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Effect of Cell Wall Destruction on Anaerobic Digestion of Algal Biomass

Jessica R. Simpson
jrsimpso@uno.edu

Effect of Cell Wall Destruction on Anaerobic Digestion of Algal Biomass—Preliminary Results

A Thesis

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Jessica R. Simpson

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Dedication

To my father,

Albert Joseph Simpson,

who has spiritually guided

me on my journey.

To my mother and step-father,

Vicki and Melvin Lowdermilk,

for their endless support, encouragement,

patience and unconditional love,

all of which helped me obtain my goal.

To my family,

for their love and support they have

given me throughout my life.

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List of Symbols and Abbreviations

WSP: Waste Stabilization Pond
EC: Electrochemical
HRAP: High-rate algal pond
CO_2: Carbon dioxide
BOD: Biological oxygen demand
CH_4: Methan
WWTP: Wastewater treatment plant
N: Nitrogen
P: Phosphorus
DC: Direct current
TSS: Total suspended solids
TS: Total solids
DO: Dissolved oxygen
UV: Ultra-violet
AIWPS: Adavanced Integrated Wastewater Pond Systems
LCFA: Long chain fatty acids
H_2O: Water
VFA: volatile fatty acids
$CaCO_3$: Calcium Carbonate
$NaHCO_3$: Sodium Hydroxide
g: grams
mg: milligrams
L: Liter
Ha: hectare
kg: kilogram

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Abstract

Research was conducted using algal biomass obtained from the surface of a secondary clarifier at Bridge City Wastewater Treatment Plant and subsequently sent through an electrochemical (EC) batch reactor at various concentrations. The first objective was to achieve maximum cell wall destruction electrochemically using the EC batch reactor and determine the optimal detention time and voltage/current relationship at which this occurred. The second objective was to subject two algal mediums to anaerobic digestion: the algal medium without electrochemical disinfection and the algal medium after disinfection. Every three days, for 12 days, total solids were measured from each apparatus to determine if cell destruction increased, decreased or did not change the consumption rate of algae by anaerobic bacteria. The consumption rate of algae is directly proportional to the production of methane, which can be used as a source of biofuel.

Keywords: algal biomass, algae, electrochemical batch reactor, electrochemical disinfection, cell wall destruction, anaerobic digestion, sludge, total solids, consumption rate, methane, biofuel, bacteria, wastewater, voltage, current, acidophilic, methanogenic

1. Introduction

1.1 Background

The use of algae in wastewater treatment systems has proven to be very useful in the removal of heavy metals along with inorganic nitrogen and phosphorus to provide a better effluent water quality (Oron, Shelef, Levi, Meydan, and Azov, 1979; Hwang, Church, Seung-Jin, Jungsu and Lee, 2016). Not only are algae very successful in removing excess nutrients in wastewater, but systems that employ this method of treatment benefit from its economical and ecological characteristics. Aerobic and facultative oxidation ponds are two types of waste stabilization ponds (WSPs) that treat municipal wastewater through the symbiotic relationship of algae and bacteria along with adequate availability of carbon dioxide (CO_2) and sunlight. High rate algal ponds (HRAPs) are also another type of WSP that treat wastewater in this manner. Other types of WSPs include anaerobic and maturation ponds, each of which play a specific role in the treatment process. When placed in a systematic arrangement, WSPs in series achieve a high degree of municipal wastewater treatment (Swiss Federal Institute of Aquatic Science and Technology and Spuhler, n.d). The final effluent from WSPs can be discharged into receiving streams or used for agriculture or aquaculture purposes.

One major goal of wastewater treatment is the removal of biodegradable organic matter, measured by the biological oxygen demand (BOD). Organic matter is abundant in wastewater and removal can be achieved by aerobic or anaerobic digestion. Both of these processes decompose organic matter and transform it into a gaseous mixture. Aerobic digestion primarily produces CO_2, while anaerobic digestion produces biogas, a combination usually consisting of 65% methane (CH_4), 35% CO_2, and trace amounts of various gases (Tchobanoglous *et al.*, 2014). Aerobic and anaerobic digestion occur in WSPs or specific structures built for such operations in

conventional wastewater treatment plants (WWTPs). Anaerobic digesters in conventional WWTPs and, less often, WSPs are designed to capture the biogas produced and further use it as a source of fuel to power operations within the WWTP (Swiss Federal Institute of Aquatic Science and Technology and Spuhler, n.d). This deems wastewater as a "renewable recoverable source of energy" (Tchobanoglous *et al.,* 2014).

As with any living matter, the protoplasm of algae consists mostly of protein, carbohydrates, lipids and nucleic acids, all of which are considered biodegradable organic matter. This characteristic of algae is especially appealing when considering co-digestion, a process that refers to the digestion of multiple substrates under anaerobic conditions. Co-digestion is an excellent way to facilitate anaerobic digestion as it has many financial, environmental, and practical benefits (Tchobanoglous *et al.,* 2014). Based on their chemical composition, algae are an excellent source of food for bacteria; however, the cell walls of algae can be extremely resilient and difficult to degrade, making it harder for bacteria to decompose and, subsequently, digest. There are various mechanical and non-mechanical methods to breakdown the cell wall of algae. Unfortunately, most of these methods are not feasible on a large scale. For instance, pyrolysis would successfully break the cell wall, but the energy required to carry out this procedure far exceeds the energy produced. Developing methods to lyse the cells of algae using electric fields are becoming more prevalent as it is an efficient approach to extract the protoplasm. Once the protoplasm is released, the biomass will settle while the lipids float to the surface (Board on Agriculture and Natural Resources, 2012).

Electrochemical disinfection is a technique that may be used as a form of wastewater treatment that works to remove pathogens by introducing an electric current to the system. This is accomplished supplying electrodes, at least one cathode and one anode, with a direct current

(DC), which results in the electrolysis of water (Kraft, 2008). Using electrochemical disinfection as a multifunctional process to treat wastewater while simultaneously lysing algal cell walls would be a resourceful way to collect biomass to further use for co-digestion. Treating wastewater containing algae using electrochemical disinfection is not only an effective way to prevent algae from being in the final effluent of a WSP, but the final product may ease the digestion of algae and, consequently, produce a higher yield of biogas during anaerobic digestion.

2. Purpose

The aim of this research was to determine the optimum operating conditions in which algal cell wall destruction occurs by utilizing the wastewater treatment technique of electrochemical disinfection. This was carried out using an electrochemical batch reactor and algae obtained from the surface of the secondary clarifier at the Bridge City Wastewater Treatment Plant. The potential for algae to be used as source of organic matter for bacteria presents opportunities for biogas production through anaerobic digestion. However, it has been demonstrated that algal cell walls are obstacle for bacterial degradation of the biodegradable protoplasm of algae. The dual function of algal cell wall destruction and pathogen removal is a possible way to achieve higher treatment of wastewater as well as biogas yield from anaerobic digestion.

3. Literature Review

3.1 Waste Stabilization Ponds

WSPs are man-made basins bounded by earthen barriers used for the treatment of wastewater in semi-centralized systems (Swiss Federal Institute of Aquatic Science and Technology and Spuhler, n.d.; Verbyla, Sperling and Maiga, 2017). The main disadvantage of using WSPs is that a large area is required for operation, limiting their use in highly populated areas. Another disadvantage is the possibility of high capital costs in areas where land is expensive; therefore, the use of WSPs for biological wastewater treatment is almost impractical unless there is an abundance of affordable land. Conversely, where land is cheap and available, the capital costs are low and the advantages of using WSPs are large. These systems are simple to construct, operate, and maintain and do not require skilled personnel. When compared with conventional WWTPs, WSPs have significantly lower operational and maintenance costs as there is no external energy needed for the system to function. As previously mentioned, a high degree of treatment is achieved when the ponds are placed in a systematic arrangement. Removal of BOD, total suspended solids (TSS), and ammonia is attained at greater than 90%, while oil removal is in the range of 50-90%. WSPs are also extremely effective when considering pathogen removal. Finally, WSPs are very resilient to large fluctuations in organic hydraulic, and heavy metal loads (Swiss Federal Institute of Aquatic Science and Technology and Spuhler, n.d).

The most common types of WSPs are anaerobic ponds, aerobic ponds, facultative ponds, HRAPs, and maturation ponds. These ponds differ from each other in their depth, loading rates, and whether or not they are aerated using mechanical equipment. The typical order of WSPs in series is an anaerobic pond followed by a facultative pond and ending with a maturation pond

(Veryla, von Sperling, Maiga, 2017). Depending on the type of receiving water, a bar racks or grit chambers may precede the first pond to remove unwanted objects (rags, grit, etc.) (Gloyna, 1971). Each type of pond along with its characteristics and purpose will be discussed in detail.

3.1.1 Anaerobic Ponds

Anaerobic ponds are most commonly the first pond in a series and their main function is BOD removal (>60%) and sludge digestion. These ponds are 2-5 m in depth and receive a high organic loading rate that exceeds 3000 kg of BOD/ha/day. As the name implies, anaerobic ponds do not contain dissolved oxygen and, therefore, do not contain algae. The process involves the sedimentation of settable solids, which forms the sludge layer. Anaerobic digestion in the sludge layer occurs through the reactions of acidophilic and methanogenic bacteria. These bacteria work together to degrade and stabilize the organic matter found in the sludge. As the organic matter is digested, biogas is formed and may be collected using a floating plastic membrane. Every 1-3 years the pond should be desludged to prevent excessive accumulation of solids (Swiss Federal Institute of Aquatic Science and Technology and Spuhler, n.d.) Figure 1 displays a cross-sectional view of an anaerobic pond.

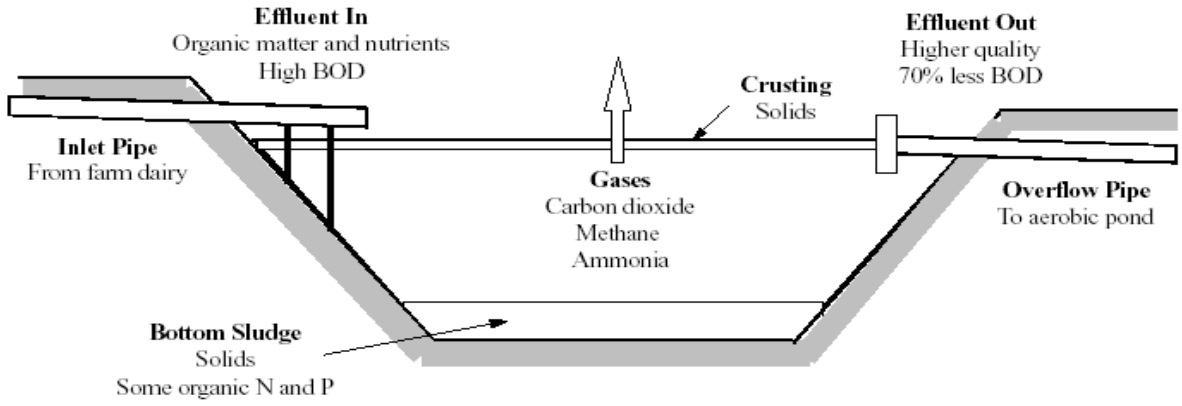


Figure 1. Anaerobic waste stabilization pond (SOURCE: http://stabilizationponds.sdsu.edu).

3.1.2 Facultative Ponds

There are two types of facultative ponds: primary and secondary. Primary facultative ponds receive raw wastewater while secondary facultative ponds receive the effluent from an anaerobic pond. These ponds are 1-2 meters in depth and the main goal is to further remove BOD. The organic loading rate is much lower (100-400 kg/ha/day), thus allowing the growth of a substantial algal population. A primary facultative pond is usually used when the entering wastewater is weak or when the odor of an anaerobic pond would be too offensive for its location. It consists of 3 zones, with the upper zone being aerobic, followed by a facultative zone and an anaerobic zone at the bottom (Figure 2). Algae will grow to the depth at which sunlight can penetrate. In the aerobic zone, photosynthesis by algae provides the oxygen needed for BOD consumption by bacteria (aerobic digestion). As a result of aerobic digestion, bacteria expire CO_2, which, along with atmospheric CO_2, aids in the growth of algae. The facultative zone occurs where the oxygen demand exceeds the supply, resulting in facultative bacteria consuming BOD by either aerobic or anaerobic digestion. The bottom of the pond accumulates a sludge

layer, which forms the anaerobic zone. Here, the sludge is further digested by the same mechanism in an anaerobic pond. Facultative ponds are very efficient and can remove pathogens as well as 80% of the BOD that entered the pond (Swiss Federal Institute of Aquatic Science and Technology and Spuhler, n.d).

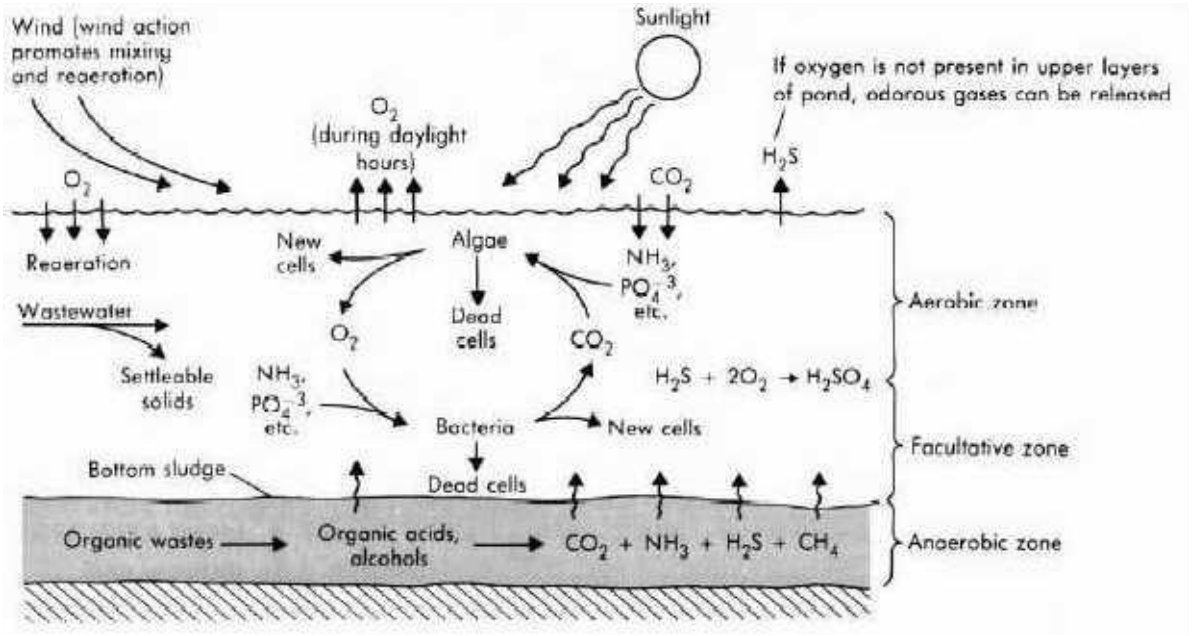


Figure 2. Process of a primary facultative waste stabilization pond (Tchobanoglous and Schroeder 1987).

3.1.3 Maturation Ponds

Generally, maturation ponds are the final pond in a series and they serve as form of tertiary treatment to remove any pathogens and nutrients that remain in the effluent from the preceding facultative pond. These ponds are aerobic, shallow ponds usually about 1 meter deep. There is typically more than one maturation pond placed in series. The total number of ponds is a function of the required degree of pathogen removal and the retention time necessary for this to

occur. For example, the higher the required pathogen removal concentration implies a longer retention time and, thus, more ponds in series.

The degree of pathogen removal required depends on where the final effluent is discharged and if public health is of concern. Bacteria and viruses that cause diseases such as cholera, gastroenteritis, typhoid fever and hepatitis can be found in wastewater. The removal of pathogens varies with retention time, temperature, pH, dissolved oxygen (DO) concentration and sunlight. *Escherichia coli*, for example, is inactivated primarily as a function of UV radiation, pH and DO concentration (Butler *et al.*, 2017). It has also been proven that increased exposure to sunlight can significantly reduce the presence of *Cryptosporidium parvum*, the cause of cryptosporidiosis (Reinoso, 2008). Maturation ponds are not necessary if the final effluent is used for restricted irrigation; however, maturation ponds will act as a buffer in the case that the preceding facultative pond fails (Swiss Federal Institute of Aquatic Science and Technology and Spuhler, n.d).

In the same manner as facultative ponds, maturation ponds utilize algae for nutrient removal, mainly nitrogen (N) and phosphorus (P). Some maturation ponds can serve a dual function of removing N and P while also removing algae (Tchobanoglous, 1985). Finding algae in the final effluent is a common problem and should be managed carefully. High concentrations of algae being discharged in the final effluent to a receiving stream may cause an influx of oxygen and organic matter (Kothandaraman and Evans, 1972). To prevent this from occurring, removal can be achieved by introducing fish in the maturation pond as the algae will serve as a food source (US EPA, 1983).

3.1.4 Aerobic ponds/Aerated Ponds

Aerated ponds are supplied with oxygen via mechanical or diffused aeration instead of algal photosynthesis and they aim to remove soluble organic matter by maximizing bacterial growth. There are two types of systems: partial mix and complete mix. Partial mix systems only supply enough oxygen to meet the requirements needed and some settling will occur. Conversely, complete mix systems use a significant amount of energy (about ten times more than partial mix systems) to keep all of the solids in suspension. Both types of aerated ponds use at least three ponds in series. Employing either system results in a high level of BOD removal with less land required. Some disadvantages are higher capital and operational and maintenance costs, more skilled personnel are needed, and desludging occurs more frequently and needs further treatment before disposal (Swiss Federal Institute of Aquatic Science and Technology and Spuhler, n.d).

3.1.5 High-Rate Algal Ponds (HRAPs)

William J. Oswald and his colleagues introduced HRAPs as a development of advanced integrated wastewater pond systems (AIWPS). The success of HRAPs in the United States is largely due to the opportunities they provide for energy recovery while efficiently treating wastewater using various naturally occurring processes. Treating wastewater using natural processes is not only cost efficient, but it also conditions the system to be more resilient to hydraulic shock and BOD loading. The general layout of an AIWPS starts with covered anaerobic ponds followed by a HRAP. Following the HRAP are algal settling ponds and, finally, maturation ponds (Figure 3). The need for further treatment depends on the final effluent water

quality. This may include multiple maturation ponds, rock filters, UV disinfection, or a membrane filter (Craggs *et al., 2017*).

The supernatant from the preceding covered anaerobic pond is conveyed and treated in a HRAP. These ponds are about 0.2-0.6 meters in depth and function under rapid hydraulic retention times, about 3-4 days (Craggs, 2014; Ramadan and Ponce, n.d.). HRAPs are characterized by a "race-track" shape with paddlewheels aiding in movement around the pond (Figure 4). This movement improves vertical

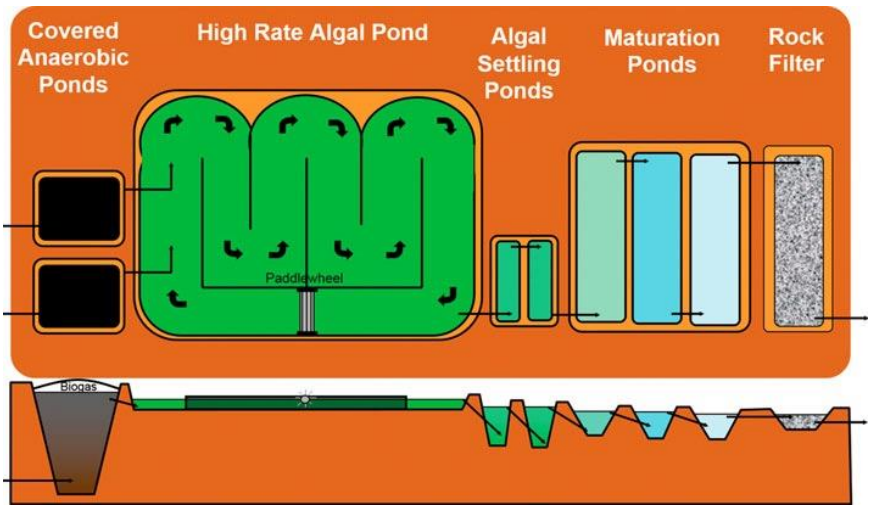


Figure 3. Schematic diagram of an AIWPS (Craggs, Park, Heubeck and Sutherland, 2014)).

Figure 4. High-rate algal pond with a paddlewheel (Chinnaswamy, 2013).

mixing, which is essential for maximum exposure of algal cells to sunlight. The use of paddlewheels also promotes the growth of colonial algae, where this type of growth is normally suppressed in facultative ponds due to a higher settling rate than unicellular algae.

HRAPs obtain many of the same advantages as any other WSP, but are more effective at producing a consistent effluent quality and removing nutrients (N and P) and pathogens. Additionally, the large amount of algal/bacterial biomass formed in the process can be further used as a source of fertilizer, feedstock, or biofuel. The mechanism occurring in a HRAP is similar to that of facultative ponds, with the exception that HRAPs are completely aerobic. As previously described, the oxygen formed as a result of algal photosynthesis aids in the growth of aerobic bacteria. This further promotes the aerobic digestion of the dissolved organic matter present in the wastewater (Craggs *et al., 2017*).

Another advantage of HRAPs is that it requires much less energy than ponds that need mechanical aeration. This is because the algae present in a HRAP will supersaturate the water with dissolved oxygen during the day; therefore, excessive external oxygen does not need to be introduced. Moreover, HRAPs can achieve partial tertiary treatment levels as found in maturation ponds since its shallow depth allows UV penetration and subsequent pathogen removal (Craggs *et al., 2017*).

3.2 Anaerobic Digestion

Anaerobic digestion describes the process in which organic matter is decomposed without the presence of oxygen. This process can also reduce some inorganic matter in this manner, too. Anaerobic digestion in wastewater treatment systems is used primarily for the stabilization of sludge to form biosolids. The term sludge is defined as a liquid or semisolid

liquid (depending on the source) that is formed as a result of the treatment of wastewater. The term biosolids is used to describe treated sludge that is applicable for reuse (e.g. land application or surface disposition). As previously stated, anaerobic digestion can also produce biogas that can be collected and used as a source of energy. Since the benefits of producing reusable biosolids and energy resource recovery are large, anaerobic digestion serves as the major form of sludge stabilization. Anaerobic digestion most commonly operates at mesophilic temperatures (30-35°C), but there has been interest in thermophilic anaerobic digestion has gained interest because of its ability to deactivate a larger number of pathogens. For simplicity, mesophilic anaerobic digestion will be solely considered (Tchobanoglous *et al*., 2014).

3.2.1 Sludge Characteristics

Although the ultimate goal of WWTPs is the same, the mechanism to obtain the desired end product may differ from plant to plant. For example, sludge stemming from a primary clarifier within a WWTP is highly putrescible, ha a slimy texture, and appears gray. On the other hand, activated sludge can resemble primary sludge or it can be browner in color, a quality indicative of more aeration and low settling (Tchobanoglous *et al.,* 2014). Table 1 shows some typical values for the chemical composition of untreated sludge.

Table 1. Typical values for the chemical composition of untreated primary and activated sludge (Tchobanoglous et al., 2014).

Item	Untreated Primary Sludge	Untreated activated sludge
Total dry solids (TS), %	3	0.8
Volatile solids (% of TS)	75	70
Grease and fats (% of TS)	6	8
Protein (% of TS)	25	36
Nitrogen (N, % of TS)	2.5	3.8
Phosphorus (P_2O_5, % of TS)	1.6	5.5
Potash (K_2O, % of TS)	0.4	0.6
Cellulose (% of TS)	10	--

3.2.2 Mechanism of Anaerobic Digestion

Hydrolysis, acidogenesis, and methanogenesis are the three fundamental stages during the anaerobic digestion of waste. Figure 5 shows the steps and the intermediates formed during each process. Each of these steps will be further described in detail.

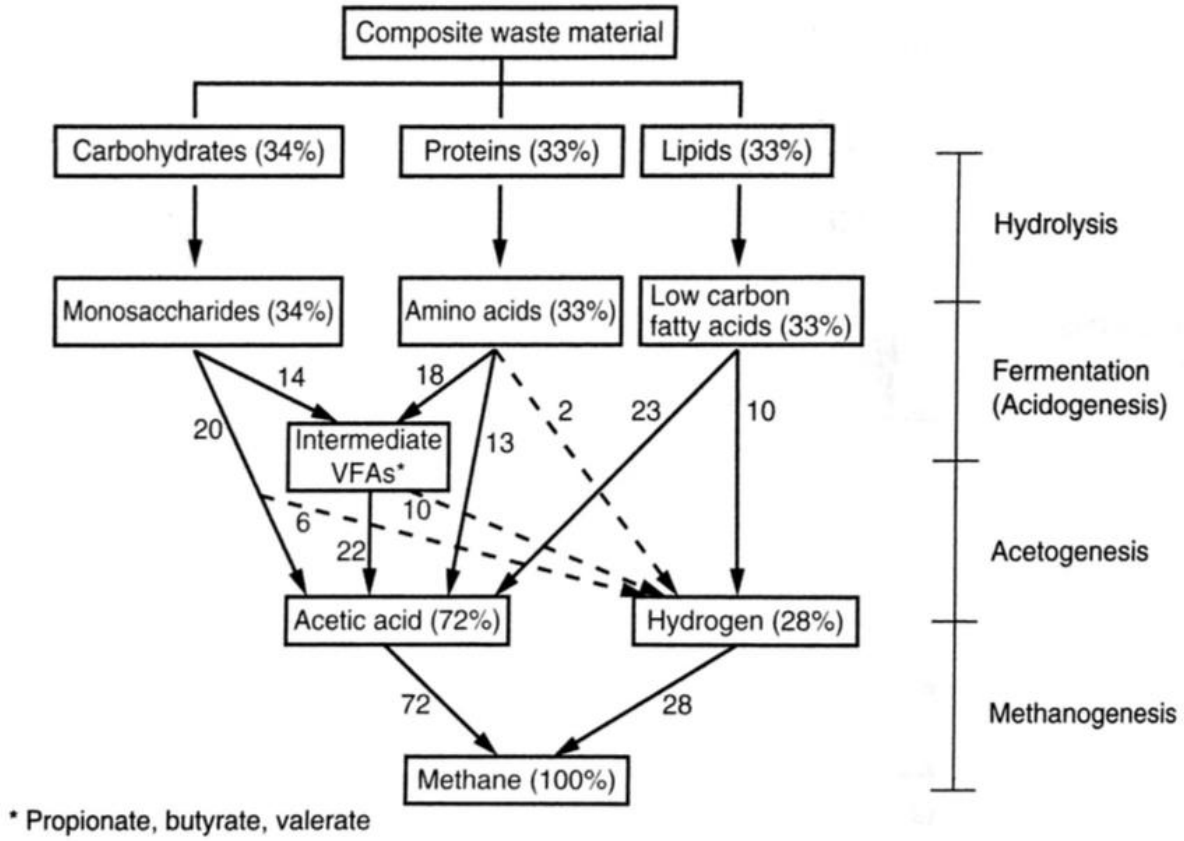


Figure 5. Schematic diagram of steps occurring during anaerobic digestion (Tchobanoglous et al., 2014).

a. Hydrolysis

Hydrolysis is generally the first step in anaerobic digestion, depending on the strength and solubility of the waste. This is the process in which organic particulate matter is converted to soluble compounds (polymers) that are further hydrolyzed (broken down) to simple monomers. This action occurs by cleaving chemical bonds in the presence of extracellular enzymes, produced by facultative and obligate anaerobes, and water (H_2O). Carbohydrates are hydrolyzed to monosaccharides (Figure 6), proteins to amino acids (Figure 7), and lipids to long chain fatty acids (LCFAs) (Figure 8) (Tchobanoglous *et al.*, 2014*).*

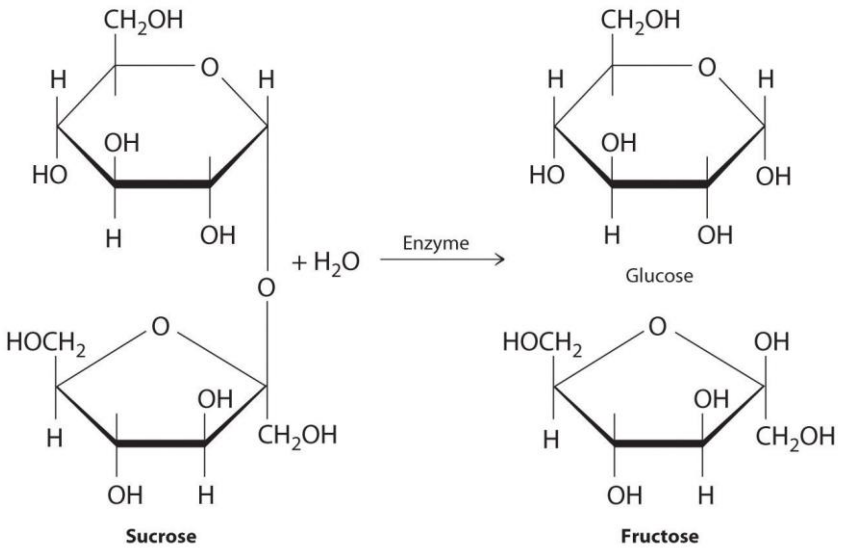


Figure 6. The hydrolysis of a carbohydrate (sucrose) to two monosaccharides (glucose and fructose) (SOURCE:https://2012books.lardbucket.org).

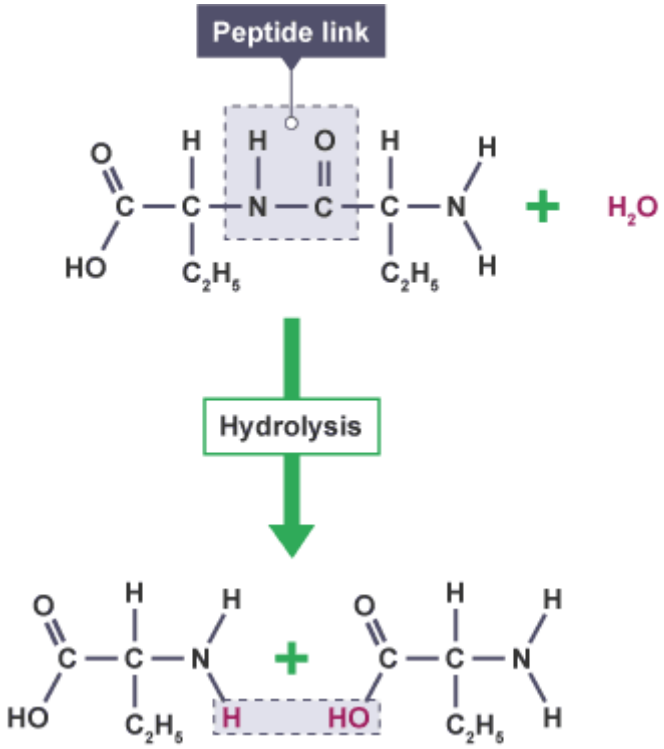


Figure 7. The hydrolysis of a protein to amino acids (SOURCE: www.bbc.co.usa).

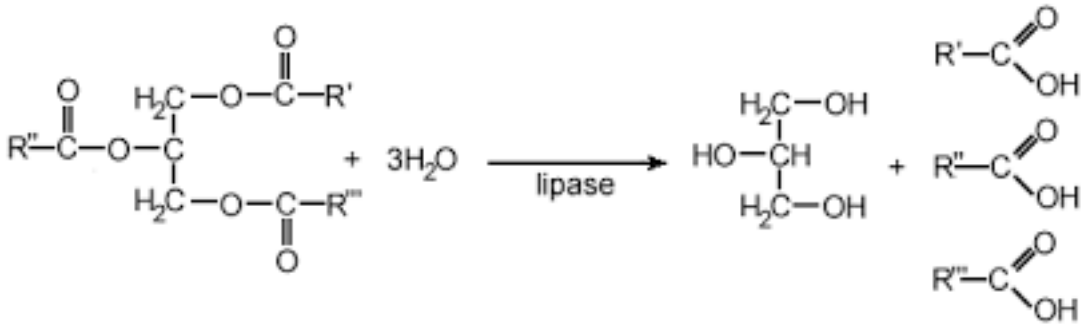


Figure 8. The hydrolysis of a lipid (triacylglycerol) to LCFAs through the action of lipase (SOURCE:www.angelfire.com).

b. Acidogenesis

Acidogenesis, more commonly referred to as fermentation, follows hydrolysis and is carried out through the action of bacteria. The products of fermentation are intermediate volatile fatty acids (VFAs), CO_2, and hydrogen. Monosaccharides and amino acids produce VFAs, CO_2, and hydrogen while LCFAs mainly form acetic acid, CO_2, and hydrogen. The most common VFAs present are acetate, propionate and butyrate (Tchobanoglous *et al.*, 2014).

c. Acetogenesis

Acetogenesis is an intermediate step that further breaks down VFAs through fermentation. Therefore, the end products of fermentation are acetate, hydrogen and CO_2, which all serve as precursors for the formation of methane (Tchobanoglous *et al.*, 2014).

d. Methanogenesis

Methanogenesis is the third and final step of anaerobic digestion and it is carried out by methanogenic bacteria called methanogens. There are two types of methanogens involved in this process: aceticlastic methanogens and hydrogenotrophic methanogens. Aceticlastic methanogens cleave acetate to form CH_4 and CO_2 (Equation 1) while the hydrogenotrophic methanogens form CH_4 by oxidizing hydrogen and using CO_2 as their carbon source (Equation 2). Another reaction

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad \textbf{(Eq.1)}$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \textbf{(Eq.2)}$$

can occur in which anaerobic bacteria called acetogens will form acetic acid using CO_2 and hydrogen, but the acetic acid formed will be further converted to CH_4. Most of the methane production stems from the cleavage of acetate, as seen in Figure 5. It should be noted that if the incoming composite waste material contains a higher lipid concentration, more methane will be formed (Tchobanoglous *et al.,* 2014).

3.2.3 Mesophilic Anaerobic Digestion Processes

This section will cover processes that take place in single stage high-rate digestion and two-stage digestion. Since the quality of sludge varies with the source it originates, so it is important that a thickening unit precede an anaerobic digester in a WWTP. This will increase the solids concentration as much as possible by removing a large portion of liquid. This can be

achieved by centrifugation, gravity settling, flocculation, and various mechanisms (Tchobanoglous *et al.*, 2014).

Single-stage high-rate digestion is the most common process used for anaerobic digestion today and is characterized by heating, auxiliary mixing, continuous feeding, and thickening of the feedstock (Figure 9). The system consists of a sludge heater to keep a desired mesophilic temperature so the anaerobic bacteria can facilitate digestion. Mixing can be accomplished by gas recirculation within the system, pumping, or draft-tube mixers. Each of these techniques prevent the formation of scum and supernatant, resulting in a completely mixed solution. The feedstock is supplied and withdrawn in a uniform manner to accomplish steady state conditions. Since complete mixing is accomplished in this type of digester, the total solids are reduced by

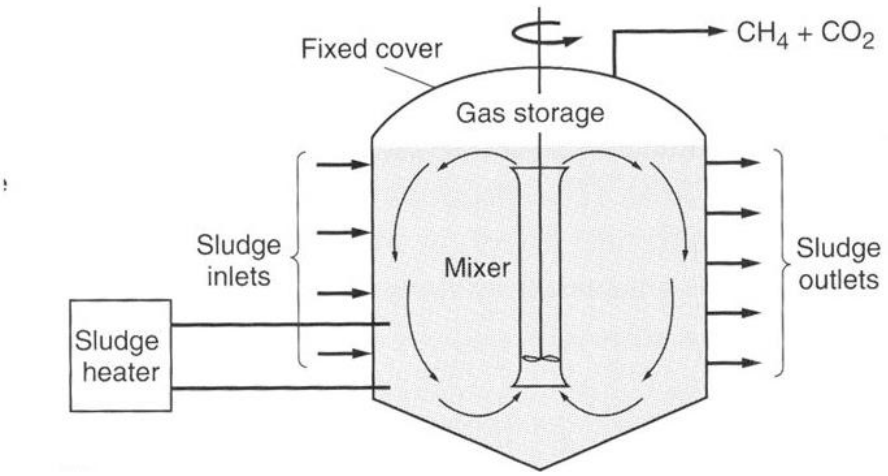


Figure 9. Schematic diagram of a single-stage high-rate digester (Tchobanoglous, et al., 2014).

45-50% and converted into biogas. Digesters that employ this mechanism max have fixed or floating roofs, with the latter accommodating for the variations in biogas production.

Two-stage digestion uses a high-rate digester followed by another tank. The first tank employs the same process as a single-stage high-rate digester while the second tank is used

primarily for storage and is unheated (Figure10). Each tank can either have a fixed or floating cover for biogas sequestration. The second tank forms three layers: digested sludge, supernatant and scum. The quality of the supernatant withdrawn is poor because it can easily contain a high concentration of solids due to poor settling characteristics of the digested sludge. Sometimes the second tank is uncovered, but this is generally not the case because anaerobic digestion may continue and release biogas into the atmosphere (Tchobanoglous *et al.,* 2014).

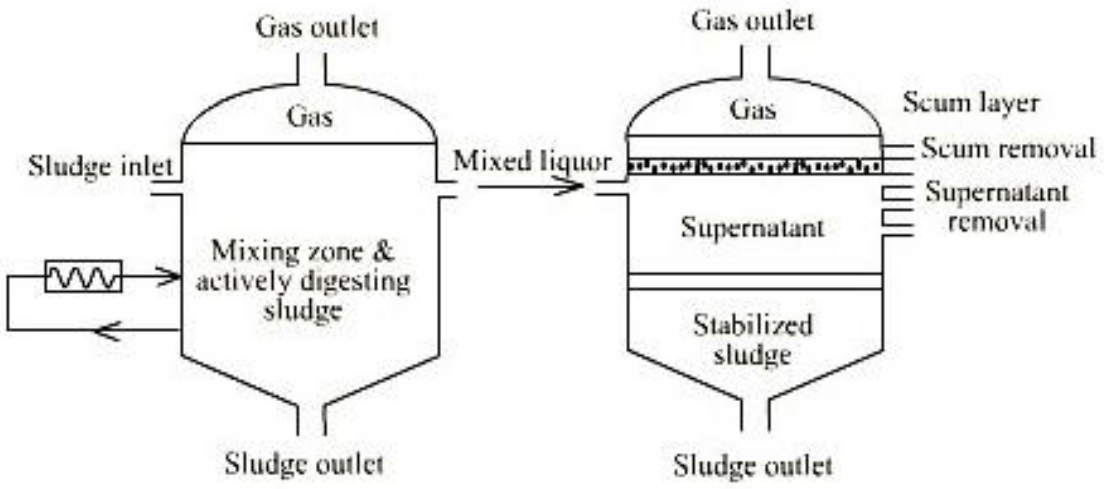


Figure 10. Schematic diagram of a two-stage digester (SOURCE:http://www.c2biotechnologies.com).

3.3 Co-Digestion

Anaerobic digestion has conventionally been used to treat a single substrate (sludge) in WWTPs; however, it has been reported that most operating wastewater treatment facilities have a surplus of digestion capacity up to 30%. If there is an additional source of organic matter, WWTPs with this excess capacity could process it along with municipal sludge. This creates an opportunity to increase biogas production significantly. The principal of an anaerobic digester processing multiple substrates is called co-digestion. The advantages of using co-digestion are outlined in Table 2, which was adapted from Tchobanoglous *et al.,* 2014.

Table 2. Advantages of co-digestion (Tchobanoglous et al., 2014).

Category	Description
Technical	• Remove nuisance wastes from the collection system, especially if a waste is causing stoppage, odor or damage • Remove organic loadings and nuisance factors from headworks and liquid treatment train. • Increase use of existing digester capacity, especially with co-digestion of wastes that are synergistic with wastewater sludge in terms of increasing the volatile solids loading rate. • Improve knowledge of how to handle organic wastes. • Provide a reliable outlet for organic wastes.
Economical	• Develop a new revenue stream from tipping fees for organic wastes. • Produce more biogas for combined heat and power systems, or thermal dryer systems, or other beneficial uses. • Reduce cost of operation, maintenance, and odor control in the liquid treatment train, from headworks to final clarifiers. • Avoid or defer construction of additional liquid train treatment capacity. • Increase the throughput rate of the sludge processing train.
Environmental	• Earn carbon credits, where applicable. • Reduce land application of organic wastes that contribute to methane production rather than carbon sequestration. • Reduce emissions of greenhouse gases, particularly methane, coincidental to increasing energy recovery from waste materials.

Typically, co-digestion operates with municipal sludge as the primary substrate and fewer amounts of secondary substrate(s). the successfulness of co-digestion is measured as a direct production of CH_4. If more CH_4 is produced using multiple substrates, co-digestion is considered

synergistic, is less CH_4 is produced, it is considered antagonistic. Co-digestion can also be neutral, with no more or less CH_4 production. With this being true, co-digestion is thought to increase the anaerobic digestion process by providing more stability (Tchobanoglous, Stensel, Tsuchihashi, 2014).

3.4 Algae

The use of algae in wastewater treatment systems is extremely useful for nutrient removal of N and P, which are consequently incorporated into the algal biomass (Butler *et al., 2017*). Although there are several different types of algae found within these systems (Figure 11), the purpose of this section is to identify and describe the general characteristics of the species most commonly found in wastewater treatments systems and explain a few techniques currently used for its conversion into energy.

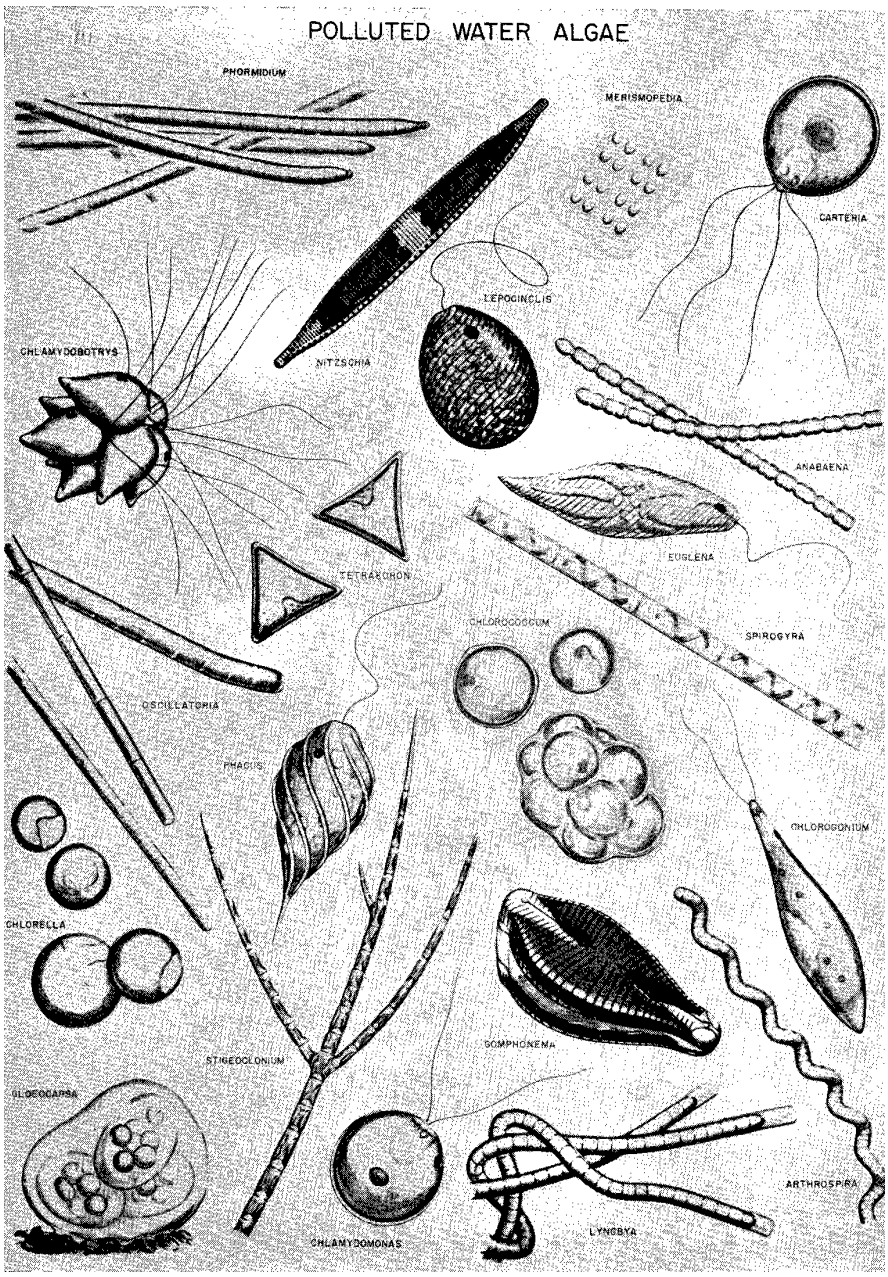


Figure 11. Algae present in WSPs. (Gloyna, 1971*).*

The most common types of algae found in WSPs are green algae, diatoms, and blue-green algae. Table 3 gives some examples of the genera of each of these types of algae that inhabit WSPs (Gloyna, 1971).

Table 3. Most common types of algae and their genera (Gloyna, 1971).

Type	Genera
Green algae	*Chlamydomonas, Chlorogonium, Pascheriella, Pandorina, Carteria, Chlorella, Golenkinia, Micractinium, Ankistrodesmus, Scenedesmus, Actinastrum, Coelastrum, Oocystis, Tetraedron, Euglena, Phacus.*
Diatoms	*Nitzschia*
Blue-green algae	*Oscillatoria*, *Anabaena*

3.4.1 Chemical Composition

The chemical composition of microalgae depends largely on the what is consumed. For instance, lower levels of phosphorus present in wastewater result in an algal biomass with a higher percentage of lipids. Additionally, trace metals or silicon can also produce a higher lipid content (Hwang *et al.*, 2016). Table 4 shows the organic contents of the algae mentioned in Table 3. It can be seen that, for most species, protein and carbohydrates make up the majority of algal matter. Lipid content is fewer, but still notable. These characteristics of algal composition make them an excellent precursor for anaerobic digestion processes. It is important to note that the cell wall of algae is also primarily made up of carbohydrates and proteins along with biopolymers that provide robustness and rigidity. Compounds that provide such structure and protection may include, but are not limited to, cellulose, hemicellulose, hydroxyproline and proline. This is especially problematic when using anaerobic digestion of algae as the cell wall is considered to be the limiting factor for efficient and successful degradation. If algal cell walls are rigid and intact, it is extremely difficult for anaerobic bacteria to access the easily degradable

inner cellular contents of the algae. Another challenge is that nonliving algae still present intact cell walls that are difficult to degrade (Torres, Fermoso and Rincon, 2013).

Table 4. Chemical composition of algal species (Source:www.oilgae.com)

Algal Species	Protein	Carbohydrates	Lipids	Nucleic Acids
Scenedesmus obliquus	50-56	10-17	12-14	3-6
Scenedesmus quadricauda	47	--	1.9	--
Scenedesmus dimorphus	8-18	21-52	16-40	--
Chlamydomonas rheinhardii	48	17	21	--
Chlorella vulgaris	51-58	12-17	14-22	4-5
Chlorella pyrenoidosa	57	26	2	--
Spirogyra sp.	6-20	33-64	11-21	--
Euglena gracilis	39-61	14-18	14-20	--
Anabaena cylindrica	43-56	25-30	4-7	--

3.4.2 Harvesting Methods

There are various methods of harvesting of algae in wastewater treatment systems to further use as a source of biofuel. However, microalgae grown in suspension are very difficult to remove as they are extremely small in size (<30 μm) and have a density comparable to water. Harvesting techniques used for algae include sedimentation, flocculation, flotation, filtration, and centrifugation. Depending on the mechanism used, there may be a trade-off between performance and economic practicality. This is a common problem for harvesting methods and may hinder the potential for use as a recoverable energy source (Hwang *et al., 2016*).

a. Sedimentation

Sedimentation relies on the natural force of gravity to separate the denser solids from the lighter liquid. This method is not typically used because the settling velocity of microalgae can be as low as 0.1 m/day; however, this is the most economical method of harvesting and can be used in conjunction with another technique to provide a desirable concentration of biomass (Hwang *et al., 2016*).

b. Flocculation

Flocculation of microalgae can be facilitated by natural processes or by the addition of a chemical coagulant. Flocculation is the process of cells clumping together, which further aids in flotation or settling rate. Chemical flocculants are very successful in promoting flocculation, but their use is limited as it can change the composition of the media. If this occurs, it is possible that the collected biomass could not be used for biofuel production.

c. Flotation

In this method of harvesting algae, mechanical aeration is used to create a foam of microalgae on the water surface. Flotation mechanisms are characterized by the way bubbles are introduced into the water. Dissolved air flotation (DAF) is the most common method used to harvest algae in WSPs. The water containing algae is brought to a flotation tank where water saturated with air is released from high to low pressure, resulting in the formation of bubbles. This process can be used in conjunction with flocculation to remove 95% of algae in a short amount of time; however, operational

costs are high because of the energy needed for DAF (Sharma *et al.*, 2013) (Hwang, *et al., 2016).*

d. Filtration

Filtration is a proficient method used for harvesting algae and it is thought to be less complex and expensive than centrifugation (Sharma *et al.,* 2013). With this being true, filtration may still have high operational costs when used on a large-scale. A pore size of 0.10-10 μm is typically used to remove algae without pretreatment. A larger pore size can be used if filtration is preceded by filtration or if larger algal cells are to be captured (Milledge and Heaven, 2013). Forward osmosis (FO) is a new technique used for filtration of algae by using osmotic pressure to facilitate separation. FO is a more economical alternative to other methods of filtration because pumps are not used. With this method come a few disadvantages such as low flux rates (Hwang *et al., 2016*).

e. Centrifugation

This is one of the more commonly used methods for algae harvesting because it is highly effective and requires a short amount of time. There are many different types and sizes of centrifuges used today, with a disc stack centrifuge being the most popular (Uduman *et al.*, 2010).

3.5 Electrochemical Disinfection

Electrochemical disinfection is a form of wastewater treatment developed over a century ago. This method employs the technique of using inert electrodes (at least one cathode and one anode) supplied with a direct current to result in the electrolysis of water. Equation 3 shoes the reaction occurring at the anode, while equation 4 shoes the reaction occurring at the cathode.

$$2H_20 \rightarrow O_2 + 4H^+ + 4e^- \qquad \text{Eq. 3}$$

$$2H_20 + 2e^- \rightarrow H_2 + 2OH^- \qquad \text{Eq. 4}$$

The result is oxygen and hydrogen gas. Electrochemical disinfection is a desirable method of wastewater treatment and disinfection because it uses minimal energy and requires no addition of chemicals for disinfection. Although this process has been around for a long period of time, the internal mechanisms are not fully understood. Just within the last 40 years, researchers developed electrodes that are very stable and efficient (titanium electrodes coated with oxide coating) (Kraft, 2008). Electrochemical disinfection can be used for polishing the final effluent of wastewater. In particular, the final effluent from WSPs may benefit not only from its treatment capabilities, but also the possibility to remove algae from the final effluent while efficiently destructing the cell wall. Simosa (2016) performed an electrochemical disinfection experiment in a EC batch reactor using an algal medium. This medium consisted of a pure culture of *Chlorella vulgaris*. Throughout the experiments, Simosa (2016) was able to successfully break the cell wall of *Chlorella vulgaris*. The resulting mass floated to the top of the

medium due to the formation of hydrogen bubbles during the electrochemical disinfection process.

4. Laboratory Methods and Equipment

This chapter will provide the laboratory equipment used for each experiment as well as the corresponding methods used for preparation and final performance of the experiments.

4.1 Laboratory Equipment

In this section, all major laboratory equipment used is described. Any equipment not mentioned in this section is considered in Appendix A.

a. Blender

This blender is made by Hamilton Beach and has the ability to operate at different speeds (Figure 12).

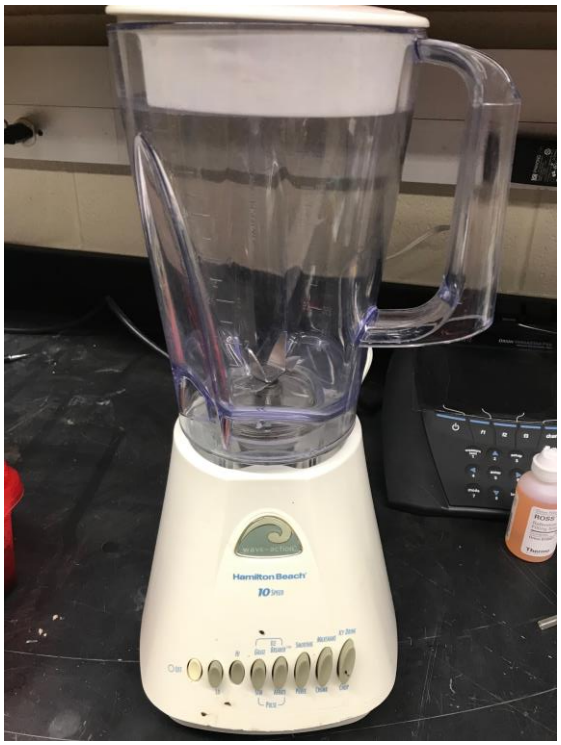


Figure 12. Hamilton Beach blender

b. Electrochemical Batch Reactor

The reactor used in this research was purchased from Ecolotron Inc. of Seabrook, TX. Its design is property of Gavrel et al. under US Patent No.: 7087176 B2, registered on August 8th, 2006 (Figure 13). This unit includes a plate and frame design. The spacer plates are used to enclose the volume of fluid, which is aided by a sealed lining. The apparatus may be closed tightly using a mechanical press. The spacers are non-electrical and are separated by electrodes that are applied with electricity. This design of this apparatus is very variable in that it will allow modifications as needed. For example, the number of spacers can be altered, electrode material can be changed, and the dimensions and the orientation of the plates can be changed all using the existing frame. (Rincon, 2013). In this particular apparatus used for this experiment, a modification was made by De Grau (2015), which created a hole for the gases formed during the reaction to escape (Figure 14). The electrodes used in this experiment are coated with iridium oxide. The dimensions and layout are shown in Figure 15. When placed in the reactor, the slits in the reactor were vertically aligned.

Figure 13. Electrochemical batch reactor (Simosa, 2016).

Figure 14. Hole for gas escape (Simosa, 2016).

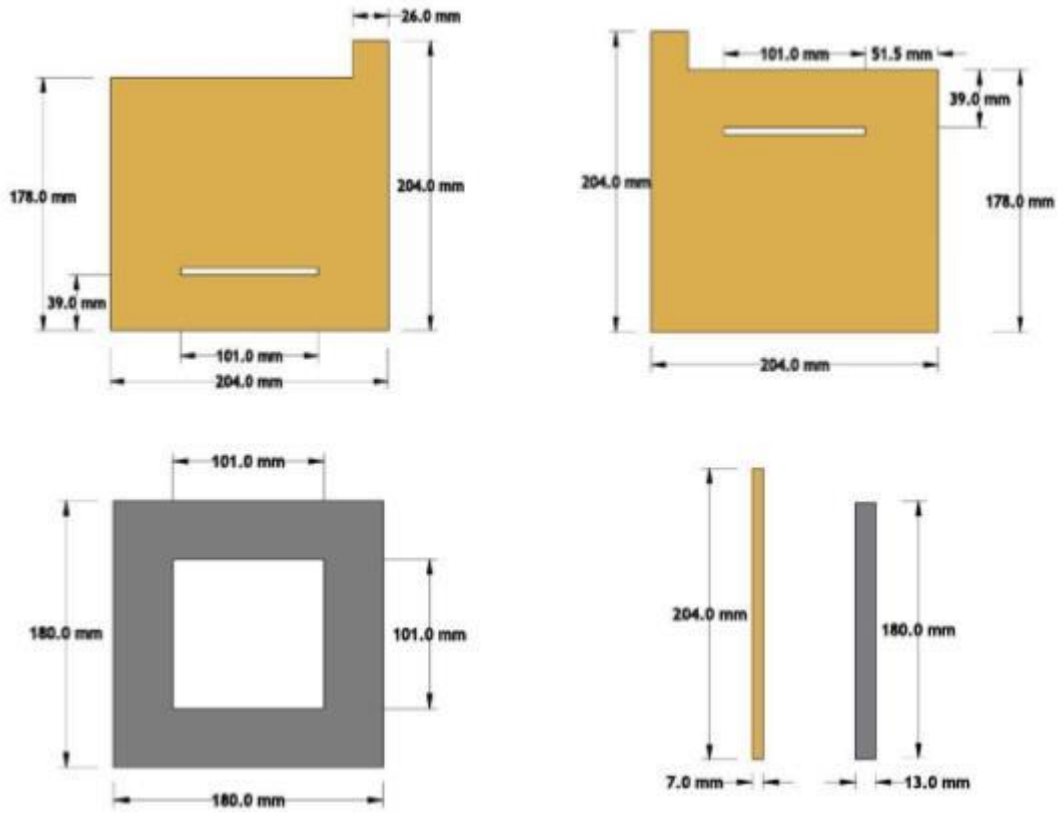


Figure 15. Electrode and spacer dimensions and layout (Rincon, 2013).

c. Shaking water bath

This water bath was purchased from General Laboratory Supply, Inc. in Pasadena, Texas. It has the ability to hold 18 liters with a tray for holding flasks. The tray can hold a total of four one liter flasks, or eight 250-mL flasks. It is also equipped with a polycarbonate lid that reduces evaporation and conserves energy (Figure 16 and 17). The dimensions of the water bath are 420 x 235 mm and the temperature can range from ambient +5 to 99°C. It also has the capability to shake at a speed ranging from 20-200 revolutions per minute (rpm). Therefore, depending on the desired operating points, the temperature and shaking speed can be altered (Simosa, 2016).

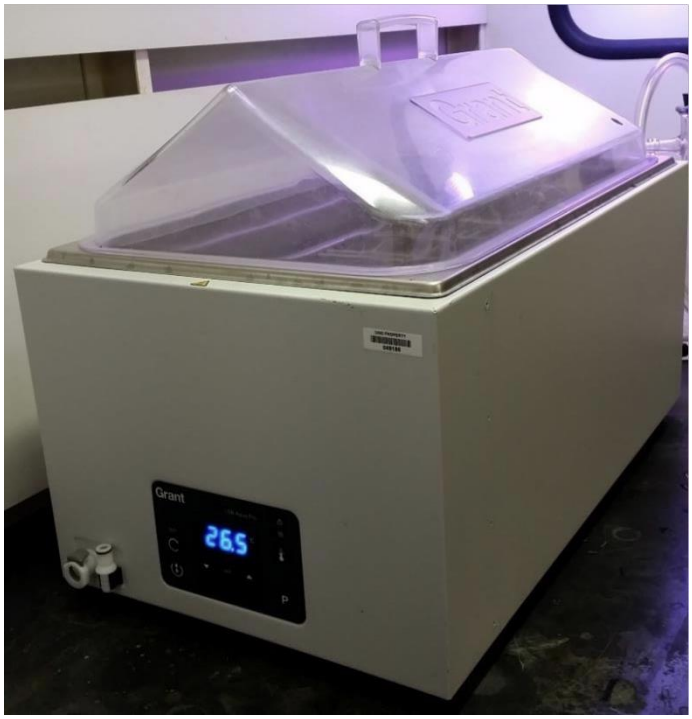


Figure 16. Shaking water bath (Simosa, 2016).

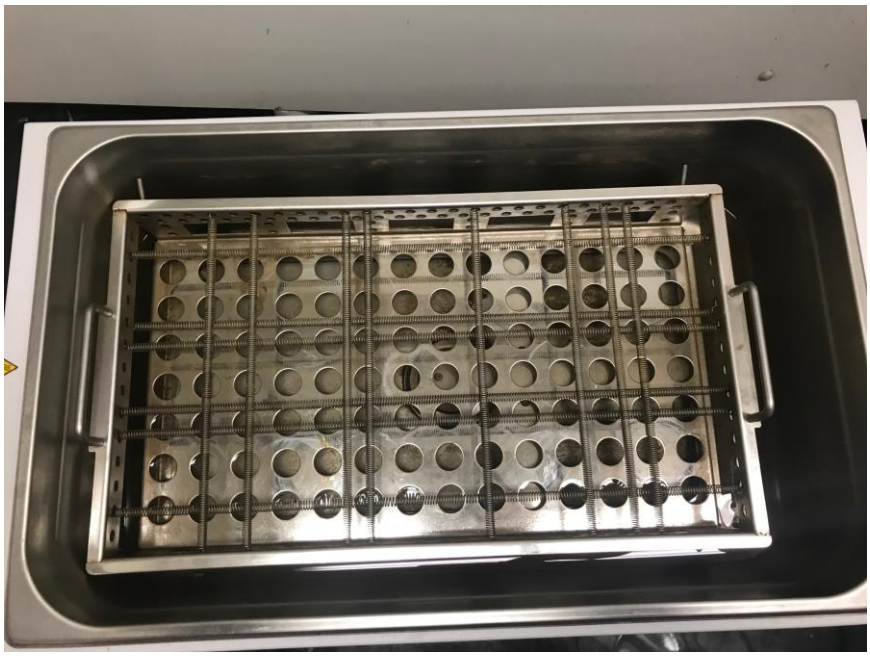


Figure 17. Plan view of the shaking water bath.

d. Glassware

One liter beakers were used for the collection of volume after being released from the electrochemical batch reactor. 250-mL flasks were used to facilitate anaerobic digestion. These flasks were equipped with one hole rubber stoppers and flexible tubing to allow the biogas formed during the reaction to escape.

e. Oven

This oven was manufactured by Fischer Scientific (Figure 18).

Figure 18. Oven used for total solids measurements.

f. Analytical balance

This analytical balance was manufactured by OHAUS (Figure 19).

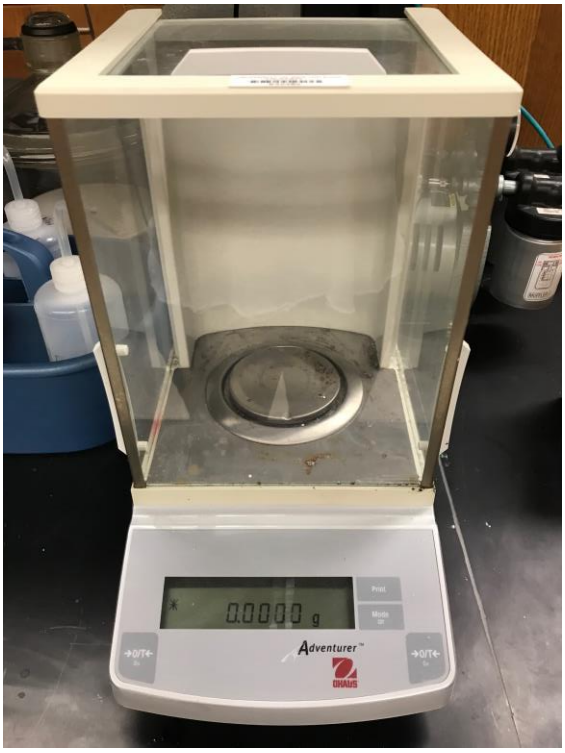


Figure 19. Analytical balance.

g. Microscope

To view algae under a higher magnification, OMANO OMFL400 Fluorescence Compound Microscope was used (Figure 20).

h. Camera

To capture all images under the microscope, Jenoptik Progres CapturePro 2.5 Camera was connected to the microscope as well as a monitor (Figure 20).

Figure 20. OMANO compound microscope assembled with a Jenoptik camera connected to a monitor (Simosa, 2016).

4.2 Laboratory Methods

This section will provide the methods used for each experiment in this research. When necessary, each method will be described in more detail in Appendix A.

a. Preparation of synthetic medium

To prepare the medium for this experiment, 10-mL of Bristol's medium were added to a one liter beaker. Then, deionized water was added to the beaker until a desired total volume of one liter was reached. To this mixture, 20 mg of calcium carbonate ($CaCO_3$) and 250 mg of sodium bicarbonate ($NaHCO_3$) were measured and added and mixed until completely dissolved.

b. Measurement of pH, conductivity and temperature

Conductivity, pH, and temperature were measured using an Orion 5 Star benchtop meter manufactured by Thermo Scientific (Figure 21). The electrode used to measure pH was an Orion 8157BNUMD Ross Ultra pH/ATC triode and the electrode used to measure conductivity and temperature was an Orion 013005MD Conductivity Cell. The pH meter was calibrated using Hydrion buffers and subsequently rinsed with deionized water and dried before taking measurements. After measurements were taken, the probe was rinsed, dried, and placed in a storage solution provided by the manufacturer. The probe used to measure conductivity and temperature was calibrated using 3163 and 3161 YSI calibrator solutions. After calibration, the probe was rinsed with deionized water and dried before taking measurements. After measurements were taken, the probe was rinsed and dried before being stored.

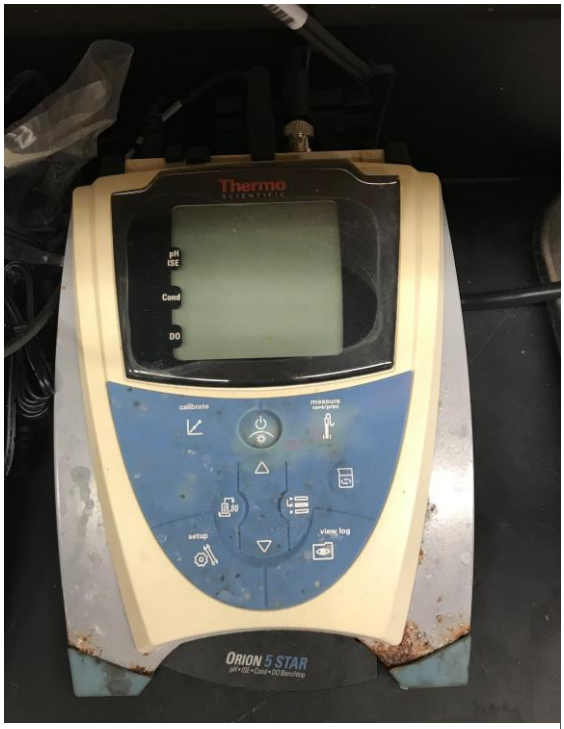


Figure 21. ORION 5 Star benchtop meter.

c. Alkalinity

To measure the alkalinity of the sample, HACH method 8221 was used.

d. Calcium

To measure calcium, HACH method 8222 was used.

e. Total solids

To measure total solids, HACH method 8271 was used.

f. Destruction of cell wall

To perform this experiment, the Ecolotron electrochemical batch reactor, BK Precision High Current DC Regulated Power Supply (Model 1791), OMANO OMFL400 Fluorescence Compound Microscope, Jenoptik ProgRes CapturePro 2.5 Camera, titanium electrodes coated with iridium oxide, and spacers were all used. A variation of spacer and electrode arrangements were used during the trial and error phase of this experiment. Constant current (CC) or constant voltage (CV) mode was then applied to the volume by connecting the power supply to each electrode with a preset maximum range for voltage and current. After subjected to the EC reactor, the algae were examined under the compound microscope and optically observed for cell wall destruction.

g. Anaerobic digestion

For this experiment, eight 250-mL flasks were used to facilitate anaerobic digestion for two sets of algal medium, with each set receiving four flasks. The first set of algal medium was collected before being sent through the electrochemical bath reactor (cell wall intact), while the second set was collected after EC (cell wall destructed). After one liter of algal medium was collected for each set, 2-mL of primary sludge were inoculated into each algal media. This sludge was obtained from East Jefferson Wastewater Treatment located in Harahan, Louisiana. Next, 200-mL of sample were divided among the flasks. These flasks were placed in a shaking water bath filled with enough water to keep the top of the sample submerged. The temperature was set at 35°C. Before covering each flask with a one-hole rubber stopper, CO_2 was injected into the flasks to displace the oxygen present and retain anaerobic conditions. Each stopper was designed to have a well fitted tube in the hole to allow the release of biogas produced during anaerobic digestion. The other end of tube stemming from each flask was fitted to another rubber stopper and flask (or bottle) filled with CO_2 to further ensure anaerobic conditions. These were 3-hole stoppers with two incoming tubes from the digester flasks and one outgoing tube (with a very small diameter) to allow for biogas escape. Every three days for 12 days, one flask was removed from the shaking water bath for each set of algal medium. Total solids were measured from the contents of each flask and recorded.

5. Experimental Design

This section is intended to give an overall description of each experiment performed for this research. Each experiment was conducted at the University of New Orleans in the Center for Energy Resource Management (CERM) located in New Orleans, Louisiana. The algae used were collected from the surface of the secondary clarifier at the Bridge City Wastewater Treatment Plant in Bridge City, Louisiana (Figures 22, 23, and 24).

Figure 22. Secondary clarifier at Bridge City WWTP.

Figure 23. Algae growing in the secondary clarifier.

Figure 24. Algae growing over the weir of the secondary clarifier.

The algal biomass was collected at the Bridge City Wastewater Treatment Plant and brought to the CERM and observed under the microscope in attempt to identify the genera (Figure 25). From the sample obtained it appeared that the genus Oedogonium was the predominant algae present in the sample. Oedogonium is an unbranched, filamentous green alga. This genus contains one layer of cells and can be found free floating or attached to another substrate. Cells of the protoplasm are circular and sometimes broader at one end than the other. This alga is commonly found in the presence of diatoms due to their touch cell wall ability to act as a substrate. Diatoms were found and are another indicator that this genus may have been Oedogonium. Areas containing loaded nutrient concentrations, as in wastewater, are the ideal location for Oedogonium to thrive (Vuuren *et al.,* 2006).

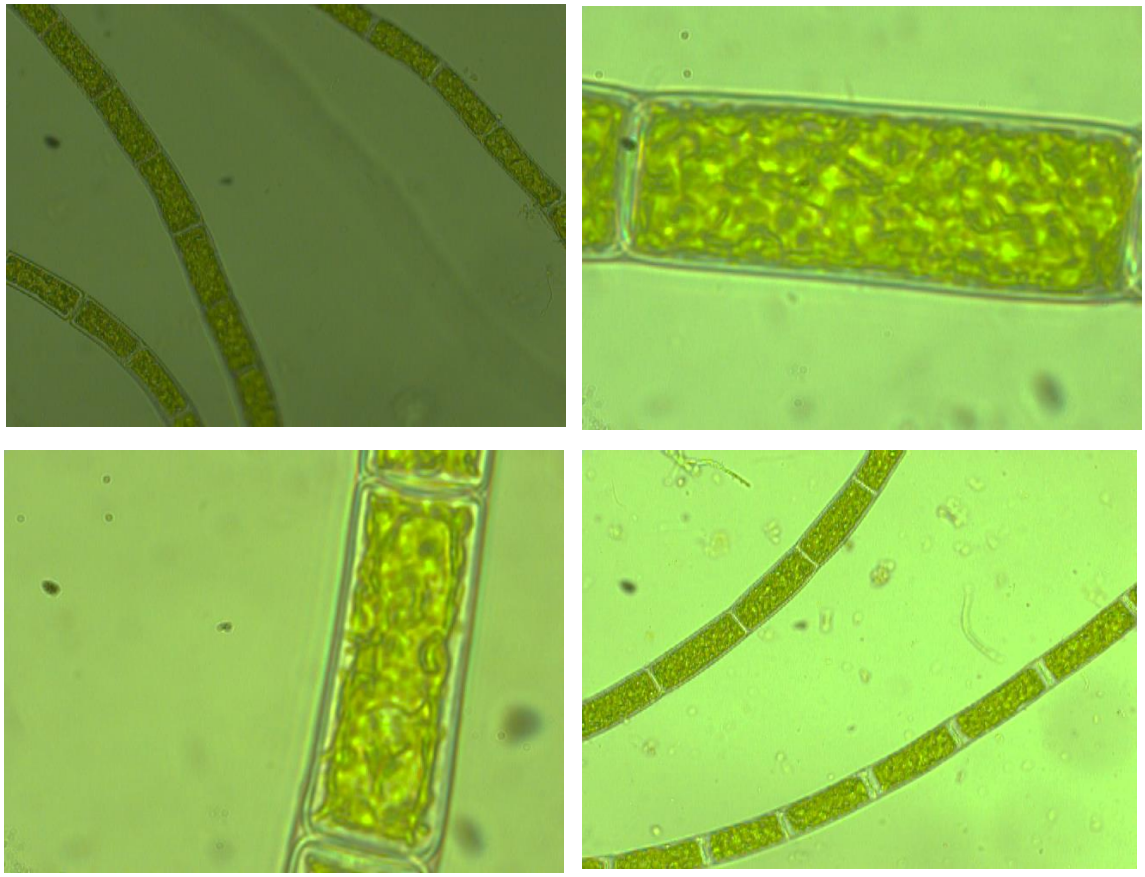


Figure 25. Algae viewed under the microscope.

5.1 Experiment One: Destruction of Algal Cell Walls Using an Electrochemical Batch Reactor

As previously described, anaerobic digestion of algae is an extremely appealing possibility as it would increase the production of biogas, which could be further used as a source of energy. However, the algal cell walls can be extremely difficult to penetrate, thereby making the anaerobic digestion process more difficult. In an experiment performed by Golueke *et al.*, (1956) it was noted that the anaerobic digestion of algae was more successful at thermophilic temperatures (50-55°C) than mesophilic temperatures (30-35°C). It was assumed that thermophilic temperatures weakened the cell wall, causing it to be susceptible to bacterial attack

(Goleuke *et al., 1956*). Facilitating anaerobic digestion at such high temperatures, however, consumes more energy than at mesophilic temperatures. Goleuke *et al.* (1956) also suggest that anaerobic digestion of algae at mesophilic temperatures may not be as successful due to the capability of algal survival in this system. Therefore, the premise for experiment one was to break the algal cell walls in preparation of anaerobic digestion at the more economical mesophilic temperature. The final products of this experiment were two samples of algae: intact algal cell walls (reserved before electrochemical disinfection) and destructed algal cell walls (obtained after electrochemical disinfection).

A sample consisting of two liters of synthetic medium and 274 mg/L of algae was prepared before using the electrochemical (EC) batch reactor for electrochemical disinfection. 2 g/L of magnesium sulfate ($MgSO_4$) were added to sample in order to generate the electrolyte necessary for constant current (CC) mode. One liter of the sample containing $MgSO_4$ was reserved before performing the EC portion of this experiment. Of the remaining one liter sample, 250-mL were withdrawn and poured into the reactor using a funnel. The reactor cell consisted of two spacers with electrodes on each side (Figure 26).

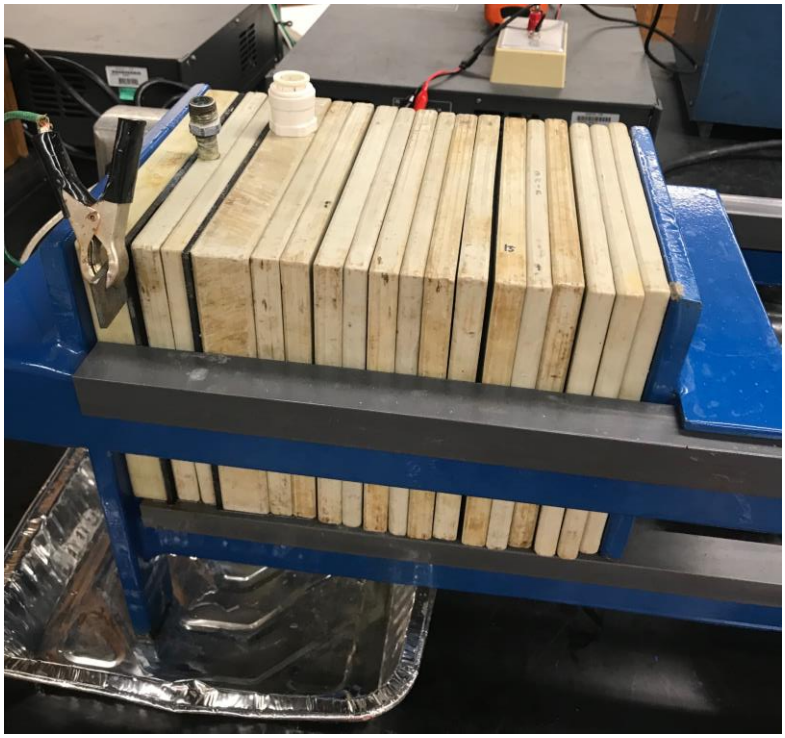


Figure 26. Electrochemical batch reactor apparatus.

The electric charge was then applied to the electrodes using direct current (DC) under bipolar conditions for various times. Once complete, the sample was collected. This was repeated until a sufficient amount of sample was collected. Figure 27 shows the reserved one liter sample before electrochemical disinfection and the volume of sample collected after disinfection. It can be seen that the sample collected after disinfection lacks the green hue found in the reserved sample. This is due to the loss of chlorophyll and is indicative of successful cell wall breakage.

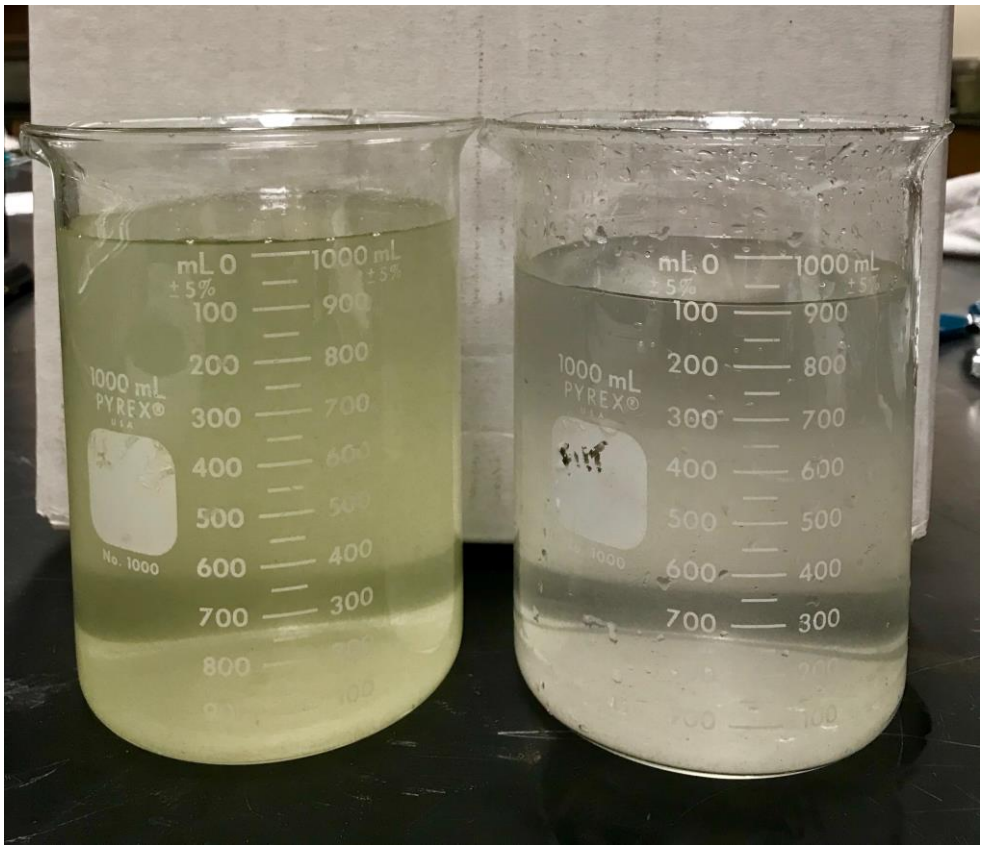


Figure 27. Algal medium before being sent through the EC batch reactor (left) and after (right).

5.2 Experiment Two: Anaerobic Digestion of Intact Algal cell walls vs. Disrupted Algae Cell Walls

The aim of this experiment was to facilitate anaerobic digestion of the reserved sample (intact algal cell wells) and the sample collected after electrochemical disinfection (destructed algal cell walls), and subsequently compare the rate of decomposition that occurred in each sample. To begin, the Series 1 volume was divided into four 250-mL flasks by placing 200-mL of the sample in each flask. This was repeated for the Series 2. Thus, a total of eight 250-mL flasks were used. The remaining sample for each set of algal medium (intact cell wall and destructed cell wall) were used to measure total solids at day zero. Next, all eight flasks were

placed in the shaking water bath at 35°C and 90 rpm. Each flask was blown out with CO_2 to facilitate anaerobic digestion (see Appendix A for details), as seen in Figures 28 and 29 This reaction occurred in complete darkness. Every three days a flask from each digester set was removed to measure total solids. This was repeated until no flasks remained (12 days). The total solids measurements were recorded and compared.

Figure 28. Experimental set-up of anaerobic digestion experiment.

Figure 29. Series 1 (back) and Series 2 (front) in the shaking water bath.

6. Results and Discussion

This chapter provides a detailed description of the results obtained from the trial and error portions of these experiments. It will also specify why the chosen parameters were used to carry out the experiments.

6.1 Experiment One Results: Destruction of Algal Cell Walls Using Electrochemical Disinfection

The first objective of experiment one was to replicate electrochemical disinfection in the EC batch reactor at the recommended voltage and current to achieve maximum cell wall destruction as performed by Simosa (2016); however, that particular experiment was performed with a pure culture of *Chlorella vulgaris* purchased from a manufacturer. Additionally, Simosa (2016) used turbidity as a method of estimating total suspended solids (TSS). This was problematic in this research because the algae were obtained from the field and contained not only multiple species of algae, but many other microorganisms as well (e.g. worms, snails, rotifers, etc.). Although the algae obtained from Bridge City WWTP is a more practical approach for the designed experiment, turbidity values and operating conditions of the electrochemical batch reactor were essentially incomparable. For this experiment, samples ranging from 200-300 mg of algae/L of medium were used when performing the trial and error procedures.

a. Trial one: Constant Voltage (CV) mode

A 4-liter sample containing 300 mg algae/L synthetic medium and 2 g/L $MgSO_4$ was used in trial one. Two experiments within this trial, each with 500-mL of sample,

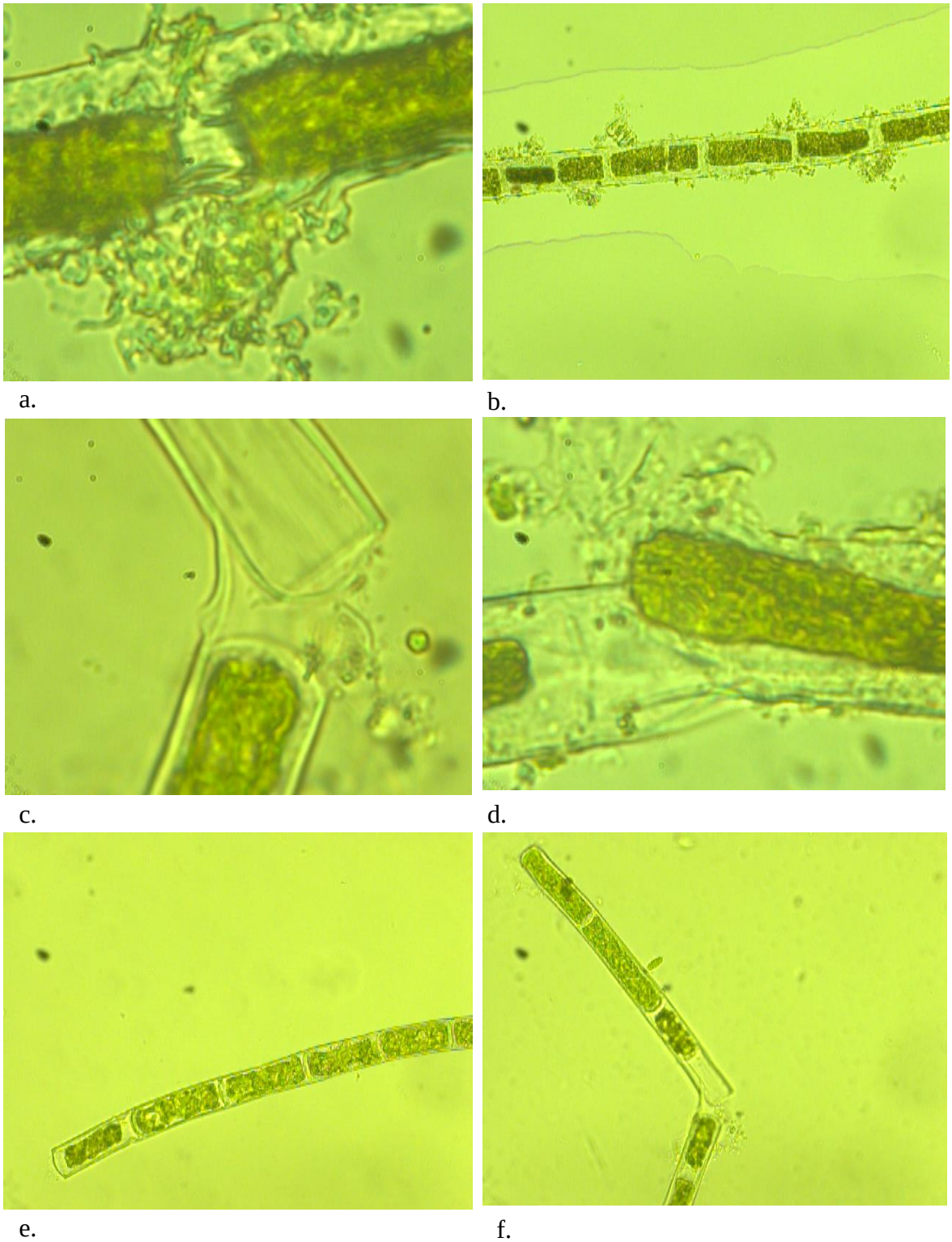
were performed. Each experiment consisted of setting the EC reactor in such a way that there were 4 spacers between 2 electrodes. Each sample batch was allowed a 30 second charge time. After the charge time, the first sample was retained in the reactor under CV for 3 minutes (Sample A) while the second sample was retained for 4 minutes (Sample B). The final voltage and current for Sample A were 64.6 V and 5.2 A, respectively, while the final values for Sample B were 64.6 V and 5.8 A, respectively. After each sample was collected, temperature, pH and conductivity were measured. Table 5 shows the before and after results of these parameters. The visual results obtained from examining the volume after being collected from the EC batch reactor on the microscope are shown in Figure 30 a-f.

Table 5. Initial and final measurements of Sample A and Sample B.

Sample	Time (mins)	Initial Conductivity (mS/cm)	Final Conductivity (mS/cm)	Initial Temperature (°C)	Final Temperature (°C)	Initial pH	Final pH
A	3	3.37	3.31	20.7	34.8	6.8	7.0
B	4	3.37	3.29	20.7	40.8	6.8	7.1

From Table 5, it can be seen that the parameter most affected is temperature. Figure 30b and Figure 30e both show that not all algal cell walls were broken in this process. Sample B obtained similar results as Sample A, and since it operates at a higher retention time and results in a higher temperature, it was determined that it should not be used as the method of cell wall destruction for anaerobic digestion.

In efforts to decrease the retention time while obtaining maximum cell wall destruction, the $MgSO_4$ concentration was increased to 3 g/L in the remaining sample volume. Again, a 500-mL sample (Sample C) was placed in the electrochemical batch reactor with the same configuration as before, but with a retention time of 2 minutes (and 30 second charge time). This trial also occurred in CV mode. The initial and final voltage and current values were recorded as 64.7 V and 7.0 A, respectively. The initial and final recorded measurements for Sample C are shown in Table 6. Once more, temperature is parameter that tends to fluctuate the most. Figure 31 shows some images of the results of this trial. Observing these results, it was concluded that increasing the conductivity (by adding more $MgSO_4$) did not improve cell wall destruction.



a. b. c. d. e. f.

Figure 30 a-f. Sample A results. Pictures b and e show no cell wall destruction.

Table 6. Sample C initial and final recorded values.

Time (mins)	Initial Conductivity (mS/cm)	Final Conductivity (mS/cm)	Initial Temperature (°C)	Final Temperature (°C)	Initial pH	Final pH
2	4.34	4.23	20.6	38.5	6.9	6.8

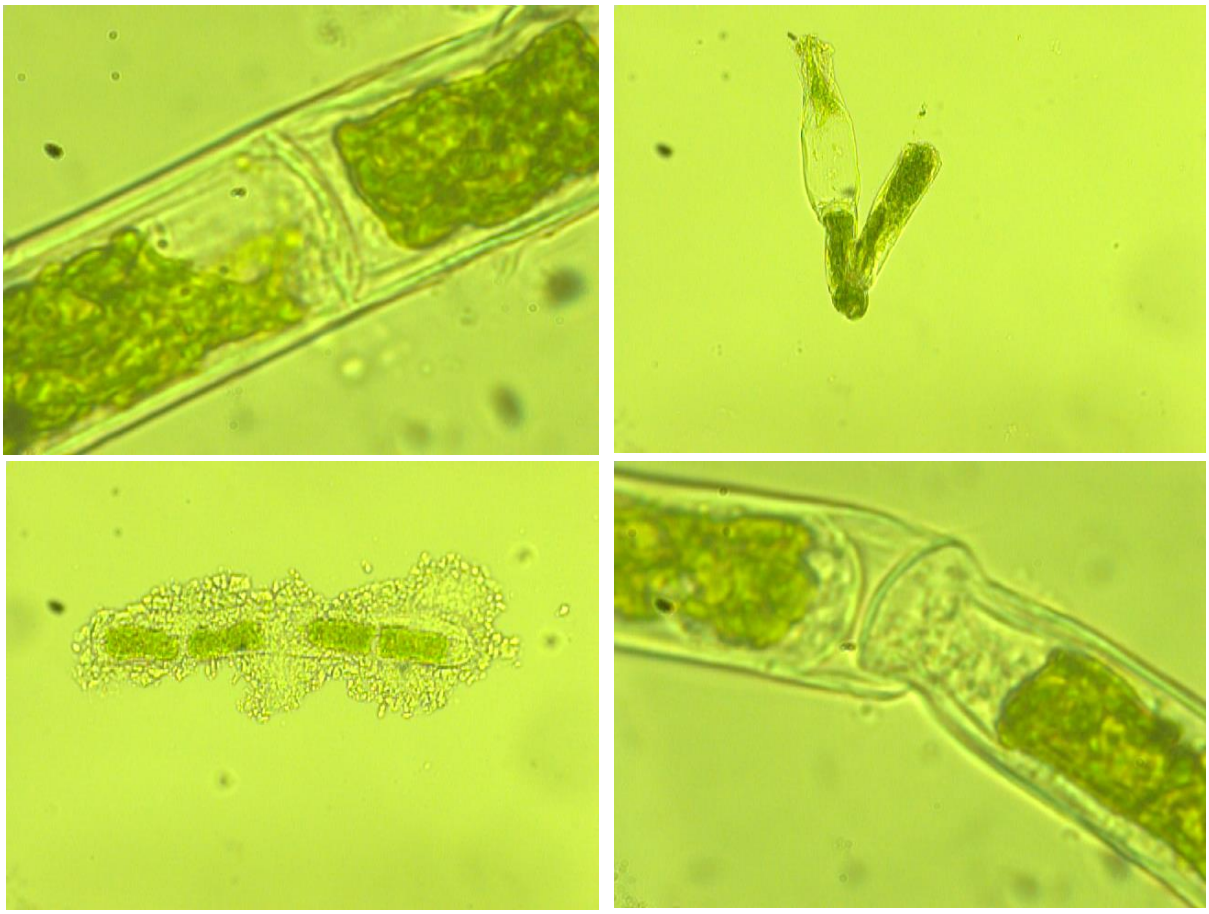


Figure 31. Results from Sample C.

b. Trial two: Constant Current (CC) mode

For this trial, 3 L of medium was prepared and contained an algal concentration of 274 mg/L. To this, 2 mg/L of $MgSO_4$ was added to increase the conductivity. In order to operate under CC mode, the volume of sample was decreased to 250-mL. By doing so, the electrodes were placed closer in proximity (two spacers in between), therefore, decreasing the resistivity. Three experiments at 8, 9 and 10 A were performed with Samples D, E, and F, respectively. Each sample was allowed a 30 second charge time. The voltage achieved with each sample and measurements taken before and after electrochemical disinfection are shown in Table 7. The images taken from the microscope are shown in Figures 32, 33, and 34. These images show physical proof of cell wall breakage as well as protoplasmic contents as the result of cell wall breakage. Sample F operated at ad detention time of 30 seconds to prevent the temperature from increasing significantly.

Table 7. Recorded values for Samples D-F.

Sample	Current	Voltage	Time (mins)	C_i (mS/cm)	C_f (mS/cm)	T_i (°C)	T_f (°C)	Initial pH	Final pH
D	8	46.7	1	3.78	3.76	21.3	37.6	7.8	7.9
E	9	40.8	1	3.78	3.73	21.3	38.4	7.8	8.1
F	10	52.1	0.5	3.78	3.68	21.3	40.1	7.8	8.3

It is important to note that the images in Figures 32, 33, and 34 all display successful examples of cell wall destruction. Throughout every experiment performed, some algal cell walls remained completely intact. Through extensive observation under the microscope of each sample from this trial, Sample E showed the greatest extent of cell wall destruction. To ensure that temperature was not responsible for cell wall

breakage, a temperature test was performed by subjecting the algal medium to a temperature of 45°C on a hot plate. The algae was then examined under a microscope and proved to still have cell walls intact.

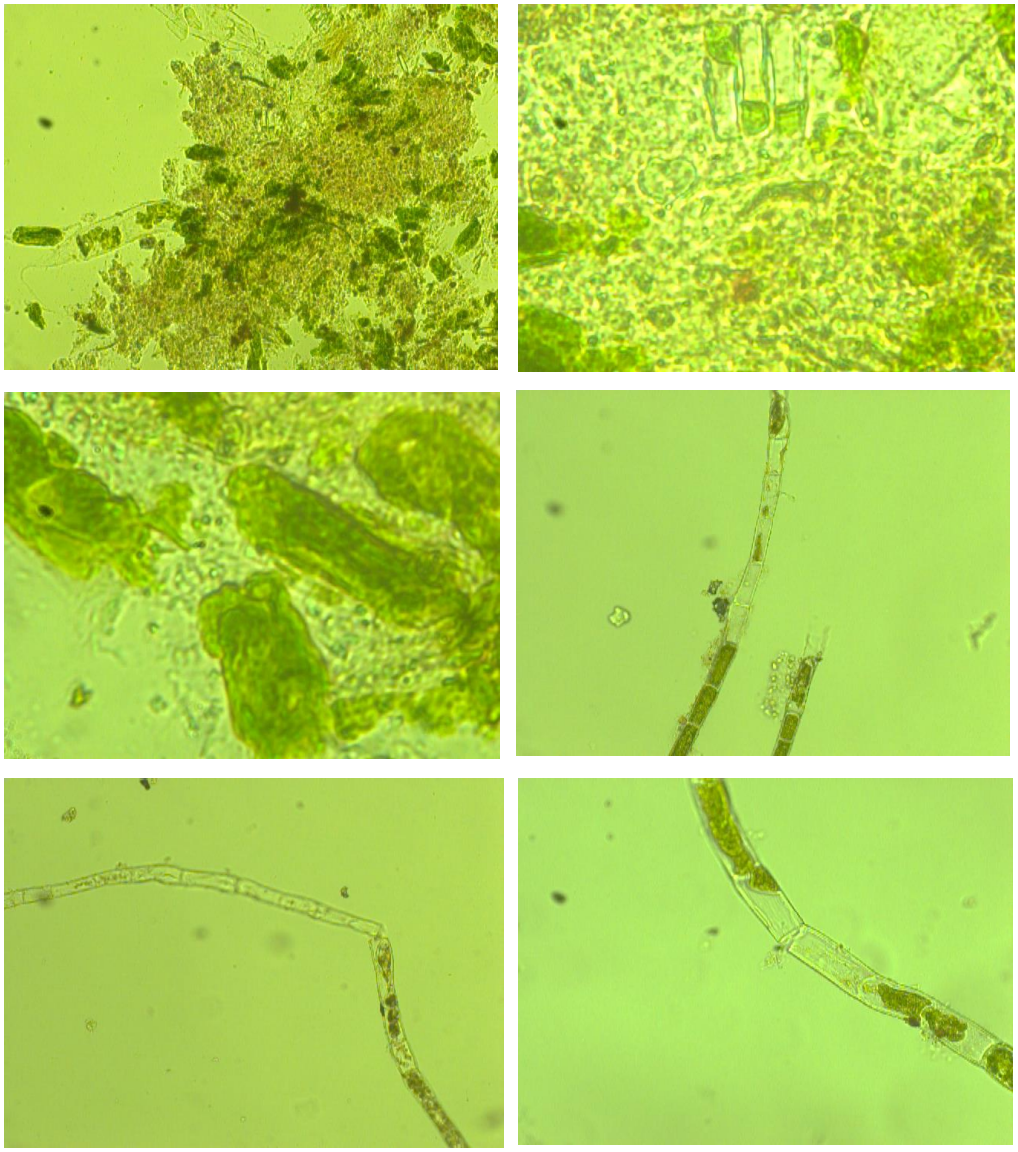


Figure 32. Images of Sample results operating at a CC of 8 amps. The first images show the cell contents escaped from inside the cell wall.

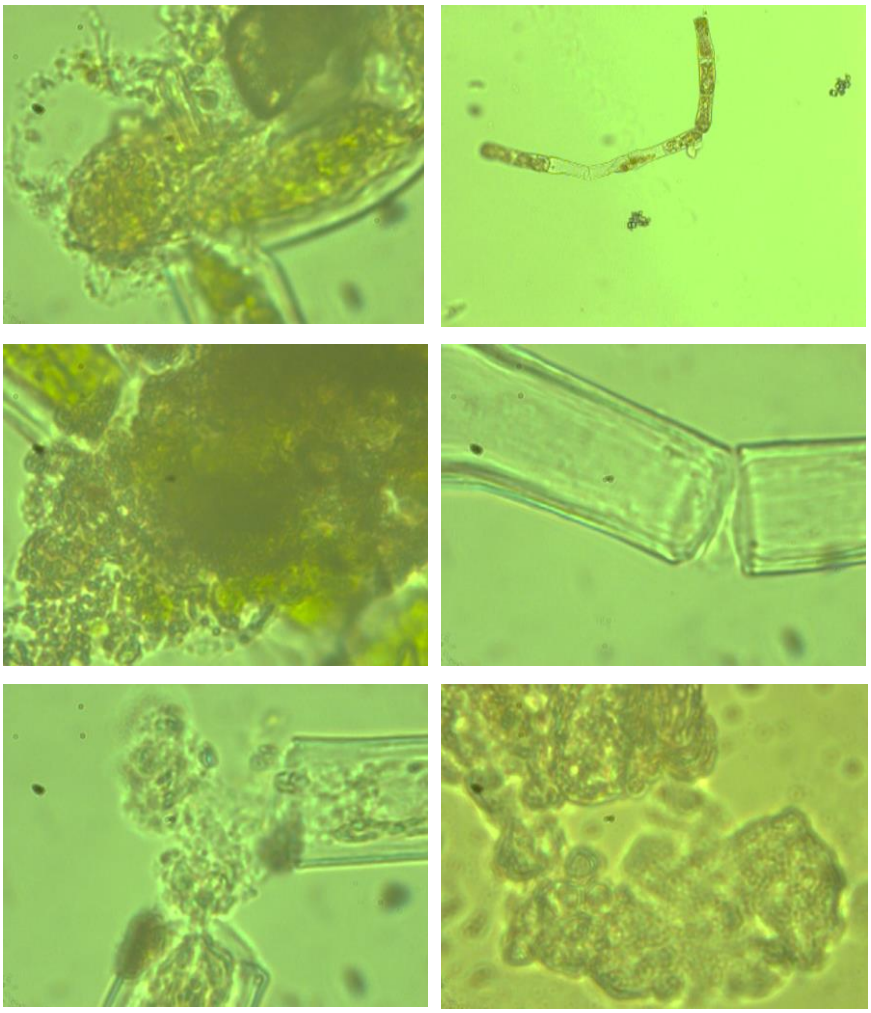


Figure 33. Images of Sample E after electrochemical disinfection at a CC of 9 amps. The cell contents can be seen as well as an algal cell with no cell contents.

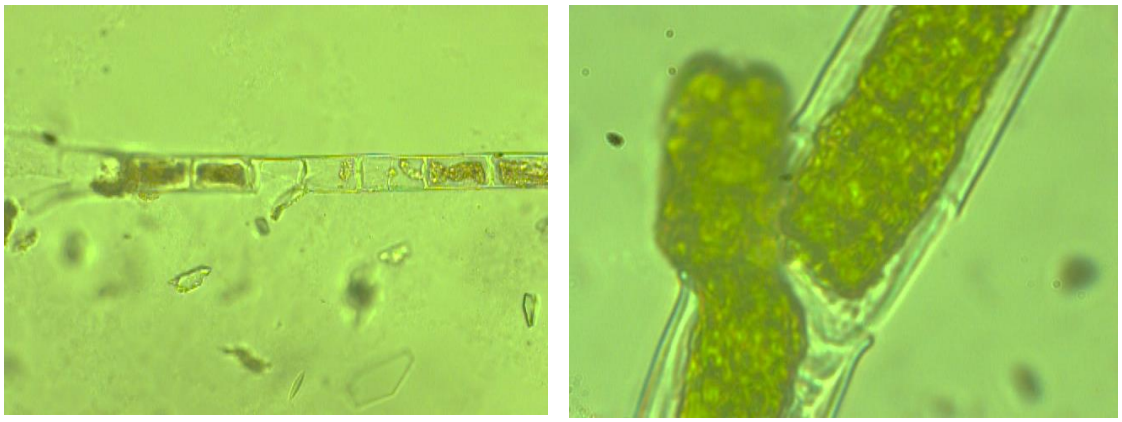


Figure 34. Sample F after electrochemical disinfection at a CC of 10 amps. Each picture displays successful cell wall destruction with the cell contents being released.

c. Design parameter selection

When choosing the best mode of operation for cell wall destruction, all parameters were considered. The best option operating in CV mode was Sample A while Sample E was chosen for CC mode. Sample E resulted in a higher temperature than Sample A. Because the temperature test proved that temperature was not the cause of cell wall destruction, this discrepancy can be omitted. Sample A operated at a retention time of 3 minutes while Sample E operated at 1 minute. Additionally, through optical observation under the microscope, Sample E appeared to achieve a higher level of cell wall destruction. For these reasons, Sample E was chosen as the primary mode of operation preceding anaerobic digestion.

Four successive electrochemical disinfection experiments using the EC bath reactor were then performed as Sample E was previously described (250-mL sample, CC

of 9 amps, 3- second charge time, and retention time of 1 minute). The measurements taken before and after being in the EC batch reactor are given in Table 8. Each sample was collected in an aluminum tray and combined in a 1-L beaker.

Table 8. Measurements taken for the chosen parameter.

Run	Initial conductivity (mS/cm)	Final Conductivity (mS/cm)	Initial Temperature (°C)	Final Temperature (°C)	Initial pH	Final pH
1	3.78	3.69	21.3	38.9	7.8	8.3
2	3.78	3.69	21.3	38.6	7.8	8.4
3	3.78	3.68	21.3	39.7	7.8	8.5
4	3.78	3.69	21.3	38.0	7.8	8.5

6.2 Experiment Two Results: Anaerobic Digestion of Algae

The results for the measured TS at days 0, 3, 6, 9, and 12 for the synthetic algal medium collected prior to electrochemical disinfection (Series 1) are shown in Table 9. These results for synthetic algal medium collected after electrochemical disinfection (Series 2) are shown in Table 10. The decrease in TS concentration was indicative that the bacteria in the anaerobic digester was consuming the biodegradable matter present.

Table 9. TS concentrations for Series 1.

Time (days)	Total Solids (g/L)
0	4.656
3	3.364
6	3.348
9	3.328
12	3.194

Table 10. TS concentration for Series 2.

Time (days)	Total Solids (g/L)
0	4.648
3	3.308
6	3.338
9	3.246
12	3.244

A nonlinear regression analysis was performed for each sample to determine the best fit curve, which compares the observed values and the calculated values (Appendix B). Figures 35 and 36 show the first-order decay curves with plateaus of Series 1 and Series 2. The R^2 value for Series 1 was 0.996 and 0.994 for Series 2. The calculations and tables are given in Appendix B.

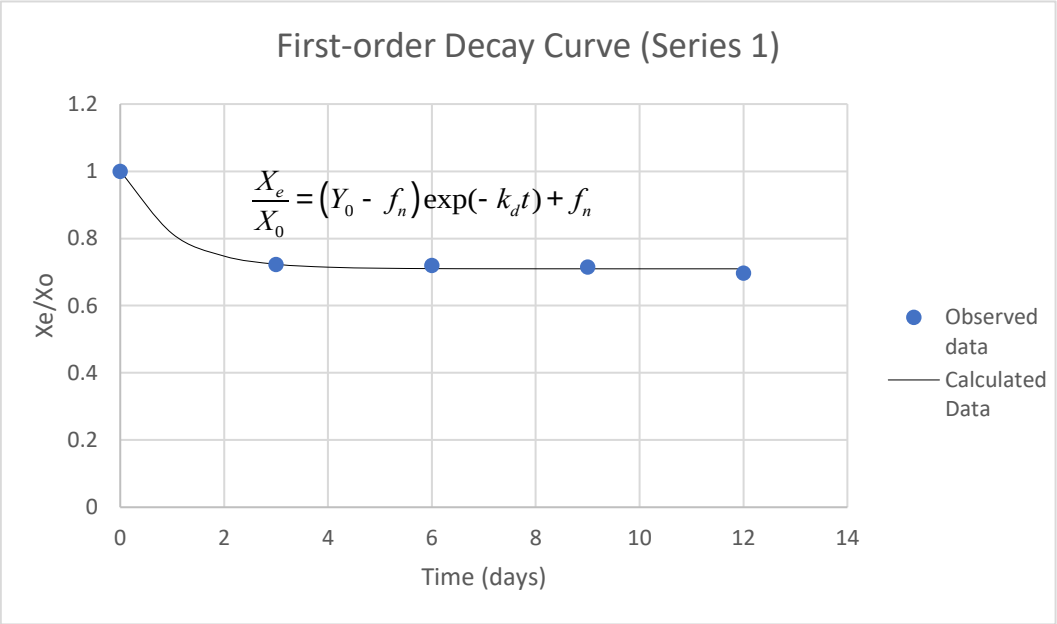


Figure 35. Non-linear regression analysis for Series 1.

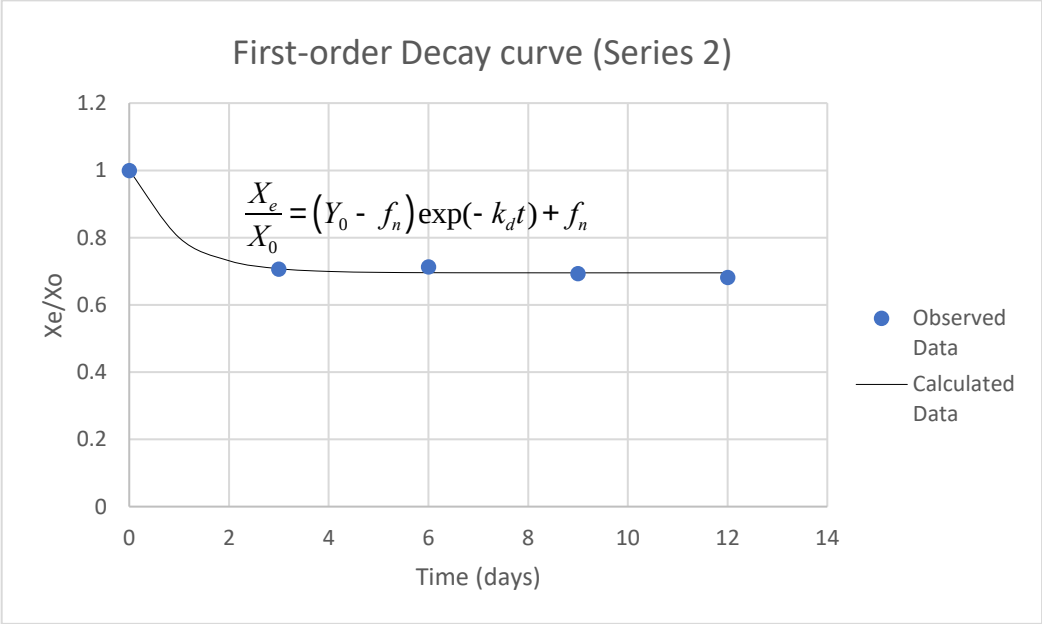


Figure 4. Non-linear regression analysis for Series 2.

For Series 1, 28% of the total solids in the anaerobic digester was consumed within the first three days. From day three to day 12, the fraction of solids remained constant and 71% of solids still remained in the digester. For Series 2, 29% of the organic matter in the anaerobic digester was consumed within the first three days. Similar to Series 1, the fraction of solids remaining began to plateau after day three and 70% of solids remained in the digester.

7. Conclusions and Recommendations

In this chapter, the results of each experiment are discussed as well as methods recommended to improve the overall process.

7.1 Conclusions

The goal of experiment one was to find the optimal parameters for maximum cell wall destruction using electrochemical disinfection. Throughout the experiment two trials were performed, one operating in constant voltage mode and the other in constant current mode. It was determined that for optimal destruction in either mode, the algal concentration be between 200-300 mg/L. Through the successive performance of each trial, it was determined that constant current mode was the better mode of operation as it provided a smaller detention time and more cell wall destruction.

Experiment 2 was performed to determine if cell wall destruction had an impact on the decomposition rate of algae. To do so, total solids were measured at three day intervals. The results of anaerobic digestion under conditions in which the algal medium did not undergo electrochemical disinfection (Series 1) were compared with the algal medium that succeeded electrochemical disinfection (Series 2). Through a non-linear regression analysis there was a slight difference in best-fit values, with Series 1 being 0.996 and Series 2 being 0.994. The calculated plateau indicated that the amount of non-biodegradable matter present in Series 1 was 71% and 70% for Series 2. This non-biodegradable matter could be due to various parameters such as the $MgSO_4$ added to each of the Series, algal cell walls, or chemicals added to the synthetic medium. The kinetic constant for Series 1 was calculated to be 1.02 while the kinetic

constant for Series 2 was 1.07. The probability value (P-value) was determined using the t-test function in excel and TS measurements over the 12-day period for Series 1 and Series 2. The calculated value was P = 0.93, which is significantly higher than 0.05. Given these values, it cannot be concluded that measured TS values are not significantly different for each series; therefore, this experiment did demonstrate that the destruction of algal cell walls changes the rate of decomposition by anaerobic digestion.

7.2 Recommendations

It is recommended that experiment one, and successively experiment two, be performed with the effluent from a HRAP to obtain results that would more closely resemble real-life conditions. The algae used for this research was collected in a clump and consisted of mostly filamentous algae, which is not ideal for the EC batch reactor. Secondly, it is recommended to use an electrochemical continuous flow reactor to prevent settling inside the reactor. This may also allow for operation under constant voltage mode, which is a better more of operation as it does not significantly increase the temperature of the solution. It also may be beneficial to use a continuous flow reactor since most of the reactions in an EC reactor take place near the electrodes. This would ensure that all of the liquid and, hence, algae, would come into contact with the electrodes (Kraft, 2008). Lastly, to determine the most successful mode of operation, it is recommended to work with one control volume instead of two different volumes. This way, the concentration of $MgSO_4$ added to the medium would determine if constant current or constant voltage mode was applied.

Experiment two was performed under unstable conditions where oxygen leaks may have occurred, so it is recommended that a more secure method of achieving anaerobic conditions be

adopted. Also, it would be highly beneficial to measure methane production as an additional parameter to determine how cell wall destruction affects anaerobic digestion. Finally, a separate digester of sludge should incubate to guarantee that methanogenic bacteria are abundant and active. It is recommended to then obtain a seed of sludge for the anaerobic digestion experiment from this batch of sludge.

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Appendix

Appendix A

A. Preparation of synthetic medium

Laboratory equipment:

OHAUS analytical balance, magnetic stirrer, Fischer Scientific Thermo® Stirrer Model 120S magnetic stirrer, 25-mL graduated cylinder, aluminum dish, 1-L beaker.

Reagents:

Bristol's algae media concentrate 100x (Flinn Scientific), EM Calcium carbonate GR 500 g, EMD® Sodium bicarbonate GR ACS 500 g, Deionized water.

Procedure:

10-mL of Bristol's algae media concentrate 100x was measure in a 25-mL graduated cylinder and subsequently added to a 1-L beaker. The beaker was then filled with deionized water to the 1-L mark. Bristol's medium provided insufficient alkalinity when compared to the alkalinity of the wastewater at Marrero WWTP located in Marrero, Louisiana (De Grau, 2015); therefore, 20 mg of $CaCO_3$ and 250 mg of $NaHCO_3$ were added. These chemicals were weighed on the OHAUS analytical balance on an aluminum dish. the 1-L beaker was placed on the stir plate with the magnetic stirrer and mixed at a sufficient speed until homogenous.

B. pH, Conductivity and Temperature

Laboratory equipment:

Thermo Scientific Orion 5 Star™ Plus Meter, Orion 8157BNUMD ROSS Ultra pH/ATC triode, Fischer Scientific Thermo® Stirrer Model 120S magnetic stirrer, 100-mL flasks, ORION 0133005MD Conductivity Cell, magnetic stirrer.

Reagents:

Hydrion buffer solutions 4, 7, and 12.

Procedure:

First, the pH meter was calibrated. While the arrow icon was pointing on pH, the "calibrate" button was pressed. The pH electrode was rinsed with deionized water and dried. The electrode was then placed in a 100-mL with 100-mL of deionized water and the 4.0 buffer. Once the pH meter read the correct pH, the calibrate button was pressed again. This step was repeated for buffer solutions 7 and 12. When taking actual readings using the pH meter, it is important to rinse the electrode before and after with deionized water and return it to the provided storage solution from the manufacturer. To measure a sample, it was placed in a beaker with a magnetic stirrer and placed on a stir place. The mixture should be mixed at all times when recording the pH. After placing the electrode in the solution, the "measure" button was pressed. The recorded value should be taken when the arrow on the screen stops blinking.

In the same manner as pH calibration, the arrow icon must be pointed to the conductivity icon before "calibrate" is pressed. Before placing the conductivity probe in the 1000 μS/cm and 10,000 μS/cm calibration standards, each probe was rinsed with deionized water and dried. The procedure with calibrating and measuring the conductivity of samples is the same as with pH calibration.

C. Alkalinity

Laboratory Equipment:

Thermo Scientific Orion 5 Star™ Plus Meter, Orion 8157BNUMD ROSS Ultra pH/ATC triode, Fischer Scientific Thermo® Stirrer Model 120S magnetic stirrer, 100-mL flask, 50-mL burette, magnetic stirrer, burette holder.

Reagents:

HACH, Cat, 20353, Sulfuric Acid Standard Solution 0.020N, 1000-mL, HACH Permachem Reagents, Cat. 94399 Pk/100, Bromcresol Green-Methyl Red Indicator Powder.

Procedure:

40-mL of sample was poured into a 100-mL flask with a stir bar inside. The flask was placed on the stir plate and continuously mixed. A known volume of sulfuric acid standard solution was poured into the burette held in place above the flask. The calibrate pH meter was carefully fixed in the flask to measure the pH throughout the procedure. One bromcresol green-methyl red indicator pillow was added to the sample, then the same was titrated very slowly until the desired indicated color (pink) and pH (4.5) was achieved. The volume of acid spent was recorded. Equation 5 gives the alkalinity in mg/L as $CaCO_3$:

$$Alkalinity\ \left(\frac{mg}{L}as\ CaCO_3\right) = \frac{V*N}{Sample\ Volume} * 50000\,\frac{mg\ CaCO_3}{eq} \qquad \textbf{(Eq. 5)}$$

where V= volume of titrant spent (mL) and N=0.02.

D. Calcium

Laboratory Equipment:

Thermo Scientific Orion 5 Star™ Plus Meter, Orion 8157BNUMD ROSS Ultra pH/ATC triode, Fischer Scientific Thermo® Stirrer Model 120S magnetic stirrer, 100-mL flask, 50-mL burette, magnetic stirrer, burette holder.

Reagents:

HACH, Cat. 205-53, TitraVer (EDTA) Standard Solution 0.010 M (0.020N), HACH, Cat. 282-32H, Potassium Hydroxide Solution 8 N, HACH Permachem Reagents, Cat. 85299 Pk/100, Calver 2 Calcium Indicator Powder

Procedure:

50-mL of sample was poured into 100-L flasks with a magnetic stirrer inside. The flask was placed on the stir plate and continuously mixed. A known volume of EDTA standard solution was poured into the burette held in place above the flask. The calibrate pH meter was carefully fixed in the flask to measure the pH throughout the procedure. The pH was adjusted to a value of 10 by adding KOH. Then, one CalVer 2 Calcium indicator pillow was added to the sample, then the same was titrated very slowly until the desired indicated color (pure blue) was

achieved. Volume spent was recorded. Calcium is calculated by multiplying the amount of spent titrant used by 20.

E. Total Solids

Laboratory Equipment:

Oven, desiccator, OHAUS analytical balance, aluminum dish, graduated cylinder, Fischer Scientific Thermo® Stirrer Model 120S magnetic stirrer, 100-mL flask.

Procedure:

First, an aluminum dish was placed in an oven set at 105ºC for one hour. The dish was the placed in a desiccator to allow it to cool to room temperature. Once cool, the dish was weighed on an analytical balance and the measurement was recorded to the nearest 0.1 mg (value B). Next, the sample was placed on a stir plate with a magnetic stirrer to obtain a homogenous mixture. When well-mixed, 50 mL of the sample was collected using a 50-mL graduated cylinder. The sample was placed in the pre-weighed aluminum dish and placed in the oven for 6 hours at 105 ºC. Once complete, the dish was placed in the desiccator until room temperature was reached. The dish was weighed on the analytical balance to the nearest 0.1 mg (value B). Equation 6 shows the calculation to get TS in mg/L.

$$\frac{[(A-B)*1000]}{50\ mL} \quad \textbf{(Eq. 6)}$$

F. Cell Wall Destruction

Laboratory Equipment:

Ecolotron reactor, BK Precision High Current DC Regulated Power Supply, Model 1791, ENERPAC P39 hydraulic jack, OMANO OMFL400 fluorescence compound microscope, Jenoptik ProgRes CapturePro 2.5 camera, Fischer Scientific, Fischerfinest Premium Cover Glass, VWR VistaVision microscope slides, electrodes, spacers, pipettes, aluminum tray, 250-mL flasks, 1-L beaker, funnel.

Reagents:

EMD® Magnesium Sulfate GR, Powder, 500 g, algae medium

Procedure:

First, algae were obtained from Bridge City WWTP in Bridge City, LA. Fragments of the algae and deionized water were blended together to form a concentrated mixture. By optical observance, the concentrated algal suspension was pipetted into a beaker of deionized water until a sufficient amount appeared to be in the beaker. This was determined by the settling characteristics of the algae since it is not desirable for settling to occur in a batch reactor. Once this was determined, TS was measured to determine the exact concentration of algae, then the remaining ingredients to prepare the medium were added. Although this is a complicated approach, it was the best way to incorporate live algae into the medium. Next, 2g/L of $MgSO_4$ were measured on an analytical balance and stirred until completely mixed. At this point, the medium was ready to undergo EC.

From right to left, 14 spacers (for the hydraulic jack to reach and exert pressure to seal the reactor completely), one film with an inlet tube, one electrode, two spacers, another electrode, and a plugged film were all place inside the reactor. 250-mL of sample was measured

into a flask and subsequently placed in the reactor by a funnel through the inlet tube. The power supply was connected to each electrode via clamps. Two different modes of power were supplied using the BK Precision 1791: CV mode and CC mode.

- CV mode

  The “POWER ON” switch was pressed and the “OUTPUT ON/OFF” switch was kept in the OFF position. Then, the “LIMIT” button switch was pressed and the voltage was adjusted; after that, the “OUPUT” switch was pressed to ON position and the CV LED light turned on.

- CC mode

  The power supply was turned off, a short circuit in the output terminals of the power supply was done and then the supply was turned on. Then the “OUTPUT ON/OFF” switch was kept in the OFF position, the “LIMIT” button switch was pressed and the current was adjusted; after that, the “OUPUT” switch was pressed to ON position and the CC LED light turned on; finally, the short circuit was removed.

Each sample was allowed a charge time of 30 seconds. When the desired retention time was accomplished, the hydraulic jack was released and the sample was collected in an aluminum dish and transferred to a beaker. The samples were all examined under a microscope to ensure cell wall destruction occurred. Pictures using the Jenoptik camera were taken.

G. Anaerobic Digestion

Laboratory Equipment:

Eight 250-mL flasks, shaking water bath, rubber stoppers, tubing, various glassware, pipette.

Reagents:

Medium will cell wall intact (Series 1) and medium collected after EC (Series 2), sludge seed, CO_2.

Procedure:

After EC was achieved, 2 mL/L of primary sludge seed (obtained from East Jefferson WWTP) were inoculated into Samples 1 and 2. Next, each sample was divided equally among eight 250-mL flasks (4 flasks for each sample) and filled to the 200-mL mark. Before tightly sealing each flask with a one-hole rubber stopper, CO_2 was blown into the flasks to displace any oxygen. This was done to ensure anaerobic conditions. Once complete, a one-hole rubber stopper with a fitted flexible tube was placed on each flask. Other flasks were filled with CO_2 and closed with a rubber stopper that allowed for the end of the tubes from the sample flasks to be fitted in and another tube with a small diameter fitted outward. This was to further ensure anaerobic digestion conditions. The flasks were placed in a shaking water bath filled with enough water to cover the medium in the flasks. The temperature of the water was set at 35°C and a speed of 90 rpm was set to enable mixing. This reaction occurred in complete darkness for 12 days. Every three days a flask from each sample was removed and total solids were measured.

H. Temperature Test

Laboratory Equipment:

Magnetic/hot plate, magnetic stirrer, thermometer, microscope, VWR VistaVision Microscope Slides, OMANO OMFL400 Fluorescence Compound Microscope, Fischerfinest Premium Cover Glass, 2-mL pipette.

Reagents:

Prepared medium, algae.

Procedure:

A temperature test was carried out to ensure that cell wall destruction was not due to increased temperatures resulting from EC. A prepared concentration of algal medium was heated on a hot plate until a temperature of 45°C was achieved. The samples were then pipetted on to the microscope slides, covered with microscope slips and examined under the microscope. No changes in cell wall formation were found to be due to a temperature increase to 45°C.

Appendix B

A. Experimental Results

The results obtained from measuring TS at days 0, 3, 6, 9, and 12 were recorded into excel. The calculated values were obtained using the first-order decay equation (Equation 7) shown below.

$$\frac{X_e}{X_0} = \left(Y_0 - f_n\right)\exp(-k_d t) + f_n \qquad \textbf{(Eq. 7)}$$

Below is Table 11 generated for Series 1 (intact cell wall)

Table 11. Data for Series 1.

		y_i	f_i		
Day	TS (g/L)	X_e/X_o	X_e/X_o calc.	$(y_i-f_i)^2$	$(y_i-y\_bar)^2$
0	4.656	1	0.99997982	4.07248E-10	0.052615847
1			0.814512609		
2			0.747541832		
3	3.364	0.722508591	0.723359201	7.23537E-07	0.002314569
4			0.71462704		
5			0.711473924		
6	3.348	0.719072165	0.710335358	7.63318E-05	0.002657031
7			0.709924231		
8			0.709775776		
9	3.328	0.714776632	0.70972217	2.55476E-05	0.003118321
10			0.709702813		
11			0.709695824		
12	3.244	0.696735395	0.7096933	0.000167907	0.005458722
	y_bar=	**0.770618557**	**SUM =**	**0.000270511**	0.066164488

Solver was used to set the sum of column 5 equal to zero by changing the parameters Y_o, K_d, and f_n. Table 12 gives the values obtained after using solver:

Table 12. Values for solved parameters (Series 1).

Y_o=	0.99997982
k_d =	1.018621737
f_n=	0.709691873

Where Y_o is the calculated initial concentration at day 0, k_d is the rate constant, and f_n is the fraction of non-biodegradable matter remaining.

Equations 8, 9, and 10 were used to calculate the R^2 value:

$$SS_{\text{tot}} = \sum_i (y_i - \bar{y})^2, \quad \textbf{(Eq. 8)}$$

$$SS_{\text{res}} = \sum_i (y_i - f_i)^2 = \sum_i e_i^2 \quad \textbf{(Eq. 9)}$$

$$R^2 \equiv 1 - \frac{SS_{\text{res}}}{SS_{\text{tot}}}. \quad \textbf{(Eq. 10)}$$

The same procedure was done for Series 2 and Table 13 shows the values.

Table 13. Data obtained for Series 2.

		y_i	f_i		
Day	TS (g/L)	X_e/X_o	X_e/X_o calc.	$(y_i-f_i)^2$	$(y_i-y\_bar)^2$
0	4.684	1	0.999972598	7.50852E-10	0.058200017
1			0.799947498		
2			0.731211672		
3	3.308	0.706233988	0.707591567	1.84302E-06	0.002758268
4			0.699474849		
5			0.696685652		
6	3.338	0.71263877	0.695727184	0.000286002	0.002126541
7			0.69539782		
8			0.695284638		
9	3.246	0.692997438	0.695245745	5.05488E-06	0.004323821
10			0.69523238		
11			0.695227787		
12	3.194	0.681895816	0.695226209	0.000177699	0.005907058
	AVG=	0.758753202	SUM=	0.0004706	0.073315705

And the values obtained for Y_o, k_d, and f_n are shown in Table 14.

Table 14. Parameters solved for Series 2.

Y_o	0.999972598
K_d	1.068172312
f_n	0.695225383

Vita

Jessica Renee Simpson was born in DeRidder, Louisiana in November of 1992. She attended Louisiana State University and graduated in 2016 with her bachelor's degree in Biological Sciences and a concentration in Marine Biology. A few months after graduating, she began attending the University of New Orleans and pursued a Master's degree in Civil and Environmental Engineering. Here, she came under the supervision of Professor Enrique La Motta and his research efforts.