestion (Dębowski et al., 2013; Mata-Alvarez et al., 2000; Wu et al., 2010). Although consensus for an optimum C/N range in feedstock for anaerobic digestion has not been reached in the literature, 20/1-30/1 is the most suitable range (Kayhanian and Tchobanoglous, 1992; Marchaim, 1992; Wang et al., 2014; Wu et al., 2010; Yen and Brune, 2007). The C/N ratio in algal
biomass is about 6/1, which is not suitable for proper anaerobic digestion (Yen and Brune, 2007).

Low C/N ratio in the substrate leads to high ammonia formation, which, as a toxic gas, would inhibit methanogenic activity and, with further accumulation, could result in the failure of the anaerobic digestion system (Yen and Brune, 2007). Excessive ammonia accumulation can be averted by increasing the C/N ratio through adding a supplemental carbon source material (Yen and Brune, 2007).

Co-digestion of cattle manure slurry, fruit and vegetable wastes with chicken manure is an example of successful co-digestion of high C/N ratio and low C/N ratio feedstock to improve the methane production rate and it was found that the methane production rate doubled when 50% of the feedstock was composed of cattle manure slurry, fruit and vegetable wastes (Callaghan et al., 2002). Anaerobic co-digestion of a mixture of 75% sewage sludge and 25% organic fraction of municipal solid waste is another example of increasing the C/N ratio of the feedstock to improve the digestion process (Sosnowski et al., 2003). Anaerobic co-digestion of algal biomass and waste paper has also been investigated. Adding waste paper as a supplemental carbon source to algal sludge feedstock increased the methane production rate to 1.2 L/L.day, as compared to 0.6 L/L.d of algal biomass digestion alone using 4 L bench-top anaerobic digesters with a hydraulic retention time of 10 days. They found that the optimum C/N ratio was in the range of 20-25/1 (Yen and Brune, 2007).

The experiments conducted by Salerno et al. (2009) showed that by blending algae, soybean oil and glycerin, the rate of biogas production could be improved by over 3 times that generated from anaerobic digestion of algae alone after 28 days incubation.
The feedstock C/N ratio was not determined in these studies. Anaerobic co-digestion of microalgae residues resulting from the biodiesel production process was also investigated using glycerol as a supplemental carbon source and a C/N ratio of 12.44 was found to be the most favorable for biogas production (Ehimen et al., 2011).

The literature reviewed has shown that by increasing the C/N ratio of algal feedstocks, the rate of methane production increases but there is no agreement among investigators on an optimum C/N ratio for anaerobic digestion of algal biomass. The variation in the reported C/N ratios may be due to the type of feedstock, measurement errors, the source of sludge seeded or the length of retention time applied.

Moreover, anaerobic algae digestion experiments reported to date have used laboratory scale, batch reactors or semi-continuous reactors. A continuous flow, high-rate UASB reactor system is expected to show desirable performance such as a high biogas production rate per unit mass of organic compound degraded (mL CH₄/g COD biodegraded), high methane composition, improved COD removal efficiency and high removal of other pollutants. This is due to their sludge retention that leads to a high concentration of suspended biomass in the reactor providing sludge-algae contact and adsorption area facilitating algae decomposition as its up-flow stream makes the sludge bed expand. The low HRT results in relatively low reactor volume; and continuous flow, removes soluble metabolic products and toxics that may inhibit microbial activity.

The UASB reactor appears to be a promising technology for anaerobic co-digestion of algae and a carbon source to produce methane because it can digest particulate substances, including algal biomass (Tartakovsky et al., 2015), and it incorporates proven gas phase separation technology. Reactors based on the UASB
concept for treating wastewater and biogas production have been widely demonstrated, both at full scale and at pilot plant scale. From what has been reviewed, the UASB reactor has several advantages over other anaerobic reactor systems (Lettinga et al., 1980; Lettinga, 1995; Li et al., 1995):

1. No packing material is required for retention of high density anaerobic sludge.
2. The simple design of UASB ensures a uniform distribution of incoming wastewater around the base of the digester minimizing channeling, and dead zones.
3. Easy to operate and represents a low cost technology.
4. Excellent sludge and wastewater contact.
5. No mechanical mixing (energy saving).
6. Three phase separator and deflectors enhance the settling properties of the sludge and enable the reactor to separate gas, water and sludge mixtures.
7. COD removal > 80% and high OLR up to 30 kg COD m⁻³ d⁻¹ and thus low HRT. The key feature of the UASB reactor to accommodate such high loading rates, compared to other anaerobic processes, is the development of dense granulated sludge that has high digestion and methanogenic capacity.
8. Capable of treating different kinds of low strength wastewater and high strength wastewaters, containing low or high levels of dissolved or suspended particulate materials.
Research Objectives

Given the advantages of the continuous flow, high-rate reactor and the three phase separating design of the UASB reactor, and since anaerobic digestion of algae has not been demonstrated using high-rate reactor systems. The principle objective of the work reported here was to assess the feasibility of implementing UASB reactor technology as a method for the co-digestion of wastewater grown algae in Logan, Utah, lagoons with sodium acetate as a readily available carbon source to produce methane. Tasks completed to achieve this objective were:

1. Evaluate the effect of finding a favorable C/N ratio and varying the organic loading rate on the biogas production rate, the methane content of the biogas, and the biodegradability of algal biomass, using batch reactor experiments.

2. Employ the organic loading rates and other design criteria obtained during batch reactor experiments to determine the feasibility of using a UASB reactor system as a method in co-digestion of algal biomass and acetate to produce methane. The design and time frame of the batch experiments performed by others (Ehimen et al., 2011; Yen and Brune, 2007) did not provide adequate information on the digestion process of algae and the removal efficiencies in terms of COD, TS, and HRT.

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CHAPTER 2

A REVIEW OF ANAEROBIC DIGESTION MECHANISMS

Abstract

Anaerobic digestion is the decomposition of organic matter by the combined action of different types of microorganisms in the absence of molecular oxygen where the main gaseous products of this process are methane and carbon dioxide. Different types of organic wastes like food waste, animal dung, dairy wastes and energy crops, algal biomass, in addition to a wide range of industrial and agricultural wastes, can be a substrate for anaerobic digestion. The literature review presented here covered the following topics: 1) Microbial ecology of anaerobic digestion of organic matter; 2) Respiratory pathways of methanogenic archaea; 3) Energetics in anaerobic digestion; 4) Environmental influence and the nature and composition of substrate on anaerobic digestion; and 5) anaerobic digestion of algae.

Microbial Ecology of Anaerobic Digestion of Organic Matter

Anaerobic digestion of the organic matter in wastewater, animal dung, food wastes and plant residues is brought about by the combined action of a wide range of anaerobic microorganisms. These microorganisms decompose organic matter that is mostly particulate, to final products, mainly carbon dioxide (CO$_2$) and methane (CH$_4$). The biochemical reactions take place in the absence of other electron acceptors such as sulfate, nitrate, Mn(IV), and Fe(III) minerals. Many kinds of microorganisms like...
archaea, bacteria, fungi and probably some protozoa participate in these processes (Bitton, 2011). Acidogenic, acetogenic and methanogenic microorganisms, which differ in their metabolic reactions, can be recognized in the anaerobic decomposition of organic matter (Demirel and Scherer, 2008; Nealson, 1997; van Haandle and Lettinga 1994). These metabolic reactions include hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 4), (WasteSUM, 2006). The whole sequence of reactions can be considered as a microbial synergistic relationship, where the products of one group of microorganisms are the substrates for the next (van Haandle and Lettinga, 1994; Shah et al., 2014).

**Hydrolysis**

Hydrolysis of polymeric materials involves primarily extra-cellular enzymatic reactions. Complex particulate matter, i.e. hydrolysable proteins, carbohydrates, and lipids, are enzymatically converted into dissolved, low molecular weight compounds that enter microbial cells. Carbohydrates are transformed into soluble sugars (Equation 1). Proteins are hydrolyzed to amino acids (Equation 2), and lipids are converted to fatty acids and glycerin (Equation 3). In practice, hydrolysis rates can be limiting to the overall rate of anaerobic digestion (Demirel, 2014; van Haandel and Lettinga, 1994).

**Sugars**

\[
\begin{align*}
(C_6H_{10}O_5)_n & \xrightarrow{\text{Exo- and endo-glucanase}} (C_{12}H_{22}O_{11})_n & \xrightarrow{\text{Endoglucanases}} & C_6H_{12}O_6 \\
\text{Cellulose} & & & \text{Cellubiose} & & \text{Glucose}
\end{align*}
\]
Figure 4—The major microbial metabolic processes in anaerobic digestion. Adapted from WasteSUM (2006).

Proteins

\[
\text{Proteins} \rightarrow \text{Amino Acids} + \text{NH}_4 \quad \text{Equation 2}
\]

Lipids

\[
\text{Fat} \rightarrow \text{Glycerol} + \text{acetate} \quad \text{Equation 3}
\]

**Acidogenesis**

The products of hydrolysis are metabolized in oxidation-reduction reactions, yielding carbon dioxide, hydrogen and volatile fatty acids (VFAs). Due to this organic acid production, the fermenting microorganisms have been called the acidifying populations. These populations are a diverse group of microbes the majority of which are obligate anaerobes (van Haandle and Lettinga, 1994). The metabolic reactions include the Strickland reaction, shown below, in the conversion of alanine and glycine to acetate (Nisman, 1954):
Alanine

\[
\text{CH}_3\text{CHNH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + \text{CO}_2 + \text{NH}_3 + 4\text{H}^+
\]

Glycine

\[
2\text{CH}_2\text{NH}_2\text{COOH} + 4\text{H}^+ \rightarrow 2\text{CH}_3\text{COOH} + 2\text{NH}_3
\]

\[
\text{CH}_3\text{CHNH}_2\text{COOH} + 2\text{CH}_2\text{NH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow 3\text{CH}_3\text{COOH} + \text{CO}_2 + 3\text{NH}_3
\]

The principle mechanism of anaerobic decomposition of long chain VFAs, followed by methanogenesis from the products, is illustrated in the following reaction steps for stearic acid (Novak and Carlson, 1970).

\[
\text{C}_{18}\text{H}_{36}\text{O}_2 + 8\text{H}_2\text{O} \rightarrow 9\text{CH}_3\text{COOH} + 32\text{H}^+
\]

Stearic acid

\[
9\text{CH}_3\text{COOH} \rightarrow 9\text{CH}_4 + 9\text{CO}_2
\]

\[
4\text{CO}_2 + 32\text{H}^+ \rightarrow 4\text{CH}_4 + 8\text{H}_2\text{O}
\]

\[
\text{C}_{18}\text{H}_{36}\text{O}_2 \rightarrow 13\text{CH}_4 + 5\text{CO}_2
\]

*Acetogenesis*

Acetogenic microbial populations metabolize the products of acidogenesis, e.g. ethanol, propionate and butyrate, to precursors for methane production such as acetate, hydrogen and carbon dioxide (van Haandel and Lettinga, 1994). These acetogenic microorganisms can only function in a syntrophic relationship with hydrogenotrophic methanogens (Bitton, 2011). Examples of acetogenic reactions (Bitton, 2011) are:

\[
\text{CH}_3\text{CH}_2\text{OH} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2
\]
Ethanol + Acetic acid
\[ \text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + \text{CO}_2 + 3\text{H}_2 \]

Propionic acid + Acetic acid
\[ \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2\text{H}_2 \]

Butyric acid + Acetic acid

**Methanogenesis**

The formation of methane from the products of acetogenesis is accomplished by the enzymatically complex decarboxylation of acetate by acetotrophic methanogens and by reduction of carbon dioxide by hydrogenotrophic methanogens (Welte and Deppenmeier, 2014). Typical reactions in acetotrophic methanogenesis and hydrogenotrophic methanogenesis are listed in Table 1.

**Respiratory Pathways of Methanogens**

The methyl group of acetate is used to produce a major part of methane in nature and two genera of archaea, *Methanosarcia* and *Methanosaeta*, use acetate to produce methane and cell growth. Aceticlastic methanogenesis can be represented simply in the following reaction (Welte and Deppenmeier, 2014):

\[ \text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2 \]  

**Equation 4**

The complex biochemistry of this process is accomplished by the enzymatic decarboxylation of acetate by acetotrophic methanogens and involves many enzymes. In *Methanosarcina*, the pathway known as the aceticlastic pathway starts with the activation of the carboxyl group of acetate by ATP-dependent phosphorylation catalyzed by an
acetate kinase (Figure 5). Then a phosphotransacetylase converts the produced acetyl-phosphate to acetyl Co-A.

In obligatory acetoclastic *Methanosaeta*, the activation of acetate is performed by an acetyl-CoA synthetase forming acetyl Co-A, AMP and pyrophosphate (PPI) from acetate, HS-CoA and ATP. A pyrophosphatase can hydrolyze PPI to drive the reaction (Figure 5). *Methanosarcinaceae* members can use compounds with one carbon atom such as methanol and methylamines as a substrate for their growth in the absence of hydrogen (Figure 5). This respiratory pathway is known as methylotrophic methanogenesis. Typically, in this pathway, only one out of four methyl groups is converted (oxidized) to CO₂ and three are converted (reduced) to CH₄.

---

**Table 1— Methanogen Reactions. Adapted from Anderson et al. (2003).**

<table>
<thead>
<tr>
<th>Reactions</th>
<th>ΔG° (KJ/mole CH₄)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate → Methane</td>
<td></td>
</tr>
<tr>
<td>HCOOH + H₂O → HCO₃⁻ + CH₄</td>
<td>-31.0</td>
</tr>
<tr>
<td>Methanol → Methane</td>
<td></td>
</tr>
<tr>
<td>CH₃OH + H₂ → CH₄ + H₂O</td>
<td>-112.5</td>
</tr>
<tr>
<td>Formate → Methane</td>
<td></td>
</tr>
<tr>
<td>HCOOH + 3H₂ + H⁺ → CH₄ + 3H₂O</td>
<td>-134.3</td>
</tr>
<tr>
<td>HCO₃⁻ → Methane</td>
<td></td>
</tr>
<tr>
<td>HCO₃⁻ + 4H₂ + H⁺ → CH₄ + 3H₂O</td>
<td>135.6</td>
</tr>
<tr>
<td>HCO₃⁻ → Acetate</td>
<td></td>
</tr>
<tr>
<td>2HCO₃⁻ + 4H₂ + H⁺ → CH₃COO⁻ + 4H₂O</td>
<td>-104.6</td>
</tr>
</tbody>
</table>
Energetics in Anaerobic Digestion

The reason for microbes to convert their substrates to their products is to gain energy in an appropriate form for growth. Energy yielding reactions are mostly oxidation reduction reactions. The energy yield of reactions is dependent on the feedstock digested and the products formed. The energy obtained from different decomposition processes, $\Delta G^\circ$, depends upon the type of feedstock, the substances formed, their concentrations, and upon the environmental conditions (DELFT, 1995). Some reactions of free energy gains under standard conditions ($25^\circ$C and pH 7.0) are presented in Table 2.

Hydrolysis reactions are performed outside the cell and, because of this, these reactions do not produce energy that can be used by microorganisms for growth. The bacteria that produce hydrolytic enzymes obtain their metabolic energy from the metabolism of the products of hydrolysis to volatile fatty acids (VFAs) along with the acidogens (DELFT, 1995). Other microorganisms may decompose polymers and use the products in anaerobic respiratory metabolism.
Table 2—Gibbs free energy of some anaerobic reactions at standard conditions. Adapted from Anderson et al. (2003).

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Δ Gº (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrate → Acetate</td>
<td>+48.1</td>
</tr>
<tr>
<td>( \text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2 \text{H}_2\text{O} \rightarrow 2 \text{CH}_3\text{COO}^- + 2\text{H}_2 + \text{H}^+ )</td>
<td>+48.1</td>
</tr>
<tr>
<td>Lactate → Acetate</td>
<td>-4.2</td>
</tr>
<tr>
<td>( \text{CH}_3\text{CHOHCOO}^- + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{HCO}_3^- + 2\text{H}^+ + \text{H}_2\text{O} )</td>
<td>+9.6</td>
</tr>
<tr>
<td>Ethanol → Acetate</td>
<td>+76.1</td>
</tr>
<tr>
<td>( \text{CH}_3\text{CH}_2\text{OH} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{H}^+ + 2\text{H}_2 )</td>
<td>+76.1</td>
</tr>
</tbody>
</table>

Another group of monomers that can be fermented to VFA, glycerin, and alcohol are the amino acids that are formed from protein metabolism. The activation energy in this metabolism is lower than that in the hydrolysis step of polymers, the acidogenic step or the methanogenesis step. Many reactions need energy (Table 2) when performed under standard condition and, therefore, they would not occur, but these reactions may occur under anaerobic conditions (van Haandle and Lettinga, 1994).

Environmental Influences on Anaerobic Digestion

The performance of anaerobic digestion depends strongly on environmental conditions and the characteristics of the material being digested. Several environmental factors such as temperature, nutrient availability, pH and toxic compounds, either enhance or inhibit anaerobic digestion, besides affecting growth rates. Methanogens typically grow more slowly than acidogens and their growth is strongly influenced by relatively small temperature changes (Chen et al., 2008; Marchaim, 1992).
A decrease in temperature leads to a decrease in the growth rate of microorganisms except psychrophiles. Therefore the temperature in mesophilic reactors should be kept between 30-35 °C. It was also reported that low temperature causes low specific methanogenic activity and slow hydrolysis. The rate of anaerobic digestion, like other biological processes, is strongly affected by temperature. The conversion rate reaches a maximum between 35-40°C in mesophilic anaerobic reactors (van Haandel and Lettinga, 1994). Research on temperature effects has shown that the mesophilic temperature range in anaerobic digestion is optimal. The mesophilic range is between 30-44°C and the thermophilic range is between 50-60 °C (Chen et al., 2008; Saleh and Mahmood, 2004; Marchaim, 1992; Hulshoff Pol, 1995).

The nutritional requirements of methanogens varies from simple to complex (Marchaim, 1992). Low concentrations of inorganic macro-nutrients (N and P) and micro-nutrients (Zn, Fe, Co, Ni, etc.) causes low methanogen growth rates (Demirel and Scherer, 2011). The resulting low population density of methanogens causes a low CH₄ production rate (Anderson et al., 2003; Chen et al., 2008; Grady et al., 2011; Krishna et al., 2014). With regard to carbon assimilation, some methanogens are autotrophs (use CO₂ as carbon source), some heterotrophs (organic carbon source), and some are mixotrophs (organic and inorganic carbon sources). In general, methanogens depend highly on other bacteria to supply essential nutrient like acetate, vitamins, amino acids or other growth factors (Whitman et al., 2006).

The pH and its variance in anaerobic digesters affects the rate of methanogenesis. It is faster when the pH is near neutral. In the acidic range (pH < 6.3) or in alkaline range (pH > 7.8), the rate of methanogenesis has been observed to decrease (van Haandel and
Acidogenic microbial populations are significantly less sensitive to low or high pH values and hence, acid formation will prevail over methanogenic respiration, which may result in a condition called “souring” of the reactor contents. This means that the pH of the reactor contents will decrease into the acidic range as a result of accumulation of VFAs (van Haandel and Lettinga, 1994).

High concentrations of suspended solids, including volatile suspended solids (VSS), cause slow hydrolysis, reduction of specific methanogenic activity, and reduction of sludge retention time (SRT) in addition to the risk of scum layer formation on the top of the reactor. Fluctuation in flow rate and concentration of the feedstock causes low effluent quality (Hawkes and Hawkes, 1987; Parawira, 2004).

Anaerobic digestion is highly influenced by toxic compounds, and the methanogens have been reported to be the most sensitive community members. It has been thought that the anaerobic digestion process cannot tolerate toxic substances, and that the microorganisms are destroyed by the toxicants. It is now known that anaerobic biomass can tolerate certain levels of toxic substances. Long generation times can extend the recovery period if the toxicant is lethal but toxicity recovery studies on certain methanogens have shown that relatively low concentrations of some toxicants were bacteriostatic and their effect was reversible. Methanogens acclimatized to some toxicants were able to tolerate concentration much higher than those causing inhibition in un-acclimatized organisms (Marchaim, 1992, Parkin and Speece, 1982). Ca++, Mg++, Na+, K+, Fe++, or NH₄⁺, which have a stimulatory effect at relatively low concentrations, can inhibit metabolism and growth at high concentrations. NO₃⁻, Fe³⁺, and SO₄²⁻, are alternative respiratory electron acceptors and can compete with and slow methanogenesis.
Sulfide ($S^{2-}$) is required for most methanogenic bacteria but becomes toxic above 200 mg/L. When polyvalent metals are in solution, sulfide may become insoluble as metal sulfides (Tugtas and Pavlostathis, 2007; Winfrey and Zeikus, 1977).

Certain heavy metals like arsenic, lead, mercury and copper are toxic to the microbial community even at low concentrations. Heavy metal ions inhibit metabolism by forming sulfur hydrol bonds (-SH) in proteins including vital enzymes (Oleszkiewicz and Sharma, 1990).

Ammonia toxicity may result when the concentration of protein is relatively high in the digester feedstock. Deamination releases ammoniacal nitrogen into solution. Ammonia ($NH_3(g)$) is toxic while the $NH_4^+$ ion is generally innocuous, hence, pH below neutrality greatly affects ammonia toxicity. Concentrations of ammonia below 80 mg/L are generally safe (Anderson et al., 1982; Bitton, 2011).

High concentrations of volatile acids such as acetic, propionic or butyric, are inhibitory to methanogenesis. Inhibitory effects have been demonstrated for propionic acid at concentrations in excess of 1,000 mg/L (Hobson and Shaw, 1976).

Management of anaerobic digestion processes requires early identification of toxicity in the reactor. Toxicity is generally indicated by two changes in behavior of the digester (Marchaim, 1992):

a. Decline in biogas production rate and methane composition, indicated by two or more consecutive decreases of more than 10% in daily production rate at a constant organic loading rate;
b. Accumulation of volatile acids, generally occurring when the total volatile acids concentration exceeds the normal range of about 250 to 500 mg/L.

The composition and nature of the feedstock being digested has an important effect on the growth rate of the anaerobic microorganisms and on the biogas production rate (Marchaim, 1992). The nitrogen in the feedstock is the source for biosynthesis of amino acids, proteins and nucleic acids. Ammonia from this nitrogen is a strong base that participated in neutralizing organic acids and maintaining pH in the neutral range. Ammonia from mineralization of abundant nitrogenous compounds can accumulate in excess of that needed for microbial assimilation and can, depending on the pH, result in toxic concentrations of NH$_3$(g) that inhibit digester performance. Therefore, it is crucial that the proper amount of nitrogen be in the feedstock (Marchaim, 1992).

Bacteria need a suitable ratio of carbon to nitrogen for their metabolic processes and nutritionally balanced growth. Macronutrients, including C and N, must be available in the correct proportions. Studies directed at finding appropriate C/N ratios for anaerobic digestion of waste materials have found that ratios higher than 25:1 were not optimal and that ratios lower than 10:1 were inhibitory (Kayhanian and Tchobanoglous, 1992; Marchaim, 1992; Wang et al., 2014; Wu et al., 2010; Yen and Brune, 2007).

**Anaerobic Digestion of Algae**

Biomass from different sources and with different characteristics represent a viable source for bioenergy production (Dębowski et al., 2013). However, some published information disagrees with this opinion in that the improper management of resources from typical energetic crops might result in the increase of greenhouse gas...
emission to the atmosphere (Dębowski et al., 2013). Some reports pointed out that the intensive use of arable lands for the cultivation of energetic crops intended for biofuel production might result in an adverse impact on the yield and prices of food on the global level (Johansson and Azar, 2007). Recently published research has been mainly focused on biodiesel production based on the high lipid content of algal biomass (Danilovic et al., 2014; Mandal and Mallick, 2009; Mata et al., 2010). Many researchers claim that anaerobic digestion of algae to produce methane is the most feasible method for the production of renewable energy from algal biomass (Dębowski et al., 2013). Anaerobic digestion as a method of algal biomass conversion to biogas is more economically feasible compared to biodiesel production based on lipid extraction and anaerobic treatment of algal residues after extraction (Sialve et al., 2009). It has also became evident that the production of biodiesel from algal biomass is not economically feasible due to the higher costs compared to fossil fuels (Harun et al., 2011)

References


CHAPTER 3

ANAEROBIC CO-DIGESTION OF ALGAL BIOMASS AND A SUPPLEMENTAL CARBON SOURCE TO PRODUCE METHANE USING BATCH REACTOR SYSTEMS

Abstract

Waste grown algae are a promising substrate for biofuel production; however, the low C/N ratio of algae is not conducive to anaerobic digestion of algae with economically attractive methane production rates. Increasing the C/N ratio in anaerobic, laboratory scale, batch reactors by blending the algal biomass with sodium acetate resulted in an increase in methane production rate as the C/N ratio increased. The highest rate of biogas production was observed when the C/N was 21/1 and gas production declined substantially when the C/N ratio was 24/1. Near the end of the experiment, the biogas methane content was 82% from the 21/1 treatment while algae alone produced 62%.

Introduction

Biomass from different sources and with different characteristics is believed by many to be one of the main sources for bioenergy production (Dębowski et al., 2013). However, improper management of resources from-energy resource crops could lead to increased emissions of greenhouse gases to the atmosphere (Dębowski et al., 2013). Some reports have pointed out that the intensive use of arable lands for the cultivation of
energetic crops intended for biofuel production might result in an adverse impact on the yield and prices of food on the global level (Johansson and Azar, 2007). Research on biofuel production from algae has been mainly focused on biodiesel production from the high lipid content of algal cells (Danilovic et al., 2014; Mandal and Mallick, 2009; Mata et al., 2010). Many researchers claim that anaerobic digestion of algae to produce methane is one of the most effective methods for energetic exploitation of algal biomass (Dębowski et al., 2013). Anaerobic digestion, as a method of algal biomass conversion to biogas, leads to higher economic benefit compared to biodiesel production based on lipid extraction and anaerobic treatment of algal residues after extraction. The production of biodiesel from algal biomass is not economically feasible in the current economic environment due to the higher cost of the fuel produced compared to conventional fuels (Bharathiraja et al., 2015; Harun et al., 2011; Sialve et al., 2009).

Waste grown algae are a promising substrate for biofuel production. There are approximately 7,000 wastewater treatment lagoons and pond systems in the US but algae harvesting is rarely done. When this is done, the algal biomass is most commonly returned to the ponds, where it is anaerobically decomposed in the sediments, resulting in methane and carbon dioxide release to the atmosphere (Salerno et al., 2009). The large amounts of algal biomass produced throughout the year, particularly in wastewater lagoons, represent a potential resource for bio-energy and recovery of fertilizers containing nitrogen and phosphorous (Mulbry et al., 2005). Anaerobic digestion of algal biomass could decrease the amount of waste to be handled and could also generate methane to offset energy demand and reduce the impact of fossil fuels on the environment (Yen and Brune, 2007).
There are two major obstacles to the anaerobic digestion of algae: the resistance of the cell envelope to decomposition and the relatively low carbon to nitrogen ratio (C/N) (Mata-Alvarez et al., 2000; Wu et al., 2010). The photosynthetic energy stored in algal biomass could be released as methane via anaerobic digestion. This was proposed by Oswald and Golueke (1960) for an algae cultivation system followed by digestion of algal biomass to methane. More recent work by Chen and Oswald (1998) found that resistance of algal biomass to hydrolysis and an improvement of the rate of methane production by 33% could be achieved by heating the biomass in a pretreatment process at 100 °C for 8 hours. However, the improvement of methane energy production would not be economically feasible because of the energy consumed in heating the algal biomass (Yen and Brune, 2007). Lee et al. (2014) found that ultra sound treatment of algae increased methane production 2.3 fold over untreated algae.

Although an optimum C/N range in feedstock for anaerobic digestion is still being debated in the literature, 20/1-30/1 based on weight ratio is generally considered the most suitable range (Kayhanian and Tchobanoglous, 1992; Marchaim, 1992; Wang et al., 2014; Wu et al., 2010; Yen and Brune, 2007). The C/N ratio in algal biomass has been found to be about 6/1 (Yen and Brune, 2007), which could result in high ammonia nitrogen production, a toxic, dissolved gas, that would decrease the methanogenic activity and, with further accumulation, cause the anaerobic digestion system to fail. Ammonia accumulation can be averted by increasing the C/N ratio via adding a high C/N material, thereby improving the digestion process. Sosnowski et al. (2003) blended high C/N municipal solid waste with sewage sludge to achieve this. Co-digestion of the high and low C/N ratios of materials in a mixture of cattle manure slurry, fruit and vegetable
wastes and chicken manure also improved the digestion process (Callaghan et al., 2002).

Anaerobic co-digestion of algae and waste paper has also been investigated. Blending certain amounts of waste paper as a carbon source with algal sludge in 4 L, semi-continuous, bench-top anaerobic digesters with a hydraulic retention time of 10 days and C/N ratio of 20-25/1, increased the methane production rate to 1.2 mL/L·d. This rate was about two times higher than the rate from algal sludge digestion alone (Yen and Brune, 2007). The experiments carried out by Salerno et al. (2009) showed that by blending algae, soybean oil and glycerin, the rate of biogas production was improved by over 3 times that from anaerobic digestion of algae alone with a 28 day detention time. The C/N ratio used was apparently not determined. Anaerobic co-digestion of microalgae residues after extraction of lipid for biodiesel production process was also investigated using glycerol as a rich carbon source revealing that a C/N ratio of 12.44 was required for optimum biogas production (Ehimen et al., 2011).

The purpose of the work described here was to evaluate anaerobic digestion of algal biomass and sodium acetate, as a supplemental carbon source. The main focus of this research was to identify the biodegradability of algal biomass, the effect of optimizing C/N ratio on biogas production using a batch reactor system, and organic loading rate needed to conduct research on co-digestion of algal biomass and supplemental carbon source material using the continuous, high-rate, up-flow anaerobic sludge blanket (UASB) reactor system.
Methods

Three batch reactor experiments were conducted. In the first experiment twelve anaerobic digesters of 500 mL each were used in duplicate. Carbon to nitrogen ratios of algae and sodium acetate of 12/1, 15/1, 18/1, and 21/1 by weight, were evaluated. Algal biomass alone (C/N = 5); a mixture of algal paste; produced by continuous flow centrifugation; sodium acetate as a co-digestion feed stock; and a mixture of anaerobic digested sludge and sediment from the Logan lagoon wastewater treatment plant were tested in duplicate.

To find out the C/N ratio of algae, triplicate samples of 50 mL of algae were dried out at 80°C until a stable weight was obtained, the total carbon of the desiccated algae samples was measured using a Skalar Primacs^{SLC} Analyzer (Buford, GA, USA). It analyzes total C by combusting the sample at 1050 °C in the presence of O$_2$ and measuring the CO$_2$ evolved with an IR detector.

Total nitrogen was measured using a Skalar Primacs^{SN} Analyzer (Buford, GA, USA). It is a combustion method (Dumas) in which the gas mixture resulting from the combustion of the sample is passed through a second oxidation oven where all the N compounds are converted into NOx. The sample then passes through a Peltier cooler to remove water, a Cu reduction column to remove excess O$_2$ and convert NOx to N$_2$, then a CO$_2$ scrubber, and finally a magnesium perchlorate H$_2$O scrubber. The resulting N$_2$ gas is measured with a thermal conductivity detector.

The chemical oxygen demand (COD) of the algae was measured by taking 0.2 mL of diluted algae suspension and placing it into Hach high range COD vials. The vials contain silver sulfate, chromic acid, and mercuric sulfate in addition to demineralized
water which all work to oxidize the organic and inorganic matter in the sample. The COD contents were then digested in a Hach COD digester at 150 °C for 2 hours. After digestion, the vials were allowed to cool to room temperature and COD values were recorded using a Hach DR/2800 spectrophotometer. Volatile suspended solids (VSS) of algae, anaerobic digested sludge and of the sediment was measured using method 2540 D from Standard Methods for the Examination of Water and Wastewater (APHA and WEF, 1995).

The metal content of algal biomass was also measured by digesting the sample using concentrated nitric acid and 30% hydrogen peroxide at 90 °C followed by inductively coupled plasma (ICP) emission spectroscopy (Thermo ICAP 6300). This was done to examine the availability of micronutrients needed for appropriate anaerobic digestion.

Each reactor received 18 mL of algal biomass obtained by continuous centrifugation, with a COD of 194.5 g/L. Reactors were inoculated with 50 mL of Logan lagoon sediment with COD of 50 g/L and VSS of 28 g/L and 250 mL of anaerobically digested sewage sludge with COD of 18 g/L and VSS of 10.5 g/L obtained from Central Valley Water Reclamation Facility (CVWRF) in Salt Lake City, Utah. Sodium acetate was added in the amounts of 2.3, 2.9, 3.45, and 4.01 g/L (COD = 0.78 g/g acetate) to produce C/N ratios of algae and sodium acetate of 12/1, 15/1, 18/1, and 21/1. The pH of the mixture was adjusted to the neutral range of 6.98-7.05 with chloric acid. The reactors were then placed in an anaerobic glove bag for 24 hours to remove oxygen. The reactors were closed to the atmosphere, put on a shaker table and incubated at a constant temperature of 30 ±1°C. The volume of biogas produced was measured 2-3 times a day in
a manometer. The manometer system consisted of a plastic 125 mL separatory funnel connected to a 50 mL graduated burette. (Figure 6) presents the batch reactor system design. The manometer fluid was water saturated with sodium chloride with a pH <1 adjusted by the addition of sulfuric acid. The biogas was evacuated from the system after each measurement using a hypodermic needle attached to a 60 mL syringe inserted through a septum and bringing the head space of the manometer to atmospheric pressure. The volume was adjusted to standard conditions based on the local barometric pressure. The biogas composition was tested near the end of the experiment using a gas chromatograph (GC) with a thermal conductivity detector (TCD), a packed column (Alltec, CTR1) 1.83 m x 6.35 mm and with a Valco injection valve with a 500 µL sample loop.

Since the highest average biogas was obtained from reactors with a C/N ratio of 21/1, a second experiment was conducted to evaluate the effect of higher C/N ratio of 24/1. The COD of algal biomass used in this experiment was of 216 g/L, so that the reactors number (7.1, 7.2 ) received 18 mL of algae (3.9 g COD) and 4.5 g acetate in addition to inoculating the reactors with the same amounts of sludge and sediment as in the first experiment. The procedure was followed in the same way as described in the first experiment. The star-tup experimental design for experiments 1 and 2 are shown in Table 3.

For more confidence that algae contributed to biogas production, a third experiment was conducted. It was hypothesized that in reactors with the same C/N ratio but decreasing amounts of COD added as acetate and algal biomass, acetate would be mineralized relatively early and that the significantly lower rate of mineralization of the
Figure 6—The batch reactor system

Table 3—The start-up experimental design of batch reactors

<table>
<thead>
<tr>
<th>Reactor No.</th>
<th>Sludge (mL)</th>
<th>Algae (mL)</th>
<th>COD of algae (g)</th>
<th>Sodium acetate (g)</th>
<th>C/N ratio (wt/wt)</th>
<th>Initial pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1, 2.1</td>
<td>300</td>
<td>18</td>
<td>3.5</td>
<td>None</td>
<td>5</td>
<td>6.98</td>
</tr>
<tr>
<td>2.1, 2.2</td>
<td>300</td>
<td>18</td>
<td>3.5</td>
<td>2.3</td>
<td>12</td>
<td>7.02</td>
</tr>
<tr>
<td>3.1, 3.2</td>
<td>300</td>
<td>18</td>
<td>3.5</td>
<td>2.9</td>
<td>15</td>
<td>7.03</td>
</tr>
<tr>
<td>4.1, 4.2</td>
<td>300</td>
<td>18</td>
<td>3.5</td>
<td>3.5</td>
<td>18</td>
<td>7.05</td>
</tr>
<tr>
<td>5.1, 5.2</td>
<td>300</td>
<td>18</td>
<td>3.5</td>
<td>4.0</td>
<td>21</td>
<td>7.00</td>
</tr>
<tr>
<td>6.1, 6.2*</td>
<td>300</td>
<td>None</td>
<td>7.0</td>
<td>None</td>
<td>NA</td>
<td>7.14</td>
</tr>
<tr>
<td>7.1, 7.2^</td>
<td>300</td>
<td>18</td>
<td>3.9</td>
<td>4.5</td>
<td>24</td>
<td>7.08</td>
</tr>
</tbody>
</table>

* A mixture of sludge and sediment only

^ Second experiment
biomass would result in a significant decrease in the rate of biogas production. Nine 500 mL reactors were used in triplicate. Triplicate reactors were seeded with varying amounts of algal biomass, with COD of 232 g/L and sodium acetate to produce a C/N ratio of 18/1 in each reactor (see Table 4).

Each reactor triplicate was inoculated with different amounts of Logan lagoons sediment and anaerobic digested sewage sludge from the same source and the same characteristics as in the first experiment. The total volume of reactor contents, COD of algae, COD of Na acetate and the amount of sludge were correlated. The same procedure and all environmental conditions were set in the same manner as in the first experiment. The start-up experimental design of these reactors is shown in Table 4.

### Results and Discussion

Water content, total solids, total carbon, total nitrogen, metal content, volatile suspended solids, and ash content of algal biomass are shown in Appendix A. Work reported by Soares et al. (2012) has shown that the micronutrient content of algae is sufficient to support anaerobic digestion without being toxic.
The results obtained during the first and second experiment are shown in (Figure 7). The four reactors with algae and sodium acetate showed an increasing trend in biogas production until the ninth day of operation, whereas the reactors with a C/N of 21 showed an increase of biogas production through the sixteenth day. The reactors with sludge alone and those with algae alone showed the least amount of biogas produced. This is consistent with what has been reported by Salerno et al. (2009) who reported higher methane production from co-digestion of algae and oil than from algae alone after 28 days incubation. They did not report the C/N ratio used in their reactors but these results indicate that by adjusting the C/N ratio in the reactor, the digestibility of algal biomass, which has been considered to be only slightly decomposable in anaerobic reactor systems, could be improved. The biogas produced was tested for its methane content on day 14 of the experiment. The total amount of biogas produced; the percentage of methane; and sludge are shown in Table 5. The reactor with a C/N of 21/1 produced the highest biogas. In the second experiment, the reactors (7.1, 7.2) with C/N of 24/1 showed much lower biogas production rates but the methane composition at day 14 was about the same in both of these reactors. These results are in agreement with the results obtained in anaerobic co-digestion of algae and waste paper (Yen and Brune, 2007) that reported an optimum C/N ratio of 21-25/1 with a hydraulic retention time of 10 days.

The pH of each reactor’s contents was measured at the end of the experiment and was found to range from 7.3 in the reactors containing algae alone to 8.0 in reactors of C/N=21/1. This indicates there was significant decomposition of fatty acids and successful anaerobic digestion of the substrates especially for reactors of higher C/N ratios.
To aid in data analysis, in experiment number 3, the biogas production rate was normalized to the total COD added as algae and acetate. The normalized biogas rate from the third experiment is shown in (Figure 8). The rate of biogas produced/gram COD in the stationary phase of gas production is not significantly different (p ≤ 0.05) for all reactors.

There was an increase of biogas produced as the mass of COD increased. The normalized, average rate of biogas production is plotted against time (Figure 8). An acceleration in biogas production rate took place for two weeks and then the rate transitioned to a more steady or stationary condition for two weeks. In the last week of operation, the rate of biogas production decreased, which implies that the decomposition of the most biodegradable fraction of the algae was complete, nutrients had become limiting and/or toxics had accumulated. The methane composition was determined one week before the end of the experiment. The results are summarized in Table 6. Reactor 1 showed only 70% methane content in biogas which may be due to dilution in the head space, which was over 350 mL. Multiway, repeated measures analysis of variance did not show a significant difference among treatments during the stationary phase which indicates that algal biomass contributed to the production of biogas in proportion to the COD amounts introduced to reactors. The amount of methane anticipated to be generated from acetate was obtained after two weeks of incubation.

Regardless, the biogas generation rates relatively steady irrespective of the amount of acetate or algae added to the reactors, reflecting the contribution of algal algal biomass to the production of biogas.
Figure 7—Average cumulative biogas produced over 16 days incubation (Error bars ± 1 - standard deviation)

Table 5—Average biogas produced over 16 days incubation

<table>
<thead>
<tr>
<th>Reactor No.</th>
<th>C/N ratio</th>
<th>Biogas (mL)</th>
<th>Methane on day 14 (%)</th>
<th>pH final</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1, 1.2§</td>
<td>5</td>
<td>567</td>
<td>62</td>
<td>7.3</td>
</tr>
<tr>
<td>2.1, 2.2</td>
<td>12</td>
<td>2,147</td>
<td>80</td>
<td>7.8</td>
</tr>
<tr>
<td>3.1, 3.2</td>
<td>15</td>
<td>2,208</td>
<td>85</td>
<td>7.9</td>
</tr>
<tr>
<td>4.1, 4.2</td>
<td>18</td>
<td>2,486</td>
<td>84</td>
<td>7.9</td>
</tr>
<tr>
<td>5.1, 5.2</td>
<td>21</td>
<td>2,858</td>
<td>82</td>
<td>8.0</td>
</tr>
<tr>
<td>6.1, 6.2†</td>
<td>none</td>
<td>499</td>
<td>49</td>
<td>7.4</td>
</tr>
<tr>
<td>7.1, 7.2</td>
<td>24</td>
<td>1,928</td>
<td>83</td>
<td>7.4</td>
</tr>
</tbody>
</table>

§ Algae without acetate

† Sediment and sludge inocula alone
Figure 8— The average, normalized rate of biogas produced per gram of COD (Error bars ± 1 standard deviation)

Table 6— The average amount of methane produced after 35 days incubation

<table>
<thead>
<tr>
<th>Reactor #</th>
<th>Total Biogas (mL)</th>
<th>Methane (%) on day 30</th>
<th>Total methane (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1, 1.2, 1.3</td>
<td>1,750</td>
<td>70</td>
<td>1,225</td>
</tr>
<tr>
<td>2.1, 2.2, 2.3</td>
<td>3,200</td>
<td>88</td>
<td>2,816</td>
</tr>
<tr>
<td>3.1, 3.2, 3.3</td>
<td>3,990</td>
<td>88</td>
<td>3,511</td>
</tr>
</tbody>
</table>

Conclusion

The experiments demonstrated that algal biomass could be digested in anaerobic reactor systems. The co-digestion of algal biomass with a supplemental carbon source material could improve the rate of decomposition and thus improve the biogas and methane production rate from algae. Increasing the C/N ratio of algal paste by blending
with a carbon source will be useful in avoiding the problem of ammonia accumulation in digesters and thus improves the digestibility of algal biomass. The optimum C/N ratio appears to be at least 21/1 based on dry weight. Gas production declined substantially when the C/N ratio was 24/1.

References


*Bioresour. Technol.*, **101** (11), 4042–4047.

CHAPTER 4

UP-FLOW ANAEROBIC SLUDGE BLANKET REACTOR CO-DIGESTION OF ALGAL BIOMASS AND ACETATE TO PRODUCE METHANE

Abstract

Anaerobic digestion of biomass is an energy generating process. In this work, algal biomass was used as a substrate for methane production in a co-digestion process with sodium acetate as a carbon source to adjust the low carbon to nitrogen (C/N) ratio of algal biomass from 5/1 to 21/1 using two duplicate, continuous flow, high-rate, up-flow anaerobic sludge blanket (UASB) reactor systems each of 34 L volume. Both reactors were seeded with equal amounts of anaerobic sediment. The reactors were incubated at a temperature of 35± 2 °C and were operated for 81 days. The feedstock was initially introduced at low organic loading rates of 0.9 g/L.d at a hydraulic retention time (HRT) of 7.2 day and then increased gradually, based on reactor performance, up to 5.4 g/L.d and a HRT of 5.5 days. These organic loading rates corresponded to an initial COD influent of 6.25 g/L that increased to 27.2 g/L with a methane production increase from 276 mL/g COD biodegraded to 349 mL/g COD biodegraded with a removal efficiency of 80% at steady state and a methane composition of 90%. By decreasing the HRT below 5.2 days, a slight decrease in COD removal efficiency, the biogas production rate and methane composition were observed. Total solids (TS), total suspended solids (TSS), and
total volatile suspended solids (VSS) showed high removal efficiencies at steady state and a slight decrease when HRT decreased below 5.2 days.

**Introduction**

Waste grown algae are a promising substrate for biofuel production (Salerno et al., 2009). Wastewater treatment lagoons and pond systems in the US and around the world could, potentially, be a source of algae biomass to be used for biogas production. The massive amounts of algal sludge, particularly in wastewater lagoons and ponds, represent a potential resource for bio-energy and recovery of fertilizers containing nitrogen and phosphorous (Mulbry et al., 2005).

All anaerobic algae digestion experiments reported to date, except the recent work by Tartakovsky et al. (2015), have used laboratory scale batch or semi-continuous reactors. These experiments provided useful information about algae digestibility, the positive effect of increasing the C/N ratio and determining the appropriate initial organic loading rate for continuous flow reactors. They did not provide information about hydraulic retention time, COD removal efficiency or effluent quality (Ehimien et al., 2011) because a mixture of untreated material together with the product is withdrawn. Tartakovsky et al. (2015) investigated methane production from algae digestion in laboratory scale UASB reactors without adjusting the C/N ratio but diluted the influent biomass concentration to avoid ammonia accumulation. Methane composition of the biogas reached 80% at a hydraulic retention time of 4 to 8 days.

A continuous flow high-rate system is expected to show better performance and higher biogas production rate per unit mass of organic compound degraded (mL CH₄/g
COD) and higher COD removal efficiency than batch reactors. This is due to the advantages of continuous flow high-rate systems like the UASB reactor. From the literature that has been reviewed, the UASB reactor has several advantages over other anaerobic batch reactor systems. High-rate reactors are characterized by their ability to accommodate very high OLR because they contain high concentrations of bacteria and provide relatively sufficient sludge-water contact (Rajeshwari et al., 2000). The biomass is generally present as biofilms and/or granular aggregates (Hulshoff Pol et al., 1983).

Among the above mentioned reactor systems (Chapter 1), the UASB reactor was reported to be the most efficient, especially as a pretreatment system. The UASB reactor is efficient in biogas generation if properly operated. The biomass from different sources and with different characteristics is believed to be one of the main sources for renewable energy production. However, some published information disagrees in that the improper management of resources of typical energetic crops could, in practice, lead to the increase of greenhouse emission to the atmosphere. Some studies pointed out that the cultivation of crops as a feedstock for biofuel product will result in lack of land intended for the cultivation of food crops and thus higher food prices (Johansson and Azar, 2007).

Research work published so far has been mainly focused on biodiesel production based on abundant lipid accumulated in algal biomass (Mandal and Mallick, 2009; Mata et al., 2010). Many researchers claim that anaerobic digestion of algae to produce methane is the most effective method for energetic exploitation of algal biomass (Dębowski et al., 2013). Anaerobic digestion is a key unit process that combines efficiency and potential environmental and economic benefits into the production of biofuels and represents an
environmentally friendly and feasible option for the production of a sustainable energy source (Ward et al., 2014).

The production of methane from algal biomass through anaerobic digestion as a primary method under controlled environmental conditions is more economically feasible compared to biodiesel production based on lipid extraction and anaerobic treatment of algal residues after extraction (Sialve et al., 2009). It also became evident that the production of biodiesel from algal biomass is not economically feasible due to the higher costs compared to fossil fuels (Harun et al., 2011).

Since anaerobic co-digestion of algal biomass has not been previously demonstrated using a high-rate, continuous flow reactor system, the objective of the present work was to assess the feasibility of implementing continuous flow, high-rate, Up-flow Anaerobic Sludge Blanket (UASB) reactor technology as a method in the co-digestion of algae grown in the Logan, Utah, wastewater treatment lagoons. Sodium acetate was blended as a supplemental carbon source material and substrate for aceticlastic methanogenesis to produce methane and to reduce the environmental impacts of algae discharged with treated wastewater.

**Methods**

Laboratory scale UASB reactors were designed and operated to evaluate the co-digestion of wastewater grown algae. An experimental plan was developed based on published design criteria and the results of the batch experiments described in Chapter 3. Initially, evaluation and refinement of the experimental methods was conducted in a preliminary experiment to assure that the apparatus would function appropriately and to
gain experience with the start-up phase for the reactors since there was considerable uncertainty about the behavior of the reactors during start-up in comparison to steady conditions.

Two, duplicate cylindrical, 34 L UASB reactors were designed and built from poly(methyl methacrylate (Plexiglass) (Figure 9) at the Utah Water Research Laboratory (UWRL). Each reactor was equipped with a three phase separator made from an inverted plastic funnel in the upper zone with a deflector beneath to reduce the up flow velocity and to help sludge coalesce back to the digestion zone. Sampling ports were made along the length of the reactor. The sampling ports were 8 mm in diameter and were closed with rubber stoppers and silicon sealant. A wastewater distributor was installed 5 cm above the reactor bottom to enable a uniform distribution of waste to the bottom of the reactor. A silicon rubber heating tape with adjustable thermostat control was wrapped in a spiral around the length of the reactor. Temperature was maintained at 35±2°C using a thermocouple temperature controller. Insulation covered the outside of the reactor. A masterflex peristaltic pump with a double channel head was used to feed both reactors. Saint-Gobain Masterflex 06508-16 PharMed tubing was used in the feed pumps with a potential flowrate range of 1.4 - 133 L/day.

In the preliminary experiment, the inner diameter of the gas tube connected to the three phase separator was 13 mm reduced to 10 mm and then to 3mm that was, in turn, connected to a gas washing bottle. The 500 mL gas washing bottle, made of glass, was immersed in ice water to facilitate condensation of water from the gas stream so that water would not condense in the gas flow meters. The biogas was measured using Cole Parmer 32707-08 digital mass flow meters with a working range of 0 to 500
sccm/minute. The flow meters were calibrated using a mixture of 80% methane and 20% carbon dioxide. The millivolt output from the flow meters was stored on a Campbell Scientific data logger type CR800 model and the biogas flowrate was then estimated using the linear equation obtained from the calibration process. The biogas samples were collected in 500 mL Tedlar gas bags every 5-6 days and the methane composition was measured using a gas chromatograph (GC) with a thermal conductivity detector (TCD) and a packed column (Alltec, CTR1) 1.83 m x 6.35 mm. The sample was introduced to the column using a Valco six port valve with a 500 µL sample loop.

Each reactor was seeded with 11 L of anaerobic sediment, obtained from the third phase of the Logan, Utah, wastewater treatment lagoons, containing 28 g/L volatile suspended solids (VSS) so that each reactor received 9.7 g VSS/L reactor volume. The feedstock was then prepared with algal biomass as the main substrate and sodium acetate as a co-digestate, carbon source material. The pH of the feedstock was adjusted to 6.8-7.0 by adding phosphoric acid.

Sodium acetate was chosen as a supplemental carbon source because it is readily available to acetotrophic methanogens and other acetotrophs. Using acetate simplified the digestion system by bypassing the processes of hydrolysis of polymeric materials (e.g. waste paper), acidogenesis and acetogenesis (Bitton, 2011) for the supplementary carbon source. These processes were anticipated to be active in the digesters as algae was decomposed. Sala and Güde (2004) found that the successional decomposition of algae detritus in aerated microcosms began with the hydrolysis of disaccharides, oligosaccharides and starch followed by hydrolysis within the much larger pool of structural polysaccharides. A somewhat similar succession might be anticipated under the
anaerobic conditions of the UASB with most of the products of enzymatic hydrolysis feeding into fermentation including acetogenesis. The nitrogen source for the reactors was provided from algal biomass through deamination of algal proteins and the decomposition of nucleic acids, etc.

Algal biomass was obtained by continuous centrifugation of Logan, Utah, wastewater from the fifth stage of the treatment lagoons and was characterized for its COD, total solids (TS), VSS, total N, total P, carbon to nitrogen (C/N) ratio and metal content. The algal biomass used to feed the reactors was harvested every two weeks, stored in a refrigerator at 4 °C and the COD of stored algae was measured every week.

![The layout of the UASB laboratory scale experimental system](image)

**Figure 9**—The layout of the UASB laboratory scale experimental system
The feed stock was initially introduced to UASB reactors at a relatively low organic loading rate. This was done to protect the successional processes and increase the desired enzymatic capacities of the microbial community under the reactor’s environmental conditions. Relatively low feeding rates during the start-up period avoids overloading that might result in the failure of the digestion process. Overloading occurs when organic loading rates cause the fermentative, acidogenic bacteria to produce VFA at a rate that exceeds the capacity of the slower growing aceticlastic methanogens to metabolize acetate to methane and of the use of VFA as carbon and energy sources by other microorganisms. This results in VFA accumulation, a drop in pH and a condition called “acidification” or “souring” of the reactor contents where acidogenic microbial populations prevail and methanogenic population activity is inhibited. Acidogenic populations are less sensitive to low or high pH values and hence acid formation will prevail over methanogenic respiration, and the start-up of the desired process fails.

The organic load was increased gradually based on the reactors’ performance in COD removal efficiency either by increasing the COD concentration of the influent or by increasing the flow rate (reducing the hydraulic retention time). This increase was done whenever the removal efficiency in COD was over 60%. The algae provided 33% -50% of the COD of the feed stock. During the course of the experiment, the pH of the effluent was monitored 2-3 times/day. Influent flow rate (L/d), rate of biogas production (L/d); COD of the influent and effluent and the COD concentration of the algae were measured periodically. Methane composition was also measured every 5-7 days. OLR was calculated using Equation 5:
$$OLR = \frac{Q \times COD_{in}}{V}$$  \hspace{1cm} \text{Equation 5}

Where $V$ is the reactor volume (L), and $Q$ is the influent flow rate (L/d). The COD removal efficiency ($\%$ Eff) was calculated by the Equation 6

$$\%Eff = \left(\frac{COD_{in} - COD_{out}}{COD_{in}}\right) \times 100\%$$  \hspace{1cm} \text{Equation 6}

In the preliminary exercise, reactors were operated for 21 days. In the first two weeks, the reactors showed a significant difference in methane composition and biogas production rate but the difference began to decrease in the third week. Frequent clogging of the gas tubing was occurring and leakage from sampling ports occurred. To allow these problems to be remedied, operation of the reactors was stopped.

Sludge was removed from the reactors and they were cleaned. All the tubing was removed and cleaned. The three-phase separator tube inside the reactor was replaced by 13 mm plexiglass tubing to minimize the transfer of solids from the three-phase separator into the biogas pathway. Biogas tubing connecting the three phase separator to the gas washing bottles, which was about 5 cm inside diameter, was replaced with polyvinyl tubing of 8 mm inside diameter to minimize clogging. The glass gas washing bottles were replaced by 2 L polyethylene bottles. The cooled biogas then passed through 3.175 mm OD flexible plastic tubing into the gas flow meters.

All sampling port stoppers, which were initially Fisher brand turnover septum stoppers size 00, were replaced with 6.35 mm OD, threaded, aluminum tubing and closed on the external end with 6 mm inside diameter, flexible plastic tubing closed with pinch clamps.
Table 7— The initial conditions of the UASB reactor

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD in (g/L)</td>
<td>6.25</td>
</tr>
<tr>
<td>Flowrate (L/d)</td>
<td>4.5</td>
</tr>
<tr>
<td>OLR (g COD/L·d)</td>
<td>0.90</td>
</tr>
<tr>
<td>Reactor working volume (L)</td>
<td>32.4</td>
</tr>
<tr>
<td>HRT (Volume/Flow Rate, days)</td>
<td>7.2</td>
</tr>
</tbody>
</table>

The reactors were then seeded with fresh anaerobic sediment that had the same characteristics from the same wastewater lagoon. They were operated as in the preliminary start-up except that during steady state, the total solids (TS), SS, and VSS of the influent and effluent were measured thus allowing calculation of their removal efficiencies. In this experiment, the reactors were operated for 81 days. The experimental design for the initial conditions of the final experiment is shown in Table 7.

Results and Discussion

From batch reactor experiments, it was concluded that the continuous flow, UASB reactors should be seeded with only a sediment obtained from the lagoons where the algal biomass was produced. Once the sediment was introduced to the digester, a secondary successional process began. It was anticipated that many of the organisms capable of decomposing the kinds of algae that grow in the lagoons would grow, maintain their populations and become part of the new community in the digester. Their presence should shorten the time needed to start-up the digester and assure that algae are decomposed at a higher rate than they would be without this source of capable organisms.
During the start-up of the UASB reactors, the feedstock composed of algae and sodium acetate, with a C/N ratio of 21/1, was introduced at a low organic loading rate initially to keep VFA and ammonia, which are inhibitory to methanogenic archaea, at low concentrations. Rapid accumulation of VFA can cause a shock decrease in pH or acidification of the reactor contents. This also helps to avoid the accumulation of ammonia from deamination of proteins, which becomes toxic to methanogenic archaea, acts as a base and leads to an increase in pH value which can also affect methanogenic activity. Starting with low OLR improves the successional processes especially during the start-up operations of the UASB reactors.

After 25 days, COD removal increased to greater than 60% and the OLR was then gradually increased from 0.9 g COD/L reactor volume/d to about 5.4 g/L·d on day 81. These organic loading rates corresponded to an initial influent COD of 6.25 g/L that increased to 27.2 g/L. The increase of organic loading rates as a function of time is shown in (Figure 10). This increase in OLR or, alternatively, an increase in COD concentration of the influent was accompanied by an increase in the removal efficiency of COD to about 80% on day 75 of the experiment. The average COD removal efficiency is shown in (Figure 11).

The biogas generated from the duplicate UASB reactors also had an increasing trend indicating the decomposition and conversion of organic compounds to biogas. This acceleration in biogas production rate as a function of time can be seen in (Figure 12). The rate began to decrease when the HRT was decreased below 5.2 days on days 76-81 of operation. The composition of methane in biogas increased as the organic loading rate increased (Figure 13), and was about 90% during steady state. During steady state, day
40 to day 75, the amount of methane produced/gram of COD biodegraded was in the range of 276-349 mL. When hydraulic retention time was decreased below 5.2 days the amount of methane produced per gram of COD biodegraded decreased to 246 mL on day 81 of the experiment.

After start-up, the UASB reactors digesting algae performed very well at a HRT of less than 7.2 days. The shortest HRT at which steady state was not affected was 5.5 days. At lower HRT, after the 75th day of operation, all monitoring parameters showed a slight decrease. Lowering the HRT down to about 5.5 days was very successful whereas at a HRT of 5.0 days, there was a slight decrease in pH to ~8.2, methane composition decreased to 85%, and COD removal efficiency decreased to 74%. The pattern of the average HRT is shown in (Figure 14).

The pH is a very important parameter in anaerobic digestion. The pH of the reactors’ effluents was monitored 2 or 3 times daily. The pH increased up to 8 during the first 40 days of the experiment. This was probably due to the increase in decomposition rate of fatty acids during the acclimatization phase. Afterwards, the pH showed an increase to above 8 indicating that anaerobic digestion, including the fermentation of fatty acids, was balanced with the rate of aceticlastic methanogenesis. From day 57 to day 75, the pH was relatively stable at ~ 8.4 with no change in relation to the increase in OLR. Buffering was complex in these anaerobic systems (Franco et al., 2007) and this increase in pH may have been due to, among other factors, the interaction of bicarbonate alkalinity with ammonium from the mineralization of protein and other nitrogenous cellular components, the decomposition of VFAs, the release of carbon dioxide from solution, and production of hydroxide from the reaction of sodium from sodium acetate
Figure 10—The average organic loading rates over time (Error bars ± 1 standard deviation (SD)).

Figure 11—The average COD removal efficiencies (%) (Error bars ± 1 SD)
Figure 12—The average biogas production rate (Error bars ± 1 SD)

Figure 13—The average methane composition in a function of time (Error bars ± 1 SD)
with water. The sodium may have reacted with VFAs to produce esters which act as a base. At these pH values, there was no decline in COD removal or the methane composition of the biogas. When the HRT was decreased below 5.2 days, there was a slight decrease in pH (Figure 15) suggesting that the accumulation of fatty acids had begun.

TS, TSS and VSS showed relatively stable removal efficiencies during steady state, where the COD removal efficiency remained relatively constant. At HRT below 5.5 days, the COD, TS, TSS, and VSS removal efficiencies decreased slightly, indicating that either wash out of sludge or overloading started to take place. The pattern of the removal efficiencies of TS, TSS, and VSS are shown in (Figures 16 to 18), respectively. This means that the continuous flow, high-rate UASB reactor was efficient not only in digesting soluble COD, but it was also capable of removing particulate matter like TS, TSS, and VSS by converting them to biogas.

**Operational Recommendations**

The anaerobic co-digestion of algae and sodium acetate as a carbon source material using UASB reactors is a technically viable option for methane production where the results showed that about 276-349 mL CH\(_4\)/g COD biodegraded was produced during the course of the experiment. COD was removed at an efficiency of about 80% while biogas methane composition was about 90% during steady state.

The experiments carried out on the laboratory scale UASB reactors involved investigating HRT, OLR, and C/N ratio that are of importance for scaling up the digestion method. From the results obtained from anaerobic co-digestion of algae using
Figure 14— The average hydraulic retention time (Error bars ± 1 SD)

Figure 15— The average pH of the effluent (Error bars ± 1SD)
Figure 16—The removal efficiency of TS (Error bars ± 1SD)

Figure 17—The removal efficiency of TSS (Error bars ± 1SD)
UASB reactor systems, and relevant literature, the following recommendation can be made:

- The most important stage in the operation is the start-up stage where succession of the microbial community to be able to digest the feedstock at a steady and relatively rapid rate is occurring. Digester operation has to be performed delicately during start-up by gradually increasing the organic loading rate to avoid overloading (Franco et al., 2007).
- Start-up took 23 days vs. several weeks to months reported with the use of other inocula. The use of wastewater lagoon sediment might have shortened this period since it was taken from the same lagoons where large amounts of algal biomass decomposition occur inherently.
• Excess biosolids should be removed periodically especially during steady state operations based on 10% conversion of the COD being degraded to biomass (Marchaim, 1992).

• The UASB is capable of decomposing waste grown algae where the removal efficiency of TSS exceeded 85% and VSS removal efficiency of about 90 % was achieved.

• The optimum HRT was 5.2 days below which, i.e. 5.0 days, all monitored parameters showed a slight decrease indicating wash out of biomass had taken place or overloading had begun.

References


CHAPTER 5

SUMMARY, OVERALL CONCLUSIONS, RECOMMENDATIONS
AND ENGINEERING SIGNIFICANCE

Summary

Rising conventional energy prices and environmental protection concerns have brought high interest to the production of bioenergy to offset the need for fossil fuels and to reduce environmental impacts. One of the main and attractive technologies for renewable energy production is anaerobic digestion of various types of organic wastes to produce methane. The success of anaerobic digestion in the last few decades is attributed to the introduction of high-rate reactor systems of which the up-flow anaerobic sludge blanket (UASB) reactor has been frequently and successfully demonstrated. Production of methane in biogas in anaerobic digesters is an attractive method for fuel production from renewable energy sources. Many kinds of microorganisms including bacteria, archaea, fungi and some protozoans participate in anaerobic digestion. Acidogenic, acetogenic and methanogenic microorganisms, which differ in their metabolic reactions, can be recognized in the anaerobic decomposition of particulate organic matter to methane and carbon dioxide.

The performance of anaerobic digestion depends strongly on environmental conditions and the characteristics of the material being digested. Several environmental factors such as temperature, nutrients, pH, C/N ratio, and toxic compounds, either
enhance or inhibit anaerobic digestion, because of their effect on microbial metabolism and growth rates.

There is a wide range of energy crops, animal waste, industrial and agro-industrial wastes and biomass that represent viable feedstocks for methane production as a renewable energy source via anaerobic digestion. Among these, waste grown algae are continuously produced in nutrient rich lagoons and ponds and are potential substrates for biogas production. However, the low C/N ratio of algae is not conducive to anaerobic digestion with economically attractive methane production rates because anaerobic digestion of algal biomass alone will lead to the formation of excessive ammonia, which as a toxic dissolved gas, inhibits methanogenic activity.

A continuous flow, high-rate UASB reactor system is expected to show desirable performance such as a high biogas production rate per unit mass of organic compound degraded (mL CH$_4$/g COD biodegraded), high methane composition, improved COD removal efficiency and high removal of other pollutants. This is due to their sludge retention that leads to a high concentration of suspended biomass in the reactor providing sludge-algae contact and adsorption area facilitating algal biomass decomposition as its up-flow stream makes the sludge bed expand. The low hydraulic retention time (HRT) results in relatively low reactor volume, and continuous flow removes soluble metabolic products and toxics that may inhibit microbial activity. Technology to capture this source of energy is being developed and demonstration of the potential for success is needed.

The principle objective of the work reported here was to provide proof of concept and assess the feasibility for implementing UASB reactor technology as a method for the co-digestion of wastewater grown algae in Logan Utah, lagoons with sodium acetate as a
readily available carbon source to produce methane. It is anticipated that the principles developed and demonstrated will be applicable to other sources of waste grown algae worldwide.

To achieve this objective it was necessary to find a favorable C/N ratio and determine the effects of varying the organic loading rate on the biogas production rate, the methane content of the biogas, and the biodegradability of algal biomass. A method to increase the C/N ratio of the feedstock is to add a supplemental carbon source. Acetate was chosen for this because it is readily available for methanogenesis by aceticlastic methanogens and as a carbon and energy source for many other anaerobic microorganisms. From batch reactor experiments, it was found that by increasing the C/N ratio by blending the algal biomass with sodium acetate there was an increase in methane production rate as the C/N ratio increased. The highest rate of biogas production was observed when the C/N was 21/1 and gas production declined substantially when the C/N ratio was 24/1. Near the end of the experiment, the biogas methane content was 82% from the 21/1 treatment while algae alone produced 62%.

Based on the results obtained from batch reactor experiments, anaerobic digestion of waste grown algae with acetate was used for methane production in a co-digestion process with a carbon to nitrogen (C/N) ratio of 21/1 using two duplicate, continuous flow, high-rate, 34 L UASB reactors. Both reactors were seeded with equal amounts of anaerobic sediment from the Logan wastewater lagoons. The reactors were incubated for 81 days at a temperature of 35 ± 2 °C. The feedstock was initially introduced at low organic loading rates of 0.9 g/L·d at a HRT of 7.2 days and then increased gradually, based on reactor performance, up to 5.4 g/L·d and a HRT of 5.5 days. These organic
loading rates corresponded to an initial COD influent of 6.25 g/L that increased to 27.2 g/L while methane production increased from 276 mL/g to 349 mL/g COD biodegraded with a removal efficiency of 80% at steady state and a methane composition of about 90% was obtained. By decreasing the HRT below 5.2 days, there was a slight decrease in COD removal efficiency, biogas production rate and methane composition. Total solids (TS), total suspended solids (TSS), and volatile suspended solids (VSS) showed high removal efficiencies at steady state but there was a slight decrease when the HRT decreased below 5.2 days.

**Overall conclusions**

Waste grown algae is a potentially important substrate for methane production via anaerobic digestion technology. However, the low C/N ratio of algal biomass may lead to ammonia accumulation that can inhibit digester performance, including decreasing the rate of methane production, below an economically feasible level.

Adjusting the C/N ratio of 5/1 by weight of algal biomass by blending with a supplemental carbon source was found to be effective in increasing the biogas production rate and its methane composition.

Using batch reactor experiments were very effective in evaluating the effect of optimizing the C/N on methane production rate from waste grown algae by blending algal biomass with sodium acetate. From these experiments, the C/N ratio with the highest methane production rate was about 21/1 and the volume of methane produced per gram of total COD was three times higher than the per gram of algae COD digested without sodium acetate.
The use of laboratory scale UASB reactors fed with a feedstock of algal biomass and sodium acetate with a C/N ratio of 21/1 were, technically, a viable option for anaerobic co-digestion of algal biomass where COD removal efficiency was about 80% and 90% methane composition. During steady state, TS, SS, and VSS removal efficiencies were about 83, 85 and 90% respectively at HRT of 5.0-5.5 days and an organic loading rates of 5.1-5.4 g/L·d with a corresponding COD of 27.2 g/L in the feed stock. From what has been learned from this experiment, the UASB reactor technology can be used to digest other types of feedstocks that have low or near optimum C/N ratios.

**Recommendations**

Since the biogas production rate and methane content increased by increasing the C/N ratio of algal biomass via anaerobic co-digestion, it is recommended to use a waste of high C/N ratio as a supplemental carbon source to be blended with algae to increase the C/N ratio to approximately 21/1. Waste paper, for example, could be economically feasible and provides a very high C/N ratio, but the rate of biogas production may be limited by the rate of paper cellulose depolymerization.

Logan lagoon sediment, used as an initial inoculum to the algal fed UASB reactor, can be used as a seed to enhance methane production from substrates of a similar composition with algal biomass. It is important to identify what microbial species contribute to the high activity of the Logan lagoon sediments and its specific potential towards algal biomass decomposition.
Engineering significance

Rising fossil fuel prices and environmental concerns have increased interest in renewable energy, and there has been experimentation with using a wide range of energy crops, animal wastes and other biomass that can be used to produce renewable energy via different technologies. Algal biomass represents a potential source of biofuel in the form of biodiesel and other liquid fuels or methane in biogas. The production of methane from algal biomass via anaerobic digestion may be more economically feasible than biodiesel production because the latter needs integrated treatment of extracted lipids and the treatment of algal residues after extraction.

In the work described here, the use of Up-flow Anaerobic Sludge Blanket (UASB) reactors in co-digestion of algae and acetate as a supplementary carbon source was found to be a technically viable option since 90% methane in biogas was produced and about 80% COD removal efficiency was achieved at a HRT of about 5.2 days.

The production of methane via anaerobic digestion of waste grown algae would benefit society by providing a clean energy source from a renewable resource, offset the need for fossil fuel, help reduce greenhouse gas emissions and reduce the amount of residual waste to be handled. Waste handling has high capital costs.

The stabilized sludge produced in UASB reactors and the treated effluent can be characterized and reused in agriculture on selected crops. Also, the anaerobic sludge from these reactors can be used to seed other reactors to treat either the same waste or other types of wastes. This enhances treatability and digestibility of the waste and shortens the start-up time of reactors due to their high concentration of biomass.
The research also provided very important information about the organic loading rate, the C/N ratio of the feed stock, and hydraulic retention time that will help design engineers scale up the UASB reactor to industrial or municipal applications. The start-up operation of these types of reactors is also important and should be performed, initially, at low organic loading rates i.e. ~ 1 g COD/L·d to prevent overloading and then increased gradually based on reactor performance. Increasing the loading rate when COD removal efficiency exceeded 60% was successful in the experiment reported here.

The research also provided essential information about the importance of adjusting the C/N ratio in anaerobic digestion of low C/N ratio material, like microbiological biomass, to increase the biogas production rate. Increasing the C/N ratio by the addition of carbon source material could prevent ammonia accumulation and its toxic effects on microorganisms.

The work will also benefit subsequent investigations of the microbiological aspects of anaerobic digestion of algal biomass. This work is the first to evaluate the feasibility of using UASB reactors for treating waste grown algal biomass with acetate as a supplemental carbon source. The optimum C/N ratio was found to be about 21/1 from batch reactor experiments. The biodegradability of algae, which has a low C/N ratio of 5/1 by weight, was enhanced and a higher methane production rate resulted when the C/N ratio of the reactor feed stock was increased to 21/1.

The UASB reactor develops a complex network of trophic relationships among interacting microbial populations that should be further evaluated in detail. Examination of the microbial community in the UASB reactor has the potential to reveal highly efficient microbial strains and their interactions, contributing to high biogas yields. A
study of this microbial ecosystem may reveal microbial ecological principles that can be applied to improving the performance of anaerobic digestion of algae in general.
Table A 1 — Total solids, water content, volatile suspended solids, and Ash concentration of waste grown algae

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (TS)</td>
<td>80 g/L</td>
</tr>
<tr>
<td>Water content</td>
<td>92%</td>
</tr>
<tr>
<td>Volatile Suspended Solids (VSS)</td>
<td>73% /SS</td>
</tr>
<tr>
<td>Ash</td>
<td>27% /SS</td>
</tr>
</tbody>
</table>

Table A 2— The carbon and nitrogen concentration based on dry weight of waste grown algae

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>TC (%)</th>
<th>IC (%)</th>
<th>OC (%)</th>
<th>TN (%)</th>
<th>OC/TN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>33.1 ± 0.9</td>
<td>2.1 ± 0.09</td>
<td>31.0 ± 0.9</td>
<td>6.4 ± 0.2</td>
<td>4.8</td>
</tr>
<tr>
<td>S2</td>
<td>31.6 ± 0.8</td>
<td>1.8 ± 0.01</td>
<td>29.8 ± 0.8</td>
<td>6.3 ± 0.2</td>
<td>4.7</td>
</tr>
<tr>
<td>S3</td>
<td>27.6 ± 2.6</td>
<td>2.4 ± 0.19</td>
<td>25.3 ± 2.8</td>
<td>5.5 ± 0.5</td>
<td>4.6</td>
</tr>
</tbody>
</table>

T = Total carbon
IC = Inorganic carbon
OC = Organic carbon
TN = Total nitrogen
TC/TN =5
**Table A 3— Metal contents of waste grown algae**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cu mg/kg</th>
<th>Fe mg/kg</th>
<th>Mn mg/kg</th>
<th>Mo mg/kg</th>
<th>Al mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algal biomass 1</td>
<td>55.0</td>
<td>4,037</td>
<td>228</td>
<td>&lt;</td>
<td>3,196</td>
</tr>
<tr>
<td>Algal biomass 2</td>
<td>57.6</td>
<td>4,142</td>
<td>236</td>
<td>&lt;</td>
<td>3,383</td>
</tr>
<tr>
<td>(Detection Limit)</td>
<td>0.4</td>
<td>0.15</td>
<td>0.05</td>
<td>7.5</td>
<td>6</td>
</tr>
</tbody>
</table>

**Table A3— continued**

<table>
<thead>
<tr>
<th>Sample</th>
<th>As mg/kg</th>
<th>B mg/kg</th>
<th>Ba mg/kg</th>
<th>Cd mg/kg</th>
<th>Co mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algal biomass 1</td>
<td>&lt;</td>
<td>6.15</td>
<td>55.0</td>
<td>0.30</td>
<td>1.48</td>
</tr>
<tr>
<td>Algal biomass 2</td>
<td>&lt;</td>
<td>6.30</td>
<td>57.5</td>
<td>0.05</td>
<td>0.25</td>
</tr>
<tr>
<td>(Detection Limit)</td>
<td>0.5</td>
<td>1</td>
<td>0.05</td>
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</tbody>
</table>

**Table A3— continued**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cr mg/kg</th>
<th>Na mg/kg</th>
<th>Ni mg/kg</th>
<th>Pb mg/kg</th>
<th>Se mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algal biomass 1</td>
<td>8.92</td>
<td>1,720</td>
<td>8.56</td>
<td>6.60</td>
<td>&lt;</td>
</tr>
<tr>
<td>Algal biomass 2</td>
<td>9.14</td>
<td>1,928</td>
<td>10.0</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>(Detection Limit)</td>
<td>0.3</td>
<td>4</td>
<td>0.15</td>
<td>1.5</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table A3— continued**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Si mg/kg</th>
<th>Sr mg/kg</th>
<th>Zn mg/kg</th>
<th>K %</th>
<th>Mg %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algal biomass 1</td>
<td>5,705</td>
<td>117</td>
<td>77.2</td>
<td>1.01</td>
<td>0.74</td>
</tr>
<tr>
<td>Algal biomass 2</td>
<td>6,055</td>
<td>120</td>
<td>76.4</td>
<td>0.76</td>
<td>0.78</td>
</tr>
<tr>
<td>(Detection Limit)</td>
<td>4.5</td>
<td>1.5</td>
<td>0.25</td>
<td>0.0023</td>
<td>0.000035</td>
</tr>
<tr>
<td>Sample</td>
<td>Ca %</td>
<td>P %</td>
<td>S %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algal biomass 1</td>
<td>4.08</td>
<td>0.75</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algal biomass 2</td>
<td>4.17</td>
<td>0.81</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Detection Limit)</td>
<td>0.0004</td>
<td>0.0004</td>
<td>0.00035</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table A3—continued
CURRICULUM VITAE

Yousef M. Soboh

CAREER OBJECTIVE
To work at a position aimed at fostering development without affecting environmental quality and public health whose requirements include leadership experience, objective oriented management, good interpersonal skills, and good written and spoken English. Areas of interest: waste water treatment with emphasis to anaerobic treatment, environmental science and environmental impact assessment, teaching, environmental and public health awareness.

EDUCATION
PhD in Biological Engineering, Utah State University, USA (08/14/2015). Dissertation Title: Anaerobic Co-Digestion of Algal Biomass and a Supplemental Carbon Source Material to Produce Methane
MSc in Environmental science and Technology, An-Najah National University, Palestine in June-2000. Theses Title: Anaerobic Treatment of Olive Mills Wastewater Treatment using UASB Reactor.
BSc in Chemical Engineering, Kosovo University, Serbia in February 1986. Thesis Title: Desiccation of Lignite of Kosovo using Fleisner Method.
EXPERIENCE

Research Assistant, Utah State University, Utah Water Research Laboratory.
Algal biomass research: collecting algal biomass samples analysis for Chemical oxygen demand, total suspended solids, Volatile suspended solids, water content, ash content, carbon to nitrogen ratio, and metal contents.
Anaerobic co-digestion of algal biomass using batch reactor systems
Anaerobic co-digestion of algal biomass using UASB reactor.

*(June 2000-September 2010).
Consultant for public environmental awareness and education. Hebron Rehabilitation Committee, Hebron, Palestine.
Participated in the design and implementation of the primary treatment plant of wastewater generated from Palestine Technical Colleges.
Lecturer and Headperson of technology education department at Palestine Technical Colleges, Arroub, Palestine.

Environmental awareness & Education Project Coordinator for Palestinian Hydrological Group (PHG) in cooperation with GVC, Italy. The project was funded by ECHO. Coordinator: Rain Water Harvesting Systems (PHG), a project funded by ICRC.
Part time Lecturer and Instructor at Al-Quds Open University Part time instructor at Palestinian Technical Colleges, Arroub, Palestine
Project Manager: Ecological sanitation with emphasis to dry sanitation- Pilot project (PHG). The project was funded by Sida-
Sweden.

Consultant at Hebron University: Reducing the impacts of Olive mills wastes. The project funded by USAID, Hebron-Palestine.


Researcher: Olive mills wastewater treatment at Hebron University, Hebron-Palestine.

Researcher: water and environment, department of planning and development, University, Graduates Union- Hebron, Palestine.

Instructor of Ceramics and Glass technology, Glass and Ceramics Engineering, Palestine Polytechnic University, University Graduates Union, Hebron, Palestine.

ACTIVITIES

- Voluntary work and lecturing of environmental awareness and education for Public Institutions.
- Voluntary work of increasing environmental awareness among students at schools.
- Field work of designing ecological sanitation systems and composting on the household levels.
- Advised NGOs and local community on proper methods for water, wastewater and solid waste management.
- Advised on water disinfection and safety measures.

PUBLICATIONS


AWARDS

- Local AWRA Student Paper Competition Doctorate award: Received an award and my research paper were selected to represent USU in the doctorate division at the American Water Resources Association Student Paper Competition (2013).USA

SKILLS

Great English communication Skills. Proposal writing, technical report writing, protection of bio-diversity, Needs assessment for rural communities in Palestine with emphasis on environmental and public health needs.