In the UK, about 11 billion litres of wastewater is treated daily in about 9000 sewage treatment works. Approximately 7700 gigawatts hour of energy is required to treat this waste. Liquor from sludge dewatering processes during sewage treatment holds a lot of potential for clean energy generation. Returning this liquor to the treatment plant increases the nutrient load of the system, thereby adding to the energy and operating cost requirement of the treatment works. The ability of Microalgae to be used as a treatment option for nutrient removal from liquor have been demonstrated in this book; thereby saving cost incurred in treating return liquor (liquor from sludge dewatering processes) by wastewater treatment plants. This book also shows how Microalgae biomass can be generated from sludge liquor for use in energy generation through anaerobic digestion. Therefore while cost of treatment is being reduced, energy can also be generated, making the entire treatment process economically viable.

Cynthia Okoro-Shekwaga is a commonwealth scholar. She had her B.Eng in Agricultural and Bioresources Engineering. She graduated top in her class during her Master's programme in Environmental Engineering and Project Management at the University of Leeds, UK in 2014. She has been involved in a number of researches in 'Waste to Energy' technologies.

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DEDICATION

This research is dedicated to my husband Chebawaza Shekwaga for his love, support and words of encouragement throughout my masters' programme.

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This research work is a success today because I had the support and professional guidance of some people. For this reason, I wish to acknowledge the following people;

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ABSTRACT

The increase in world population resulting in increased global energy demand has consequently led to the extensive use and global depletion of fossil fuel sources. Environmental menaces resulting from the extensive use of fossil fuel as primary energy; such as the accumulation of anthropogenic carbon dioxide in the atmosphere giving rise to global warming stimulates the need for clean energy systems. Liquor from sludge dewatering processes holds a lot of potential for clean energy generation. Returning this liquor to the treatment plant increases the nutrient load of the system, thereby adding to the energy and operating cost requirement of wastewater treatment plants (WWTPs).

In this research, *Chlorella vulgaris* (*C.vulgaris*) strain of the microalgae was cultivated in sludge liquor to achieve three purposes; treat sludge liquor, generate biomass and generate energy. *C.vulgaris* was first cultured in Bolds Basal Media (BBM) and the after four days (during exponential growth phase) it was extracted and cultivated in liquor samples. All culture and cultivation was done using cotton plugged conical flasks. Liquor was obtained from four sludge samples using different chemical treatments. The liquor obtained was then used to cultivate *C.vulgaris.* Sample1 was obtained from sludge conditioned with zetag66 polyelectrolyte at 0.6g/l sludge, sample2a was obtained using 27.5g lime/l sludge, sample2b was obtained using 1.28g lime/l sludge and lastly sample3 was obtained using 35g ferric chloride/l sludge. Each sample was then characterised and necessary dilutions were made and used to cultivate *C.vulgaris.* 50ml of *C.vulgaris* was used for each media and the ratio of *C.vulgaris* : liquor was 5:1, 1:1, 5.6:1 and 10:1 for media1, media2a, media2b and media3 respectively. At the end of a 14 day cultivation period, the following results were obtained;

- Ammonia and phosphate removal of 97.4% and 91.5%, 84.2% and 89.3%, 88.9% and 88.3%, and 91.1% and 83.3% for media1, media2a, media2b and media3 respectively.
- Biomass yield of 1.36kgDS/m^3 with 65%VS, 2.94kg/m^3 with 24%VS, 1kg/m^3 with 72%VS and 0.87kg/m^3 with 60%VS was obtained from media1, media2a, media2b and media3 respectively.
- The energy and financial worth of 1m^3 of each media was estimated based on the methane potential of *C.vulgaris* biomass obtained. Therefore media1 was worth 3.07kWh about £1.12, media 2a and 2b 2.51kWh about £0.91 each and media3 1.81kWh about £0.66.
- Additional digestate of 0.83kgDS, 2.51kgDS, 0.57kgDS and 0.56kgDS can be generated from media1, media2a, media2b and media3 respectively with ammonia within the range of 24.70 – 47.52 mgNH_3-N/m^3media.

Therefore growing microalgae in sludge liquor will help to meet stringent effluent standards by WWTPs and generate income. The ammonia and phosphate required for the cultivation are readily available in the dewatered sludge liquor, but for liquor from chemical treatment process, the nutrients might be insufficient. Dewatering liquor from raw sludge therefore provides a suitable option for energy generation using *C.vulgaris* and also saves the operating cost associated with the use of chemicals and aeration by conventional treatment methods.

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CHAPTER ONE

1. GENERAL INTRODUCTION

1.1 GLOBAL ENERGY DEMAND

The world of engineering is transiting from the use of fossil fuel for energy generation to the use of products from natural biological processes. This is influenced by the global need to reduce greenhouse gas emissions to the atmosphere, with the advent of global warming.

Energy is a primary need for humans. For decades, fossil fuel has been the primary energy source by humans. In the International Energy Outlook 2013 (IEO2013) by the US Energy Information Administration, it was reported that the global energy consumption will increase by 56 percent between the year 2010 and 2040 (Sieminski, 2013). It was also projected that the global energy use will rise from 524 quadrillion British thermal units (Btu) in 2010 to 630Btu in 2020 and 820Btu in 2040. This increase is as a result of fast growing economies especially in non-OECD (Organization for Economic Co-operation and Development) countries and increasing population (Sieminski, 2013).

1.1.1 ENERGY SOURCES AND GREENHOUSE GAS EMISSIONS

Fossil fuel was recorded to account for 88 percent of global primary energy consumption in 2008 (Singh *et al.*, 2011). Although renewable energy and nuclear power are both increasing by 2.5 percent annually; making them the worlds' fastest growing energy sources (Sieminski, 2013), a large fraction of the worlds' primary energy is still from fossil fuel as only 13 percent of the global energy demand is met by renewable energy (International Energy Agency, 2012). The IEO2013 projects 80 percent dependence on fossil fuel as a source of primary energy through 2040 (Sieminski, 2013). With the ever growing world population and consequent economic development, an increase in primary energy demand of about 1.5 to 3 times the current demand is expected by 2050 (Stambouli and Traversa, 2002). This pressure

exerted on fossil fuel resources has consequently resulted in a reduction in the amount available to meet global energy needs (Ahmad *et al.*, 2013; Sacasa Castellanos, 2013; Singh *et al*., 2011).

The use of fossil fuel also influences the increasing levels of anthropogenic CO_2 accumulated within the atmosphere (Fon Sing *et al.*, 2013; Parmar *et al.*, 2011; Sacasa Castellanos, 2013; Singh *et al.*, 2011; Stambouli and Traversa, 2002). CO_2 levels have been estimated to increase from 280ppm in the preindustrial times to 350ppm currently, with 1.8ppm annual growth rate (Li *et al.*, 2014). Global energy-related CO_2 emissions is expected to increase by 46 percent in 2040, reaching an approximate of 45 billion metric tons by 2040 (Sieminski, 2013). The increasing levels of CO_2 in the atmosphere has been identified as the major contribution to global warming (Li *et al*., 2014).

The need for sustainable energy from other sources with minimal CO_2 emission to the environmental is therefore imperative (Fon Sing *et al*., 2013; Stambouli and Traversa, 2002; Wang *et al*., 2010).

1.1.2 MICROALGAL BIOMASS

Biofuel generation were first achieved from food and oilcrops (such as maize, sugarcane, rapeseed, wheat) and also animal fats (Singh *et al*., 2011) and are termed first generation biofuels. The competition imposed on food and fibre production for arable land, fertilizer and water demand raised criticisms on the generation and use of such fuels. These led to the production of second generation biofuels from lignocellulosic materials such as waste residues and can be grown on abandoned land, thereby improving rural development and economic conditions in developing countries (Singh *et al*., 2011). Biomass supply to the commercial plant and cost effective conversion technologies available poses some barriers to the production of second generation biofuels (Singh *et al*., 2011). The need therefore for biofuel production with minimal land use, low cost and environmental

improvement by CO_2 sequestration gave rise to the exploitation of microalgae for biofuel production. The third generation technologies therefore focused on microalgae or cyanobacteria, containing high oil contents and grown in ponds (Singh *et al.*, 2011). The microalgae have been reported to be the only oil plant that can conveniently displace fossil fuel oil (Singh *et al.*, 2011).

Microalgae are single-cell, photosynthetic organisms (Stephenson *et al.*, 2010), with rapid growth rate and high energy content (Singh *et al.*, 2011; Stephenson *et al.*, 2010). The approximate molecular formula of the microalgae, $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ shows that minimal nutrient is required for cultivation (Chisti, 2007). Microalgae in contrast to fossil fuel can fix CO_2 in the atmosphere through photosynthesis, therefore reducing CO_2 levels (Syed Shabudeen *et al.*, 2013; Wang *et al.*, 2010) and the use of biofuels or biogas from algae sources does not add to the CO_2 in the atmosphere, these makes the algae a suitable option for renewable energy production and CO_2 sequestration.

Microalgae production for energy has a number of advantages over other energy crops;

a) Microalgae has high growth rate; doubling its biomass within 24 hours (Singh *et al.*, 2011; Chisti, 2007). Chisti, (2007) reported that microalgae doubling time can be as low as 3.5 hours during its exponential growth phase.

b) Microalgae can be cultivated on abandoned non-arable land, thereby eliminating competition with food crops (Stephenson et al., 2010; Parmar et al., 2011)

c) Microalgae fixes CO_2; 50% of the microalgal biomass is carbon by dry weight, which comes from CO_2 (Becker, 1994). For 1kg of microalgal biomass, 1.83kg of CO_2 is required (Chisti, 2007), CO_2 from flue gas plants and other industrial could be used as a carbon source (Stephenson *et al.*, 2010).

d) Freshwater, seawater, brackish water and wastewaters can be use to grow microalgae as a source of nutrients required (nitrogen and phosphorus) (Parmar *et al.*, 2011); thereby reducing inputs such as fertilizer and also enhancing wastewater treatment from sewage treatment and other industrial plants (Stephenson *et al.*, 2010).

e) Also the biochemical composition of microalgae cells can be modified to suit desired outcomes such as high lipid contents, by varying the growing conditions (Stephenson *et al*., 2010).

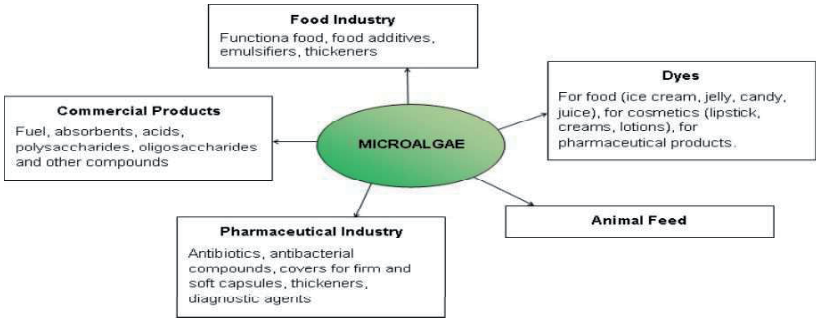


Figure 1. Commercial options for microalgae production (Sacasa Castellanos, 2013).

1.2 WASTE WATER TREATMENT IN UK

The water industry currently ranks fourth in terms of energy consumption (Caldwell, 2009). In the UK, 11 billion litres of wastewater is treated daily in about 9000 sewage treatment works (DEFRA, 2002). Approximately 7700 gigawatts hour of energy is required to treat this waste, accounting for about 1% of the daily energy consumption in the UK (England and Wales) (Parliamentary Office of Science and Technology, 2007). The final effluent is then discharged into estuaries, inland waters and the sea (DEFRA, 2002). Wastewater treatment works generate a considerable amount of sludge daily. This is usually paste-like, composed of approximately 5-6% solids.

1.2.1 SLUDGE TREATMENT

Currently in the UK about 1.2 megatonnes of dry solids of wastewater sludge is produced annually and approximately 6.5 megatonnes of dry soilds

by the EU as a whole (Garg, 2009). One of the most difficult problems faced with wastewater treatment plants (WWTPs) is how to conveniently dispose of the sludge (Garg, 2009). Sludge makes up the largest fraction of by-products from wastewater treatment (Garg, 2009). Generated sludge consists of organic and inorganic matter with 60 percent or less arising from the raw waste water and 40 percent or more grown during biological processes (Evans, 2011a). Sludge usually takes the form of liquid or semi-solid containing about 1 to 5 percent dry solids after gravitational thickening (Saveyn *et al.*, 2005) and up to 12 percent dry solids, depending on the dewatering process further used (Garg, 2009).

Sludge contains nutrients such as ammonia, potassium and phosphorus and can therefore be used on agricultural lands for soil enrichment. Due to the large amount of moisture in the sludge, the volume of the sludge is usually high, incurring high cost of transportation during transfer to agricultural land. Also following some sludge regulations and directives, such as the Urban Wastewater Directive, the sludge has to be treated in order to minimise adverse effects on the environment before spreading on land. For these reasons a number of approaches are used by different WWTPs to reduce volume thereby minimising handling and cost of transportation, destroy pathogens in the sludge and also reduce smell (Evans, 2011a). Sludge treatment processes include thickening, dewatering, biological treatment (anaerobic digestion and composting), and chemical stabilization with quicklime, thermal treatment and drying.

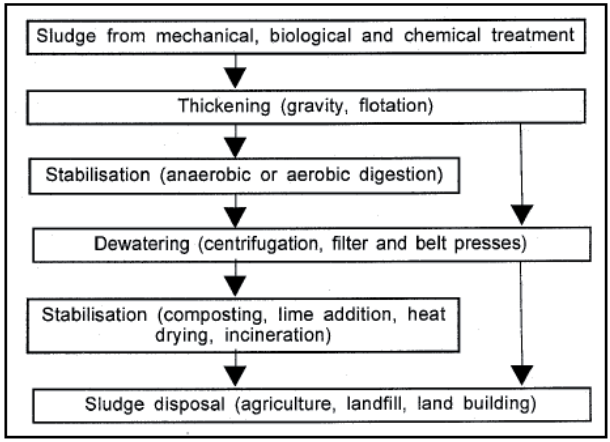


Figure 2. Different options for sludge handling (Hultman *et al.*, n.d.).

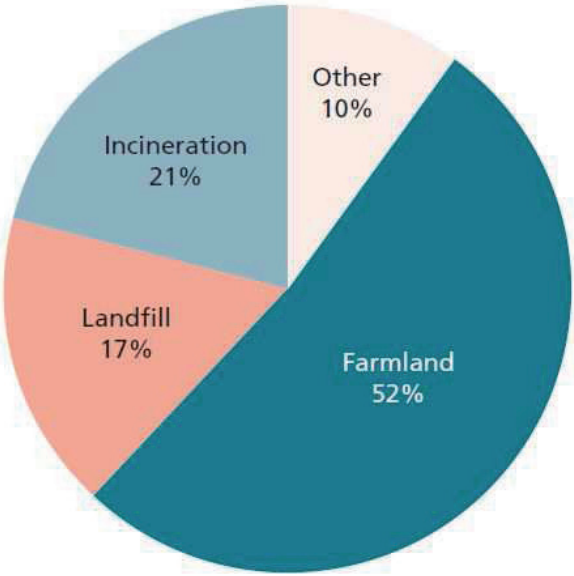


Figure 3. Sewage sludge disposal outlets 1999/00 (DEFRA, 2002).

1.2.2 LIQUOR TREATMENT

During sludge treatment liquor is produced; that is the reject water gotten from the treatment process. Some of the nutrients in the original sludge are then passed to the liquor. For many years treatment of this liquor has been a great problem in WWTPs. Because of the nutrient in the liquor it has to be treated before it can be disposed of to water bodies.

By convention sludge liquor is returned to the wastewater treatment works (Evans, 2011b; Ocansey, 2005) and removal of ammonia and phosphorus is achieved during side stream wastewater treatment (Boonchai *et al*., 2012). This causes an additional nutrient load (Demooij and Thomas, 2010) of up to 25 percent (Evans, 2011a, 2011b; Jardin *et al*., 2006) to 30 percent (Ocansey, 2005; Willie and Reitsma, 2011). In most cases, the initial design capacity of wastewater treatment plant is unable to treat this additional load; therefore expansion of the system (Demooij and Thomas, 2010; Evans, 2011b; Jardin *et al*., 2006). The high cost involved in treating sludge liquor when returned to treatment works, led to the decision by most wastewater treatment plants to adopt sustainable and less expensive methods for treating sludge liquor (Ocansey, 2005). Methods for separate treatment of sludge liquor and nutrient recovery have therefore become most suitable option (Jardin *et al*., 2006).

1.2.2.1 AMMONIA REMOVAL/RECOVERY

A separate treatment of sludge and digestate liquors is the best option for ammonia removal/recovery, in order to meet stringent effluent standards (Gustavsson, 2010). The conventional approach is by biological nitrification and denitrification (Ocansey, 2005). Other biological processes include nitritation/denitritation and deammonification. The recent approach is to use physico-chemical processes to recover ammonia solution (Evans, 2011b). This is the process of ammonia stripping, whereby ammonium first gets to be converted to slightly volatile gaseous ammonia which is readily soluble in

water and then the gas is physically stripped off (Jardin *et al*., 2006). These processes have been discovered to be more expensive than biological processes (Gustavsson, 2010), but are said to have lesser global warming potentials (Evans, 2011b). Ammonia solution have both agricultural and industrial uses (Evans, 2011a). Recovery of ammonia for use as fertilizer is therefore a definite move from disposal to recycling economy (Evans, 2011b).

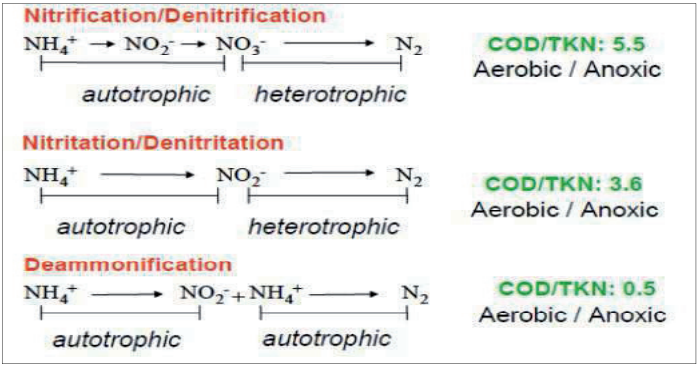


Figure 4. Biological processes for ammonia removal (Jardin et al., 2006)**.**

1.2.2.2 PHOSPHORUS REMOVAL/RECOVERY

Phosphorus removal technologies began in 1950s following eutrophication problems (Morse *et al*., 1998). Precipitation by metal salts such as calcium, aluminium, iron and magnesium was the first and has been the most widely used method for removing phosphorus from wastewater. Recently, the use of microorganisms such as microalgae and bacteria (de-Bashan and Bashan, 2004; Morse *et al*., 1998), crystallization technologies and technologies that extend chemical precipitation for nutrient removal are also available (Morse *et al*., 1998).

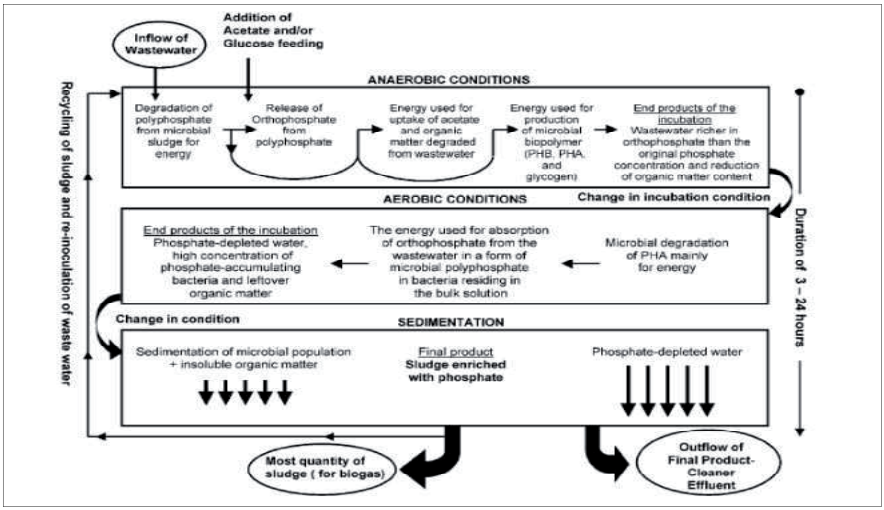


Figure 5. Biological phosphorus removal from wastewaters (de-Bashan & Bashan, 2004)**.**

1.2.3 MICROALGAE IN WASTEWATER TREATMENT PLANTS

The use of microalgae for wastewater treatment purposes began only in recent years (1970s) (Wang *et al*., 2010). The eutrophication of many waters from effluent discharges opened up the potential for microalgae to be used in treating wastewater (Wang *et al*., 2010). At the same time cultivation of microalgae for biomass production can be achieved from nutrients in the wastewater (Boonchai *et al*., 2012). More so, the microalgae can be used to destroy pathogens in the wastewater, by increasing the pH when CO_2 is consumed (Ahmad *et al*., 2013).

Table 1. Typical energy requirements from wastewater treatment plants (Caldwell, 2009)

Stage	Energy need (%)	Rank / comments
Inlet pumping and headworks	4.9	
Primary clarifier and sludge pumps	10.3	2
Activated sludge aeration	55.6	1 Main power use
Secondary clarifier and RAS	3.7	

Thickener and sludge pump	1.6	
Effluent filters and process water	4.5	
Solids dewatering	7.0	4
Tertiary treatment	3.1	
Heating	7.1	3
Lighting	2.2	

Approaches to generate energy from wastewater treatment is on the increase, between the years 2005-2006, water industry recorded renewable energy generation of about 6.4% of the energy required on site (Parliamentary Office of Science and Technology, 2007). The microalgae can be the most suitable option for nutrient removal in terms of energy savings. External oxygen supply for aeration used in biological nutrient removal, which constitutes the largest energy cost (as seen from Table1) can be eliminated, as the microalgae produces oxygen which can be used by other bacteria in the wastewater treatment plant for biomass (Ahmad *et al*., 2013).

This study shows the ability for microalgae to be used as cost effective sludge liquor treatment alternative (Figure 6). It also shows the amount of energy that can be generated from using this process, as the biomass produced can be harvested and used for other energy generation processes, such as the anaerobic digestion.

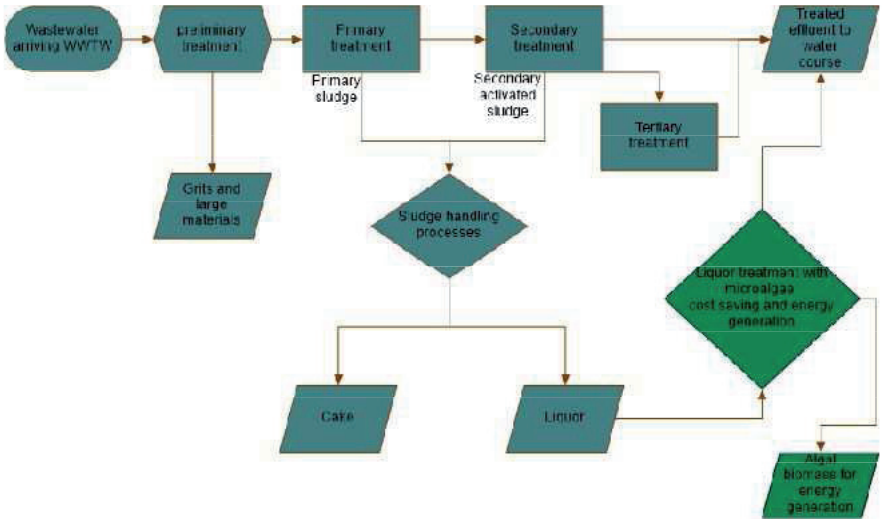


Figure 6. Microalgae as a suitable liquor treatment alternative to current approaches.

CHAPTER TWO

2. AIM AND OBJECTIVES

2.1 GENERAL RESEARCH AIM

This research work is part of a broad project aim for optimising the resources and energy recovery from organic waste (Figure 7).

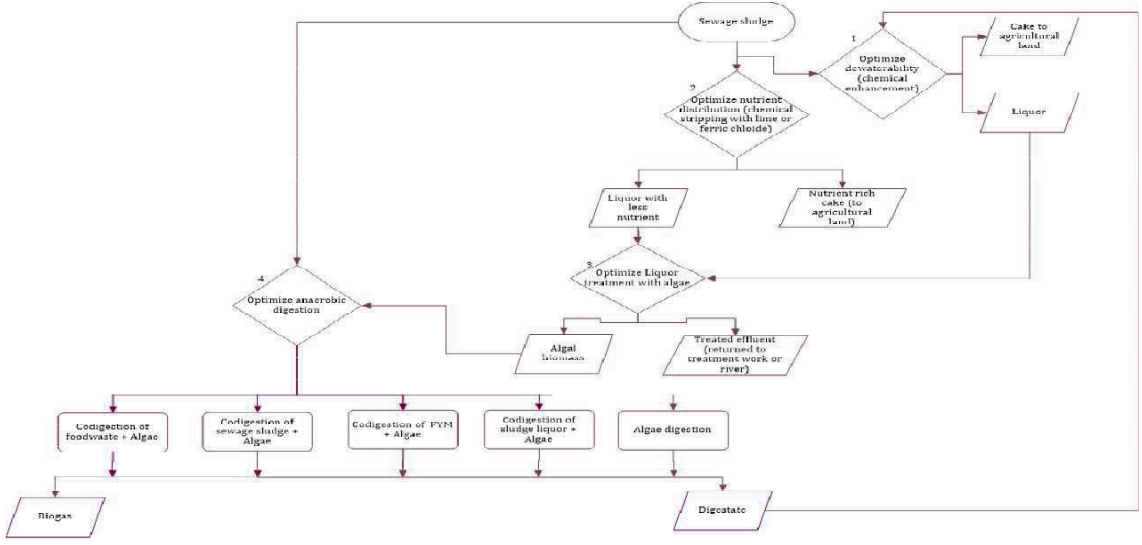


Figure 7. Broad aim of research study by a team of seven.

2.2 PERSONAL RESEARCH AIM

The aim of this study is to optimize microalgae (*Chlorella vulgaris*) for sludge liquor treatment, biomass production and energy generation from anaerobic digestion.

2.3 OBJECTIVES

The objectives of this study are;

1. To use *Chlorella vulgaris* in treating sludge liquor.
2. To grow microalgae on sewage sludge liquor, thereby maximising the nutrients in liquor for algal biomass production.
3. To determine the biomass yield of *Chlorella vulgaris* in sewage sludge liquor.
4. To measure the nutrient reduction effect from growing *Chlorella vulgaris* on sewage sludge liquor.
5. To determine the theoretical methane and energy yield of generated biomass from the liquor media.
6. To estimate the economic advantage of treating sewage sludge liquor using microalgae, over the conventional method of return to the treatment works.

2.4 SCOPE OF STUDY

This scope of this study (Figure 8) involves the cultivation of *Chlorella vulgaris* (henceforth referred to as *C.vulgaris)* using sludge liquor from different sludge pre-treatment processes (ammonia and phosphorus). It demonstrates the ability of *C.vulgaris* to be used as a treatment option for nutrient removal from liquor; thereby saving cost incurred in treating return liquor by wastewater treatment plants. This study also shows how C.*vulgaris* can be cultivated during sludge liquor treatment for use in energy generation by anaerobic digestion. Therefore while cost of treatment is being reduced, energy can also be generated, making the entire process economically viable.

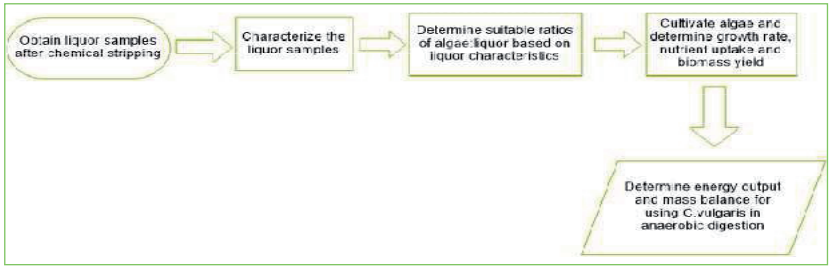
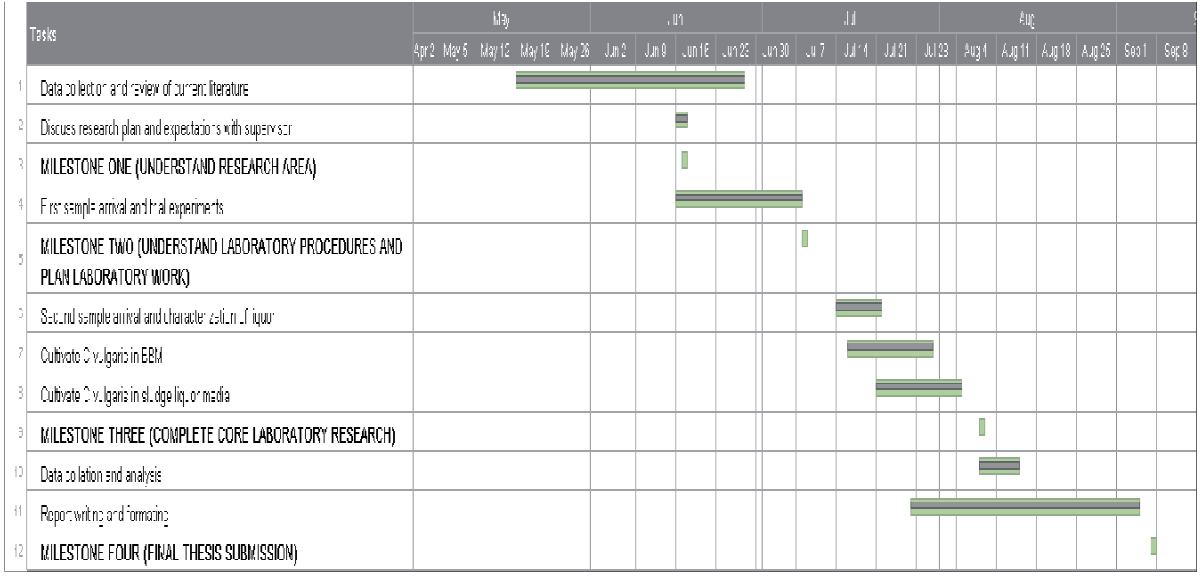


Figure 8. Scope of research

2.5 RESEARCH GANTT CHART



CHAPTER THREE

3. LITERATURE REVIEW

The need to generate energy with minimal greenhouse gas emissions has become important. The comfortable exploitation of fossil fuel resources for primary energy needs is fast becoming a burden, as these resources are fast diminishing and the environment at large is not any better, with increasing accumulation of carbondioxide. A number of renewable energy sources are now being exploited for environmental and economic sustainability. Although this is so, energy conversion, access and utilization of these renewable energy sources is still a major drawback (Singh *et al*., 2011). Renewable energy sources are mainly biomass, solar, wind, geothermal and hydropower energies. These sources also produce greenhouse gases and other pollutants, but only negligible amounts in comparison to the traditional fossil fuel sources (Demirbas, 2008).

3.1 ENERGY FROM BIOMASS

Biomass simply refers to all of earth's living matter. Biomass includes all plants and animals and any form of waste resulting from them (Demirbas, n.d.). Bioenergy is therefore renewable energy derived from biological (plant and animal) sources. Bioenergy comprises a wide range of energy fuels, derived from variety of materials with biological origin using numerous conversion methods to generate power, heat, gaseous biofuels and liquid biofuels (World Energy Council, 2013a). Traditional biomass includes agricultural residues, fuelwoods and charcoal used for lighting, space-heating and cooking in developing countries (World Energy Council, 2013a).

Biomass energy supply increased from 38 to 52EJ between 1990 and 2010 owing to increased energy demand and policies developed to increase the use of renewable energy (World Energy Council, 2013a). Biomass is at present the largest contributor of world renewable energy, with even more potentials to exploit for electricity, heat and vehicle fuels (World Energy

Council, 2013b). 10 percent of the global annual primary energy consumption is from biomass, majorly from municipal and agricultural residues, forestry and wastes (World Energy Council, 2013b).

3.2 THE ALGAE

The term algae refers to a collection of large variety of organisms, which are polyphyletic, mostly photosynthetic and have different origins (Domínguez, 2013; Stengel *et al.*, Connan and Popper, 2011). Algae are majorly aquatic plants (Myerson, 1976). Like every other plant, they contain chlorophyll and converts inorganic compounds to organic biomass through photosynthetic processes by means of light (Myerson, 1976). Algae are primary producers (Stengel *et al.*, 2011), although this makes them have similar ecological and biological functions to plants, their biochemistry and evolutionary background differs significantly from that of terrestrial plants (Kumari *et al.*, 2013; Myerson, 1976; Stengel *et al.*, 2011).

3.2.1 ALGAE VS. TERRESTRIAL PLANTS

Algae are not as specialized as terrestrial (higher) plants, lacking the elaborate transport and reproductive system that is common to most terrestrial plants (Myerson, 1976). In other words materials produced in one part does not readily move to other parts, therefore functions peculiar to leaves, stems and roots are not found in the algae (Myerson, 1976). Most of the higher plant structure serves mechanical purpose; root for anchor and extraction of food and water from the soil, leaves for exposure to sunlight for photosynthesis, and stems to provide support for the leaves and fruits (Myerson, 1976). Unlike the higher plants, algae in its entirety is nutritious, with very little of it used for indigestible purposes (Myerson, 1976). Therefore over 50 percent of protein is obtainable from the dry algae when grown under favourable conditions, compared to the higher plants (Myerson, 1976). As a result of the algal ability to survive extreme conditions, they produce an array of bioactive nutrients such as fatty acids and exotic acyl lipids, which are

generally not present in terrestrial plants (Kumari *et al.*, 2013). They are great sources of carbohydrates, proteins and lipids (Sankar and Ramasubramanian, 2012). They are therefore vital sources of food, fertilizers, animal feed, pharmaceuticals and much more (Stephenson *et al.*, 2010).

3.2.2 ALGAL DIVERSITY

Algae basically fall within the two domains of life which have extremely complex evolutionary interrelationships; the bacteria and eukaryotes (Stengel *et al.*, 2011). The algae comprise of diverse sizes and forms; from the colonial or unicellular microalgae to the multi-cellular, macro-algae (seaweeds and macrophytes) (Domínguez, 2013; Myerson, 1976). There is great diversity existing amongst the algae, which can be described based on their phylogenetic relationships or taxonomy, morphology, life stage, chemical diversity or the habitats they occupy (Stengel *et al*., 2011; Domínguez, 2013).

There are broadly two types of algae; macro-algae (also known as seaweed) that grow very large, about 60m in length and the micro-algae, which are more or less the most primitive and simple plants (Domínguez, 2013). Domínguez (2013) reported the total estimate of algae as 72,500, of which 20,000 are diatoms.

3.2.3 CURRENT RESEARCH ON THE ALGAE

For decades the potential of the algae have been investigated. A wide range of species of algae have been used for various research purposes such as food production, pharmaceuticals, agriculture, horticulture and renewable fuel production (Stengel *et al.*, 2011). In order to fully exploit the diversity and complex nature of algae, a profound understanding of environmental impacts (Domínguez, 2013) and other factors affecting the levels of bioactive compounds is important (Domínguez, 2013; Stengel *et al*., 2011).

However the microalgae have been more extensively used for many studies as a result of its ability to grow under a wide range of environmental

conditions. Microalgae has been used for many purposes such as transesterification to biodiesel (Stephenson *et al.*, 2010; Ahmad *et al.*, 2013), anaerobic digestion to biogas (Bohutskyi *et al.*, 2014), gasification to methane or hydrogen, pyrolysis gas or liquid fuels and also burning to generate heat and electricity (Stephenson *et al.*, 2010). Microalgae have also been used extensively in the medical sector for purposes like cancer treatment (Boopathy and Kathiresan, 2013), anti-inflammatory drugs (Abad, 2013), anti-oxidants (Silva, n.d.), and many more. In the environment and engineering sector, microalgae have been harnessed for heavy metal biosorption from aqueous media (Davis *et al.*, 2003; Liu *et al.*, 2009; Romera *et al.*, 2007).

3.2.4 MICROALGAE CULTIVATION

Microalgae are single-cell, photosynthetic organisms (Stephenson *et al.*, 2010), with rapid growth rate and high energy content (Singh *et al.*, 2011; Stephenson *et al.*, 2010). They are naturally found in benthic and littoral waters (Domínguez, 2013). Micro-algae have been the focus of research for biomass production for renewable fuels such as biodiesel, methane, biohydrogen and bioethanol (Yeh and Chang, 2012). Different media compositions have been proposed and used for growing microalgae, from investigations of the chemical environment of the natural occurring microalgae (Mandalam and Palsson, 1998). The purpose for which the microalgae are intended also influences the media composition.

3.2.4.1 MICROALGAE CULTIVATION REQUIREMENTS

A lot of factors affect the cultivation of microalgae both in the laboratory and large (industrial) scale. A major factor to consider is the algal growth rate. Different types of algae have different growth rates. The ideal selection of the algae will determine the resultant biomass yield. Other factors include the nutrient concentration in the media, CO_2, light intensity for photosynthesis, hydrogen potential (pH), temperature and mixing (Barsanti and Paolo, 2006).

3.2.4.1.1 CARBON DIOXIDE

Carbon dioxide is essential for microalgae growth and must be continuously fed during daylight hours (Chisti, 2007). As the microalgae biomass is 50% carbon by dry weight, it is important that CO_2 is supplied in sufficient quantity (Becker, 1994). The CO_2 can come from the atmosphere, flue gases from power plants or other fossil fuel combustion processes or from biological processes (Oilgae, 2013a). The amount of CO_2 used can be controlled by pH sensors, so the optimal pH is not exceeded (Chisti, 2007). Supplementary CO_2 addition is necessary for dense cultures, as the CO_2 from air (0.03% CO_2) introduced via bubbling is insufficient for microalgae growth (Barsanti and Paolo, 2006; Becker, 1994). CO_2 addition also helps to correct pH due to the CO_2/HCO_3^- balance.

3.2.4.1.2 NUTRIENT

The basic elements for microalgae cultivation are nitrogen, phosphorus, iron (and silicon in some cases) (Chisti, 2007). Excessive or deficient nutrients can both cause morphological and physiological changes in the microalgae (Rowley, 2010). Nitrogen is the second most important element required after CO_2, as it is about 10% of dry microalgae biomass (Becker, 1994). The forms of nitrogen consumed by microalgae are nitrate, organic urea and ammonia. Ammonia and urea are quite cheap and easy to metabolize by microalgae, but the nitrate-N have to be converted first to ammonia before it can be assimilated by the microalgae (Becker, 1994; Rowley, 2010). Therefore where both forms of nitrogen are present, the ammonia is first consumed, inhibiting the nitrate-N reduction (Rowley, 2010).

Phosphorus is the second most limited macronutrient for plant growth (Rowley, 2010). It is needed for almost all the cellular processes such as energy transfer and biosynthesis of nucleic acid (Becker, 1994). The concentration of phosphorus in aqueous habitat is growth limiting, but inorganic phosphate is the form of phosphorus consumed by microalgae

(Becker, 1994). Therefore phosphorus has to be supplied in excess as not all the phosphorus is bio-available for microalgae growth (Chisti, 2007). (Becker, 1994) reported that most algae can tolerate phosphorus within the range of 50µg/l to 20mg/l.

3.2.4.1.3 LIGHT

Light is what drives the photosynthetic reactions in microalgae, therefore the spectral quality, intensity and photo-period have to be given great consideration (Barsanti and Paolo, 2006). Light intensity varies with the depth and density of microalgal culture; at higher cell concentrations and depth, the light intensity should be increased for ideal penetration. Typically the light intensity employed is between 100 to 200 µE sec^{-1} m^{-2} that is about 5 to 10% of natural full daylight (2000 µE sec^{-1} m^{-2}) (Barsanti and Paolo, 2006). Light intensity should not be too high to avoid photo inhibition and excessive heat. This can be supplied naturally or with the use of florescent tubes either in the blue or red spectrum, which is the most active for photosynthesis (Barsanti and Paolo, 2006). The photo-period should be adjusted, as many microalgae does not grow well under constant light, as such light/dark cycle is employed usually 14:10 and 12:12, but should not exceed 16:8 (Barsanti and Paolo, 2006).

3.2.4.1.4 pH

The pH range adopted for most microalgae cultures is in the range of pH7 – 9, with an optimum range of pH8.2 – 8.7, although some microalgae species survive in more basic or acidic environments (Barsanti and Paolo, 2006). Depending on the species, the pH of the culture media can always be adjusted by aeration or the addition of CO_2

3.2.4.1.5 TEMPERATURE

Ideally the temperature used for microalgae cultures should be very close to that of the environment from which the microalgae was collected; <10˚C, 10 - 25˚C and >20˚C for polar, temperate and tropical environments

respectively (Barsanti and Paolo, 2006). Most microalgae species can tolerate a temperature range of 16 - 27˚C, typically 18 - 20˚C is used. The temperature should generally be maintained between 20 – 30˚C (Chisti, 2007; Oilgae, 2013a); below 16˚C, microalgae will experience poor growth and above 35˚C, most species die off (Barsanti and Paolo, 2006; Oilgae, 2013a).

3.2.4.1.6 MIXING

Adequate mixing is very important in order to ensure that all the microalgae cells are equally exposed to light and nutrients, to avoid sedimentation and thermal stratification (in open ponds) and to improve the exchange of gas between the media and the air (Barsanti and Paolo, 2006; Chisti, 2007). This can be achieved by bubbling air through the media from a compressor or air blower, which also serves as a CO_2 source, by continuous shaking or continuous stirring.

3.2.4.2 MICROALGAE CULTIVATION SYSTEMS

A number of technologies are available for algae cultivation, which includes open ponds, photo-bioreactors, fermentation tanks, hybrid systems and some combination of two or more of these systems (Oilgae, 2013b). The desired end product is a major factor for the type of system used (Oilgae, 2013b).

Autotrophic microalgae can be cultivated in open pond (raceway pond) or closed photo-bioreactors with the use of enriched CO_2 (Oilgae, 2013a). Flue gas from power plants, other fossil fuel combustion as well as biological processes can be good sources of the required CO_2. This makes the microalgae a good CO_2 sequestration and greenhouse gas reduction agent. Heterotrophic microalgae are usually grown in large fermenters with the use of starch or sugar in a similar fashion as the corn fermentation is done (Oilgae, 2013a). This system can be in form of batch cultures or continuous cultures.

3.2.4.2.1 AUTOTROPHIC SYSTEMS

- **OPEN SYSTEMS (RACEWAY PONDS)**

The open ponds can be grouped into natural lakes (lagoons, ponds and lakes) and artificial containers or ponds (Oilgae, 2013c). They are simple, requiring minimal production and operation cost. Not all algae can grow in these ponds, due to contamination by other algae and bacteria present. The temperature and amount of light are usually impossible to control (Oilgae, 2013c). The most commonly used systems include raceway ponds, circular ponds, circular big ponds and tanks. Raceway pond is the common term given to the ponds in which microalgae are cultivated. The microalgae, nutrients and water circulate around a racetrack, paddlewheels provide the flow. The microalgae remain suspended in the water and circulated back to the surface regularly (Oilgae, 2013c). For microalgae exposure to sunlight, the ponds are usually shallow, as the sunlight can only penetrate to a limited depth. CO_2 and nutrients are fed constantly to the ponds and the water containing algae is removed at the other end (Oilgae, 2013c).

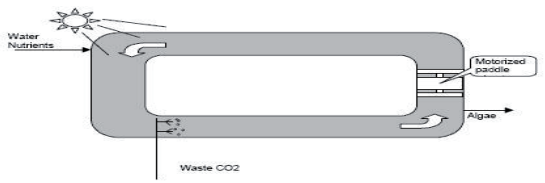


Figure 9. Raceway pond system (Oilgae, 2013c)

- **CLOSED SYSTEMS (PHOTOBIOREACTORS)**

Unlike the raceway ponds, these are closed systems with an inbuilt light source to grow the algae. It provides a controlled environment for microalgae growth, thereby enabling high productivity (Oilgae, 2013d). All requirements for growth are introduced into the system at a controlled rate. They therefore enhance better control of the culture environment such as water supply, exposure to light, CO_2 supply, optimal temperature, pH, mixing, and culture densities and so on (Oilgae, 2013d).

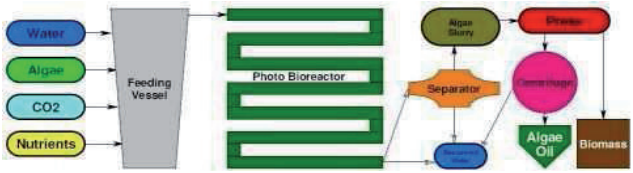


Figure 10. Working principle of the photo-bioreactor (Oilgae, 2013d).

There are different types of photo-bioreactors; tubular, column and flat plate photo-bioreactors. Although a better yield is obtainable from photo-bioreactors, they are more expensive to install and operate compared to open ponds (Oilgae, 2013d). Table 2 shows different cultivation systems with respective prospects and limitations.

Table 2. Different algae cultivation systems' prospects ad limitations (Ugwu *et al*., 2008).

Cultivation systems	Prospects	Limitations
Open ponds	Easy to clean up after cultivation, good for mass algae cultivation and relatively economical	Difficulty in growing algae for long periods, little control over culture conditions, poor productivity, large land area required, cultures are easily contaminated, limited to a few strains of algae
Flat-plate photobioreactors	Suitable for outdoor cultures, large illumination surface area, good for algae immobilization, good light path, relatively cheap, easy to clean up,	Difficulty in controlling culture temperature, some degree of wall growth, hydrodynamic stress possibility to some algae strain, scale up

	good biomass yield, low oxygen build up, readily tempered.	requires large compartments and support.
Tubular photobioreactors	Suitable for outdoor cultures, fairly good biomass yield, relatively cheap, large illumination surface area.	Fouling, some degree of wall growth, gradients of pH, dissolved oxygen and CO_2 along the tube, requires large land space.
Vertical-column photobioreactors	Good mixing with low shear stress, low energy consumption, high scalability potentials, good mass transfer, reduced photo-inhibition and photo-oxidation, readily tempered, easy to sterilize, good for algae immobilization	Sophisticated materials required for construction, small illumination surface area, decrease of illumination surface area with scale up, shear stress to algal cultures.

3.2.4.2.2 HETEROTROPHIC SYSTEMS

Heterotrophic systems provides opportunity for microalgae to completely metabolize in the dark, grow in an inexpensive and sterile media an also withstand hydrodynamic stresses (Agwa *et al*., 2013). The system eliminates the need for light source thereby reducing the cultivation cost, and the high biomass yield reduces harvesting cost (Agwa *et al*., 2013).

- **BATCH CULTURE**

This is a closed system where there is no exchange of elements between the media and the environment; the resources are finite (Barsanti and Paolo, 2006). Due to its simplicity and low cost, it is the most common used culture system. In a batch culture, the microalgae follow a growth path in a series of phases; lag phase, acceleration phase, declining log growth phase, stationary phase, accelerated death phase and log death phase (Becker, 1994). During the lag phase, microalgae tries to adapt to its new environment, with little or no growth observed. Once they are used to the culture environment, they multiply and grow exponentially (Becker, 1994) with the biomass doubling within 24 hours (Chisti, 2007). As the cell multiplies, light penetration reduces, therefore causing a log decrease in growth. Other factors such as build-up of toxic metabolite and reduction in amount of nutrients also influences this phase (Becker, 1994). Afterwards the microalgae stabilizes (stationary phase) with zero net growth. When nutrients in the culture become insufficient for cell growth and other growth inhibition factors set in, the cells begin to rapidly die off (death phase).

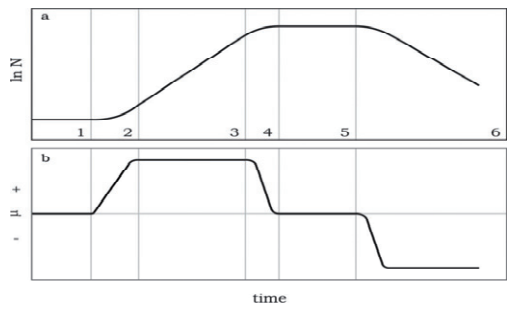


Figure 11. Microalgae growth profile in a batch culture (a) and the corresponding growth rate variations (b) (Barsanti and Paolo, 2006).

- **CONTINUOUS CULTURE**

This is a closed system with relatively infinite resources. In this system, nutrients are added to the culture media at a constant rate, with equal amount of cell culture removed in order to maintain a constant volume (Barsanti & Paolo, 2006). Regulated addition of fresh culture medium makes it possible to maintain the culture at a chosen point on the growth curve (Barsanti and Paolo, 2006). This helps to produce microalgae of a more predictable quality. There are two categories of continuous culture; turbidostat culture whereby when the cell density of the culture reaches a predetermined point, as measured extinction of light passing through the culture, then is the fresh medium introduced and a chemostat culture fresh medium is added to the culture at a steady predetermined flow rate (Barsanti and Paolo, 2006).

3.3 SEWAGE SLUDGE-LIQUOR TREATMENT

Liquor from sludge and digestate dewatering has small volumes but high nutrient concentration (Demooij and Thomas, 2010; Evans, 2011b; Suschka and Poplawski, n.d.). Return liquors to treatment works can add about 25 percent (Evans, 2011a) to 30 percent to the initial nutrient concentration but only 1 percent of the wastewater volume (Gustavsson, 2010). Depending on the treatment process sludge liquors can have up to 500mg/l to 3500mg/l of ammonium-nitrogen (Demooij and Thomas, 2010). The EC Urban Wastewater Treatment Directive (UWWTD) demands that WWTP treat effluent phosphorus and nitrogen down to 2 mgP/l and 15mg/l respectively for a population between 10,000 to 100,000 p.e. and 1 mgP/l and 10 mg/l respectively for greater than 100,000 p.e. (Council Directive, 91/271/EEC). While it is energy and cost consuming to treat the arriving wastewater to the plant to meet this standard, an addition of up to 30 percent increase to the initial nutrient concentration of the wastewater would therefore require greater cost. For this reason, ways are sought to treat this additional nutrient load and still meet the required effluent standard.

The conventional method is the most widely applied method of nutrient removal in WWTP. This method involves returning the liquor to the WWTP. In this approach existing processes are expanded and new tanks built to accommodate the extra load introduced (Jardin *et al*., 2006; Evans, 2011b).

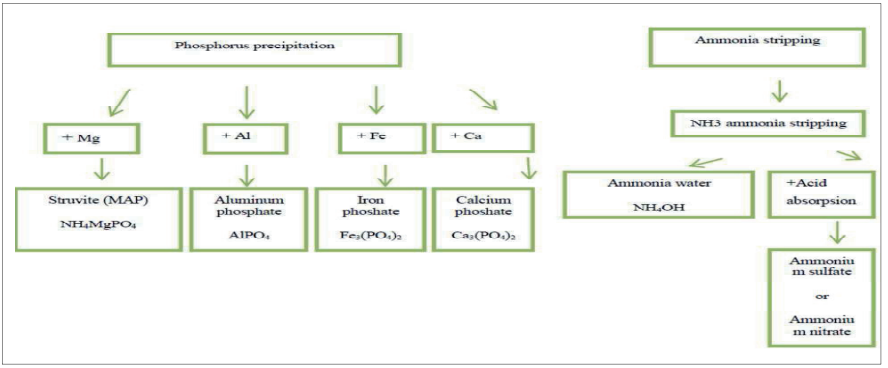


Figure 12. Options for nutrient recovery from sludge liquor (Moldakhovskaia, 2013).

3.3.1 AMMONIA REMOVAL/RECOVERY

3.3.1.1 BIOLOGICAL AMMONIA REMOVAL/RECOVERY

There are basically three biological methods for ammonia removal; firstly nitrification and denitrification, secondly nitritation and denitritation and lastly deammonification (Jardin *et al*., 2006).

Nitrification/denitrification involves first converting ammonia to nitrate with the use of oxygen (Nitrition) and then nitrate to nitrogen gas (denitrification) (Ocansey, 2005). Nitrification/denitrification requires in most cases additional carbon sources such as raw waste water, primary sludge or sludge filtrate, in order to meet the necessary C/N ratio (Jardin *et al*., 2006) or the extension of the anoxic zone (Ocansey, 2005). In a side stream treatment at WWTPs, the reaction occurs as a two-stage process in two separate reactors and can also be operated as a single stage process in a sequence batch reactor (SBR) (Ocansey, 2005).

To save operating costs resulting from high oxygen and carbon demand, nitritation/denitritation process is employed. The nitritation/denitritation

process involves ammonium oxidation to nitrites (nitritation) and then to dinitrogen gas (denitritation). Nitritation prevents the complete oxidation of ammonium, by inhibiting nitrite oxidation to nitrate or washing out the nitrite oxidizers, achieved by either increasing the ammonium concentration or limiting oxygen supply (Jardin *et al*., 2006). This process requires high temperatures and pH. It is the major process currently employed in full scale liquor treatment (Ocansey, 2005) saving about 25 percent of the oxygen demand and 40 percent of carbon demand required in the nitrification and denitrification processes (Gustavsson, 2010; Jardin *et al*., 2006). A major setback with this process is fine tuning to inhibit nitritation, therefore an additional expenditure for measuring and control instruments is required (Jardin *et al*., 2006).

Deammonification process makes it possible to completely eliminate any carbon dosage required for denitrification (Jardin *et al*., 2006). This involves a two-stage process, whereby the ammonium is almost completely converted to molecular nitrogen directly (Jardin *et al*., 2006). Firstly a fixed amount of ammonium in the waste water is fully oxidized to nitrite and then the ammonium elimination occurs under anoxic conditions, simultaneously reducing the nitrite and production of nitrogen gas as an end product (Jardin *et al*., 2006).

3.3.1.2 PHYSICO-CHEMICAL AMMONIA REMOVAL/RECOVERY

Dissociation equilibrium will be completely on the side of ammonia at a pH of 10 and a temperature of 70°C; leaving no ammonium in the water phase. Therefore a pH of more than 11 and a temperature of 20°C is usually typical (Jardin *et al*., 2006). The ammonia dissolved in the water is then transferred to gas phase (desorption). Air and steam stripping in packed columns have been the widely used industrial methods for this step. The desorption process is also temperature dependent, as such the entire process requires high energy demand typical of steam generation (Jardin *et al*., 2006). The stripped

ammonia is then converted into a recyclable or disposable product for ecological reasons. Acidic scrubbing to produce ammonium sulphate and rectification to aqueous ammonia are the options employed in large scale production (Jardin *et al*., 2006).

3.3.2 PHOSPHORUS REMOVAL/RECOVERY

The rate at which the earth's phosphorus is depleting is such that by the end of the century mankind might run out of phosphorus (Evans, 2011b). Phosphorus is an essential element of life, which is part of the DNA, cells energy pathway and cannot be substituted (Evans, 2011a, 2011b). About 9 percent of the world's phosphorus is imported by the EU_{27} equivalent to 3,400,000 tonnes of P_2O_4. About 34 percent of the imported phosphate (1,145,000 tonnes) by the EU_{27} ends up in WWTPs (Evans, 2011a, 2011b). Although about 595,000 tonnes of the phosphate in WWTPs ends up in the sludge, only approximately 220,000 tonnes have been recorded to be recycled to farmlands (Evans, 2011a). Therefore about 48 percent of the phosphate entering WWTPs is wasted in landfills or incinerator bottom ash (Evans, 2011a). Some countries with large phosphorus reserves like China (3.6%) and the USA (11%) have now put measures to restrict the exportation of phosphorus (Evans, 2011a). For these reasons phosphorus is the most important compound that can be recovered from liquor (Evans, 2011a).

3.3.2.1 BIOLOGICAL PHOSPHORUS REMOVAL

This process optimizes the ability of activated sludge to take up phosphorus more than it normally requires for biomass growth under certain conditions (Morse *et al*., 1998). Although this technology helps to avoid the use of chemicals and production of excess sludge, it requires more complex plant design and operation (Morse *et al*., 1998). A more recent method is the use of microorganisms such as bacteria and microalgae (de-Bashan and Bashan, 2004).

3.3.2.2 CHEMICAL PHOSPHORUS REMOVAL/RECOVERY

- **Natural aging**

Battistoni *et al*., (2000) reported in a research on anaerobic supernatant that when the supernatant is enriched with Na_3PO_4 and kept in a thermostatic room, natural aging of phosphorus would occur. Figure 13 shows that at the right pH and temperature, phosphorus removal can occur naturally.

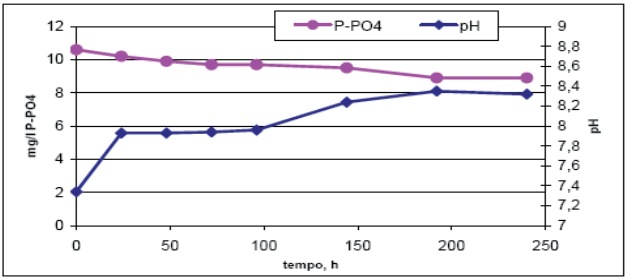


Figure 13. Decreasing P with increase in pH (Ocansey, 2005).

- **Struvite (MAP) precipitation**

Struvite precipitation involves the use of magnesium salts ($Mg(OH)_2$) to recover phosphorus (de-Bashan & Bashan, 2004). The formation of struvite (NH4MgPO4·6(H2O)) a magnesium-ammonium-phosphate (MAP) mineral (Moldakhovskaia, 2013) by the chemical composition of the liquor can also be extracted for use as fertilizer (Ocansey, 2005). Struvite recovery is effective in both ammonia and phosphorus removal (Moldakhovskaia, 2013; Ocansey, 2005) following the equation below

$$Mg^{2+} + PO_4^{3-} + NH_4^{+} \longleftrightarrow MgNH_4PO_4.6H_2O$$

- **Calcium Phosphate- hydroxyapatite (HAP) crystallization**

Calcium- phosphorus precipitation is a commonly used method for phosphorus removal, mainly due to its low cost and it is easy to handle (de-Bashan and Bashan, 2004). Phosphorus removal is achieved by the addition of calcium salts (Hultman *et al*., n.d.) and direct calcium phosphate

(hydroxyapatite, $Ca_5OH(PO_4)_3$) removal (de-Bashan and Bashan, 2004). The formation of HAP follows the following equation

$$5Ca^{2+} + 7OH^- + 3H_2PO_4^- \longrightarrow Ca_5OH(PO_4)_3$$

3.4 ANAEROBIC DIGESTION OF MICROALGAE

Anaerobic digestion of microalgae can be economical for methane generation using low cost and low quality microalgae cultivated on wastewater or harvested from water bodies (Bohutskyi *et al*., 2014). Unlike biofuel production from algae that depends on the algae lipid content, methane generation process recovers more energy than available in cell lipid (Sialve *et al*., 2009). With a cell lipid content below 40%, AD proves to be the optimal choice in terms of energy balance (Sialve *et al*., 2009). Generation of biogas by AD eliminates a lot of steps that could be energy intensive such as drying and extraction (Bohutskyi *et al*., 2014). Although the use of microalgae for AD holds a lot of prospects, biochemical composition of biomass affects methane and biogas yield from AD and the amount of VS destroyed during AD is critical to the entire process. These factors put some huddles in biogas production from algae biomass as algae have limited biodegradability (Bohutskyi *et al*., 2014; Sialve *et al*., 2009). For most macro- and microalgae, only about 20 to 60% VS destruction can be achieved and as a result of algae cell wall, there is limited accessibility of enzymes to the substrates; therefore biodegradation is limited, leading to a longer retention time of about 20 to 30 days for the conventional AD process (Bohutskyi *et al*., 2014). Also high cellular protein leads to release of ammonia, which in turn could result in toxicity (Sialve *et al*., 2009).

Therefore to make AD of microalgae more economically viable by maximizing biomass conversion to biogas, some pre-treatment (hydrolysis) is

important in order to increase biodegradability, improve the rate of production and consequently methane yield (Bohutskyi *et al*., 2014).

Also AD of microalgae for biogas can be combined with the production of biodiesel using the post-extracted algal biomass (Bohutskyi *et al*., 2014; Sialve *et al*., 2009). Harun *et al.* (2011) reported that the conversion of low lipid content algae to biogas has more potential energy than conversion to biodiesel alone and a combination of both yields the greatest energy output.

Table 3. Theoretical methane potential and ammonia release during anaerobic digestion of total biomass for different microalgae species (Sialve *et al*., 2009).

Species	Proteins (%)	Lipids (%)	Carbohydrates (%)	CH4 (L CH4 /g VS)	N–NH3 (mg /g VS)
Euglena gracilis	39 - 61	14 - 20	14 - 18	0.53 - 0.8	54.3 - 84.9
Chlamydomonas reinhardtii	48	21	17	0.69	44.7
Chlorella pyrenoidosa	57	2	26	0.8	53.1
Chlorella vulgaris	51 - 58	14 - 22	12 - 17	0.63 - 0.79	47.5 - 54.0
Dunaliella salina	57	6	32	0.68	53.1
Spirulina maxima	60 - 70	6 - 7	13 - 16	0.63 - 0.74	55.9- 66.1
Spirulina platensis	46 - 63	4 - 9	8 - 14	0.47 - 0.69	42.8 - 58.7
Scenedesmus obliquus	50 – 56	12 - 14	10 - 17	0.59 - 0.69	46.6 - 42.2

CHAPTER FOUR

4. MATERIALS AND METHOD

The following flowchart (Figure 14) illustrates the flow of events adopted in achieving the objectives of this study.

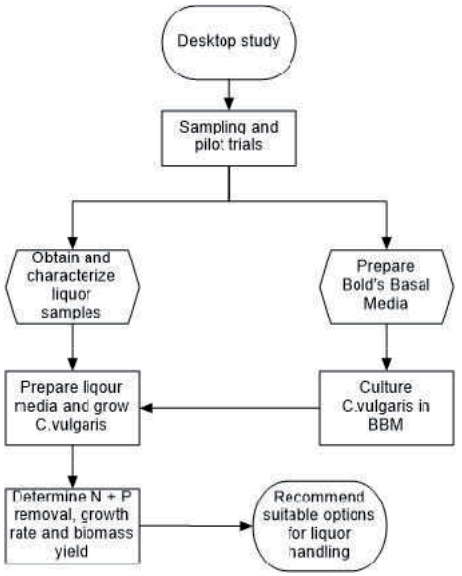


Figure 14. Research flow chart

4.1 SAMPLING

Sludge samples used in this research was a mixture of primary sludge and secondary activated sludge, obtained from Esholt wastewater treatment plant, Bradford. *C.vulgaris* was obtained from a running photo-bioreactor in Leeds University laboratory; 200ml was obtained and cultured in conical flasks using BBM.

4.2 BOLDS BASAL MEDIA (BBM) PREPARATION

BBM was prepared to culture *C.vulgaris*. 1litre Stock solution was prepared, which was used to prepare 1litre BBM according to the recipe shown in table 4 and table 5 respectively. It is important that the media used

for microalgae cultivation be free from any form of contamination that could inhibit the optimal growth of the microalgae, or even allow the growth of other bacteria which could in turn feed on the microalgae. Therefore the BBM was transferred to a 1l Duran bottle and autoclaved at 120˚C and 2.5bar pressure for 40minutes, cooled at room temperature and stored in a refrigerator.

Table 4. Preparation recipe for 1litre BBM stock solution

Component	1 litre stock solution
Major stock solutions	
Sodium nitrate ($NaNO_3$)	25.00 gl^{-1} dH_2O
Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)	2.50 gl^{-} dH_2O
Magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$)	7.50 gl^{-1} dH_2O
Dipotassium sulphate (K_2HPO_4)	7.50 gl^{-1} dH_2O
Potassium dihydrogen sulphate (KH_2PO_4)	17.50 gl^{-1} dH_2O
Sodium chloride (NaCl)	2.50 gl^{-1} dH_2O
Alkaline EDTA Stock solution	
EDTA anhydrous	50.00 gl^{-1} dH_2O
Potassium hydroxide (KOH)	31.00 gl^{-1} dH_2O
Acidified iron stock solution	
Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$)	4.98 gl^{-1} dH_2O
Sulphuric acid (H_2SO_4)	1.0 ml
Boron stock solution	
Boric acid (H_3BO_3)	11.42 gl^{-1} dH_2O
Trace metal stock solution	
Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$)	8.82 gl^{-1} dH_2O
Manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$)	1.44 gl^{-1} dH_2O
Molybdenum trioxide (MoO_3)	0.71 gl^{-1} dH_2O
Copper (II) sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$)	1.57 gl^{-1} dH_2O
Cobalt nitrate ($Co(NO_3)_2 \cdot 6H_2O$	0.49 gl^{-1} dH_2O

Table 5. Preparation recipe for 1 litre BBM

Component	Quantity per litre of medium (ml)
Sodium nitrate ($NaNO_3$)	10
Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)	10
Magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$)	10
Dipotassium sulphate (K_2HPO_4)	10
Potassium dihydrogen sulphate (KH_2PO_4)	10
Sodium chloride (NaCl)	10
Alkaline EDTA stock solution	1
Acidified iron stock solution	1
Boron stock solution	1
Trace metal stock solution	1

4.3 C.VULGARIS CULTURE IN BOLDS BASAL MEDIA

Carbon source for the culture was prepared using 45g of sodium bicarbonate ($NaHCO_3$) dissolved in 1litre of distilled water. 100ml *C.vulgaris* cultures were prepared composed of 10ml *C.vulgaris* (10% of media), 7.5ml sodium bicarbonate and 82.5ml BBM in a volumetric flask. The cultures were then transferred to 250ml conical flasks and inoculated in an incubator (INFORS HT Multitron Pro) for 14 days.

4.3.1 GROWTH RATE IN BOLDS BASAL MEDIA

Chlorophyll-a and turbidity tests were conducted on three cultures to measure the growth rate of *C.vulgaris* in BBM over 14 day culture period. Five point measurements were done in total on day1, day4, day7, day11 and day14, which was used to plot a growth graph based on the average increase in chlorophyll-a and turbidity from the three cultures.

4.3.2 BIOMASS YIELD IN BOLDS BASAL MEDIA

At the end of the 14 days culture, total suspended solids (TSS) and volatile suspended solids (VSS) tests were conducted. These were used to estimate

the biomass yield in BBM after the 14 day period. The percentage biomass yield was then calculated both on TSS and VSS basis using the equations 1 and 2 below.

$$\boldsymbol{TSS\ Yield}\ (\%) = \frac{\boldsymbol{TSS_{14} - TSS_0}}{\boldsymbol{TSS_{14}}} \times \mathbf{100} \qquad 1$$

The VSS yield was calculated as a percentage of the TSS

$$\boldsymbol{VSS\ Yield}\ (\%) = \frac{\boldsymbol{VSS_{14} - VSS_0}}{\boldsymbol{TSS_{14} - TSS_0}} \times \mathbf{100} \qquad 2$$

Where TSS_{14} and VSS_{14} are the TSS and VSS values after 14 days respectively and TSS_0 and VSS_0 are the initial TSS and VSS values after media preparation before culture.

4.4 LIQUOR MEDIA PREPARATION

Sludge liquor was obtained from four different sludge treatment (chemical treatment), which were centrifuged to obtain the supernatant. The first sample was prepared by adding 0.6g zetag66 polyelectrolyte per litre of sludge. The second and third samples were prepared by adding lime at different concentrations in order to optimize the phosphorus distribution to the sludge cake. Optimization was done at varying concentrations, but only the liquor from the highest and least concentrations of lime was used. Therefore the second sample was prepared using 27.5g lime per litre of sludge, while the third was prepared using 1.28g of lime per litre of sludge. And lastly the fourth sample was prepared by adding 35g of ferric chloride to the sludge to also optimize the phosphorus distribution to the sludge cake. The samples were labelled sample1, sample 2a, sample 2b and sample3 respectively.

Sludge samples were centrifuged using Eppendorf centrifuge5810 at 4000rpm for 30minutes. Liquor samples obtained were too concentrated; still having high amount of suspended solids. As this will not allow adequate light penetration for *C.vulgaris* photosynthesis, each sample was further centrifuged using 50ml centrifuge tubes at 4000rpm for 40minutes. The supernatant was then filtered using Whatman GF/C 90mm filter paper, to obtain clear liquor. Liquor samples were thereafter autoclaved at 120˚C and

2.5 bar pressure to destroy any bacteria or pathogen that could inhibit the growth of the algae in the media. Samples were then cooled at room temperature and stored in a refrigerator.

Liquor samples were characterized for phosphate, pH, alkalinity and ammonia-nitrogen; these parameters influenced the dilutions done for each sample and the volume of *C.vulgaris* used in preparing the culture media.

4.5 *C.VULGARIS* CULTIVATION IN THE LIQUOR MEDIA

The liquor samples prepared from the sludge were used to grow the microalgae. *C.vulgaris* was obtained from the culture in the BBM after 4 days of culture (at the exponential growth phase). 200ml of liquor media was prepared from each sample and cultivated in 500ml cotton plugged conical flasks. Aeration was achieved by the shaking action of the incubator. Equal volume of *C.vulgaris* (50ml) was used in each media. Media1 was prepared using 1/15ml dilution of sample1, media2a was prepared using 1/3ml dilution of sample2a, media2b was prepared using 1/16.7ml dilution of sample2b and lastly media3 was prepared using 1/30ml dilution of sample3. The dilution was done in order to control the nutrient in the culture, so the cultures do not get over- or under-dosed. The overall percentage ratio of liquor to *C.vulgaris* in each media was 5:25, 25:25, 4.5:25 and 2.5:25 for media1, media2a, media2b and media3 respectively. The cultures were then inoculated in the incubator (as in the BBM).

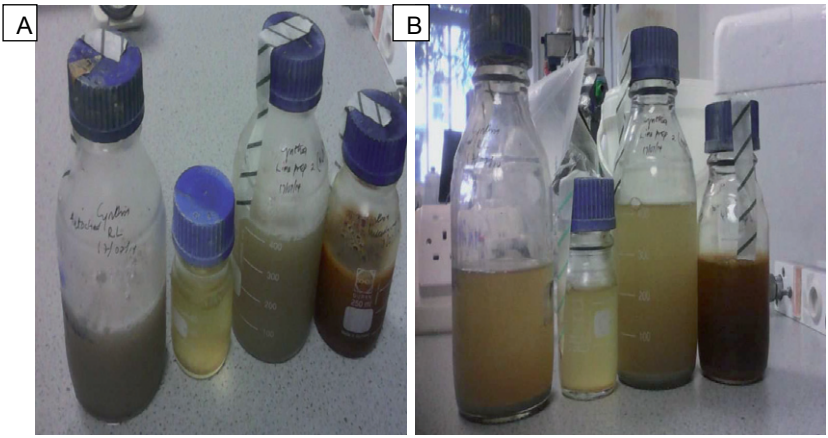


Plate 1. (A) Initial liquor samples obtained from chemical treatment of sludge, from the right; ferric chloride treated, lime treated with minimal concentration, lime treated at higher concentration and polyelectrolyte (zetag66) enhanced dewatering liquor. (B) Final liquor after autoclaving used in the media preparation.

4.5.1 DETERMINATION OF C.VULGARIS GROWTH RATE IN SLUDGE LIQUOR MEDIA

Using a spectrophotometer, the absorbance (optical density) at 540nm wavelength was carried out on each media to determine growth rate. Five point measurements were done on day1, day4, day7, day11 and day14 and the increase in the absorbance was used to determine the growth rate of the algae in each media. The growth rate was then calculated using the equation below,

$$\boldsymbol{Growth\ rate}\ (/\boldsymbol{day}) = (\boldsymbol{OD_t} - \boldsymbol{OD_0})/\boldsymbol{t} \qquad 3$$

4.5.2 NUTRIENT REDUCTION EFFECT (NUTRIENT UPTAKE)

Ammonia-nitrogen (NH_3-N) and reactive phosphorus (PO_4-P) are the forms of ammonia and phosphorus consumed by microalgae respectively. Nutrient uptake by the *C.vulgaris* was determined by carrying out ammonia and phosphate test on the media. As in the growth rate, five point measurements were done on each sample. The tests were carried out on same days as the

test for growth rate. At the end of the 14 day cultivation period, the percentage nutrient uptake was then calculated by,

$$\boldsymbol{Nutrient\ uptake}\ (\%) = \frac{\boldsymbol{initial\ concentration - final\ concentration}}{\boldsymbol{initial\ concentration}} \times \mathbf{100} \quad 4$$

4.5.3 BIOMASS YIELD IN LIQUOR MEDIA

At the end of the 14days cultivation period, the percentage biomass yield was calculated as in the case of the BBM culture described in section 3.3.2. *C.vulgaris* yield per m^3 of media was also calculated for all liquor media using the equations below

$$\boldsymbol{Yield}\ (\boldsymbol{kgDS/m^3}) = \frac{\boldsymbol{final\ TSS\ (mg/l) - initial\ TSS(mg/l)}}{\mathbf{1000}} \quad 5$$

4.5.4 METHANE AND ENERGY WORTH OF C.VULGARIS FROM LIQUOR MEDIA

The theoretical methane that can be achieved from the amount of *C.vulgaris* obtained from equation 5 was calculated. A Biochemical Methane Potential (BMP) test was carried out on digesting *C.vulgaris*. The result was then used to estimate how much methane can be obtained from *C.vulgaris* cultivated in the liquor media. To calculate this, the amount of volatile solids generated was first determined by the equation below;

$$\boldsymbol{Biomass}\ (\boldsymbol{kgVS/m^3 media}) = \frac{\boldsymbol{final\ VSS\ (mg/l) - initial\ VSS\ (mg/l)}}{\mathbf{1000}} \quad 6$$

Afterwards, the methane yield per m^3 of media was calculated using the equation below;

$$\boldsymbol{Methane\ yield}\ (\boldsymbol{m^3/m^3 media}) = \boldsymbol{BMP}\ (\boldsymbol{m^3 methane/kgVS_{added}}) \times \boldsymbol{Biomass\ VS} \quad 7$$

The energy worth of $1m^3$ of liquor media was then analysed. Horan (2013) reported that $1Nm^3$ of methane is equivalent to 9.97kWh of energy. Therefore

from the methane yield calculated in equation 7, the amount of energy per m^3 of media was estimated by the following equation;

$$\boldsymbol{Energy\ (kWh/m^3 media) = methane\ yield \times 9.97 kWh} \qquad 8$$

The use of anaerobic digestion technology to generate energy in the UK has been greatly encouraged by some government incentives. These incentives are avenues for income generation from the energy derived from any AD plant. Figure 15 below shows the options for the methane generated from AD and their respective incentives.

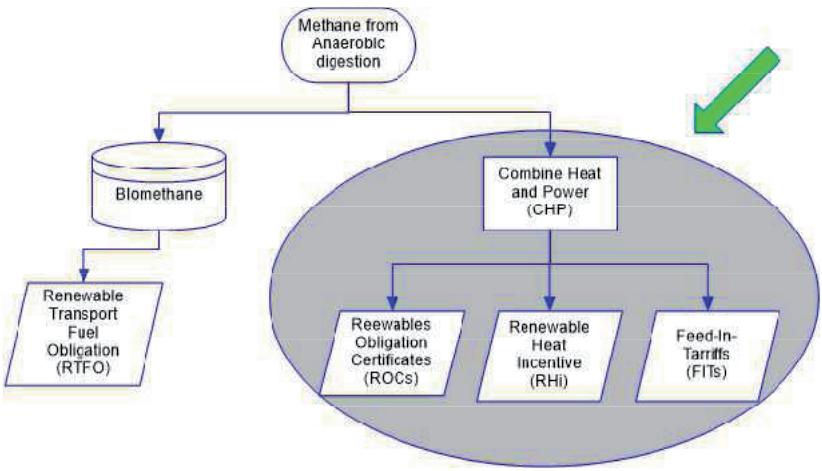


Figure 15. Options for Methane from AD and their respective incentives in the UK.

For the sake of this study, the use of methane for income generation from combined heat and power was analysed. Defra (2011) reported that using AD to generate renewable energy attracts 2RoCs/MWh. A typical value of £80/MWh was assumed for 1RoC. FiTs for small scale AD with capacity of up to 250kW is 14p/kWh and 13p/kWh is provided for capacities between 250kW and 500kW. Therefore 14p/kWh was used for FiTs. Lastly the RHi provides

6.5p/kWh over a 20 year period for biogas combustion in installations below 200kWh and biomethane injection into the gas grid at all scales. These incentives were then used to estimate the financial worth of the methane generated from liquor media.

4.5.5 MASS BALANCE FOR C.VULGARIS ANAEROBIC DIGESTION

The mass balance across the anaerobic digestion plant was analysed to estimate the additional digestate produced from the cultivated *C.vulgaris* as well as the nutrient accumulation in the digestate. VS destruction of 60% was assumed following an estimate of 20 – 60% for microalgae by Bohutskyi *et al*. (2014). Ammonia-nitrogen accumulation in the digestate was estimated using the range of 47.5 – 57.4 mgNH_3-N/gVS, when the total biomass from *C.vulgaris* is digested, which was calculated by Sialve *et al*., (2009) based on Symons and Buswell formula for calculating methane and ammonia yield from organic matter as follows;

$$C_aH_bO_cN_d + \left(\frac{4a-b-2c+3d}{4}\right)H_2O \rightarrow \left(\frac{4a+b-2c-3d}{8}\right)CH_4 + \left(\frac{4a-b+2c+3d}{8}\right)CO_2 + dNH_3 \qquad 9$$

4.6 ANALYTICAL PROCEDURES

4.6.1 TURBIDITY

The turbidity was measured using DR/890 colorimeter. 10ml of the sample was transferred into a 10ml turbidity tube; 10ml of a blank sample containing distilled water was also transferred into a 10ml turbidity tube. The colorimeter was first zeroed using the blank sample and then the turbidity (in Nephelometric Turbidity Units, NTU) of each sample was read from the colorimeter; the colorimeter was zeroed before each sample reading.

4.6.2 CHLOROPHYLL-a

4.6.2.1 MATERIALS

Materials used for the chlorophyll-a test include; 25mm and 47mm Whatman GF/C filter papers, 25mm and 47mm filtration rigs, water bath set at 60°C, measuring cylinder, glass rod, hand vacuum pump, 50ml graduated

tubes with caps, tweezers, 5ml pipettes and tips and spectrophotometer with 1cm cells.

4.6.2.2 REAGENTS

90% methanol reagent was prepared using 225ml methanol diluted in 25ml distilled water. 1% magnesium carbonate solution was also prepared by diluting 2.5g magnesium carbonate in 250ml distilled water.

4.6.2.3 PROCEDURE

The water bath was turned on and allowed to heat up to 60°C. Using the 47mm filter paper, a filter apparatus was set up and 2.5ml of magnesium carbonate was filtered through, this was done in order to increase the surface area of the filter paper. Successive 2.5ml volume of the sample was then filtered through until pumping became difficult or the green colour became intense. The final volume used was then recorded. Using tweezers, the filter paper was folded and placed into a graduated boiling tube pushed down to the bottom. 90% methanol was then added to the tube up to the 10ml mark and capped. The graduated tube was placed in the water bath and allowed to heat up for at least 2 minutes. Once the filter paper in the boiling tube completely turned white, it was removed from the bath and allowed to cool. The sample was then filtered through a 25mm GF/C filter. The spectrophotometer was zeroed at 663nm using the methanol solution and the absorbance for each sample at this wavelength was recorded. The spectrophotometer was then re-zeroed at 750nm and again the absorbance of each sample at this wavelength was recorded.

Chlorophyll-a for each sample was then calculated using the formula below;

$$\boldsymbol{Chlorophyll-a\ (mg/L)} = \frac{\boldsymbol{Abs663-Abs750}}{\boldsymbol{77}} \times \frac{\boldsymbol{10}}{\boldsymbol{V}} \times \boldsymbol{10^3} \qquad 10$$

Where Abs663 = absorbance at 663nm wavelength,

Abs750 = absorbance at 750nm wavelength and

V = volume filtered measured in ml

4.6.3 TOTAL SUSPENDED SOLIDS (TSS) AND VOLATILE SUSPENDED SOLIDS (VSS) PROCEDURE

4.6.3.1 MATERIALS

The ESS method 340.2 (USEPA, 1993) was used to determine TSS and VSS. Materials used for these tests include filtration apparatus, 9cm glass fibre filter paper, vacuum pump, oven, furnace, desiccators and watch glass.

4.6.3.2 PROCEDURE

The glass fibre filter paper was weighed and recorded. The filter paper was then placed on the filtration apparatus, with the vacuum pump in place. 10ml of sample was filtered through. The filter paper was then placed in an oven drying tray and dried in the oven (Gollenhamp Hotbox oven) at 105°C for 24hours. The filter paper was then placed in a crucible and kept in a desiccator to cool. After 10minutes the filter paper was weighed at an interval of 10minutes, until a constant weight was recorded. The TSS was then calculated using the formula below

$$\boldsymbol{TSS\ (mg/L)} = \frac{\boldsymbol{dried\ wt.(mg) - initial\ wt.(mg)}}{\boldsymbol{volume\ used\ (ml)}} \times \mathbf{1000} \qquad 11$$

The volatile suspended solid (VSS) was then determined by placing the filter paper from the TSS in a crucible and transferring it to furnace (Carbolite furnace) at 560˚C for 2 hours, to remove all volatiles. Afterwards it was allowed to cool in a desiccator and weighed again at 10 minutes interval until a constant weight was observed. The VSS was then calculated by the equation below

$$\boldsymbol{VSS\ (mg/L)} = \frac{\boldsymbol{dried\ wt.from\ TSS\ (mg) - final\ wt.(mg)}}{\boldsymbol{volume\ used\ (ml)}} \times \mathbf{1000} \qquad 12$$

4.6.4 pH MEASUREMENT

The pH of each sample was measured using a Hach HQ40d pH meter. The pH probe was dipped into the sample and the meter reading was allowed to stabilize and recorded.

4.6.5 ALKALINITY

The Mettler Toledo apparatus was used to measure the alkalinity. The test was set up using 1ml volume of sample dissolved in 50ml of distilled water. The corresponding reading as measured by the apparatus was then recorded as total alkalinity in mg$CaCO_3$/l.

4.6.6 REACTIVE PHOSPHORUS

4.6.6.1 MATERIALS

The apparatus used for this test include spectrophotometer, 1cm cuvettes, nessler tubes, measuring cylinders, pipette and pipette tips.

All the glassware, measuring cylinders and pipette tips used were acid washed in 1M hydrochloric acid before use. This was done in order to remove any phosphate contamination from detergent residues.

4.6.6.2 REAGENT PREPARATION

The following reagents were used in this test and their preparation have been described in table 6 below; 1M hydrochloric acid solution, ammonium molybdate solution, potassium antimonyl tartrate solution, 0.1M ascorbic acid solution, 2.5M sulphuric acid, standard phosphate solution.

Table 6. Reagents prepared for phosphate analysis

Reagent	Preparation
1M hydrochloric acid solution	86ml concentrated hydrochloric acid in 1l distilled water
Ammonium molybdate solution	10g ammonium molybdate dissolved in 250ml distilled water
Potassium antimonyl tartrate solution	1.372g potassium antimony tartrate dissolved in 500ml distilled water
0.1M ascorbic acid solution	1.76g ascorbic acid dissolved in 100ml distilled water

2.5M sulphuric acid	68ml concentrated sulphuric acid in 500ml distilled water
Standard phosphate solution	0.22g anhydrous potassium dihydrogen orthophosphate in 1l distilled water

100ml of combined reagent was prepared according to the following proportions; 50ml of 2.5M sulphuric acid, 5ml of potassium antimonyl tartrate solution, 15ml ammonium molybdate solution, 30ml ascorbic acid solution, mixed in that order. The mixture produced a pale yellow liquid, which was used within 4hrs of stability.

4.6.6.2 PROCEDURE

4.6.6.2.1. STANDARD PHOSPHATE CURVE

A standard curve was prepared every time the phosphate test was carried out. 10ml of standard phosphate solution prepared in table 6 above was placed in a 100ml volumetric flask. Distilled water was then added to make up to 100ml. This gave a 5mg P/l phosphate standard. Using a pipette, volumes of the 5mg P/l was added to the Nessler tubes as outlined in table 7. Distilled water was then added to each tube to make up to 50ml. The cuvette was then filled with the sample and allowed to develop colour (allowed for 10 minutes) and the absorbance at 880nm recorded using the spectrophotometer. A calibration curve of the absorbance was then plotted against phosphate concentration (mg P/l).

Table 7. Preparation for standard phosphate curve

Volume of 5mg P/l phosphate standard added to the Nessler tube (ml)	**Phosphate concentration (mg P/l)**
2.5	0.25
5	0.50
7.5	0.75
10	1.00
12.5	1.25

4.6.6.2.2 SAMPLE ANALYSIS

Depending on each sample, the anticipated reactive phosphorus in each sample was used to select the sample size from Table 8. Using a measuring cylinder, the selected volume of each sample was placed in a Nessler tube and distilled water was added to make up to 50ml. A control tube was prepared using 50ml of distilled water (to replace sample). 8ml of combined reagent was then pipette into all tubes and swirled to mix. With the spectrophotometer set to 880nm wavelength, the control sample was used to zero the spectrophotometer by filling the cuvette with the control sample and measuring the blank at 880nm. The cuvette was then filled with the sample in the Nessler tube and after 10 minutes (but not exceeding 30 minutes) the absorbance of the developed colour was read using the spectrophotometer. This was repeated for all samples.

Table 8. Sample size selection for reactive phosphorus analysis.

Reactive phosphorus in sample ($mgPO_4$-P/l)	Sample size (ml)
Up to 1.25	50
0.5-2.5	25
2.5-12.5	5
12.5-62.5	1

From the calibration curve, the absorbance of each sample initially recorded was used to determine their corresponding phosphate concentration (mg P/l) and corrected using the formula below,

$$\boldsymbol{Corrected\ phosphate\ concentration}\ (\boldsymbol{mg\ P/l}) = \boldsymbol{phosphate\ concentration\ as\ measured\ from\ the\ garph}\ (\boldsymbol{mg\ P/l}) \times \left(\frac{\mathbf{50}}{\boldsymbol{actual\ volume\ of\ sample\ used}}\right) \quad 13$$

4.6.7 AMMONIA-NITROGEN

4.6.7.1 MATERIALS

Materials used were volumetric flasks, beakers, Duran bottles, pipette, magnetic stirrer and bar, 6 × 300ml distillation tubes with rack and stand, measuring cylinders, Buchi distiller, 50ml burette and burette stand and funnel to fit burette.

4.6.7.2 REAGENT PREPARATION

4.6.7.2.1 MIXED INDICATOR SOLUTION

200mg methyl red indicator was weighed into a 100ml beaker, with a little quantity of propan-2-ol to dissolve. The sample was then transferred into a 100ml volumetric flask and made up to 100ml with propan-2-ol. 100mg of methylene blue indicator was weighed into a beaker and dissolved with a little quantity of propan-2-ol. The sample was then transferred to a 50ml volumetric flask and made up to 50ml with propan-2-ol. The two solutions were then transferred into a 250ml Duran bottle.

4.6.7.2.2 INDICATING BORIC ACID SOLUTION

20g boric acid was weighed into a 1l beaker and dissolved in 500ml of distilled water using a magnetic stirrer and bar. The solution was then transferred into a 1l volumetric flask. Using a pipette, 10ml of mixed indicator solution was added to the solution and made up to 1l with distilled water. The solution was then transferred into a Duran bottle.

4.6.7.2.3 10mM SULPHURIC ACID

1litre volumetric flask was half filled with distilled water and 0.55ml concentrated sulphuric acid was then carefully added to the flask. The sample was mixed thoroughly and topped up to 1litre with distilled water. The mixture was then transferred into a 1l Duran bottle labelled and stored.

4.6.7.3 DISTILLATION PROCEDURE

2.5ml of each media sample was pipette into separate distillation tubes and made up to 100ml with distilled water respectively. A control tube

containing 100ml of distilled water was prepared. The control tube was then loaded on to the distiller. 50ml of indicating boric acid was transferred into a 500ml Duran bottle and placed under the receiving arm of the distiller. The distillation took approximately 9 minutes, after which the Duran bottle was removed and titrated using 10mM sulphuric acid. The distillation process was repeated for all three media samples and titrated.

4.6.7.4 TITRATION

Using a 100ml beaker and funnel, the 50ml burette was half filled with 10mM sulphuric acid and run to waste accompanied by large amounts of water. The burette was then set up at an easily reachable height and refilled with 10mM sulphuric acid with all air bubbles evacuated. The samples in the Duran bottles were then titrated until a pale lavender endpoint was reached and the volume of the titrant used was recorded (A for samples and B for the control).

The final ammonia concentration was then calculated using the following equation

$$\boldsymbol{mg\ NH_3 - N/L = \frac{(A-B)\times 280}{Volume\ used\ (ml)}} \qquad 14$$

CHAPTER FIVE

5. RESULTS AND DISCUSSION

This chapter shows the results obtained from the research carried out on *C.vulgaris.* The outcome of all laboratory experiments has also been discussed here in comparison with existing data from literature.

5.1 RESULTS

The results obtained for the experimental period have been presented in this section. Standard international units have been used to represent the data for easy understanding and comprehension.

5.1.1 C.VULGARIS CULTURE IN BBM

Chlorophyll-a and turbidity tests were used to measure the growth of *C.vulgaris* in BBM. A number of tests can be used to quantify microalgae growth rate, but the Chlorophyll-a test was used considering the fact that BBM is a controlled and standard media used to culture microalgae. The relative increase in chlorophyll gives a representative result of the growth rate determination. Figure 16 therefore represents the growth rate in BBM based on the increase in Chlorophyll-a for the 14 days culture period.

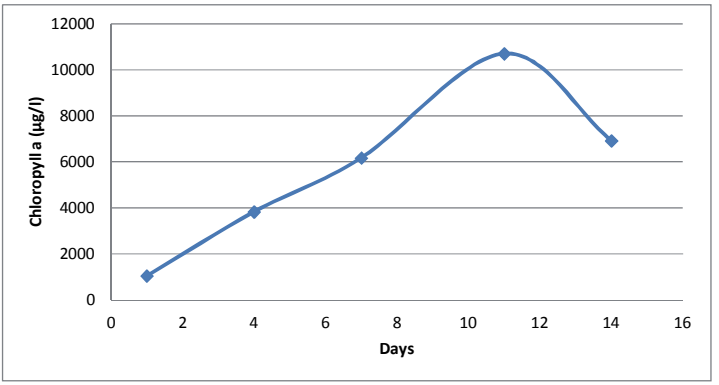


Figure 16. Growth rate of *C.vulgaris* cultured in BBM for 14 days

Turbidity was only carried out to act as a check and the result is presented in figure17; a graph of chlorophyll against turbidity should ideally give a straight line graph.

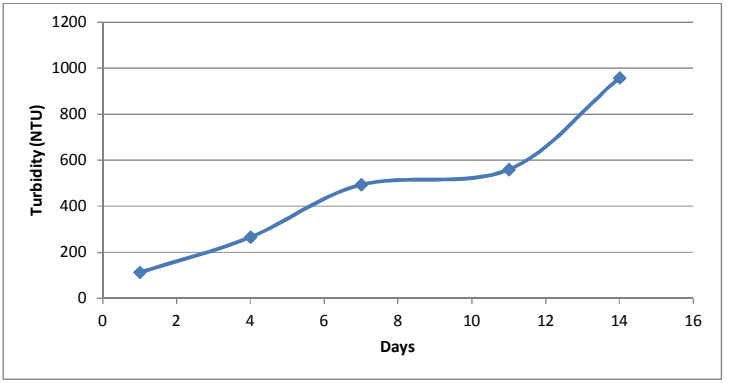


Figure 17. Turbidity curve for microalgae growth in BBM culture.

The biomass generated in BBM was 1.34kgDS/m^3 with a percentage yield of 98.2% total suspended solids and 94% volatile suspended solids (%of TSS), calculated from equations 1 and 2. Plate 2 shows the final outcome of *C.vulgaris* cultured in BBM for 14 days.

5.1.2 SLUDGE-LIQUOR AND MEDIA CHARACTERIZATION

Table9 shows the characteristic of each liquor sample used.

Table 9. Characteristic of sludge liquor from the three different streams before introduction of microalgae

Sample	Colour	pH	Alkalinity ($mgCaCO_3/l$)	Ammonia (mg NH_4-N/l)	Phosphate (mg P/l)
Sample1	Grey	5.09	4994	2240	380
Sample2a	Light gold	11.27	5305	5040	20
Sample2b	Light Grey	5.92	5540	3080	120
Sample3	Brown	**	NA	2240	110

** Outside pH range

NA: No alkalinity

From table 9, realistic dilutions of liquor in distilled water were made and a calculated amount of *C.vulgaris* introduced into each diluted sample. The resultant characteristics of each media after respective dilutions and addition of *C.vulgaris* are in table 10.

Table 10. Media characteristics for microalgae cultivation

Sample	C.vulgaris: liquor	pH	Alkalinity ($mgCaCO_3/l$)	Ammonia (mg/l)	Phosphate (mg/l)	TSS (mg/l)	VSS (mg/l)
Media 1	5:1	9.39	1730.0	212.8	16.00	60	450
Media 2a	1:1	9.09	2805.0	212.8	7.50	100	710
Media 2b	5.6:1	9.76	1985.0	302.4	10.25	200	400
Media 3	10:1	7.50	789.2	515.2	12.00	410	470

At the end of the 14 days cultivation of *C.vulgaris* in liquor media, each media was characterised to estimate the amount of ammonia and phosphate removed therefrom. The percentage biomass yield was also calculated from equations 1 and 2 as described in chapter four. The final characteristics of each media are shown in table11.

Table 11. Characteristics of liquor media after 14 days cultivation period.

Sample	pH	Alkalinity ($mgCaCO_3/l$)	Ammonia (mg/l)	Phosphate (mg/l)	TSS (mg/l)	VSS (mg/l)
Media1	7.92	215.0	5.6	1.36	1420	1330
Media2a	7.82	2245.0	33.6	0.80	3040	1430
Media2b	7.82	2507.7	33.6	1.20	1200	1120
Media3	7.82	1045.00	44.8	2.00	1280	990

5.1.3 C.VUGARIS GROWTH RATE IN LIQUOR MEDIA

Optical density (absorbance) is the log ratio of radiation falling upon a material to the radiation transmitted through it. Optical density at 540nm wavelength was used to analyse the growth rate of *C.vulgaris* in each liquor media. At a low wavelength such as 540nm used in this research, a higher

absorbance can be measured. Absorbance has been used to estimate growth rate in microalgae cultivation within the range of 540nm to 680nm. Figure 18 shows the absorbance of each media at 540nm for the 14 days cultivation period.

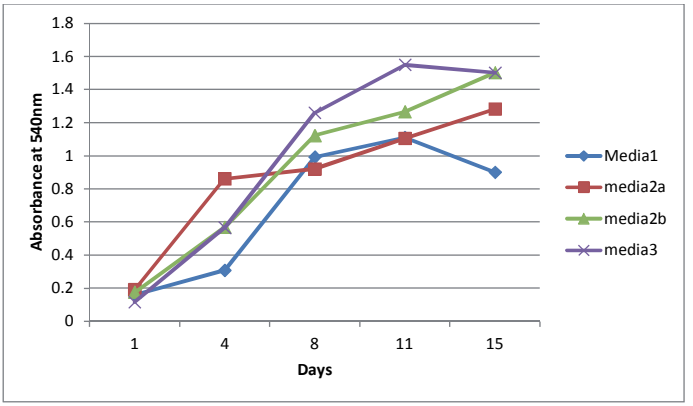


Figure 18. Growth curve for *C.vulgaris* in liquor media

5.1.4 NUTRIENT UPTAKE BY C.VULGARIS IN LIQUOR MEDIA

Phosphate and ammonia concentrations measured on five occasions during the cultivation period are shown in figures 19 and 20 respectively. The trend shows relative reduction in phosphate and ammonia concentrations in each media with time, over the 14 day cultivation period.

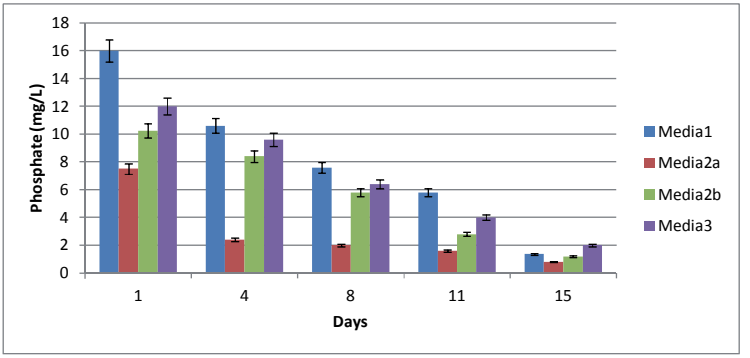


Figure 19. Phosphate concentration in liquor media (error bars at 5% error).

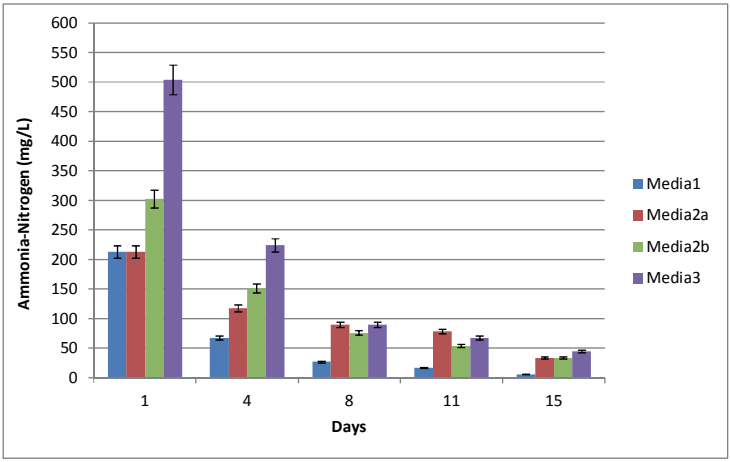


Figure 20. Ammonia- Nitrogen concentration in each liquor media (error bars at 5% error).

The effectiveness of nutrient removal by *C.vulgaris*, *C.vulgaris'* growth rate and biomass yield are presented in table12. The pictorial outcome of the liquor media after the cultivation period is shown in plate 2.

Table 12. Percentage nutrient removal by *C.vulgaris* and biomass yield after 14days of cultivation

Sample	Ammonia removal (%)	Phosphorus removal (%)	Growth rate (/d)	Biomass yield (%)	
				TSS	VSS (% of TSS)
Media1	97.4	91.5	0.05	95.8	65
Media2a	84.2	89.3	0.08	96.7	24
Media2b	88.9	88.3	0.09	83.3	72
Media3	91.1	83.3	0.10	68.0	60

Plate 2. Outcome after 14 days culture in liquor media; from the left media1, media2a, media2b and media3.

5.1.5 METHANE YIELD AND ENERGY POTENTIAL

The biomass yield and amount of methane that could be generated from *C.vulgaris* biomass cultivated in liquor media was calculated as described in sections 4.5.4 and 4.5.5 of the methodology respectively. The methane potential from the BMP test on *C.vulgaris* was 0.35m^3/kgVS. Figure 21 shows the gross methane yield from the respective biomass recovered from each liquor media.

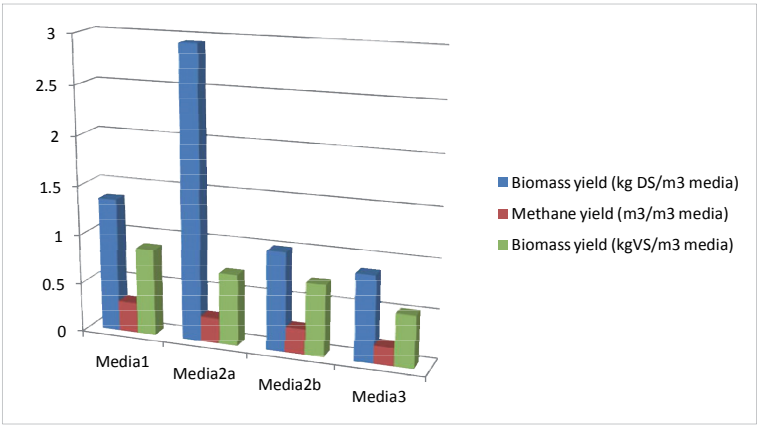


Figure 21. Methane yield per cubic meter of liquor media in comparison with biomass yield.

The essence of estimating the methane yield is to know the energy and economic worth of sludge liquor when *C.vulgaris* is cultivated on it. Horan (2013b) reported that 1Nm^3-methane is equivalent to 9.97kWh energy. A sum of 36.5p/kWh is available for energy generated from AD using the incentives from section 4.5.4. The resultant energy and economic worth of 1m^3 of each liquor media used have been presented in table13. For each media, a different amount of ammonia and phosphate was taken up by *C.vulgaris* in order to achieve the energy produced from each media. The ammonia and phosphate required by each media are also presented in table13.

Table 13. Energy and economic value of a 1m^3 of liquor media and the associated ammonia and phosphate required.

Sample	Energy yield (kWh/m^{3}media)	Economic worth (£/m^{3}media)	Ammonia required (mg/l)	Phosphate required (mg/l)
Media1	3.07	1.12	207.2	14.64
Media2a	2.51	0.91	179.2	6.70
Media2b	2.51	0.91	268.8	9.05
Media3	1.81	0.66	459.2	10.00

5.1.6 C.VULGARIS MASS BALANCE ACROSS ANAEROBIC DIGESTION PLANT

Table 14 below shows the mass balance across an AD plant assuming the whole *C.vulgaris* biomass was digested alone to generate energy.

Table 14. Mass balance across AD plant with *C.vulgaris* cultivated in liquor media

Sample	Feedstock		Digestate	
	Total biomass ($kgDS/m^3media$)	Volatile Solids ($kgVS/m^3media$)	Ammonia ($mgNH_3$-N/m^3media)	Biomass ($kgDS/m^3media$)
Media1	1.36	0.88	41.80 – 47.52	0.83
Media2a	2.94	0.72	34.20 – 38.88	2.51
Media2b	1.00	0.72	34.20 – 38.88	0.57
Media3	0.87	0.52	24.70 – 28.08	0.56

5.2 DISCUSSIONS

This section discusses the results obtained, as presented in section 5.1 above.

5.2.1 C.VULGARIS IN BOLDS BASAL MEDIA

Microalgae batch culture in controlled media is known to follow a standard pattern; lag phase, exponential phase, stationary phase and then the downward growth phase. The culture of *C.vulgaris* in BBM has shown this pattern as demonstrated by increase in Chlorophyll-a versus days of culture (figure16). Becker (1994) reported that the lag phase of a batch culture could be by-passed, if the culture is prepared using the inoculum from a running culture in its exponential phase. That explains why the growth curve shows no lag phase. The turbidity curve (figure18) shows a slightly different pattern, which could be due to error in pH during measurement.

5.2.2 EFFECT OF CHEMICAL STRIPPING ON LIQUOR SAMPLES

Quicklime (CaO) and ferric chloride are well established chemicals for removing phosphorus from sludge by precipitation into the sludge cake where it can be used on land; thereby reducing the phosphate in the liquor that

needs to be treated. Although this is so, liquor from chemical treatment processes do not still meet required standards by UWWTD. Therefore further treatment of the effluent from these chemical processes is done. The characterization of the liquor obtained from all treatment processes; summarised in table 9, shows that more than just removing the phosphorus from the liquor, it affects the liquor in other ways, although this is dependent on the downstream dewatering method used to separate the sludge cake from the supernatant.

5.2.2.1 POLYELECTROLYTE (ZETAG66)

It has been established that the dewaterability of sewage sludge is very poor (Saveyn, Pauwels, *et al*., 2005; Tuan *et al*., 2012). Since mechanical dewatering processes are only effective in removing free bound water within the sludge, measures to enhance floc formation and settle ability of sludge cake during dewatering have been proposed (Mahmoud *et al*., 2010). Therefore sludge is treated with some pre-determined amount of charged organic polymers known as polyelectrolyte (Saveyn, Pauwels, *et al*., 2005). Although the use of polyelectrolyte is said to enhance dewaterability, the supernatant of the sewage sludge treated with Zetag66 was opaque and greyish in colour. The fact that the amount of Zetag66 used was not predetermined using a jar test for the sludge sample in particular before use could be responsible for the nature of the liquor. Saveyn, Meersseman, *et al*. (2005) reported that the combination of sludge composition and the polyelectrolyte characteristics would determine the sludge dewaterability performance. Also the speed of the centrifuge used (4000rpm) and the time (30 minutes) could be factors to consider. Perhaps at a higher speed of 15,000rpm and longer time, it would have been better. That notwithstanding, since all the samples were subject to the same centrifugation treatment, it would have been expected that the liquor from the polyelectrolyte treated sludge would be clearer than the others. In line with this, (Saveyn, Pauwels,

et al., 2005) reported that sludge with biological origin have poor dewaterability even after polyelectrolyte conditioning.

Polyelectrolyte conditioning does not affect the nutrient distribution between the solid-liquid phases. But with more solids settling, it can be assumed that more nutrients will also be available in the solid cake after dewatering compared to sludge dewatered using mechanical method only. The liquor from the polyelectrolyte conditioning was therefore assumed to be representative of raw liquor obtained from mechanical dewatering only. Dewatering liquor have been reported to contain ammonia between 500 to 3500mg/l (Demooij and Thomas, 2010), this is evident from the ammonia concentration in sample1 (table 9).

5.2.2.2 LIME CRYSTALLIZATION

The liquor from the lesser lime concentration (sample2b) produced opaque grey liquor; therefore it was further centrifuged and filtered to clearer liquor that can allow for light penetration. The addition of more lime gave transparent light-golden liquor (sample2a). Thus it did not require any further centrifugation to remove solids just filtration. It can therefore be established that sludge dewatering can be enhanced with increase in lime concentration. In this light, it has been observed that the addition of calcium ions to sludge does reduce bound waters to a great extent (Nguyen *et al*., 2008). Tuan *et al*. (2012) also stated that to achieve high dewatering after sludge treatment with inorganic salts, dosage in the range of 20 – 40% of the dry weight of the sludge would be needed.

Addition of lime to the raw sludge increases the pH and consequently the alkalinity of the liquor. Evans (2011a) reported that lime addition to moist sludge increases pH of the mixture and consequently the alkalinity. Literature has it that the optimal pH for the removal of phosphorus with lime is 12 (Hultman *et al.* n.d.). This is evident from the result obtained, with sample2a resulting in a pH of 11.27 and lesser phosphate concentration of 20mg PO_4-

P/l; thereby achieving 94.7% phosphate transfer to the cake. On the other hand liquor with the pH of 5.92 (prepared from 1.28g of lime per litre of sludge) had a phosphate concentration of 120mg PO_4-P/l achieving only 68.4% phosphate transfer to the cake.

The addition of lime on the other hand increases the ammonia concentration in the liquor as observed from table 9 increasing the lime concentration further increases the ammonia in the liquor. Lime addition liberates the ammonia from the sludge (Evans, 2011a), which is by convention air-stripped (Jardin *et al*., 2006). Since the lime was added prior to centrifugation without provision for ammonia stripping; the liberated ammonia gets dissolved in the supernatant. Therefore the more the lime added, the more the ammonia in the liquor. Sample2b had 37.5% increase in ammonia concentration, while sample2a had 125% increase in ammonia concentration, compared to the raw liquor. The use of lime to precipitate phosphorus from sludge has been discovered to also achieve some amount of ammonia removal by stripping. Therefore where this is the case, the increase in ammonia concentration observed in the liquor of the lime treated sludge would possibly have been the opposite.

5.2.2.3 FERRIC CHLORIDE CRYSTALLIZATION

The addition of ferric chloride produced densely brown liquor; even after further centrifugation and filtration the liquor was still very brown; leaving a brown stain on the Duran bottle in which it was stored. Therefore care was taken when using it to prepare the *C.vulgaris* cultivation media. Nguyen *et al*., (2008) reported that multivalent elements such as Iron salts have been greatly used in WWTPs as coagulants and phosphate-removing agents. The dense brown colour could therefore be attributed to the amount of ferric chloride used and perhaps the chemical reaction within the sludge during dewatering and not poor dewatering. Although Tuan *et al.* (2012) reported

that mechanical sludge dewatering especially centrifugation, after treatment with inorganic salts such as ferric chloride, is usually poor.

The pH of the resulting liquor was out of range, meaning it could either be too acidic or too alkaline. The alkalinity test further conducted on the sample showed that there was no alkalinity, as such the liquor was therefore assumed to be too acidic. Ferric chloride therefore increases the acidity of liquor; which again is worthy of consideration if it is to be used to cultivate microalgae.

Addition of ferric chloride seemed to have no effect on the ammonia concentration in the liquor as shown in table 9 but was able to transfer 71% of the phosphate to the sludge cake.

5.2.3 C.VULGARIS CULTIVATION IN THE SLUDGE LIQUOR MEDIA

The characteristic of the liquor as influenced by the different chemical treatment option, determined the ratio of *C.vulgaris* to liquor used in preparing each media. In order to obtain a conducive growth media, the ratio of *C.vulgaris*:liquor of 5:1, 1:1, 5.6:1 and 10:1 was prepared (table10) for media1, media2a, media2b and media3 respectively.

5.2.3.1 EFFECT OF MEDIA DILUTIONS ON BIOMASS YIELD

Media1 in figure 18 demonstrates a standard growth curve of *C.vulgaris* in batch cultivation. On the other hand, the high absorbance observed in the media from chemical treatments (media2a, 2b and 3) could be due to a number of factors. Firstly liquor sample1 was coloured and it was initially assumed that *C.vulgaris* would not grow in it. Also the residual effect of the chemicals used during treatment might have influenced the increase in absorbance.

The outcome of *C.vulgaris* cultivation showed that media2a with equal amount of *C.vulgaris* and liquor had the highest biomass yield of 2.94 kgDS/m^3 followed by media1 with 1.36 kgDS/m^3, media2b with 1 kgDS/m^3 and finally media3 with 0.89 kgDS/m^3. This is a reflection of the increasing

order of *C.vulgaris* in media sample. Therefore increasing the amount of *C.vulgaris* in the media will give rise to decrease in the biomass yield (dry solids). Also as all media had suitable pH and no media was starved of nutrient; having sufficient ammonia and phosphate, the ratio of *C.vulgaris :* liquor can be said to be the primary influence for the biomass yield observed in figure 21.

Of greater importance is the amount of volatile solids generated, as this is what generates methane. Media1 showed highest amount of volatile solids of 0.88kg/m^3, media2a and 2b had the same amount of volatile solids of 0.72kg/m^3 while media3 had the least amount of volatile solids of 0.52kg/m^3. This shows that the media1 has the highest energy potential. Liquor from lime treatment; regardless of the amount used, will generate the same amount of energy, making excessive lime dosing an unnecessary cost burden. Also upon digestion the nutrient dissociates and is then transferred to the digestate. Thus media1 saves extra nutrient burden that would have been transferred to the digestate liquor by the reduced 1.58kgDS/m^3 biomass compared with media2a.

The low growth rate of *C.vulgaris* observed in all media was due to the fact that it was calculated over the entire cultivation period. The major growth occurs during the exponential phase (Chisti, 2007), therefore if calculated over this period exponentially, higher growth rate would have been observed. In line with this, Wang *et al*. (2010) reported an average specific growth rate of 0.948d^{-1} for *Chlorella sp.* when cultivated in sludge liquor media.

Although microalgae from all liquor sources seemed to have a good growth, it is important to consider that chemical precipitation of nutrients into the sludge cake depends on specific soil characteristics and the availability of plant to use up the nutrient (Jardin *et al.*, 2006). Therefore if there is insufficient landbank or stringent regulations regarding the spreading on land, the process becomes futile. As such since *C.vulgaris* showed an impressive growth in the raw liquor media, it would be better to use just microalgae for

treating sludge liquor. However, wherever it is necessary to use chemicals, only little chemical dosing should be employed in order to save cost.

5.2.3.2 NUTRIENT REDUCTION BY MICROALGAE IN THE SLUDGE LIQUOR MEDIA

Table 9 shows final phosphate concentrations of 1.36 mgPO_4-P/l, 0.8 mgPO_4-P/l, 1.2 mgPO_4-P/l and 2 mgPO_4-P/l for media1, media2a, media2b and media3 respectively and final ammonia concentrations of 5.6 mgNH_3-N/l, 33 mgNH_3-N/l, 33 mgNH_3-N/l and 44 mgNH_3-N/l for media1, media2a, media2b and media3 respectively. *C.vulgaris* can therefore be used to achieve the stringent effluent consent of 2mg P/l phosphorus and 15mg N/l for a p.e. of 10,000 to 100,000 and to an extent the more stringent 1mg P/l and 10mg N/l for above 100,000 p.e. The effluent from *C.vulgaris* treatment returned to the treatment plant would add only very little nutrient load to the system; perhaps insignificant compared with the untreated return liquor.

Although the liquor from the chemical treatment processes show high ammonia concentration, if ammonia stripping was done during the chemical treatment as usual, the liquor would not have so much ammonia left in it and the ammonia concentration in the liquor might turn out to be insufficient for *C.vulgaris* cultivation. In which case a different ratio of *C.vulgaris* : liquor would be determined. Overall media1 had the highest ammonia and phosphate removal of 97.4% and 91.5% respectively. Wang *et al*. (2010) reported 85.6% phosphate removal by *Chlorella sp.* from sludge supernatant. Ahmad *et al*. (2013) also reported 99.96% phosphate removal from wastewater by *C.vulgaris*. The three media from chemical treatment also showed impressive nutrient removal.

For the biomass yield observed in section 4.2.3.1 above, the amount of ammonia and phosphorus required by each media to achieve the respective yields were 207.2 mgNH_3-N/l and 14.64 mgPO_4-P/l, 179.2 mgNH_3-N/l and 6.7 mgPO_4-P/l, 268.8 mgNH_3-N/l and 9.05mgPO_4-P/l and 459.2 mgNH_3-N/l and

10mgPO_4-P/l for media1, media2a, media2b and media3 respectively. For media1, the ammonia and phosphate required can conveniently be obtained from the dewatering liquor, in line with Demooij and Thomas (2010) report that sludge liquor contains about 500 to 3500 mgNH_3-N/l. But for media2a and media2b the ammonia from dewatering liquor after chemical treatment might be insufficient, as this process is also effective in ammonia removal. Media3 however would have sufficient ammonia and phosphorus that is required, as it shows no significant effect on ammonia. It suffices therefore to say that sludge dewatering liquor holds ample potential for *C.vulgaris* cultivation.

5.2.4 METHANE AND ENERGY POTENTIAL OF GENERATED BIOMASS IN ANAEROBIC DIGESTION

The gross methane yield presented in figure21 shows that media1 from the raw liquor have the highest methane yield of 0.308 m^3/m^3media, media2a and media2b from the lime treatment liquor both yielded 0.252 m^3/m^3media, while media3 had the least methane yield of 0.182 m^3/m^3media.

As discussed in section 5.2.3 above, media1 with the highest amount of volatile solids saves 1.58kgDS/m^3 of additional biomass and yields an additional 0.16kgVS/m^3 in comparison with media2a that had the highest yield on dry solids. This resulted in an increase of 0.056 m^3methane/m^3media in media1 compared with media2a. With an energy equivalent of 9.97kWh per Nm^3-methane, dewatering liquor from WWTPs can yield 3.07kWh/m^3media prepared. Therefore in cultivating *C.vulgaris* for methane generation from AD, more attention should be paid to factors that enhance volatile solids than dry solids yield.

Raw liquor from dewatering process has good methane and energy potential. Lime treatment liquor would generate about the same amount of methane (regardless of the amount used), this should be considered when

using such treatment. Ferric chloride treatment seems to have significant effect on the methane generated from the liquor media.

5.2.5 ENERGY AND COST SAVINGS

The conventional oxidation of ammonium ions to nitrate (NO_3^-) and then denitrification to nitrogen gas as a treatment option in WWTPs requires a lot of energy for pumping and aeration and therefore cost. For this process, 4.57 gO_2/gNH_3-N is required for complete oxidation. It therefore means that for the 2240 $mgNH_3$-N/L (2.24gNH_3-N/L) in raw liquor obtained, an additional 10.24 gO_2/L (10.24 kg O_2 m^{-3}) of liquor is required. On the other hand, the photosynthetic activity of the microalgae produces oxygen. Chisti (2007) reported that a maximum of 10 gO_2 m^{-3} min^{-1} (14.4 kgO_2 m^{-3} d^{-1}) can be generated by microalgae in a typical photobioreactor. Therefore apart from the microalgae consuming about 97.4% of the ammonia in the raw liquor, 141% of the oxygen required to treat the liquor by conventional process would be produced daily. This can be extracted and used in WWTPs for other necessary bacterial activities. The energy and cost of aeration as required by the conventional process would therefore be greatly saved.

Also from table 13, $1m^3$ of media has a financial worth of £1.12. The ammonia available in the raw liquor from dewatering is about 11 times the ammonia required to prepare the raw liquor media (media1). Therefore, 11 times the financial worth of $1m^3$ of media can be generated. In essence, the raw liquor from sludge dewatering is worth an approximate of £12.32.

5.2.6 THEORETICAL MASS BALANCE FROM ANAEROBIC DIGESTION OF GENERATED BIOMASS

Anaerobic digestion breaks down organic compounds, thereby converting the nitrogen in proteins that has been held by peptide links into soluble ammonium ions (NH_4^+) (Evans, 2007). *C.vulgaris* is known to have high composition of protein; about 51 to 58 percent protein (Sialve *et al*., 2009) depending on the medium of cultivation. This is evident in the results

presented in table 14, which shows that biomass generated from $1m^3$ of media when digested would yield up to 47.52 mgNH_3-N in the digestate.

Phosphorus can also be retained in biomass under aerobic conditions, but in anaerobic conditions such as the AD, phosphorus is released (Evans, 2007). A typical scenario was reported of increase in phosphate concentration from 10mg/l in the influent to 60mg/l after anaerobic digestion (Momberg and Oellermann, 1992, cited in Ocansey, 2005).

CHAPTER SIX

6. CONCLUSIONS AND RECOMMENDATION

After the research process, the following conclusions and recommendation have been drawn.

6.1 CONCLUSIONS

1. *C.vulgaris* demonstrates excellent growth and nutrient removal in sewage sludge liquor.

2. Regardless of the liquor source; whether directly from sludge dewatering or chemical treatment, the liquor can still be used to grow microalgae, as long as appropriate liquor/microalgae ratio is achieved.

3. The operating cost of introducing oxygen into the conventional liquor treatment process to remove nutrients will be saved when the microalgae is used as a treatment option.

4. Chemical treatment of sludge liquor is cost intensive. This cost can be avoided by treating sludge liquor with microalgae. But in cases where the nutrient in sludge cake is of interest in terms of agriculture, then the liquor from chemical treatment processes could still be used to grow microalgae.

5. Methane and energy generated from the *C.vulgaris* biomass produced from sludge liquor makes the use of *C.vulgaris* for sludge liquor treatment more economical than conventional nutrient removal processes. Also, the oxygen released during microalgae photosynthesis provides supplementary oxygen for other microbial processes.

6. The use of *C.vulgaris* in anaerobic digestion will not only generate biogas, but will produce a digestate rich in nitrogen and phosphorus that can be used on agricultural land.

6.2 RECOMMENDATION

It is recommended that the use of microalgae for liquor treatment be given more attention by WWTPs if environmental sustainability, clean energy development and cost effective systems are to be obtained.

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