Design and Optimization of Process Parameters in Bio-Gas Production Systems

BY

FATMA A. ALFARJANI, B. Sc

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Supervisor

Dr. Abdul Ghani Olabi

School of Mechanical and Manufacturing Engineering

Dublin City University

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DECLARATION

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Master of Engineering M.Eng is entirely my own work, that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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FATMA ALFARJANI

Student ID Number: 58116885

Date : January 2012

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Design and Optimization of Process Parameters in Bio-Gas Production Systems

Fatma A. Alfarjani, B. Sc.

Abstract

Concerns about the environmental impacts associated with using fossil fuels are ever increasing, with a particular focus on the global climate change that is caused by increasing concentrations of greenhouses gases (CO_2 among others).

Biogas production is a key technology in the development of sustainable energy supply systems that aims to cover the energy demand using renewable sources and to mitigate greenhouse gas emissions. Biogas production can be achieved through anaerobic digestion (AD), a biological process, which uses biomass as a renewable energy source. Pre-treatment is the first stage of the AD process. The main goal of pre-treatment is to change the biomass properties in order to prepare lignocelluloses for enzymatic attack; which in turn enhances biogas production and anaerobic digestion performance.

In this work beating treatment as a new mechanical treatment was applied to grass silage to improve the anaerobic digestion combined with sludge to produce biogas. One of the objectives is to investigate the effect of beating treatment on the AD process to produce biogas from grass silage. The other objective is to optimise the condition of the process and develop a mathematical model of this process. Techniques such as Design of Experiment (DOE) and Response Surface Methodology (RSM) have been used to optimise the mean factors of the process (temperature and the time of beating). A Bioreactor has been designed and built to conduct the experiments that were designed by using RSM. The use of numerical and graphical optimisation methods have been presented in this thesis and a mathematical model has also been developed.

It was reported in this research that the anaerobic digestion process using the beating pre-treatment method produces a higher volume of biogas. It was concluded that the beating treatment achieves 12.65% increase in biogas production from grass silage and the maximum biogas yield is 3410 cc, which can be achieved at the optimal condition: temperature of 37.3 °C and beating time of 2 min 42 s.

Table of Contents

CHAPTER 6

List of Figures

LIST OF TABLES

CHAPTER 1
INTRODUCTION

1. INTRODUCTION

With the increasing demand for energy, the limited resource of fossil fuels amid growing concerns for the environment, the development of an alternative energy source has become the forefront of research. In recent years biogas has been receiving increasing attention as an alternative to fossil fuels in solving the problems of rising energy prices, waste treatment/management and creating a sustainable development. [1, 2].

Biogas is a product of anaerobic digestion processes in biomass by certain bacteria. Biogas mainly consists of methane and carbon dioxide, with trace amounts of other gases.

Biogas technology plays an important role in producing energy from renewable and clean resources, in addition to its application to treat animal manure and organic waste from the industry and household sectors [3]. Furthermore, it is a flexible form of renewable energy that can produce heat, electricity and is commonly used for cooking and lighting and also serves as a vehicle fuel [4].

Over the years, the trend of biogas plant building has increased in many countries. Among the European countries that have most developed agricultural biogas plants are Germany, Denmark, Austria and Sweden. In Germany, the number of biogas plants have increased from 139 in the year of 1992 to 3891 biogas plants by the end of 2008[5].

Wide ranges of feedstock can be used for the production of biogas, these include: organic fractions of municipal solid wastes, animal manure and slurries, agricultural residues, organic wastes from dairy production, food industries and agro industries, wastewater sludge, organic wastes from households and from catering business as well as energy crops [6, 7].

Biogas can be produced through various types of energy crops. The most commonly used crops are maize, sunflower, grass and Sudan grass [8].

The energy crop selected for this experiment was grass silage. Grass silage is the second most frequent crop used as feedstock (50%) after maize silage (80%), this is shown in the assessment of recent biogas plants in Germany and Austria [9, 10], these types of crops can generate large amounts of energy through anaerobic digestion AD processes.

In recent decades, several biological processes have been studied to convert biomass to energy [11,12]. One of the most important processes for this proposal is AD, which has been defined as the biological breakdown of organic material by micro-organisms in an air tight environment with no oxygen present to obtain biogas. Another product is digestat that consists of nutrient-rich solids and fluid left after digestion; this can be used as fertilizer [13]. Compared with other conversion technologies, AD has all the advantages to increasingly become one of the most efficient and economical sources of renewable fuel.

The AD process can be described in four stages. These are:

- Hydrolysis;
- Acidogenesis;
- Acetogenesis;
- Methanogenesis.

The hydrolysis stage is considered the limiting step of anaerobic digestion, where the cell wall is broken down allowing the organic matter inside the cell to become available for biological degradation. The AD process may therefore be improved if the hydrolysis step can be enhanced [14,15]. There are many studies that have been conducted in recent years in order to improve the performance of AD through methods of pre-treatment of various wastes, especially solids, owing to the positive relationship between successful pre-treatments and improved yields. These treatments can be biological, mechanical, thermal or chemical [16,17].

In this work, beating treatment has been applied by employing Hollander Beater to treat grass silage as lignocellulosic material. Beating lignocellulosic materials will result in disruption of the crystalline structure of the cellulose cells and the assist in the break down of the lignin component, thus improving hydrolysis and overall methane yield [18].

AD processes in stage 1 (pre-treatment) and stage 2 (digestion) are very much related in terms of efficiency. In order to obtain high production levels of biogas from any AD process, pre-treatment processes must take place. There are a range of factors that affect

the AD process; the most important being temperature and pH. Additionally, the degree of beating associated to the first stage of the process is also imperative. Several researchers have identified that the optimum value of pH varies within the ranges of ± 7 [19] and [20]. For the optimization of the other two factors, Design of Experiment (DOE) as a statistical technique has been used in this thesis, this has been used instead of traditional methods which are criticised because they depend on trial-and-error, are time consuming and increase the overall cost of the process.

1.1Thesis Objectives

The first main objective of this work is to prove that beating techniques as a mechanical pre-treatment method are effective and accelerate the degradability of grass silage as one of the important source of biomass energy.

The second objective is to apply responses surface methodology RSM to develop mathematical models in the form of function showing the relationships between input and output of anaerobic digestion processes. These models would add significant knowledge in assisting scientists and researchers in conducting experiments. Also, the developed models would be useful in predicting responses, and thus would allow the selection of optimal settings of the process input parameters to minimise or maximise certain responses. The aims of applying RSM in this work can be summarised in the following key points:

1. To build up mathematical models using RSM with the aid of Design-Expert version-7 statistical software to predict the production of biogas every three days for 21 days;
2. To identify the most influential anaerobic digestion parameters (beating time and temperature);
3. To present the developed models in 3D plots and contour graphs;
4. To identify the optimal combinations of the process input parameters, using numerical and point prediction optimisation, to achieve a specific target criterion.

1.2Thesis Outline

The thesis is divided into six chapters. A brief summary of each chapter is shown below:

Chapter 1: The general introduction, the thesis objectives and thesis outline.

Chapter 2: An overall literature review. This chapter divided into two parts:

Part 1, shows the renewable and non-renewable energy sources in general;

Part 2, concerned with the theoretical background of anaerobic digestion, the important factors affecting the process, available feedstock for anaerobic digestion, mechanical pre-treatment and a summary of biogas technology.

Chapter 3: A discussion of the statistical Design of Experiment (DOE) method used in this work and the optimisation method details.

Chapter 4: A description of all materials and equipment which was used during the experimental procedures and testing carried out.

Chapter 5: A description and discussion of the results obtained from the experiments. Also the numerical and point predication optimization have been presented.

Chapter 6: A conclusion and recommendations for future work have been outlined here.

CHAPTER 2
LITERATURE REVIEW

2. LITERATURE REVIEW

2.1Energy

Energy is in everything. It makes everything happen. We buy energy, sell energy, eat energy, waste energy, talk a little about conserving energy, fight over energy – and kids use it to chase pigeons. Whatever happens it is caused by energy. In physics, energy is the capacity of a physical system to perform work. Energy exists in several forms such as heat, kinetic or mechanical energy, light, potential energy, electrical, or other forms [21].

2.2 Energy production and consumption

Between 1996 and 2006, the world's total output of primary energy -- petroleum, natural gas, coal, and electric power (hydro, nuclear, geothermal, solar, wind, and wood and waste)--increased at an average annual rate of 2.3%. World production increased from 373 quadrillion BTU in 1996 to 469 quadrillion BTU in 2006.

In 2006, petroleum (crude oil and natural gas plant liquids) continued to be the world's most important primary energy source accounting for 35.9 % or 169 quadrillion BTU of world primary energy production. Between 1996 and 2006, petroleum production increased by 11.7 million barrels per day, or 16.9 %, rising from 69.5 to 81.3 million barrels per day. Coal ranked second as a primary energy source, accounting for 27.4 % of world primary energy production. Dry natural gas ranked third as a primary energy source, accounting for 22.8 % of world primary energy production, while hydro, nuclear, and other (geothermal, solar, wind, and wood and waste) electric power generation ranked fourth, fifth, and sixth, respectively, as primary energy sources in 2006, accounting for 6.3, 5.9, and 1.0 %, respectively, of world primary energy production [22]. Figure 2.1 shows the evolution of energy supply in the world from 1971 until 2007 [23].

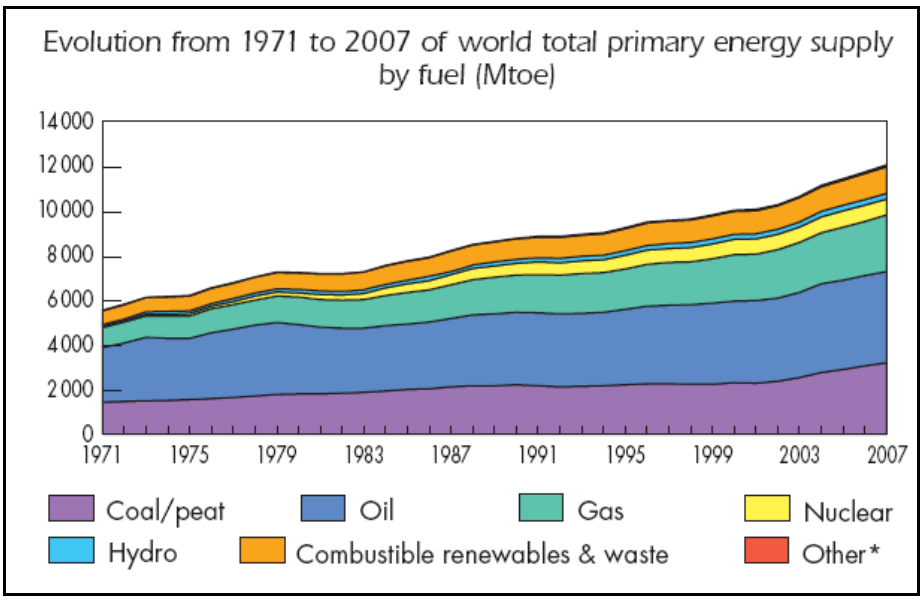


Fig. 2.1: Evolution of energy supply in the world from 1971 until 2007 [23].

2.3 Energy classification

Energy can be generally classified as non-renewable and renewable. Over 85% of the energy used in the world is from non-renewable supplies [24]. Figure 2.2 illustrates the two different energy recourses [25].

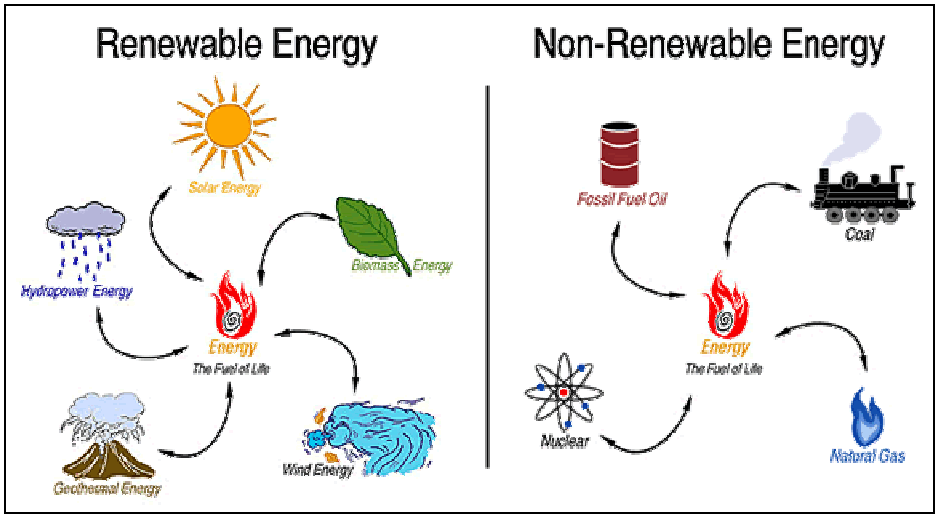


Fig.2.2: Classification of energy [25].

Most developed nations are dependent on non-renewable energy sources such as fossil fuels (coal and oil) and nuclear power. These sources are called non-renewable because they cannot be renewed or regenerated quickly enough to keep pace with their current usage. Some sources of energy are renewable or regarded as potentially renewable. Examples of renewable energy sources are: solar, geothermal, hydroelectric, biomass, and wind. Renewable energy sources are more commonly used in developing nations. Industrialized societies depend on non-renewable energy sources. Fossil fuels are the most commonly used types of non-renewable energy. They were formed when incompletely decomposed plant and animal matter was buried in the earth's crust and converted into carbon-rich material that is useable as fuel. This process occurred over millions of years. The three main types of fossil fuels are coal, oil, and natural gas. Two other less-used sources of fossil fuels are oil shale and tar sands [25].

2.3.1 Non-renewable energy

2.3.1.1 Oil

Crude oil or liquid petroleum is a fossil fuel that is refined into many different energy products (e.g., gasoline, diesel fuel, jet fuel, heating oil) figure 2.3 shows a schematic of a fractional distillation column. Oil forms underground in rock such as shale, which is rich in organic materials. After the oil forms, it migrates upward into porous reservoir rock such as sandstone or limestone, where it can become trapped by an overlying impermeable cap rock, figure 2.4 illustrates typical formation.

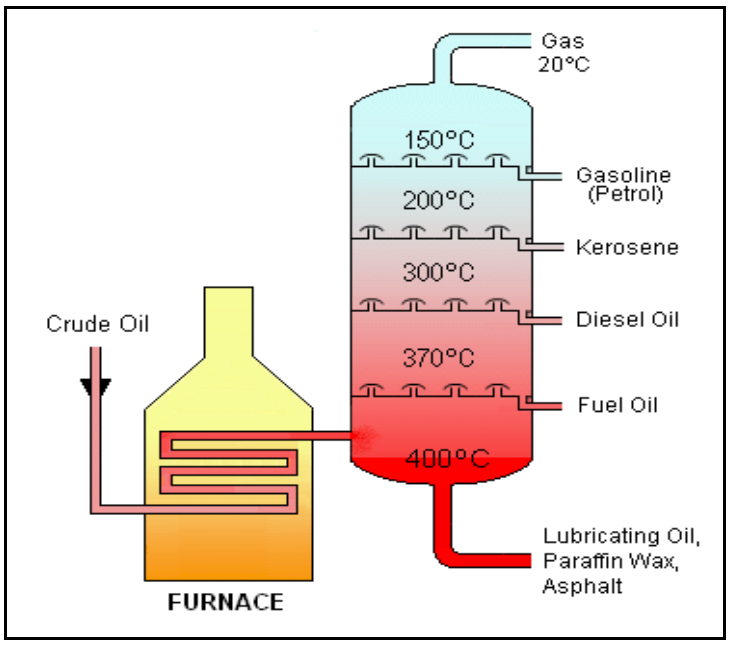


Fig.2.3: Schematic of a fractional distillation column [26].

Wells are drilled into these oil reservoirs to remove the gas and oil. Over 70 % of oil fields are found near tectonic plate boundaries, because the conditions there are conducive to oil formation.

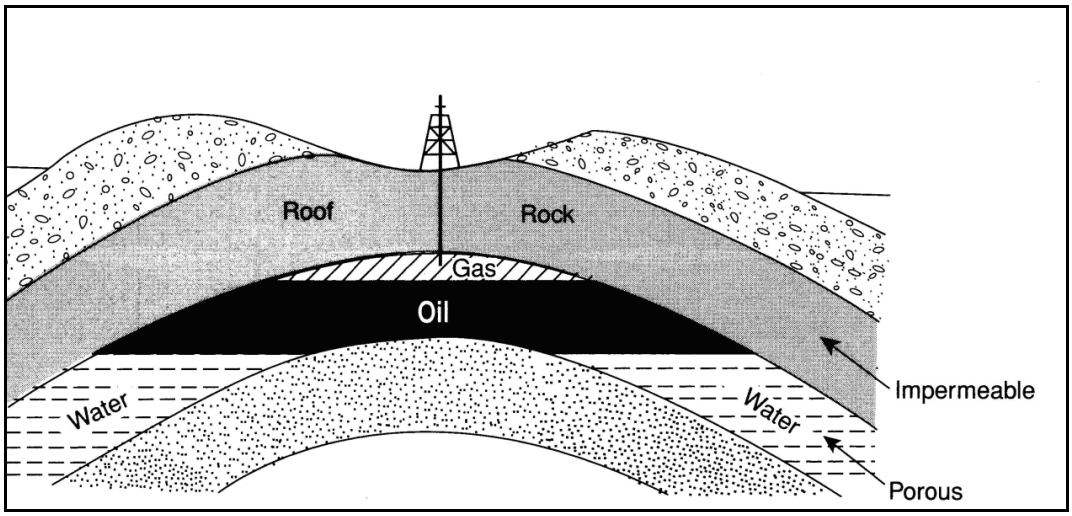


Fig. 2.4: A typical oil and natural gas deposit. [27]. Original source [28]

Oil recovery can involve more than one stage. The primary stage involves pumping oil from reservoirs under the normal reservoir pressure. About 25 % of the oil in a reservoir can be removed during this stage. The secondary recovery stage involves injecting hot water into the reservoir around the well. This water forces the remaining oil toward the area of the well from which it can be recovered. Sometimes a tertiary method of recovery is used in order to remove as much oil as possible. This involves pumping steam, carbon dioxide gas or nitrogen gas into the reservoir to force the remaining oil toward the well. Tertiary recovery is very expensive and can cost up to half of the value of oil removed. Carbon dioxide used in this method remains sequestered in the deep reservoir, thus mitigating its potential greenhouse effect on the atmosphere. The refining process required to convert crude oil into useable hydrocarbon compounds involves boiling the crude and separating the gases in a process known as fractional distillation. Besides its use as a source of energy, oil also provides base material for plastics, provides asphalt for roads and is a source of industrial chemicals [24] and [27].

The modern oil era was started by Colonel Edwin L. drake in 1859 in Titusvill, Pennsylvania, USA, when he drilled to just over 20 meters and plumbed the first large quantities of oil to the surface. By 1862, Pennsylvania production had risen to three million barrels per annum from 75 different wells. In 1909, US production stood at half a million barrels per day. During the 1920s and 1930s large fields were discovered and developed in the Middle East. Over 50 % of the world's oil is found in the Middle East; sizeable additional reserves occur in North America. Most known oil reserves are already being exploited, and oil is being used at a rate that exceeds the rate of discovery of new sources. If the consumption rate continues to increase and no significant new sources are found, oil supplies may be exhausted in another c.a. 30 years from 2011, figure 2.5 illustrates the annual regional oil production and consumption (2004).

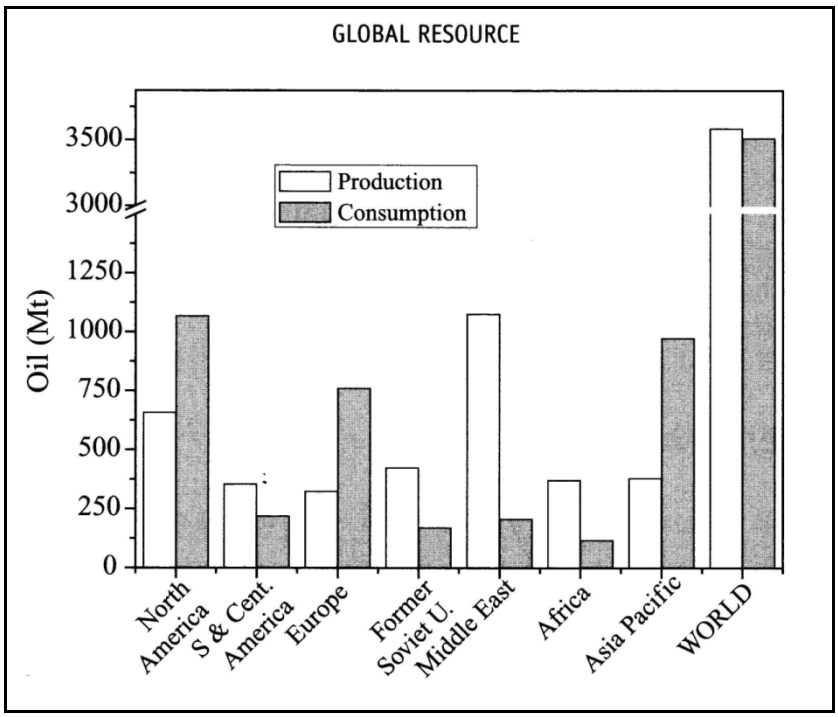


Fig.2.5: the annual regional oil production and consumption [27].

Until 1973, the price of crude oil was controlled by the large European and American producing companies and price had been stable since the end of nineteenth century (figure 2.6). However, in 1973 the dominant force became the producing nations in the form of the Organisation of Petroleum Exporting Countries (OPIC), which had come into existence in 1960 to represent the 12 countries, which together are responsible for almost half of world oil production.

Despite its limited supply, oil is a relatively inexpensive fuel source. It is a preferred fuel source over coal. An equivalent amount of oil produces more kilowatts of energy than coal. It also burns cleaner, producing about 50 % less sulphur dioxide. Oil, however, does cause environmental problems. The burning of oil releases atmospheric pollutants such as sulphur dioxide, nitrogen oxides, carbon dioxide and carbon monoxide. These gases are smog-precursors that pollute the air and greenhouse gases that contribute to global warming. Another environmental issue associated with the use of oil is the impact of oil drilling. Substantial oil reserves lie under the ocean. Oil spill accidents involving

drilling platforms kill marine organisms and birds. Some reserves such as those in northern Alaska occur in wilderness areas. The building of roads, structures and pipelines to support oil recovery operations can severely impact the wildlife in those natural areas [24] and [27].

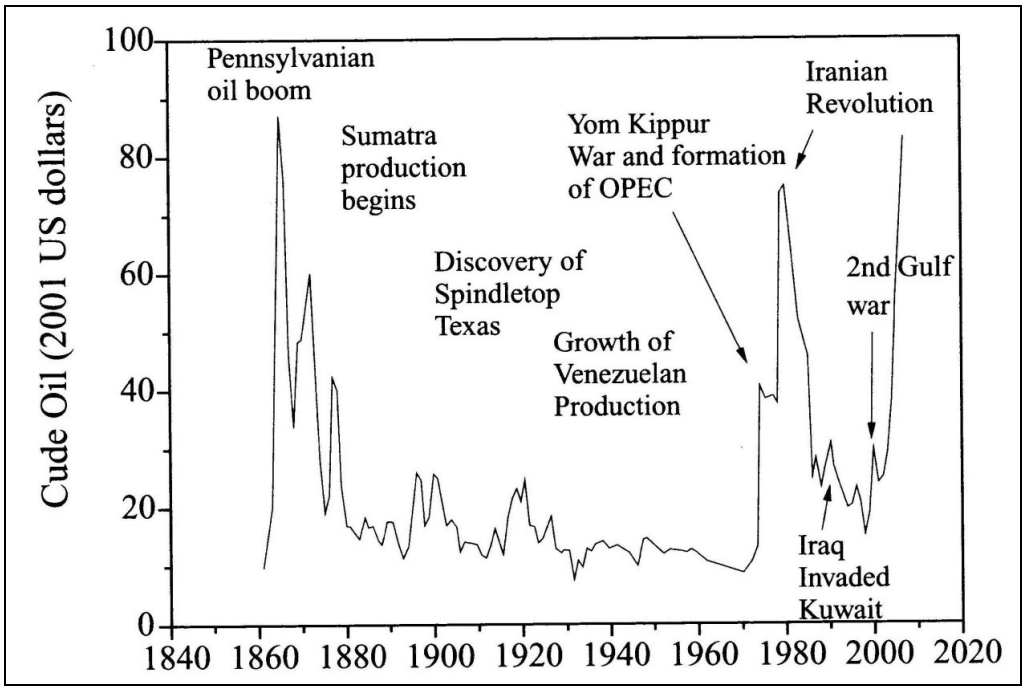


Fig.2.6: History of world oil prices in real terms.

2.3.1.2 Natural gas

Natural gas was used as early as the sixth century BC, in China and Japan. The gas was used primarily for lighting and was distributed via bamboo-constructed pipelines. Modern usage started in 1821 in New York on a small scale and then expanded in 1883 in Pittsburgh, USA, with the creation of the first modern natural gas pipeline. Natural gas production is often a by-product of oil recovery, as the two commonly share underground reservoirs. Such gas is found above the oil deposits, from which it derives; see figure 2.4. Gas can also be found remote from oil source, in which case its origin is usually lower-lying coal deposits that have been degassed through high temperature stresses. Natural gas connected with oil deposit is termed *associated gas* or *condensate*,

unconnected is termed *non-associated*. When extracted it is usually around 80 to 90 % methane (CH4). It also contains some ethane (C2H5), propane (C3H8), and butane (C4H10). Natural gas is not usually contaminated with sulphur and is therefore the cleanest burning fossil fuel. After recovery, propane and butane are removed from the natural gas and made into liquefied petroleum gas (LPG). LPG is shipped in special pressurized tanks as a fuel source for areas not directly served by natural gas pipelines (e.g., rural communities). The remaining natural gas is further refined to remove impurities and water vapour, and then transported in pressurized pipelines (figure 2.7). The United States has over 300,000 miles of natural gas pipelines [24].

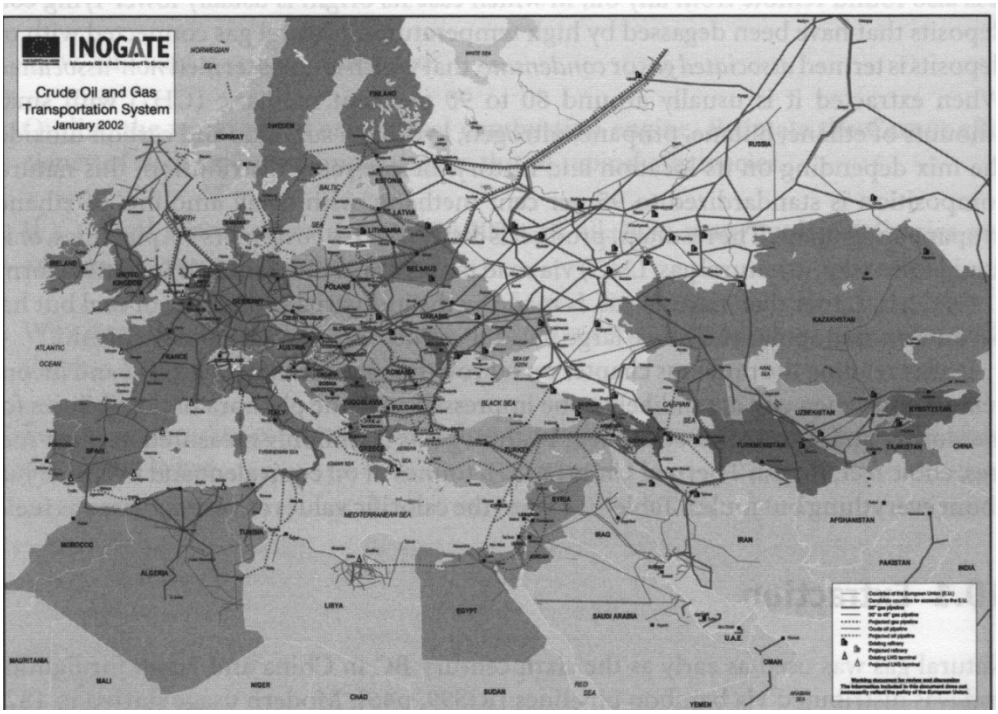


Fig. 2.7: The large scale gas and oil pipelines of Europe and nearby State [27].

Natural gas is highly flammable and is odourless. The characteristic smell associated with natural gas is actually that of minute quantities of a sulphur compound (ethyl mercaptan), which is added during refining to warn consumers of gas leaks.

The use of natural gas is growing rapidly. Besides being a clean burning fuel source, natural gas is easy and inexpensive to transport once pipelines are in place. In developed countries, natural gas is used primarily for heating, cooking, and powering vehicles. It is also used in processes for making ammonia fertilizer, figure 2.8 details the regional annual natural gas production and consumption together with their excess production globally, noting the USA has large negative excess, the sign of the excess is reversed in the graph by the excess production of Canada. In addition, the figure indicates that the production and consumption are somewhat balanced [27].

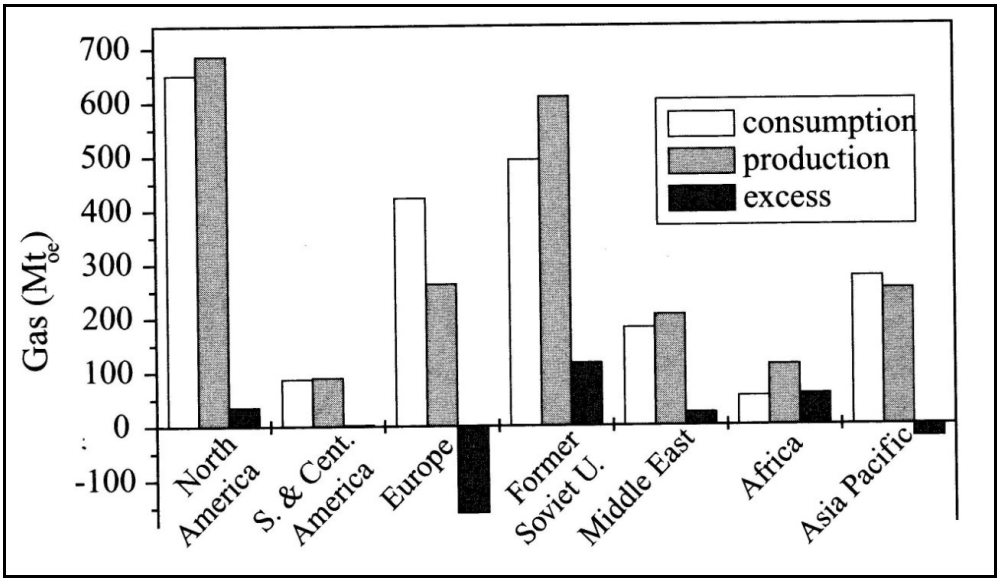


Fig. 2.8: Regional annual natural gas production and consumption together [27].

The current estimate of natural gas reserves is about 142400 $Mt_{oe.}$ At current levels of usage, this supply will last an estimated 100 years (figure 2-9). Most of the world's natural gas reserves are found in Eastern Europe and the Middle East, where Russian Federations among other states of the former Soviet Union hold over one third of global reserves. The price of natural gas is proven to be variable (figure 2.10) [24] and [27].

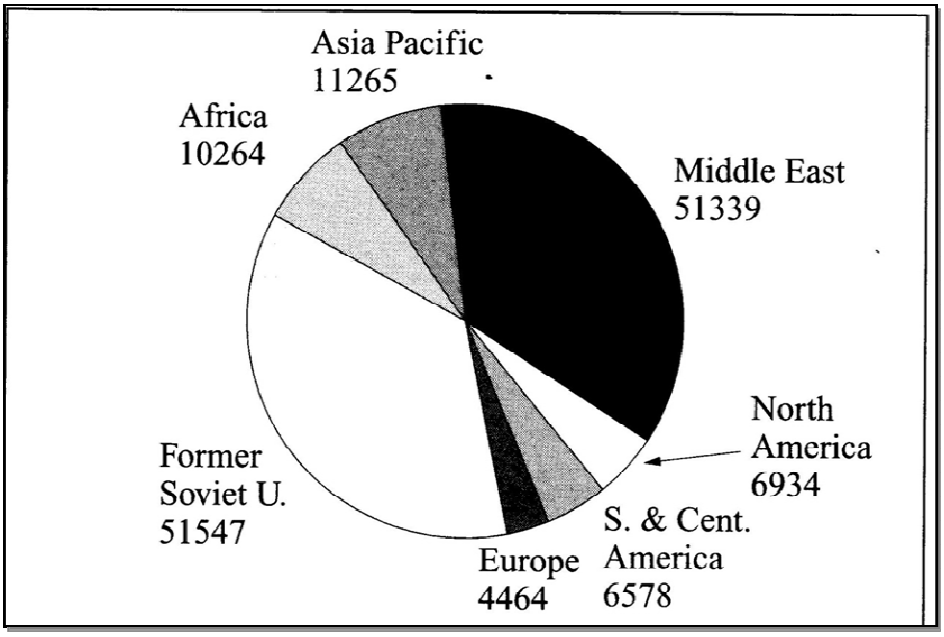


Fig. 2.9: Pie chart showing regional natural gas reserves (trillion cubic metres) [27], original source [29].

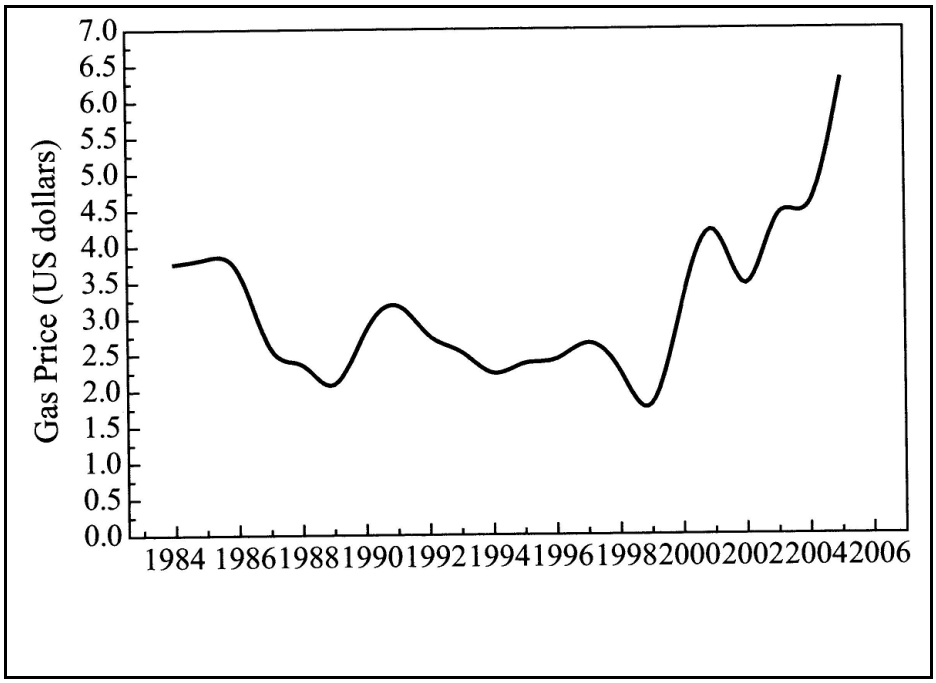


Fig. 2.10: Trends in natural gas price per million BTU [27].

2.3.1.3 Coal

Coal is a fossil fuel created from the remains of plants that lived and died about 100 to 400 million years ago when parts of the earth were covered with huge swampy forests. Coal is classified as a non-renewable energy source because it takes millions of years to form [30]. Coal is one of the world's most important sources of energy, fuelling almost 40% of electricity worldwide. In many countries this good evening value is much higher: Poland relies on coal for over 94% of its electricity; South Africa for 92%; China for 77%; and Australia for 76%. Coal has been the world's fastest growing energy source in recent years – faster than gas, oil, nuclear, hydro and renewable [31].

Coal formed slowly over millions of years from the buried remains of ancient swamp plants. During the formation of coal, carbonaceous matter was first compressed into a spongy material called "peat," which is about 90% water. As the peat became more deeply buried, the increased pressure and temperature turned it into coal. Different types of coal resulted from differences in the pressure and temperature that prevailed during formation. The softest coal (about 50% carbon), which also has the lowest energy output, is called lignite, it has the highest water content (about 50%) and relatively low amounts of smog-causing sulphur. With increasing temperature and pressure, lignite is transformed into bituminous coal (about 85% carbon and 3% water). Anthracite (almost 100% carbon) is the hardest coal and also produces the greatest energy when burned. Most of the coal exists, particularly in the United States is bituminous. Unfortunately, bituminous coal has the highest sulphur content of all the coal types. The burning of coal results in significant atmospheric pollution. The sulphur contained in coal forms sulphur dioxide when burned. Harmful nitrogen oxides, heavy metals, and carbon dioxide are also released into the air during coal burning. Installing scrubbers and electrostatic precipitators in the smokestacks of power plants can reduce the harmful emissions. The toxic ash remaining after coal burning is also an environmental concern and is usually disposed into landfills [24]. Coal mining creates several environmental problems. Coal is most cheaply mined from near surface de-posits using strip mining techniques. Strip-mining causes considerable environmental damage in the forms of erosion and habitat destruction. Sub-surface mining of coal is less damaging to the surface environment, but

is much more hazardous for the miners due to tunnel collapses and gas explosions [24]. Coal is the most abundant fossil fuel in the world with an estimated reserve of 984 million tonnes (figure 2.11) [27].

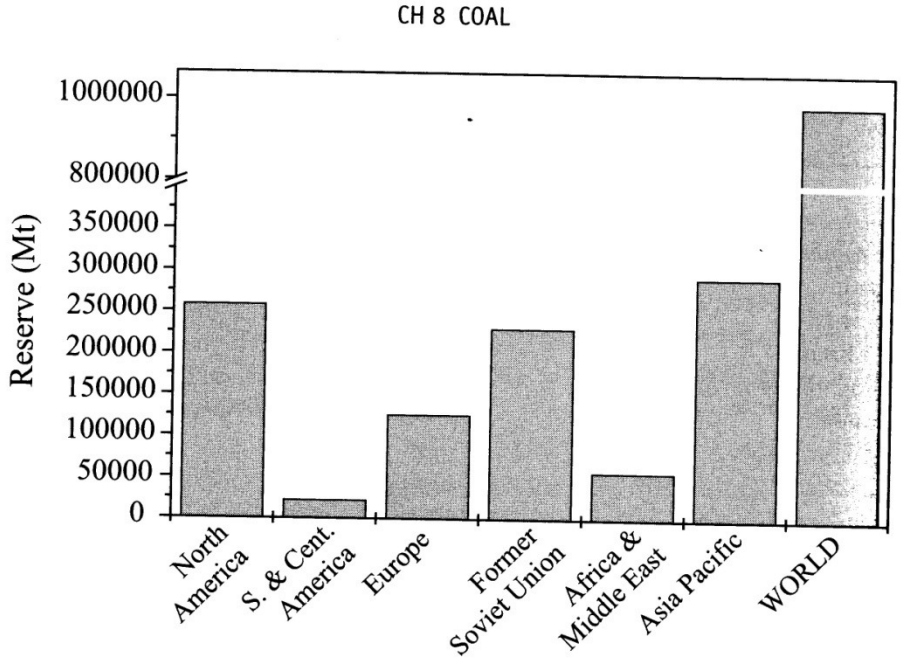


Fig. 2.11: Bar plot showing world coal reserves [27], original source [32].

Most of the world's coal reserves exist in Eastern Europe and Asia, but the United States also has considerable reserves. Currently, the world is consuming coal at a rate of about 5 billion metric tons per year. The main use of coal is for power generation, because it is a relatively inexpensive way to produce power. In addition to electricity production, coal is sometimes used for heating and cooking in less developed countries and in rural areas of developed countries [27].

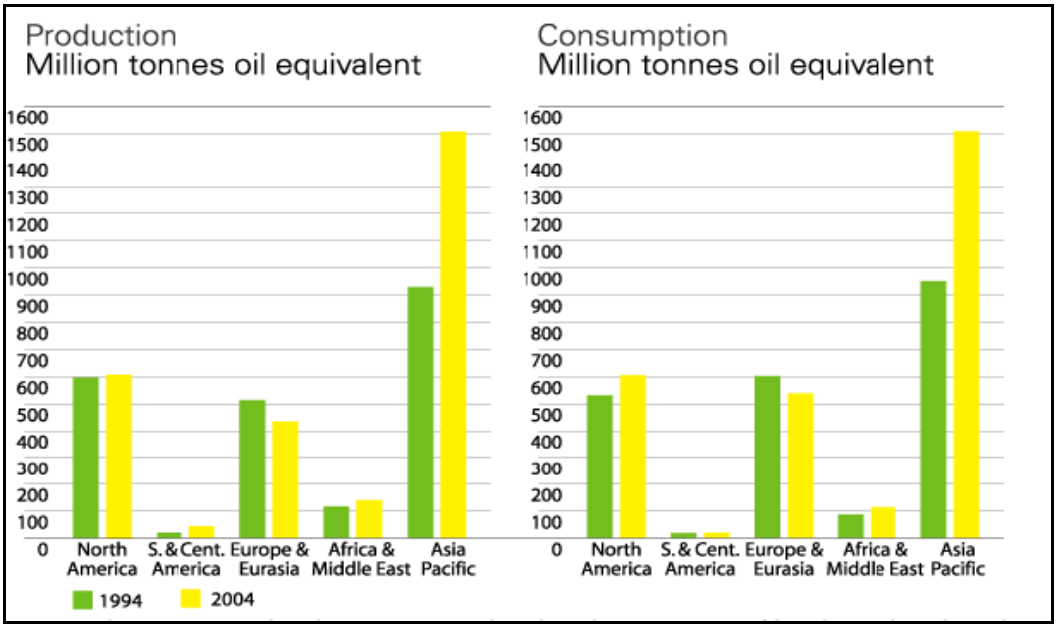


Fig. 2.12: Annual regional coal production, consumption and its growth [33].

If consumption continues at the same rate, the current reserves will last for more than 200 years, figure 2.12 illustrates the production and consumption of coal over the world, the figure demonstrates that the production and consumption reciprocate each other, also indicating that the growth of both production and consumption were strongest in Asia Pacific regions. In the past, the fluctuations in the world coal price have been small and thus no marked progression in price is realised, figure 2.13 illustrates the trends in coal price over c.a. 20 years.

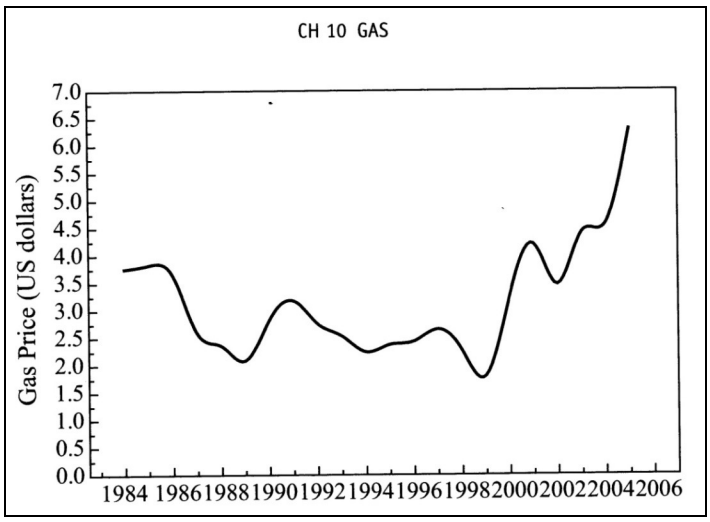


Fig. 2.13: Trends in coal prices (based on North Western European prices) [27].

2.3.1.4 Nuclear power

Nuclear power is generated using uranium, which is a metal mined in various parts of the world. The first large-scale nuclear power station opened at Calder Hall in Cumbria, England, in 1956 [34]. In 1957, the first commercial nuclear power station started production of electricity at a shipping port in Pennsylvania, USA. The development of the technology had been surprisingly rapid [27]. In most electric power plants, water is heated and converted into steam, which drives a turbine-generator to produce electricity. Fossil-fueled power plants produce heat by burning coal, oil, or natural gas. In a nuclear power plant, the fission of uranium atoms in the reactor provides the heat to produce steam for generating electricity. Several commercial reactor designs are currently in use in the United States where in figure 2.14 a model of commercial reactor is shown. The most widely used design consists of a heavy steel pressure vessel surrounding a reactor core. The reactor core contains the uranium fuel, which is formed into cylindrical ceramic pellets and sealed in long metal tubes called fuel rods. Thousands of fuel rods are contained in the reactor core. Heat is produced in a nuclear reactor when neutrons strike uranium atoms, causing them to split in a continuous chain reaction [24].

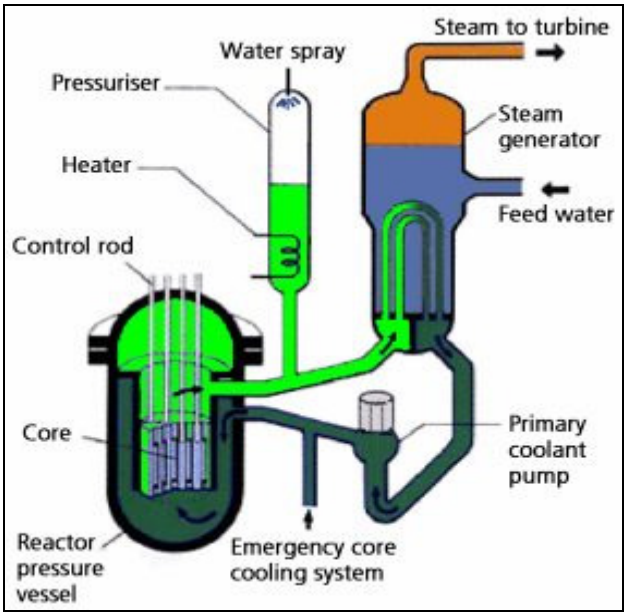


Fig. 2.14: Model of commercial reactor core [35].

Control rods, which are made of a material such as boron that absorbs neutrons, are placed among the fuel assemblies. When the neutron-absorbing control rods are pulled out of the core, more neutrons become available for fission and the chain reaction speeds up, producing more heat. When they are inserted into the core, fewer neutrons are available for fission, and the chain reaction slows or stops, reducing the heat generated. Heat is removed from the reactor core area by water whereby it is contained in a closed pressurized loop. The heat is transferred to a second water loop through a heat exchanger. The water also serves to slow down, or "moderate" the neutrons that are necessary for sustaining the fission reaction. The second loop is kept at a lower pressure, allowing the water to boil and create steam, which is used to power the turbine-generator and produce electricity. Originally, nuclear energy was expected to be a clean and cheap source of energy. Nuclear fission does not produce atmospheric pollution or greenhouse gases and it proponents expected that nuclear energy would be cheaper and last longer than fossil fuels. Unfortunately, because of construction cost overruns, poor management, and numerous regulations, nuclear power ended up being much more expensive than predicted. The nuclear accidents at Three Mile Island in Pennsylvania

and the Chernobyl Nuclear Plant in the Ukraine raised concerns about the safety of nuclear power. Furthermore, the problem of safely disposing spent nuclear fuel remains unresolved [24].

2.3.2. Renewable energy sources

Renewable energy is derived from natural processes that are replenished constantly. In its various forms, it is derived directly from the sun, or from heat generated deep within the earth. Included in the definition is electricity and heat generated from solar, wind, ocean, hydropower, biomass, geothermal resources [36]. The potential remains enormous; they can enhance diversity in energy supply markets, secure long-term sustainable energy supplies, and reduce local and global atmospheric emissions. They can also provide commercially attractive options to meet specific needs for energy services (particularly in developing countries and rural areas), create new employment opportunities, and offer possibilities for local manufacturing of equipment [37]. In 2006, renewable energy accounted for 14.7% of total energy production in the EU; figure 2.15 demonstrates the percentage of each source of renewable energy [38].

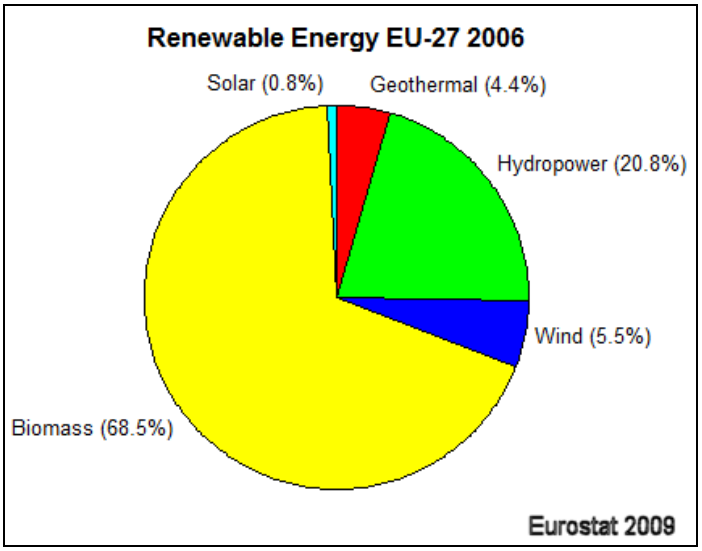


Fig. 2.15: Pie chart highlighting renewable energy EU-2006 [38].

2.3.2.1 Solar energy

Solar energy is electricity created using the sun. When the sun shines, it is possible to capture the sunlight using solar panels and turn the sun's energy into electricity. Once it is converted into electricity, it can power items in the home (or businesses) that require electricity to function [39], for this a series of processes are required [40]:

- The sun;
- Solar panels (to convert energy (sunlight) in to DC electricity);
- An inverter (to convert DC to AC for use in the home). Figure 2.16 illustrate how solar energy is working.

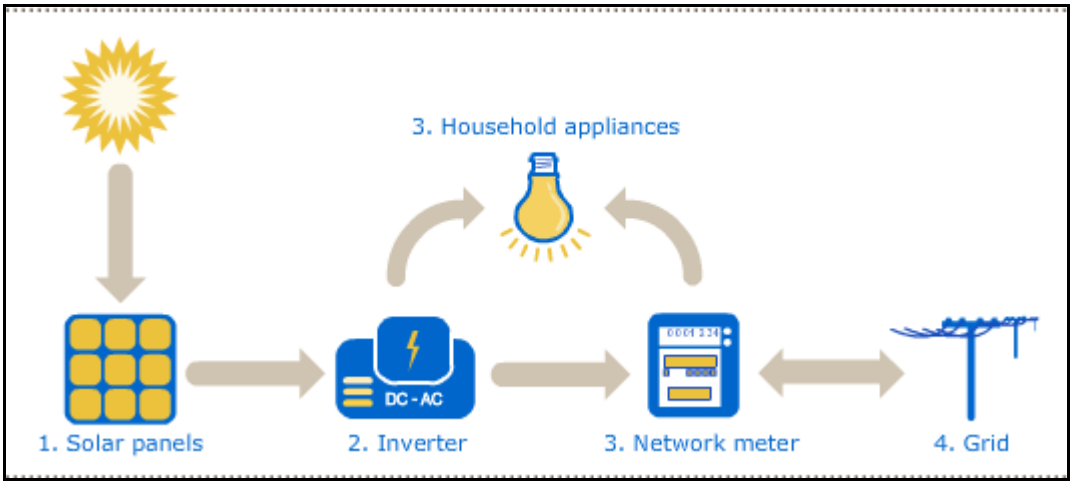


Fig. 2.16: The way that solar energy works [40].

Solar energy is the most abundant inexhaustible form of energy. Solar energy is a clean, inexpensive alternative to other energy sources that burn fossil fuels and emit greenhouse CO_2 gasses into the air. Solar radiation is an agglomeration of light, heat, and other radiation that is emitted from the sun. Solar radiation contains impressive quantities of heat that reaches the Earth's atmosphere and facilitates most life processes on the planet. Until recently, it has been difficult to harness the sun's energy to power and heat items, for example; homes and cars. Recent innovations in technology have enabled the utilization of more of the sun's rays than ever before.

There are two categories of solar energy: thermal and light. Light solar energy can be harnessed by photo-voltaic cells (PV) using semiconductor-based technology to convert light energy directly into an electric current that can either be used right away, or stored for later use in battery form. PV panels are becoming widely used, as they are now multipurpose. PV panels can be mounted on residential and commercial roofs or on the sides of buildings where they can efficiently catch the sun's rays. They provide a clean renewable energy resource, which supplements the heating sources currently available in homes. PV can provide a viable energy alternative to regions with limited or non-existent mains electricity supply. The disadvantage of PV energy panels and solar energy heating systems are the high costs and relatively low energy conversion rate. The conversion rates of most PV energy systems are around 16%. The energy conversion rate of thermal solar energy can be as high as 50-60%, and is more energy efficient. Thermal energy can be used to heat the interior of buildings in a number of ways. For instance, the use of certain types of building materials can be used to absorb the heat from the sun's rays and minimize the amount of energy that is reflected out. With solar water heating systems, solar energy is used directly to heat water for applications such as radiant floor heating. In regions where thermal energy panels are able to catch the sun's rays unobstructed, solar water heaters can be a viable alternative or supplement to conventional heating. Thermal energy can be utilized for many household purposes including hot water used for cooking or bathing, and space heating [27]. Stored thermal energy may even be used for cooling in a process known as absorption chilling technology. Flat panel solar collectors have been in use for several decades, but only of late have technological advances yielded such a high-energy conversion rate along with ease of installation. With these technological advances, the number of households using thermal solar energy has increased steadily [27,41].

2.3.2.2 Wind Energy

Wind energy concerns the use of natural wind as an energy source. A wind energy system transforms the kinetic (moving) energy of the wind into a useful form of energy, such as using wind turbines to make electricity, wind mills for mechanical power, wind pumps for pumping water or drainage, or sails to propel ships [42]. Wind energy has been used for thousands of years for milling grain, pumping water and other mechanical power applications. The first windmills were used mainly for grinding grain and pumping water in Persia about 500-900 A.D. these were vertical-axis systems [43]. Today there are several hundred thousand windmills in operation around the world [44]. At the end of 2009, worldwide rated capacity of wind-powered generators was 159.2 GW. Energy production was 340 TWh, which is about 2% of worldwide electricity usage (figure 2-17 illustrates the world installed capacity) [45]. The highest wind velocities are generally found on hilltops, exposed coasts and out at sea. Mountains, oceans, valleys and other features of the terrain create local wind patterns that change from season to season, and even hour to hour. The wind energy system mainly depends on the extraction of energy from the wind by the wind turbine. The main components of a wind turbine are; the rotor, the transmission system, the generator, the yaw and control systems [46]. The environmental impact of wind power is relatively minute. Wind power consumes no fuel, and emits no air pollution, unlike fossil fuel power sources. The energy consumed to manufacture and transport the materials used to build a wind power plant is equal to the new energy produced by the plant within a few months of operation. One disadvantageis danger to birds and bats which forms a primary concern in some locations; some studies have shown that the number of birds killed by wind turbines is very low compared to the number of those that die as a result of certain other ways of generating electricity, especially where the environmental impacts of using non clean fuel sources are described [47].

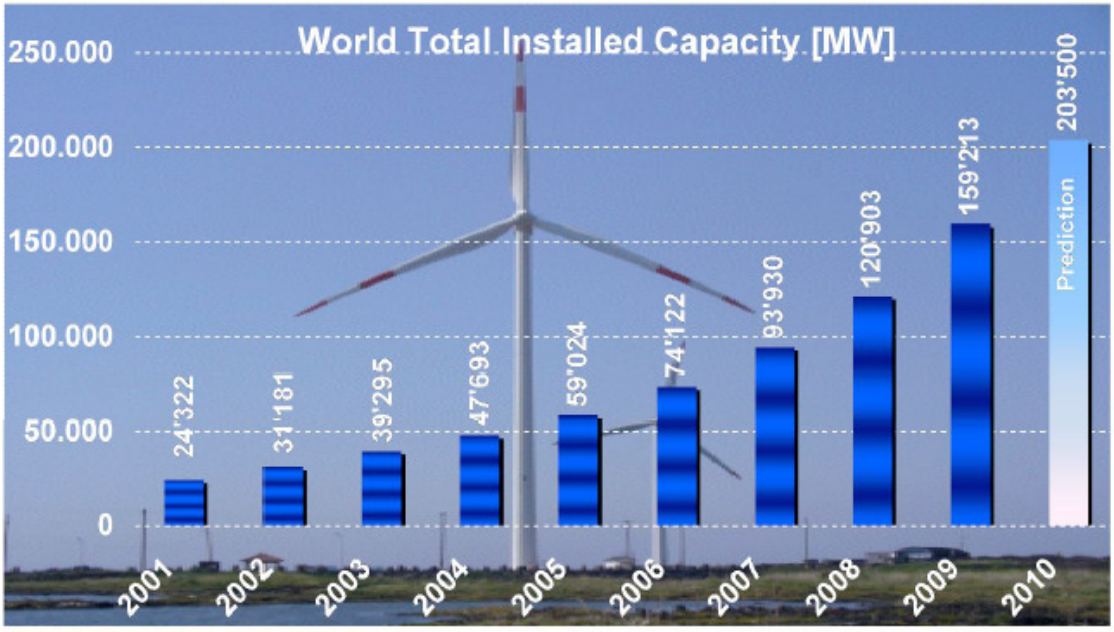


Fig. 2.17: World total installed capacity of wind energy [45].

2.3.2.3 Hydroelectric Energy

Hydroelectricity is electricity produced by the movement of fresh water from rivers and lakes (see figure 2.18) [48]. This water comes to the rivers as runoff from rainfall. Rainfall is powered by solar energy, which drives complex energy transfer processes in the atmosphere and between the atmosphere and oceans. The potential (gravitational) energy associated with this water matrix causes it to flow downwards. This downward motion of water contains kinetic energy that can be converted into mechanical energy, and then from mechanical energy into electrical energy in hydroelectric power stations [49].

The first proposals to use the rivers and lochs of south-west Scotland for hydro power appeared in the 1890s, but the scheme became feasible only with the establishment of a National Grid in the 1920s[44].

Fig. 2.18: Hydroelectricity energy [48].

2.3.2.4 Geothermal Energy

Geothermal energy comes from the natural heat of the Earth, originating deep in its molten interior (see figure 2.19) [50]. This heat is stored in rock and water within the Earth (which is forced to the surface in certain areas in the form of hot steam or water, e.g. hot springs and geysers) and can be extracted by drilling wells to tap irregular concentrations of heat at depths shallow enough to be economically feasible. Low enthalpy resources (50°C to 150°C) can be used for heating purposes: large base load demands such as district heating, horticulture, recreational uses such as spas. It are these medium and high enthalpy resources (> 150°C) that are used for electricity production. Some geothermal resources may be regarded as renewable because they are derived from energy sources deep within the Earth's interior. This energy source is so large that the rate of depletion by a geothermal energy extraction project is negligible. However, projects based on using the remainder of the heat stored in shallowly placed igneous rocks may be non-renewable [41]. Like many energy resources, humankind's use of

geothermal energy has a longer history than is probably realised. Roman spa towns were an early example as were Polynesian settlements in New Zealand 1000 years ago [27].
If environmental impact geothermal energy is to be considered, it is given that the carbon emission levels are well below the figures for natural gas, oil or coal-fired power stations per kWh generated, such as; 0.01 – 0.4 kg/kWh of carbon dioxide compared to 0.5 – 1.1 kg/kWh of carbon dioxide from fossil fuels [46].

Fig. 2.19: Geothermal energy [50].

2.3.2.5 Wave/Tidal Energy

Wave power results from the harnessing of energy transmitted to waves by winds moving across the ocean surface. Ocean waves are caused by winds as they blow across the surface of the sea. The energy that waves contain can be harnessed and used to produce electricity. Due to the direction of the prevailing winds and the size of the

Atlantic Ocean, the UK and north western Europe have one of the largest wave energy resources in the world.

Tidal energy is the energy dissipated by tidal movements, which derives directly from the gravitational and centrifugal forces between the earth, moon and sun. The tidal phenomenon occurs twice daily. A tide is the regular rise and fall of the surface of the ocean due to the gravitational force of the sun and moon on the earth. Tidal power is not a new concept; it has been used since the 11th Century in Britain and France for the milling of grains. Tidal energy offers a vast and reliable open energy source. Currently, harnessing tidal energy from the rise and fall of the tides has been exploited on a commercial scale using tidal barrage systems (see figure 2.20). Recent efforts to exploit this predictable energy source have been directed towards kinetic energy from tidal currents [51, 41,44].

Fig. 2.20: Tidal Energy.

2.3.2.6 Biomass energy

Biomass is defined as contemporary plant matter formed by photosynthetic capture of solar energy and stored as chemical energy [52].

$$CO_2 + 2H_2O \rightarrow CH_2O + H_2O + O_2$$

CO_2 from the atmosphere and water from the earth are combined in this photosynthetic process to produce carbohydrates that form the essential building blocks for biomass. The solar energy that drives photosynthesis is stored in the chemical bonds of the structural components of biomass. The process is cyclic because the biomass absorbs the same amount of CO_2 in growing that it releases when burned as a fuel in any form (figure 2-21 illustrate the CO_2 cycle). As a consequence, global warming and the contribution of biomass are minute. In addition, biomass fuels contain negligible amounts of sulphur, thus their overall contribution to acid rain remains largely not scalable [53].

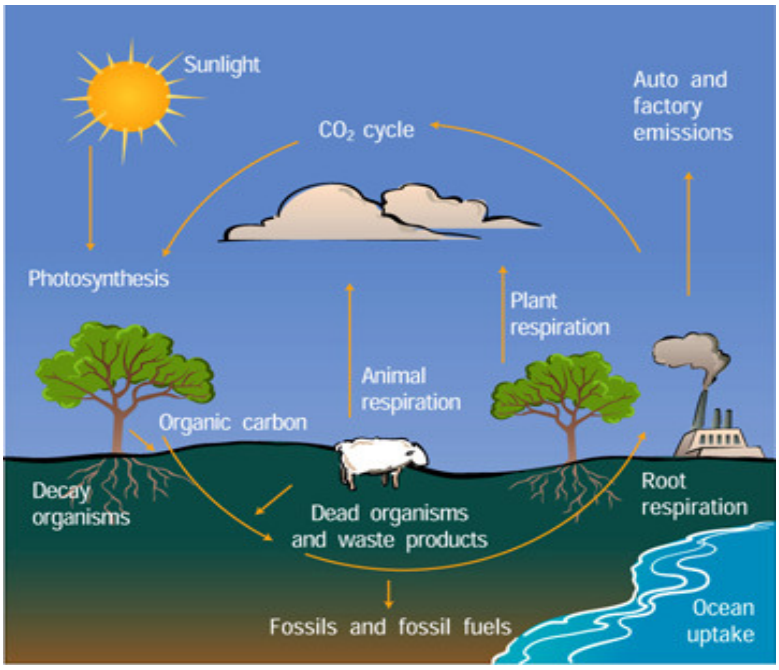


Fig. 2.21: Carbon cycle [54].

Biomass is a simple term for all organic material that has been derived from plants or animals such as trees, crops (including algae), agricultural and industrial residues along with forestry processes, human or animal wastes (figure 2-22). In nature, all biomass ultimately decomposes to its elementary molecules with the release of heat. Therefore, the release of energy from the conversion of biomass into useful energy imitates natural processes (but at a faster rate), and this energy can be considered renewable energy. Converting biomass to fuel can be as simple as cutting trees into small pieces so they can be burned to produce heat or electricity, or as complicated as converting it into a liquid or gaseous fuel (e.g. sugar-cane or cereal crops to liquid fuels such as ethanol). Unlike any other energy resource, using biomass to produce energy is often a way of disposing of biomass waste materials that otherwise would create environmental risks [55 ,56].

Fig. 2.22: Biomass sources [57].

The main biomass utilization technologies that produce energy from biomass that are useful are:

- Direct Combustion;
- Gasification;
- Anaerobic Digestion;
- Pyrolysis.

2.4 Environmental Impacts

The extraction, conversion, and utilization of various forms of energy is recognized as one of the major contributors to environmental degradation at a global and local level; be it greenhouse gas emissions and local air pollution due to combustion of fossil fuels, coal, nuclear energy and deforestation [58]. Burning fossil fuels increase the effect of greenhouse gases in the atmosphere, this is also known as Global Warming. Global warming is threatening animal extinction, extending pollution, human migration and sudden climate changes [59]. There appears to be an agreement among the world's leading environmental scientists that there is a discernable level of activity attributed to human influence on the climate where a direct link between the concentration of greenhouse gases and the increase in global temperatures is prominent. Gases such as carbon dioxide, nitrous oxide, ozone, chlorofluorocarbon (CFCs) and methane, allow the Sun's energy to penetrate the Earth's atmosphere and at the same time act as a blanket, trapping the heat radiated from the Earth's surface, (figure 2.23) demonstrating the growth rate of those gases since 1979 to 2009. Human activities such as using fossil fuels as energy sources, has increased the amount of greenhouse gases (especially CO_2), thereby amplifying the greenhouse effect [55,60]. Therefore, the issue of global climate change is gaining greater interest in the scientific community. To address this phenomenon, the Kyoto Protocol was introduced in 1997. The purpose of the Kyoto Protocol was to reduce the total greenhouse gas emissions of developed countries (and countries with economies in transition) to 5% below the level they were in 1990 (United Nations, 1998). The protocol sets targets for greenhouse gas emissions of developed countries for the period 2008 to 2012. EU have specifically set a target to tackle climate change by reducing greenhouse gases emissions by 8% between 2008-2012 and target further reduction of CO_2 by 20 % in 2020 [61].

In order to achieve such an ambitious target, motivation for renewable energy has become a principle for sustaining energy in the future. Therefore the need for renewable energy technologies has emerged significantly in recent years derived from; wind, solar, geothermal, biomass and hydro power technology.

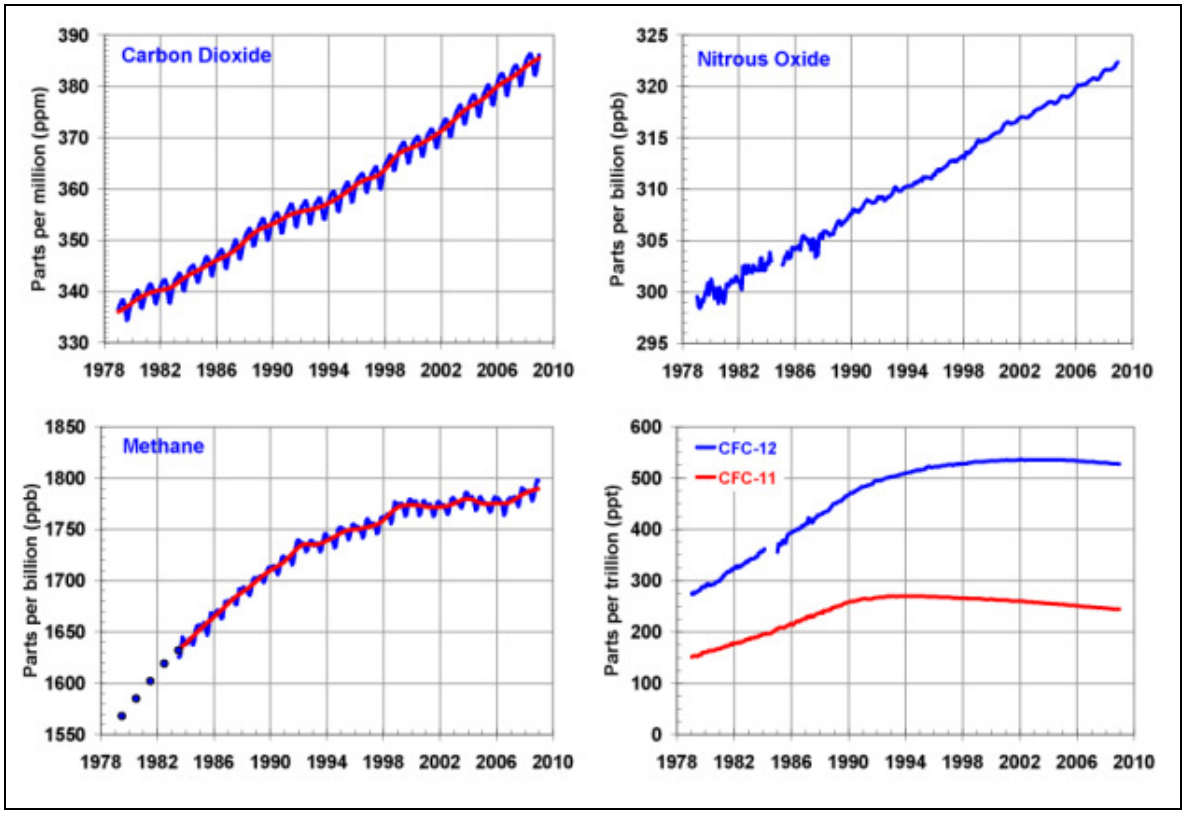


Fig. 2.23: Global averages of the concentrations of the major greenhouse gases from the NOAA global flask sampling network since the beginning of 1979 [62].

2.5 History of anaerobic digestion

The process of breakdown of organic material by micro-organisms in the absence of oxygen is commonly known as AD resulting in biogas (methane and carbon dioxide). This can then be used as chemical feedstock or as a fuel. In 1860, Louis Pasteur discovered all fermentation processes are resultant from microbial activity. Louis Pasteur also defined the process of fermentation as life without oxygen [63]. Anaerobic digestion has long been exploited by human beings for brewing alcoholic drinks, bread making and food preservation [64] however, anecdotal evidence indicates that biogas was used for heating bath water in Assyria 3,000 years ago [65]. In the 17th century, Jan Baptita Van Helmont Flammable described the release of combustible gas evolving from

decaying organic matter [66 ,67]. In 1776, Alessandro Volta examined the relationship between the amount of decaying organic matter and the amount of flammable gas released [66]. In 1808, Sir Humphrey Davy concluded that methane was present in gases produced during the anaerobic digestion of animal manure [66]. The first anaerobic digestion plant occurred in 1859 in Bombay, India [66]. In 1895, anaerobic digestion was introduced in England; biogas released from the sewage treatment plant was used to light street lamps in Exeter [66]. Buswell (1936) and Boyle (1977) developed a scientific model describing the composition of biogas (CH4, CO2, H2S and NH3) following anaerobic digestion and the chemical composition of organic substrates: C, H, N and S the chemical formula illustrates the biodegradability and the composition of methane yielding after anaerobic digestion [68]. The Buswell and Boyle scientific chemical formula [69]:

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3.d}{4} + \frac{e}{2}\right)H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} + \frac{3.d}{8} + \frac{e}{4}\right)CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3.d}{8} + \frac{e}{4}\right)CO_2 + d\,NH_3 + e\,H_2S$$

2.6 The Anaerobic Digestion (AD) Process

Nature has a provision of destroying and disposing of wastes and dead plants and animals. Tiny microorganisms called bacteria carry out this decay or decomposition. Manure and compost can also be obtained through the decomposition of organic matter. AD is defined as the biological breakdown of organic material by the microorganisms in an airtight environment with no oxygen present [70]. The AD process can be used to turn residues from livestock farming, food processing industries, waste water treatment sludge, water treatment plant sludge among other organic wastes into biogas and digestate. The biogas can be used to generate heat and/or electricity; fibre. The biogas produced in AD plants is comprised largely of methane (60-80%) and carbon dioxide (20-40%) but also usually contains a small amount of hydrogen sulphide (H_2S) and ammonia (NH_3), as well as traces of other gases [71].

2.7 Biochemical Reactions in anaerobic digestion

AD is a series of chemical reactions during which organic material is decomposed through the metabolic pathways of naturally occurring microorganisms in an oxygen-depleted environment. AD can be used to process any carbon-containing material, including food, paper, sewage, yard trimmings and solid waste, with varying degrees of degradation. The organic fraction of municipal Solid Waste, for example, is a complex substrate that requires an intricate series of metabolic reactions to be degraded. This section describes the reactions pertaining to AD and the reactions detailing the intermediary products formed and the types of bacteria involved [72].

The full process can be considered to occur in four stages as illustrated in Figure 2-24. Hydrolysis is the process in which complex molecules are broken down to constituent monomers; acidogenesis, in which acids are formed; acetogenesis, or the production of acetate; and methanogenesis, the stage in which methane is produced from either acetate or hydrogen. Digestion is not complete until the substrate has undergone all of these stages, each having a physiologically unique bacteria population responsible that require dissimilar environmental conditions [73].

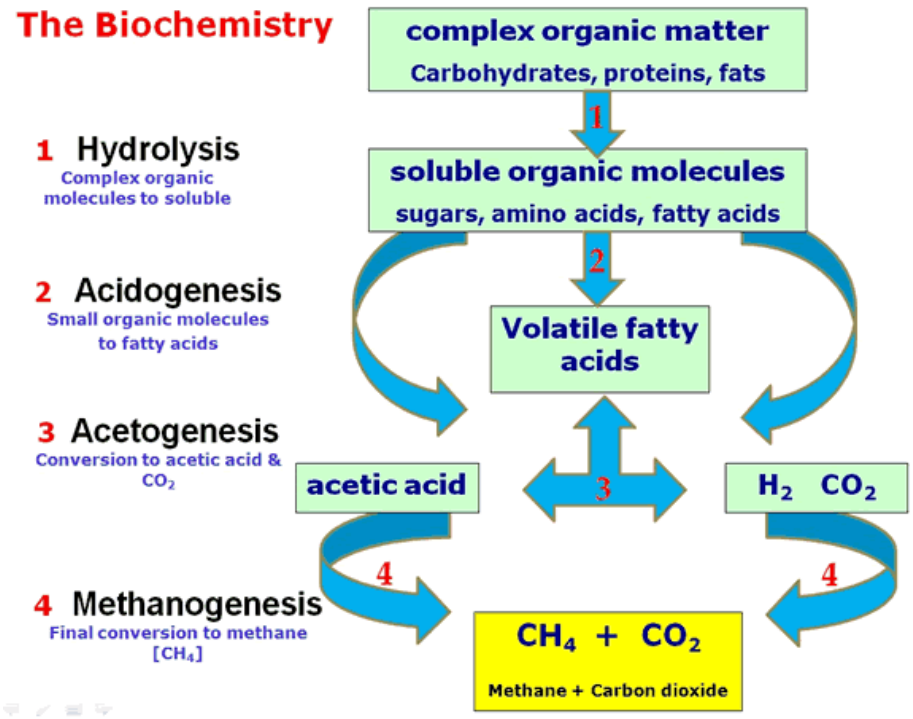


Fig. 2.24: The anaerobic digestion biochemical conversion pathways [74].

2.7.1 Hydrolysis

In the first stage, complex organic materials are broken down into their constituent monomers in a process known as hydrolysis. The result is the creation of soluble monomers: proteins are converted to amino acids; fats to fatty acids, glycerol and triglycerides; complex carbohydrates such as polysaccharides, cellulose, lignin, starch and fibre converted to simple sugars, such as glucose. Fermentative bacteria are responsible for the creation of monomers, which are then available to the next group of bacteria. Hydrolysis is catalyzed by enzymes excreted from the bacteria, such as cellulase, protease, and lipase. If the feedstock is complex such as raw cellulolytic waste, which contains lignin, the hydrolytic phase is relatively slow [75]. For this reason, woody waste is not an ideal feedstock for the AD process. Carbohydrates, on the other hand, are known to be more rapidly converted via hydrolysis to simple sugars and subsequently fermented to volatile fatty acids (VFA). An approximate chemical formula

for the mixture of organic waste is $C_6H_{10}O_4$. A hydrolysis reaction where organic waste is broken down into a simple sugar, in this case glucose, can be represented by the following [73]:

$$C_6H_{10}O_4 + 2H_2O \rightarrow \ C_6H_{12}O_6 + 2H_2 \qquad (1)$$

2.7.2 Acidogenesis

Hydrolysis is immediately followed by the acid-forming phase of acidogenesis. In this process, acidogenic bacteria turn the solubilised monomers produced by hydrolysis into simple organic compounds, mostly short chain volatile fatty acids (e.g., propionic, formic, lactic, butyric, or succinic acids), ketones (e.g., ethanol, methanol, glycerol, and acetone) and alcohols. The specific concentrations of products formed in this stage vary with the type of bacteria as well being influenced by the culture conditions, such as temperature and PH [75]. Typical reactions in these stages are shown below. In equation 2, glucose is converted to ethanol and equation 3 shows glucose is transformed to propionate.

$$C_6H_{12}O_6 \leftrightarrow 2\ CH_3CH_2OH + 2CO_2 \qquad (2)$$

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2\ H_2O \qquad (3)$$

2.7.3 Acetagenesis

The next stage of acetagenesis is often considered with acidoenesis to be part of a single acid forming stage. The long chains volatile fatty acids (VFA) formed during acidogenes are oxidized to acetate or propionate and hydrogen gas by the acetogenic bacteria. These bacteria require a low H_2 partial pressure in order to conserve energy for growth. The role of hydrogen as an intermediary is of critical importance to AD reactions to support the growth of methogenic bacteria for the conversion of CH_4 [76]. The free energy value of the reaction that converts propionate to acetate, shown in equation 4, where acetate and hydrogen are consumed by bacteria, however, the free energy becomes negative. In

general, for reactions producing H_2, it is necessary for hydrogen to have a low partial pressure for the reaction to proceed [75].

$$CH_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \qquad (4)$$

2.7.4 Methanogenesis

The methanogenic anaerobic bacteria involved in the final stage, known as methanogenesis or methane fermentation can produce methane in two ways: either by means of cleavage of acetic acid molecules to generate carbon dioxide and methane, or by reduction of carbon dioxide with hydrogen [77]. About 2/3 of the methane produced in an anaerobic digester comes from acetate [76]. The reactions that occur during this stage are as follows.

Acetate conversion:

$$2CH_3CH_2OH + CO_2 \leftrightarrow 2CH_3COOH + CH_4$$

Followed by: $CH_3COOH\ CH_4 + CO_2$

Methanol conversion:

$$CH_3OH + H_2 \leftrightarrow CH_4 + H_2O$$

Carbon dioxide reduction by hydrogen

$$CO_2 + 4H_2 \leftrightarrow CH4 + H_2O$$

2.8 General Process Description AD

The process of AD can be further divided into four stages: pre-treatment, digestion, and gas upgrading and digestate treatment (see figure 2.25).

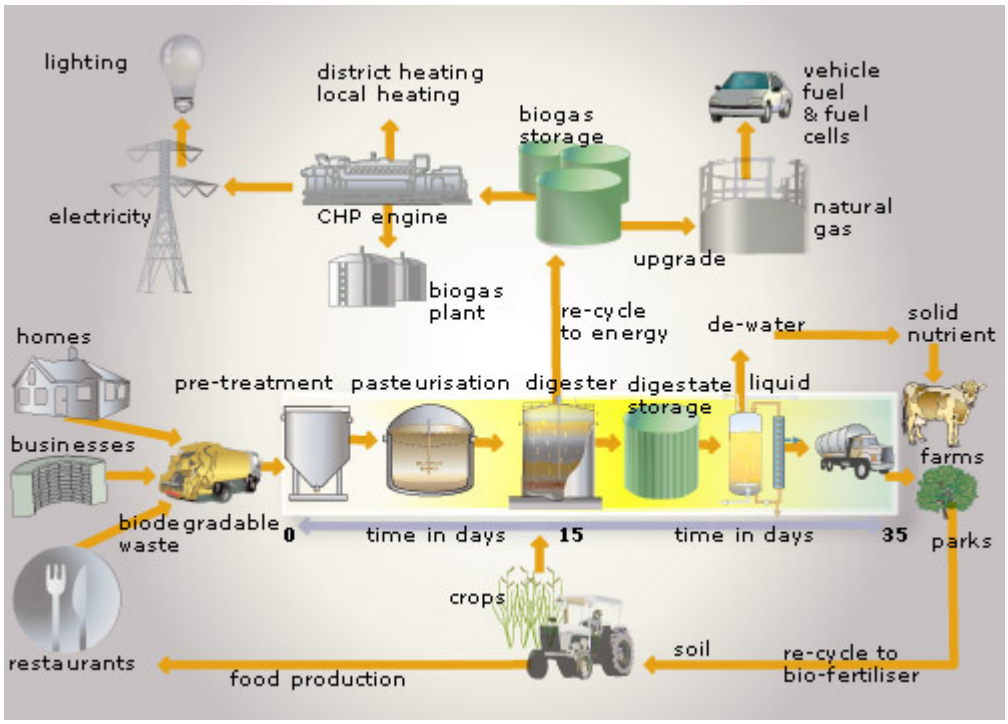


Fig. 2.25: General Process stages of AD [78].

2.8.1 Pre-treatment

Pre-treatment is used extensively to improve degradability and rate of hydrolysis material being fed into digesters to increase the methane yield in the anaerobic digestion process [79]. Some biomass wastes which are composed of cellulose, hemicelluloses and lignin, have evolved to resist degradation. Pre-treatment therefore is needed to alter or remove structural and compositional impediments to the hydrolysis process and subsequent degradation processes in order to enhance digestibility, improve the rate of enzyme hydrolysis and increase yields of intended products [80] (see figure 2.26).

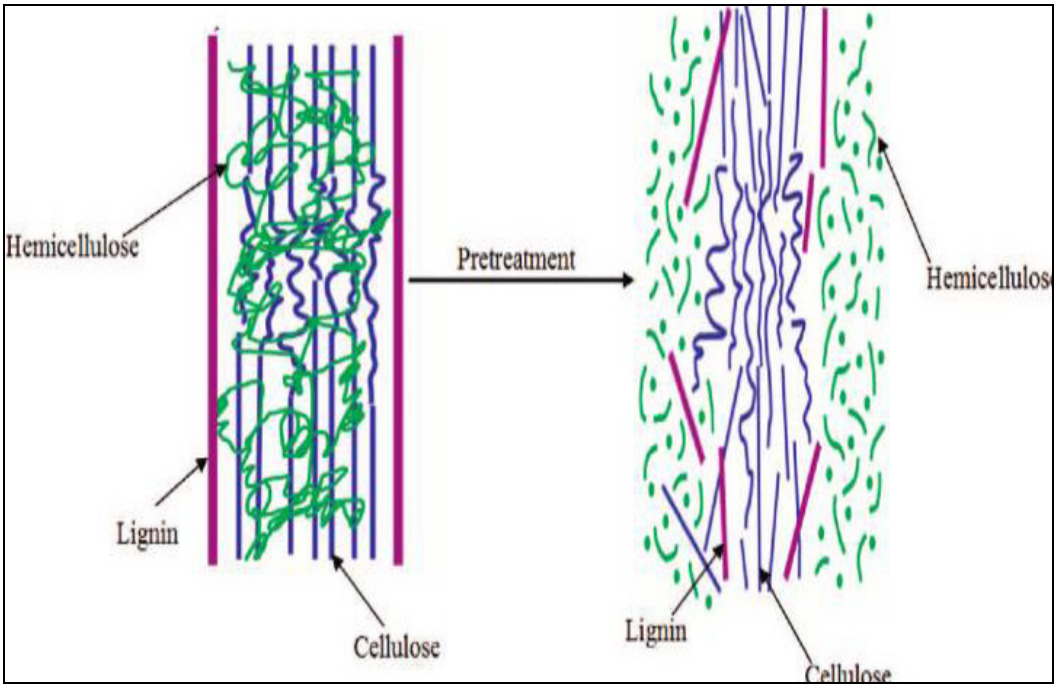


Fig 2.26: Schematic of the role of pre-treatment in the conversion of biomass to fuel [81].

Carrere *et al.*, pointed out that pre-treatment could be done in any of the following ways [82]:

- Biological treatment methods;
- Chemical treatment methods;
    - Oxidation;
    - Alkali treatment;
- Thermal hydrolysis;
- Mechanical treatment.

2.8.2 Digestion

The digestion stage takes place in a digester. There are many types of digesters that can operate within two temperature ranges, either at 35 °C (mesophilic) or 55 °C (thermophilic). The digestion can be either dry or wet depending on the solid content [83]. Thus, the feedstock can be mixed with water and other appropriate liquid wastes such as sewage sludge or re-circulated liquid from the digester effluent [77].

2.8.3 Gas upgrading

The biogas produced during the digestion stage has to be upgraded because it contains impurities that can damage boilers and combined heat and power units. Removal of carbon dioxide will be required if the gas is to be used as natural gas or vehicle fuel [84].

2.8.4 Digestate

Digestate is the residual fibrous material left at the end of processing End-use ranges from landfill cover, land spread for agriculture or the production of a high quality soil conditioner after an additional maturation process. The quality of the original input bio waste determines the quality of the digestate at the end of the process. The digestate produced by most operational plants is destined for use as a soil conditioner and most have a useful level of nutrients resulting in less demand for inorganic fertilisers. There is also evidence that using digestate on land has the benefit of suppressing normal pathogen and parasite levels [83, 84].

2.9 Mechanical pre-treatment

Mechanical pre-treatment is aimed at reducing the particle size and crystallinity of the substrate thus increasing the digestibility of cellulose and hemicellulose in the biomass material. This increases the digestion performance and biogas yield [85]. The following mechanical pre-treatment methods are given from Section 2.9.1 to 2.9.6.

2.9.1 Ultrasonic treatment.

The major effect of ultrasonic treatment is in the disruption of the physical, chemical and biological properties of sludge, reduction of floc size, and biodegradability improvement. So, an ultrasonic pre-treatment of sludge could increase sludge biodegradability through enhancing the hydrolysis stages and thus leads to enhanced anaerobic digestion [86,87]. The ultrasound treatment is cyclic sound pressure

(compression and rarefactions) with low frequencies (20–40 kHz); these frequencies are common and extremely efficient in generating high-frequency waves. When these ultrasound waves propagate in sludge medium, it generates a repeated pattern of compressions and rarefactions in the medium. The compressions cycle makes positive pressure on the liquid by pushing the molecules together and the rarefaction cycle makes a negative pressure by pulling the molecules from one another. Micro bubbles (cavitations) are generated from large negative pressure in rarefaction region. As a result of alternating expansion and compression cycles, these bubbles expand and implode, at very extreme conditions of temperature (5000 K) and pressure (500 bar) (see fig. 2.27) and produces hydro mechanical shear forces, rupturing the cell wall and membranes [86, 88 , 89, 90].

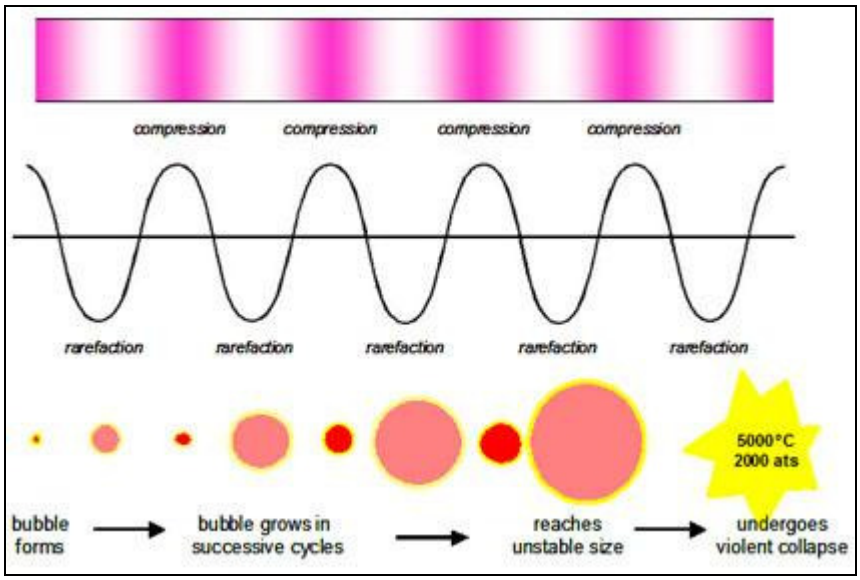


Fig. 2.27: The illustration shows how a cavity builds up successively until it implodes [91].

Kameswari *et al*., observed that during the optimum contact times of 2 and 1 min, increases in the soluble chemical oxygen demand COD of 85 and 97% were observed for the primary and the secondary sludge samples using ultrasonication where it was observed that, during 6 weeks of residence time The increase in biogas generation was

observed for ultrasonicated pre-treated primary and secondary sludge along with fleshings was 53% [90].

2.9.2 Grinding

Another predominantly used pre-treatment technique is milling, or more specifically a grinding technique. The aim of milling is to improve susceptibility of enzymatic hydrolysis by reduction of particle size and crystallinity of lignocelluloses in the material, These parameters lead to the increase of the total hydrolysis yield by 5–25 %, but also reduce the digestion time by 23–59%, thus increasing the digestion efficiency and biogas yield [92, 93, 94]. Several milling technologies were experimented with ranging from, mechanical chopping, hammer milling, roll milling, colloid mill, vibratory milling and ball milling [85,95 ,93]. All these techniques have increased surface area and confirm successes as a low cost pre-treatment strategy [85,96], It has been reported that the ball mill is the best performing and the most common form of milling treatment, though many disadvantages make it not economically feasible for large-scale applications, especially due to high energy requirements, long process times, and feed rate of material [97].

Ball mills consist of a cylindrical chamber (vertical or horizontal) which is almost completely filled with grinding beads by diameter (0.2–0.25mm) into the agitator disc that generate kinetic energy to small beads in the chamber, this energy creates shear forces and compression loading between the grinding agents to break the cell walls (see Fig. 2.28) [89, 98, 99 , 100].

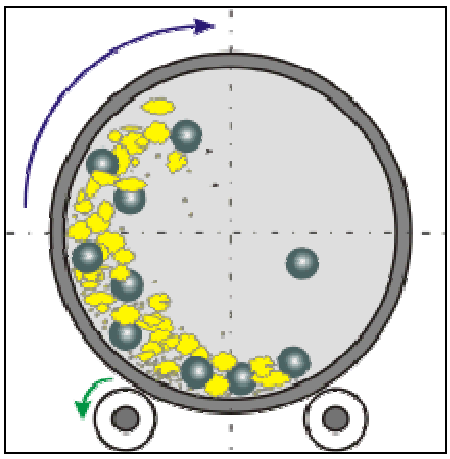


Fig. 2.28: Horizontal section of ball mill [101].

The size of the substrate is usually 0.2-2 mm after milling or grinding [102], while extremely reducing the size of the substrate this has little effect on the hydrolysis rate of the biomass. It is caused by the accumulation of volatile fatty acid (VFA), resulting in decreased methane production and decreased solubility in the anaerobic digestion process [103,104]. The energy requirement for mechanical commination depends on the final particle size and materials characteristics and can be one of the most important parameters describing the economical side of this physical pre-treatment [97, 105].

Mshandete *et al.* [1] used sisal fibre waste and found a reduction in grinding to 2 mm particle size using a laboratory mill with 2 mm sieve and demonstrated that smaller particles increased the surface area available to the microorganisms. This resulted in increased food availability to bacteria; thus, anaerobic biodegradability increases and mentioned that the methane yield increased by 23% with decreasing particle size from 100 mm to 2 mm and the fibre degradation increased from 31% to 70%. Izumi *et al.* [103] studied grinding pre-treatments on food waste by a bead mill process and found that particle size of the substrates decreased from 0.843 to 0.391 mm, respectively, at 20,000 total revolutions where methane production increased by 28% when the particle size was decreased from 0.888 to 0.718mm. Kratky *et al.* [97] pointed out that Koullas reported the dependence of process time on the hydrolysis effectiveness for wheat straw.

The results of this work demonstrated that the conversion of saccharides for untreated straw was 17.7% and after ball milling with a process time of 2 h there was an increase in conversion up to 61.6%. Baier *et al.* [106] reported that during anaerobic digestion of sludge, which had been disintegrated by stirred ball mills by diameter 0.25 mm, grinding was more beneficial on digested sludge (increase of batch biogas production by 60%).

2.9.3 High pressure homogeniser

A high pressure homogenizer is one of most widely used methods in large-scale operations; disruption in this method is worked out through pumping the sludge under high pressure (400-900 bars) through a homogenizing valve at high velocity against an impaction ring with a decrease in pressure, (See fig. 2.29) this will generate intense energy which lead to the formation of cavitation bubbles [89, 100, 107, 108] . Rai *et al.* [109] studied the disintegration of sewage sludge by employing high-pressure homogenizers with disk valves from 150 to 750 bars, they found out that the degree of disintegration increased to 29% and increasing in particle size reduction was observed. Engelhart *et al.* [110] studied the effects of mechanical disintegration (by a high-pressure homogenizer) on anaerobic biodegradability of sewage sludge. A 25% increase in volatile solids where a reduction was achieved, also resulting in a higher specific biogas production. Onyeche *et al.,* [111] conducted mechanical disruption by using a high-pressure homogeniser for sewage sludge at 500 bar where it was demonstrated after 20 days, improved anaerobic digestion could be realised thereby increasing the biogas production.

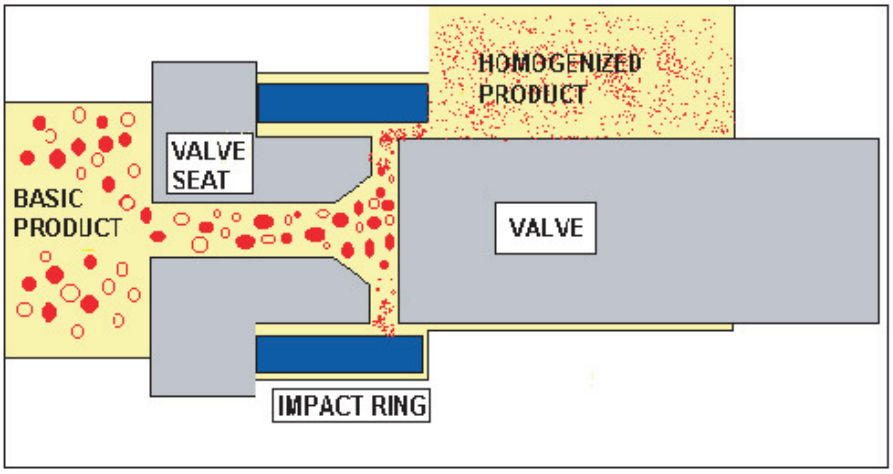


Fig. 2.29: Cross-section of high pressure homogeniser [108].

2.9.4 Collision plate

This technique is commonly used in the treatment process of wastewater, where sludge is pressurised to 30–50 bar by a high pressure pump and jetted to the collision plate after going through a nozzle (see Fig. 2.30). Thus, sludge undergo a rapid depressurisation and then jetted on to a plate with velocities of 30–100ms^{-1}. This process has only been applied at laboratory scale and allowed to decrease in Hydraulic Retention Time (HRT) from 14 to 6 days without affecting anaerobic digestion performance [82]. Nah *et al.* [112] examined the mechanical pre-treatment of Waste Activated Sludge (WAS) and determined that jetting to and colliding with a collision plate at 30 bar to solubilize the sludge, thus enhanced volatile mass reduction to 30% and unit gas production and decrease the anaerobic digester SRT from 13 to 6 days.

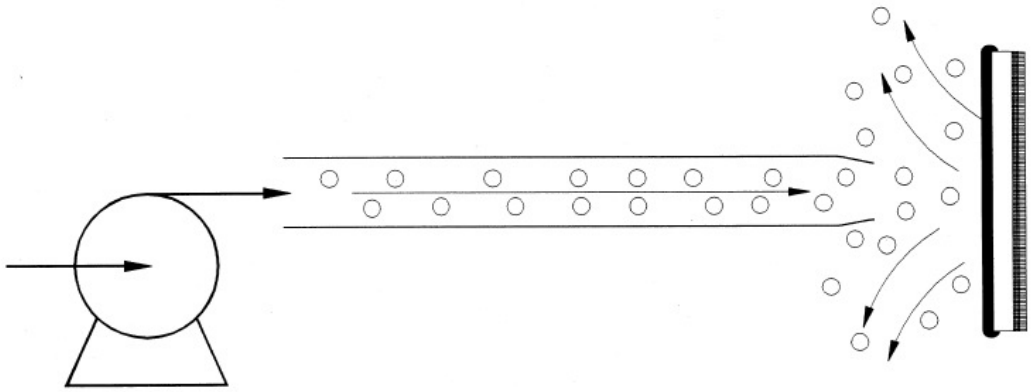


Fig. 2.30: Schematic diagram of the Collision plate mechanical pre-treatment of WAS [112].

2.9.5 Lysis-centrifuge.

Lysis-centrifuge works by directly operation on the thickened sludge stream in a dewatering centrifuge. The goal of this method is the partial disintegration of cells during the thickening with the centrifuge through kinetic energy generated by the centrifuge without any additional energy [99] (see Fig.2.31). Zabranska *et al.* [113] proved that anaerobic digestion of sewage sludge can be improved by this process, organic matter in digested sludge significantly decreased to 48-49% and an increase in biogas production by 15–26%. In similar studies, Delgenés *et al.* [99] mentioned that Dohanyos *et al.* [114] reported that the improvement of methane yield from thickened activated sludge, in comparison with untreated activated sludge, was 84.6%. The extent of increase in methane production was found to depend on sludge age and the content and type of organic material in mixed raw sludge, and the hydraulic retention time in digesters [113,115].

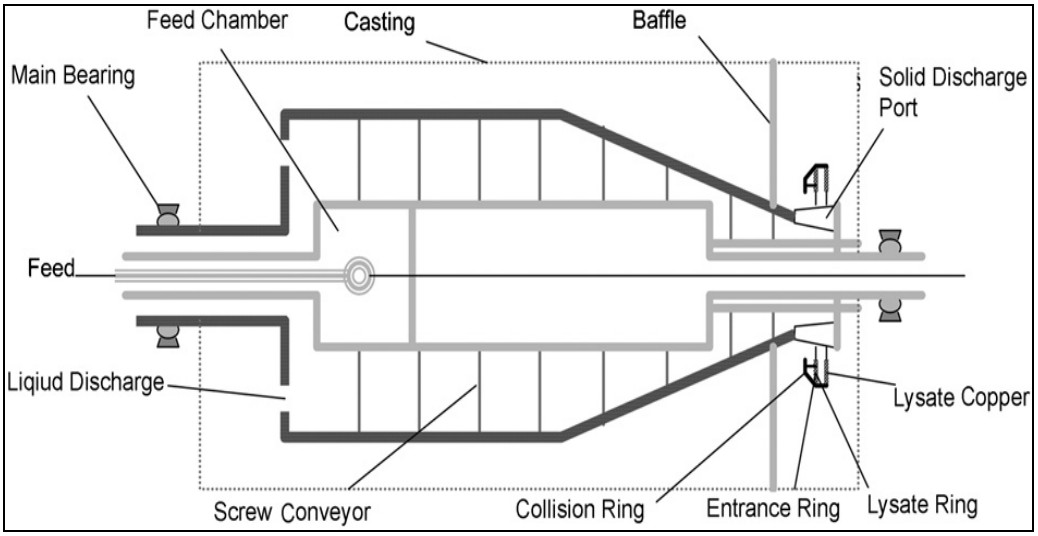


Fig. 2.31: Schematic of a lysate-thickening centrifuge [89].

2.9.6 Beating treatment

The latest mechanical treatment method is called ***beating treatment***, which has been introduced by the biomass research team in the school of mechanical, and manufacturing engineering in Dublin City University (DCU) based on employing Hollander beater device [116].

The Hollander Beater was developed by Dutch scientists sometime between 1660 and 1682. The purpose of the machine was to produce paper pulp from cellulose containing plant fibres. The Hollander beater (see Fig. 2.32) consists of an ovoid raceway with a beater wheel placed at a single point along the raceway. The beater wheel is made up of a number of paddles mounted on a shaft. The beater wheel is similar in appearance to a water wheel. The raceway is usually filled with a solution of water and plant fibres.

The fibrillated fibres created by beating are abraded to the extent that many partially broken off fibrils extend from the main fibre, increasing the fibres surface area and therefore its potential for hydrogen bonding [117].

Beating treatment employs Hollander Beater to treat lignocellulosic materials. Beating lignocellulosic materials will result in disruption to the crystalline structure of cellulose cells and break down the lignin component, this will improve hydrolysis and methane

yield [116] more details about beating mechanical treatment and Hollander Beater device is presented in chapter 4.

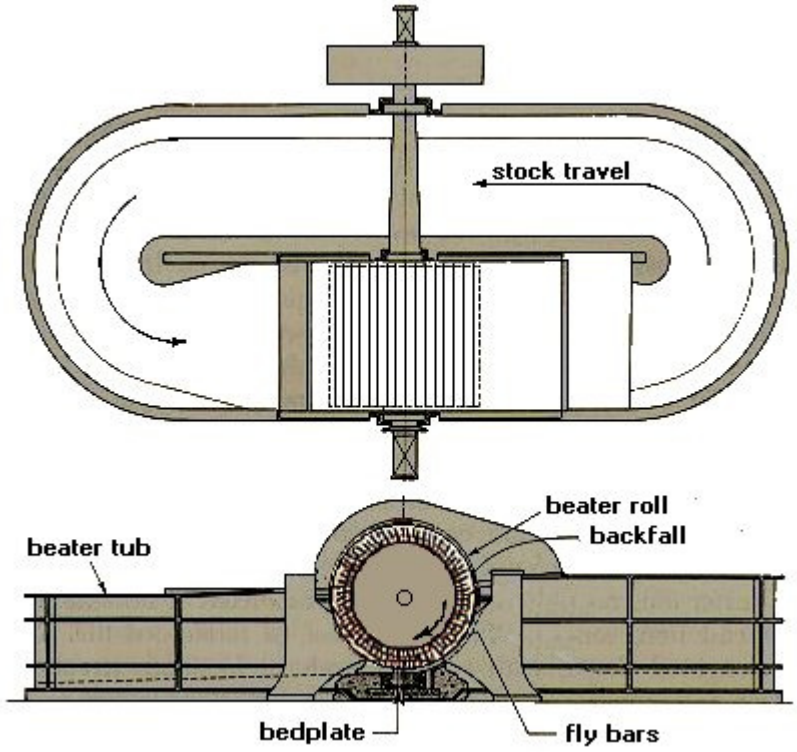


Fig. 2.32: Hollander Beater with the main parts illustrated and named [118].

2.10 Conditions and variables influencing AD

The complete process of anaerobic digestion requires a complex interaction of several varieties of bacteria that must be in equilibrium in order for the digester to remain stable [56]. The methanogenic phase is normally considered the limiting step of the process due to the slow growth rate of the methanogenic bacteria. [119]. Various physical–chemical conditions affect the production of methane, and inhibition of bacterial activity by either substrate or product may be expected when their concentrations are increased to certain extremities. These extremes must be monitored and maintained using the following parameters within acceptable ranges: pH, temperature, C/N ratio, retention time, volatile fatty acids VFA**,** bacterial competition, nutrient content, the presence of

toxicants and solids content. The optimal ranges and importance of these parameters are discussed below.

2.10.1 Temperature

Temperature is considered as the most important operational digestion parameter in AD processes. There is a linear relationship between the temperature and the rate of metabolic reaction during AD [120]. It is well known that there are three ranges of anaerobic degradation temperatures: degradation at ambient temperature (psychrophilic range) at 0-20 °C, mesophile degradation at 33-40 °C and thermophilic degradation at 50-60 °C. Typically, temperature ranges that at higher temperature decomposition take place quickly. Technically, only the mesophilic and thermophilic range is interesting with optimum at 35 °C and 55 °C, respectively, since at the ambient temperature the anaerobic degradation is extremely slow [121, 122]. It has been observed that higher temperatures in the thermophilic range reduce the required retention time. In fact, the greater gas production can be obtained if a digester operates in thermoplilic conditions as shown in Figure 2.33 [123]. However, this is rarely performed because the energy requirement in maintaining the temperature is more expensive than the biogas yields. Moreover, the thermoplilic bacteria are more sensitive than that of mesoplilic bacteria, so higher costs are needed to control the temperature in the thermophilic range [124,125]. Bolzonella, *et al.* [126] reported that all digestion plants were initially operated at mesophilic temperatures. Therefore, it was recommended to preserve digestion system at mesophilic level, in order to maintain the feasibility of utilizing AD to produce alternative source of renewable fuel [127].

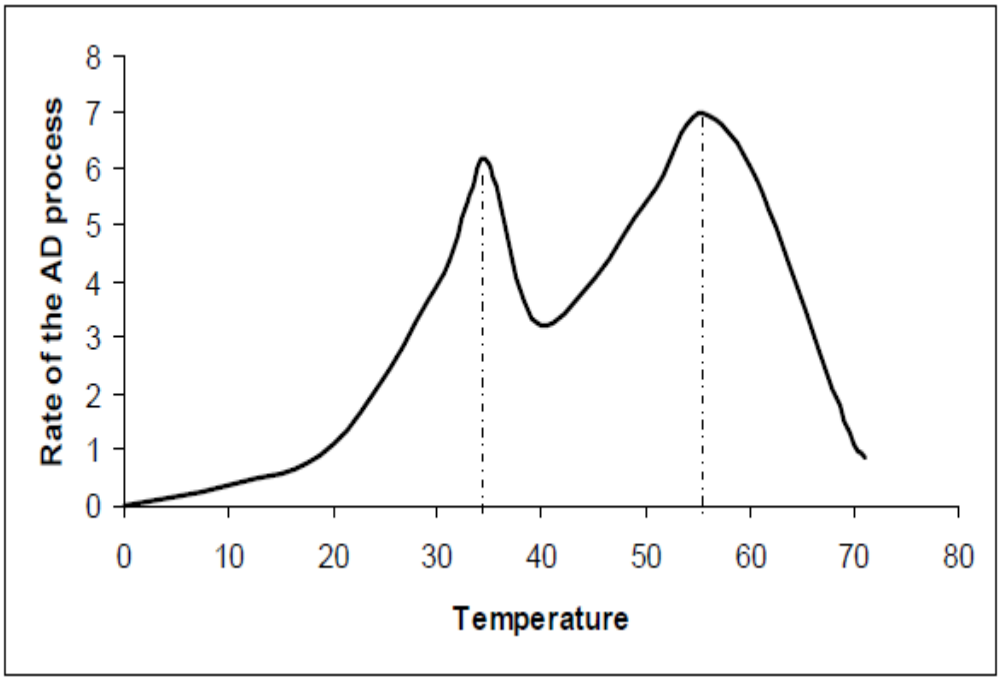


Fig. 2.33: Temperature range for anaerobic digestion [123].

2.10.2 PH

The pH values indicate the acidity/alkalinity of a given solution. A stable pH indicates system equilibrium and digester stability. A falling pH can point toward acid accumulation and digester instability [56]. Anaerobic bacteria, especially the methanogens, are extremely sensitive to pH in the reaction. During digestion, the two processes of acidogenesis and methanogenesis require different pH levels for optimal process control [128]. Alastair *et al.* [129] pointed out that the optimal pH of methanogenesis is around pH 7.0, the optimum pH of hydrolysis and acidogenesis is between pH 5.5 and 6.5 as reported by [130,131]. This is an important reason why some designers prefer the separation of the hydrolysis/acidogenesis and acetogenesis/methanogenesis processes in two-stage processes. It was suggested that pH value below 7 could prevent methanogenesis from acetate and also inhibit the microbial population that is in the system. The reported optimal pH range appears to be at neutral state from 7 to 7.2 [56].

2.10.3 Volatile fatty acids (VFA)

Volatile fatty acids (VFA) are intermediate compounds (acetic HAc, propionate HPa, ethanol Het, lactate LA and butyric HBu) that are produced during acidogenesis. Volatile fatty acids can be inhibitory to the production of methane. The increase concentration of acid exhibits the effect of fermentation digestion [132,133]. Hydrogen plays a significant role in preventing the formation of methane if the accumulation of acids is out of control. The high concentration of VFA will decrease the pH value and indirectly disrupt the fermentation process. AD processes will not work below certain pH value as mentioned above [134,135]. It has been shown that fermentation of glucose is inhibited at total VFA concentrations above 4 g l^{-1} [119]. Acetic acid is usually present in higher concentrations than other fatty acids during anaerobic digestion [132], but propionic and butyric acids are inhibitory to the methanogens. Propionic acid concentrations over 3000 mg l^{-1} have previously been shown to cause digester failure [136]. In a more recent study, it was found that propionic acid was an effect rather than a cause of inhibition of anaerobic processes [137].

As shown in many studies, the conversion rates of VFAs to methane vary in the order of acetic acid HAc > ethanol (HEt) > butyric acid (HBu) > propionic acid (HPa) [138]. Lactic acid, which has the potential to be converted to HPa, is an undesirable terminal fermentation product. Therefore, accumulation of HPa always results in failure of methanogenesis [139]. Y. Wang *et al.* mention that when the highest concentrations of ethanol, acetic acid and butyric acid were 2400, 2400 and 1800 mg L_1, respectively, there was no significant inhibition of the activity of methanogenic bacteria. However, when the propionic acid concentration was increased to 900 mgL_1, significant inhibition appeared, the bacteria concentration decreased from 6 _ 107 to 0.6–1_ 107 ml_1[132].

2.10.4 Carbon to Nitrogen Ratio (C/N).

The relationship between the amount of carbon and nitrogen present in organic materials is represented by the C/N ratio. A high C/N ratio is an indication of rapid consumption of nitrogen by methanogens and results in lower gas production. On the other hand, a

lower C/N ratio causes ammonia accumulation and pH values exceeding 8.5, which is toxic to methanogenic bacteria [128]. Optimum C/N ratios in anaerobic digesters are between 20 – 30 in order to ensure sufficient nitrogen supply for cell production and the degradation of the carbon present in the process, and in order to avoid at the same time excess nitrogen, which could lead to toxic ammonium concentrations [140]. Thus, the optimum C/N ratios of the digester materials can be achieved by mixing materials of high and low C/N ratios, such as organic solid waste mixed with sewage or animal manure [56,77].

2.10.5 Effect of toxicity on digestion

Toxic compounds affect digestion by slowing the rate of metabolism at low concentration, or by poisoning or killing the organisms at high concentration. The methanogenic bacteria are generally the more sensitive, although all groups involved in digestion can be affected. In order to control and adjust operation, to minimize toxic effects, it is important to identify inhibition in its early stages. The two main indicators of inhibition are [141 ,142]:

- Reduction in methane yield, indicated by two or more consecutive decreases of more than 10% in daily yield at a constant loading rate;

- Increase in volatile acids concentration, generally occurring when the total volatile acid (expressed as acetic acid) exceed the normal range of about 250 to 500 ppm (mg/L).

The major toxicants usually encountered with natural feed stocks are ammonia, Hydrogen sulphide, volatile acids, and heavy metals.

Ammonia (NH_3) is derived from digestion of protein during the hydrolysis step in AD process. NH_3 is an important source of nutrients for growing plants, thus this compound can be used as fertilizers [120]. High concentration of ammonia is toxic or inhibitory to anaerobic microbial populations, methanogens [143].

Hydrogen sulphide (H_2S) originates from the primary raw materials such as silage and sewage sludge, in which high concentration of sulphide is present. If sulphide

concentration is the dominant composition during AD process, it may avoid biomethanization in favour of sulphide production. Sulphide is important in the production of sulphur amino acids in bacteria and it also acts as a chemical reducing agent allowing growth of anaerobic microorganisms [120].

Heavy metals can be present in significant concentrations in municipal sewage and sludge. The heavy metals identified to be of particular concern include chromium, iron, cobalt, copper, zinc, cadmium, and nickel. An advantage of heavy metals is that, unlike many other toxic substances, they are not biodegradable and can accumulate to potentially toxic concentrations. The concentration of these heavy metal ions must be kept low, in order to maintain the growth of certain bacteria and to support methanogenesis [144,120].

Nutrients are essential for the growth of bacteria. Municipal wastewater sludge usually contains all the nutrient quantities that is require for optimal growth. These macronutrients are carbon, nitrogen, phosphor and sulphur. The optimal ratio for (C:N:P:S) is considered 600:15:5:1. Nutrients must be sufficient to maintain the growth of bacteria. Insufficient elements and nutrients may lead to inhibition effect and cause disruption to AD process [120].

2.10.5 Organic loading rate (OLR).

The organic loading rate (OLR) is the quantity of organic matter fed per unit volume of the digester per unit time, (e.g., Kg VS m^{-3} $d^{-1)}$. OLR plays an important role in anaerobic wastewater treatment in continuous systems and is a useful criterion for assessing performance of the reactors. A higher OLR feed rate may cause crashing of anaerobic digestion if the acidogenic bacteria multiply and produce acids rapidly. Many industrial plants have reported system failures due to overloading. Maximum OLR for an anaerobic digester depends on a number of parameters, such as reactor design, wastewater characteristics, the ability of the biomass to settle, and activity among others [145].

2.10.6 Retention time.

The Hydraulic Retention Time (HRT) is the time needed to achieve the complete degradation of the organic matter. The retention time varies with process parameters, such as process temperature and waste composition. The retention time for waste treated in a mesophilic digester ranges from 15 to 30 days and 12-14 days for thermophilic digester [77,128]. Reducing HRT reduces the size of the digester, resulting in cost savings. Therefore, there is an active incentive to design a system that can achieve a complete digestion in shorter HRT. A shorter HRT will lead to a higher production rate per reactor volume unit, but a lower overall degradation. These two effects have to be balanced in the design of the full-scale anaerobic digester [73].

2.10.7 Mixing.

The objective of mixing in a digester is to improve the contact between the microorganisms and substrate. Mixing distributes the heat and bacteria uniformly in the digester; furthermore, mixing prevents scum formation and avoids temperature gradients within the digester. However, excessive mixing can disrupt the microbes thus slow mixing is preferred [128], also Alastair *et al.,* [129] noted that evidence suggests that minimal mixing in the digester is preferable unless there is some form of microbial support material used which prevents the lost of active microbial biomass. However, the optimal mixing pattern is still a topic of debate. Mixing can be achieved through several methods, including mechanical mixers, recirculation of digester contents, or by recirculation the produced biogas to the bottom of the digester using pumps [146].

2.11 Available Feedstock for AD.

Various types of feedstock can be used for the production of biogas: animal manure and slurries, crop residues, organic wastes from dairy production, food industries and agro industries, wastewater sludge, organic fraction of municipal solid wastes, organic wastes from households and from catering business as well as energy crops. Biogas can also be collected, with special installations, from landfill sites. One main advantage of biogas production is the ability to use "wet biomass" types as feedstock, all characterised by moisture content higher than 60–70% (e.g. sewage sludge, animal slurries, flotation sludge from food processing etc.). In recent years, a number of energy crops (grains, maize, grass silage), have been largely used as feedstock for biogas production in countries like Austria or Germany. Besides energy crops, all kinds of agricultural residues, damaged crops, unsuitable for food or resulting from unfavourable growing and weather conditions, can be used to produce biogas and fertiliser. A number of animal by-products, not suitable for human consumption, can also be processed in biogas plants [147].

2.12 Advantages of anaerobic digestion process.

Some advantages of the AD process can be summarised in the following:

- AD contributes to reducing the greenhouse gases. A well-managed AD system will aim to maximise methane production without release any gases to the atmosphere, thereby reducing overall emissions [77];
- The feedstock for AD is a renewable source, and therefore does not deplete finite fossil fuels [77];
- The slurry produced (digestate) is an improved fertiliser in terms of both its availability to plants [148];
- AD leads to a reduction up to 80% of the odour associated with animal slurries, included volatile compounds that are oxidatively decomposed upon combustion, e.g. H2S forms SO2 [77];

- On a financial aspect, the advantage of AD is to convert residues into potentially saleable products: biogas, soil conditioner, liquid fertilizer [77];
- Successful in treating wet wastes of less than 40% dry matter [149];
- AD destroys a wide range of pathogenic and faecal micro-organisms [150].

2.13 Biomethanation of grass silage.

In general, grass silage is one of the most widely used agriculture crop by the majority of existing biogas plants after maize silage.
One of the advantages of digestion of grass silage for bioenergy and biorefinery systems is due to its high yield potential in terms of methane production per hectare [151]. Also, in general usage of grassland as a renewable source of energy through biogas production will contribute significantly to the protection of the environment, due to the ability of grass to sequester carbon into the soil matrix [152].

Grass silage is wet (less than 20% dry solids content) or dry (20– 40% dry solids) depending on whether it is wilted, weather conditions at the time of harvesting and storage conditions. 75% of dry matter of grass silage comprise of cellulose, hemi-cellulose and lignin. The crystalline structure of cellulose and the non-water soluble nature of lignin are resisting of microbes and enzymes during anaerobic digestion. Hydrolysis is often assumed to be a rate-limiting step in anaerobic digestion particularly with materials like grass silage that contain high structural carbohydrates. Treating lignocelluloses biomass prior to anaerobic digestion can accelerate hydrolysis and improve biogas yields [153].

2.14 Biodegradability Effects.

Plants stored energy in the cell wall as starch. The cell wall is composed of polysaccharide that consists of long chain of glucose produced by the plants during

photosynthesis. High concentrations of glucose can be found in plants and it can be converted to produce energy. The production of the methane from the organic substrates mainly depends on the composition of the substrate that can be degraded to methane and carbon dioxide. The composition and biodegradability are the two main factors of the organic substrates for the energy [68].

2.14.1 Lignocellulose structure.

Lignocelluloses are combination of cellulose, hemicellulose and lignin that strengthen the woody plant cells. There are three main constituents in the plant cell wall: cellulose, lignin and hemicelluloses [68].

2.14.2 Cellulose structure.

Cellulose is an organic compound consisting of long chain of glucose molecules bonded by glycosidic linkage. Celluloses comprise a large fraction of green component in the structure of the plant cell wall. The chemical formula for cellulose is $C_6H_{10}O_5$, thus this can be converted to energy by the action of enzyme [68].

2.14.3 Hemicellulose structure.

Hemicelluloses are made up of different small sugar units called monomers. The sugar units consist of branched polymers of glucose, xylose, galactose, mannose, arabinose and rhamnose. In hemicelluloses there are large amount of xylose, this type of polymer strengthen the plant structure and increase the resistance to microbial degradation. Hemicellulose is like a physical barrier, which protects the cellulose fibre from enzymatic attack [120].

2.14.4 Lignin.

Lignin is a complex chemical component and holds cellulose and hemicellulose together. The lignin structure creates the rigid structure of the plant and it prevents the swelling of lignocelluloses. The enzymatic action will have no effect on the breakdown the plant cell wall due to the structural rigidity of lignin. Lignin is the cause of using lignocellulosic materials in anaerobic digestion, as it makes lignocellulose resistant to enzymatic degradation. Thus, on-going improvements in utilising pre-treatment processes are under investigation. The aim of pre-treatment is to change the properties of

lignocellulosic materials for greater exposure to chemical and biological degradation [81,119].

2.15 Biogas Technology.

The production of biogas from AD is widely used by modern society for the treatment of livestock manure and slurries. The aim is to produce renewable energy and to improve their fertiliser quality. In countries with significant agricultural production, the strengthening of environmental legislation and regulation of manure and vegetable wastes recycling increased the interest for AD as a cheap and environmental friendly solution. Latest developments in Europe, USA and other parts of the world have shown increasing interest among farmers to cultivate energy crops, used as feedstock for biogas production. AD is today standard technology for stabilisation of primary and secondary sewage, sludge, for treatment of organic industrial waste from food-processing and fermentation industries as well as for the treatment of the organic fraction of municipal solid waste. A special application is biogas recovery from existing landfill [147].

The trends of biogas plants have increased in many countries over the years. The EU-countries where the agricultural biogas plants are most developed are Germany, Denmark, Austria and Sweden and to certain level the Netherlands, France, Spain, Italy, United Kingdom and Belgium. The technology is under current development in countries like Portugal, Greece and Ireland as well as in many of the new, Eastern European, member states, where a large biomass potential is identified [154]. Germany is the largest producer of biogas on the continent of Europe. Figure 2-34 [155] illustrates the trend of increasing biogas plant in Germany. The numbers of biogas plants have increased from 139 in the year of 1992 to 3891 biogas plants in Germany by the end of year 2008. There, the largest increase in biogas production has taken place on agricultural plants [156]. The steady increase in energy crop digester applications in many European countries are supported by the European subsidiaries scheme, paid for renewable energy [157]. However, increased prices on energy crops have affected the production of new biogas plants. Therefore, improvement in biogas technology is

essential to preserve the attractiveness of biomethanisation and to maintain the balance of producing economical clean fuel.

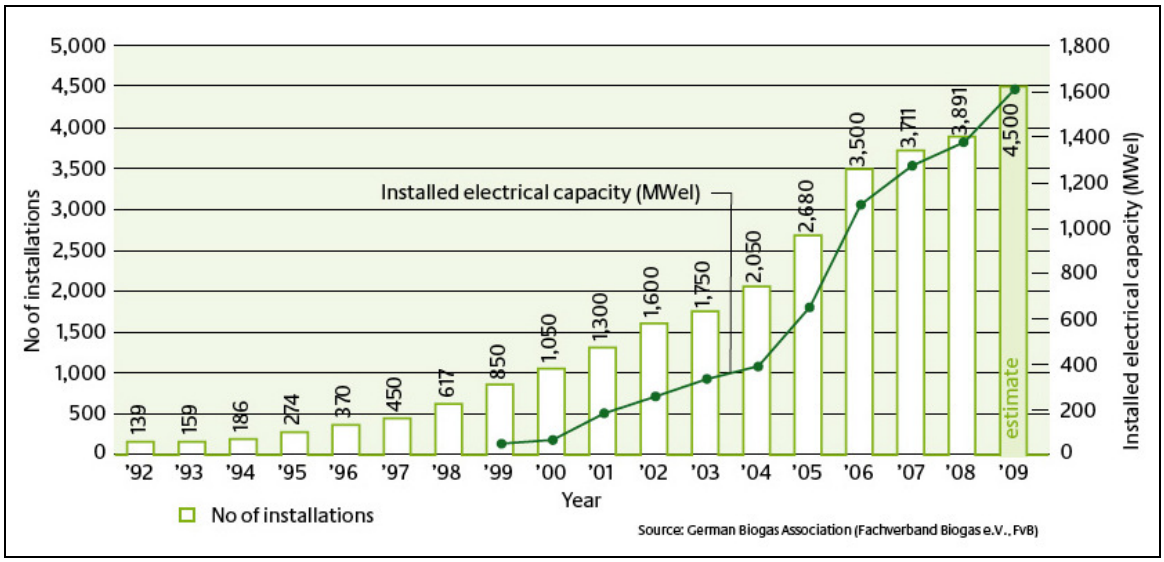


Fig. 2.34: Development in the number of biogas plants in Germany and their combined installed electrical capacity in megawatts (MW) [155].

2.15.1 Biogas composition and properties.

Biogas is regarded as the most important product of fermentation digestion. Biogas is a flammable gas and the quality of which is defined by its composition. Biogas consisting of primarily of CH_4 (50-70%) and CO_2 (30-50%), In addition small traces of other gases Nitrogen (N2), Hydrogen (H2), Hydrogen sulphide (H2S) and ammonia. The small percentage of oxygen and nitrogen present in biogas is relatively common due to the natural characteristic of microorganisms have variation. The exact composition of biogas depends on the feedstock composition, the process conditions and the type of digester used [76,73]. A Summary of the percentage mixture of different gases in biogas is estimated in the table 2.1 below [147,158].

Table 2.1: Physical and Chemical properties of biogas production.

Property	CH_4	CO_2	H_2S	H_2	Typical biogas ($60\%CH_4/40\%CO_2$)
% by volume	54-84	20-45	1/10	0.0-10	100
Energy value (kcal/litre)	9.0	—	—	2.9	5.4
Explosive range (% by vol. with air)	5-15	—	4-46	6-7.1	6-12
Density (g/litre) °C 760mm	0.72	1.98	1.54	0.99	1.22
Specific gravity (relative to air)	0.55	1.5	1.2	0.07	0.93
Critical temperature (°C)	-82.5	31.1	100.4	-239.9	
Critical pressure (Atm.)	45	73.0	88.9	12.8	
Odour	None	None	Present	None	

CHAPTER 3

DESIGN OF EXPERIMENT (DOE)

3 DESIGN OF EXPERIMENT (DOE).

3.1 Introduction.

DOE was introduced by Sir R. Fisher in the early 1920s to determine the effects of various fertilizers on different ranges of plots of land. Since then, DOE has been utilized in many disciplines such as biological, pharmaceutical, or engineering to name but a few. In the last two decades, the use of DOE has grown rapidly and been adapted for many processes in industry such as machining, chemical mixing and biochemical processes to find out the optimal conditions. Responses surface methodology (RSM) is the best known type of DOE designs; the concept of RSM was introduced in the early 1950's by Box and Wilson [159].

Among the RSM designs, two most popular types of experimental designs exist for developing second-order models: central composite design (CCD) and Box-Behnken design (BBD).

3.2 Response Surface Methodology (RSM).

Engineers often search for the conditions, which would optimize the process of interest. In other words, they want to determine the values of the process input parameters at which the responses reach their optimum. The optimum could be either a minimum or a maximum of a particular function in terms of the process input parameters. RSM is one of the optimization techniques currently in large-scale usage in describing the performance of the biochemical process and finding the optimum of the responses of interest.

RSM are a set of mathematical and statistical techniques that are useful for modelling and predicting the response of interest, affected by several input variables with the aim of optimizing this response [160]. RSM also specifies the relationships among one or more measured responses and the essential controllable input factors [161]. If all independent variables are measurable and can be repeated with negligible error, the response surface can be expressed by:

$$y = f(x_1, x_2, \ldots x_k) \quad (3.1)$$

Where: k is the number of independent variables

To optimize the response "y", it is necessary to find an appropriate approximation for the true functional relationship between the independent variables and the response surface. Usually a second order polynomial Eq.3.2 is used in RSM.

$$y = b_o + \sum b_i \chi_i + \sum b_{ij} \chi_i \chi_j + \sum b_{ii} \chi_{ii}^2 + \varepsilon \quad (3.2)$$

In this work the RSM design central composite design (CCD) has been used.

3.3 Central composite design (CCD).

The most popular RSM design is CCD and has three associated groups of design points: two-level factorial or fractional factorial design points, axial points (sometimes called star points) and centre points. CCD's are designed to estimate the coefficients of a quadratic model. All point descriptions will be in terms of coded values of the factors [160,162].

3.3.1 Factorial points.

The two-level factorial part of the design consists of all possible combinations of the +1 and -1 levels of the factors. In the two factors case there are four design points: (-1, -1) (+1, -1) (-1, +1) (+1, +1). In general, the number of factorial points equal to 2^k.

3.3.2 Star or axial points.

The star or axial points all have factors set to 0, the midpoint, except one factor, which has the value +/-α. For a case with two factors, the star points are: (-α, 0) (α, 0) (0, -α) (0, α). The value for α is calculated in each design for both rotatability and orthogonality of blocks. A design is rotatable if the variance of the predicted response, at any point x, depends only on the distance of x from the design centre points and a design is orthogonal if the effects of any factor balance out (sum to zero) across the effects of

the other factors. The experimenter can choose between these values or enter a different one. The default value is set to the rotatable value and can be calculated by: $\alpha = (2^k)^{1/4}$. The number of star points is equal to 2k.

3.3.3 Centre points.

Centre points, as implied by the name, are points with all levels set to coded level (0) the midpoint of each factor range: (0, 0). Centre points are usually repeated 4-6 times to get a good estimate of experimental error (pure error). These points are shown in Fig. 3.1.

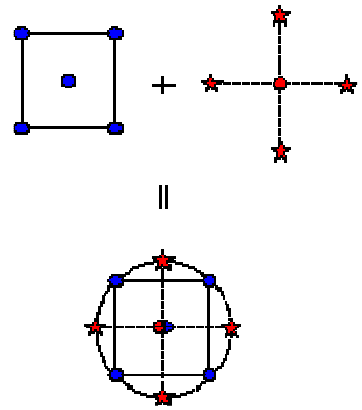


Fig. 3.1: Generation of CCD for two factors [163].

3.4 Analysis for the design

The sum of squares of the model and each term is given by Eqs. 3.3- 3.9

Sum of Squares –total = $SS_T = \sum_{i=1}^{n} (y_i - \bar{y})^2$ (3.3)

Sum of Squares- model = $SS_M = \sum_{i=1}^{n} (\hat{y}_i - \bar{y})^2$ (3.4)

Sum of Squares –Residuals = $SS_R = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2$ (3.5)

Sum of Squares-Pure error = $SS_{PE} = \sum_{i=1}^{n_o} (y_i - \hat{y}_i)^2$, for centre points only (3.6a)

$$SS_{lof} = SS_R - SS_{PE} \quad (3.6b)$$

$$SS_{b_i} = b_i \sum_{i=1}^{n} x_i y_i \quad (3.7)$$

$$SS_{b_{ij}} = b_{ij} \sum x_i x_j y_i \quad (3.8)$$

$$SS_{b_{ii}} = b_0 \sum_{i=1}^{n} y_i + b_{ii} \sum_{i=1}^{n} x_i^2 y_i - (\sum y_i)^2 / n \quad (3.9)$$

3.5 Advantage of Central Composite Design.

1. Created from a 2-level factorial design, improved with centre points and axial points.
2. Normally has 5 levels for each factor, this can be modified to a face-centred CCD by choosing α =1.0. The face-centred design has only three levels for each factor.
3. Created for estimating a quadratic model.
4. Rather insensitive to missing data this makes them more robust to problems.
5. Replicated centre point provides excellent prediction capability near the centre of the design space.
6. Region of operability must be greater than region of interest to accommodate axial runs.

3.6 General Steps in RSM.

RSM is usually carried out as a problem that is considered in sequential steps. The following steps are performed in order to develop a mathematical model in the case of anaerobic digestion:

1. **Identifying the critical process variables (or factors).**

These critical factors may be defined from past literature or by conducting a preliminary study (i.e. screening study) based on factorial design or partial factorial design. In a present case, vital process factors are determined from historically published articles. The process input factors are: beating time and temperature.

2. **Finding the limits of each factor.**

To find the limits of each factor, the trial beating of grass silage as main substrates in our work was performed for different times. The particle size of grass silage was a criterion of selecting the range of beating time factor.

As the anaerobic digestion process was at a mesophilic range (33-40) ° C [121], thus a range of temperatures were selected inside this range. Although Design-Expert V7 software was used to code the data, develop the design matrix and analyze the case, the limits for each factor were coded via this relationship $X_I = 2(2X - (X_{max} + X_{min}))/ (X_{max} - X_{min})$. Where: X_i is the required coded value, X is any value of the factor that requires coding and X_{max}, X_{min} are the upper and lower limit of the factor being coded respectively [160].

3. **Design matrix development.**

The matrix depends on the type of RSM design selected, for CCD the design matrixes in coded values are shown in Table 3.1. As previously stated in current work carried out experimentally, the matrix for each experiment was developed using the same statistical software.

Table 3.1: Design matrix for CCD, coded values.

Run	Std	A: beating time	B: temperature.
1	12	0	0
2	6	1	0
3	11	0	0
4	9	0	0
5	3	-1	1
6	2	1	-1
7	4	1	1
8	10	0	0
9	1	-1	-1
10	8	0	1
11	7	0	-1
12	5	-1	0

4. Performing the experiment.

The anaerobic digestion experiments were accomplished according to the design matrix, Table 3.1 and in a random order to avoid any systematic error in the experiment.

5. Recording the responses.

All responses mentioned earlier in chapter one were measured in sequential order for each experiment following the measuring procedure of each response. Usually, the first response measured is residual stress if it is of interest in the active experiment. If applicable, an average of at least three recorded measurements are calculated and considered for further analysis.

6. Development of mathematical model.

The functional relationship representing any response of interest can be expressed as y = *f* (t, T) and Eq. 3.2 becomes as follows:

$$Y = b_0 + b_1 t + b_2 T + b_{11} t^2 + b_{22} T^2 + b_{12} tT \qquad (3.10)$$

7. Estimation of the coefficients in the model.

Regression analysis is applied in order to specify the values of the coefficients in Eq. 3.10. Nevertheless, computer software was used to evaluate the coefficients for all responses of each experiment.

8. Testing the adequacy of the models developed.

An analysis of variance (ANOVA) was used to test the adequacy of the models developed. The statistical significance of the models developed and each term in the regression equation were examined using the sequential F-test, lack-of-fit test and other adequacy measures (i.e. R^2, Adj- R^2, Pred. R^2 and Adeq. Precision ratios) using the same software to obtain the best fit. The prob.>F (sometimes it is called the *p*-value) of the model and each of the term in the model can be computed by means of ANOVA. If the Prob.> F of the model and of each term in the model does not exceed the level of significance (say α= 0.05) then the model may be considered adequate within the confidence interval of (1- α). For the lack-of-fit test, the lack of fit could be considered insignificant if the Prob.>F of the lack of fit exceeds the level of significance. Table 3.2 below is a summary of the ANOVA table. The equations by which the adequacy measures can be calculated are shown below Eqs. 3.11 to 3.15 [160,162].

Table 3.2: ANOVA table for full model:

Source	SS	df	MS	$F_{cal.}$- Value	p-value or Prob > F
Model	SS_M	p	Each SS divided by its df	Each MS divided by MS_R	From table or software library
A-Laser power	SS_1	1			
B-Welding speed	SS_2	1			
C-Focused position	SS_3	1			
AB	SS_{12}	1			
AC	SS_{13}	1			
BC	SS_{23}	1			
A^2	SS_{11}	1			
B^2	SS_{22}	1			
C^2	SS_{33}	1			
Residual	SS_R	N-p-1			-
Lack of Fit	SS_{lof}	$N - p - n_0$			From table
Pure Error	SS_E	n_{0-1}			-
Cor Total	SS_T	N - 1	-	-	-

Where:

P: Number of coefficients in the model.
N: Total number of runs.
n_0: Number of centre points.
df: Degree of freedom.
MS: Mean square.

$$R^2 = 1 - \left[\frac{SS_R}{SS_R + SS_M} \right] \tag{3.11}$$

$$AdjR^2 = 1 - \left[\left(\frac{SS_R}{df_R} \right) \times \left(\frac{SS_R + SS_M}{df_R + df_M} \right)^{-1} \right] \tag{3.12}$$

$$\Pr edR^2 = 1 - \left[\frac{PRESS}{SS_R + SS_M} \right] \tag{3.13}$$

$$PRESS = \sum_{i=1}^{n} (y_i - \hat{y}_{i,-i})^2 \tag{3.14}$$

$$Adeq.precision = \left[\frac{Max(\hat{Y}) - Min(\hat{Y})}{\sqrt{\frac{p \times MS_R}{n}}} \right] \quad (3.15)$$

Where:

p: Number of model parameters (including intercept b_0)
n = number of experiments

9. Model reduction

Usually, the full model Eq. 3.13 consists of an insignificant model of terms that need to be eliminated, terms that have a p-value greater that the level of significance α. This elimination can be done manually or automatically. The three automatic procedures of evaluating all possible regression equations (or selection of variables) are [162,164]:

a) Forward selection procedure:

This procedure begins with the constant term only, and the first variable added is the one with the highest simple correlation with y. If the regression coefficient of this variable is significant, it remains in the equation and a new search for the second variable with highest correlation with y commences, after y has been adjusted for the effect of the first variable and the significance of the regression coefficient for the second variable is then tested. If the regression coefficient is significant, a search for a third variable is made in the same manner, and so on. The procedure is completed when the last variable entered to the equation has insignificant regression coefficient or all variables are included. The test statistic for this selection procedure is the standard t or F-statistic, which equal to t^2.

b) Backward elimination procedure:

In this procedure, the full equation is fitted and sequentially eliminates one variable each time. The variable with the smallest contribution to the reduction of error is eliminated first, or the variable with the smallest t ratio (i.e. the ratio of the regression coefficient to its standard error) is eliminated and so on. In the case of more than one variable that have insignificant t ratios, the procedure operates by dropping the variable with the

smallest insignificance for t ratio and the equation with the remaining variable is then fitted where the ratios for the new regression coefficient are then tested. The procedure stopped when all the t ratios are significant or all but one variable has been deleted.

c) Stepwise regression method:

This method is a forward selection. However, the possibility of eliminating a variable that might be added in an earlier stage, as in backward procedure, is considered. The calculation made for inclusion and deletion of variables are the same as the forward and backward procedures. This procedure has the advantage of assuming different or similar levels of significance for inclusion or deletion of variables from the regression equation.

10. Development of the final reduced model.

At this stage, the final reduced model as determined by applying the above steps can be built upon. This model contains only the significant terms and the terms that are necessary to maintain hierarchically. Furthermore, a reduced quadratic ANOVA table can also be produced.

11. Post analysis.

As the final model was tested and checked and was found to be adequate. Then, predicting the response at any midpoints using this adequate model is possible at this stage. In addition, producing plots such as 3D graphs, contours and perturbation plots in representing the factors that affect how they contribute in the response. Moreover, possibility of employing the developed model for finding the optimal condition for optimised anaerobic digestion processes.

3.7 Optimization.

3.7.1 Desirability approach.

Considerable techniques are available in the statistics science for solving multiple response problems like overlaying the contours plot for each response, constrained

optimization problems and desirability approaches. The common statistical software packages such as GPSS, NEMROD and Design-Expert include multiple response optimization techniques. The desirability method is a method that is recommended owing to its simplicity, availability in the software and also provides flexibility in weighting and giving importance of individual responses. Solving such multiple response optimization problems using this technique consists of using a technique for combining multiple responses into a dimensionless measure of performance called overall desirability function. The desirability approach consists of transforming each of the estimated responses, Y_i, into a unit less utility bounded by $0 < d_i < 1$, where a higher d_i value indicates that response value Y_i is more desirable, if $d_i = 0$ this means a completely undesired response or vice versa when $d_i = 1$ [165]. In the current work the individual desirability for each response d_i was calculated using Eqs.3.16 - 3.19. The shape of the desirability function can be changed for each goal by the weight field 'wt_i'. Weights are used to give added emphasis to the upper/lower bounds or to emphasize the target value. Weights could be ranged between 0.1 and 10; where weight greater than one gives more emphasis to the overall goal, while weight that is less than one gives less emphasis toward the goal. With a weight value of one, this will make the d_i's vary from zero to one in a linear mode. In the desirability objective function (D), each response can be assigned an importance (r), relative to other responses. Importance varies from the least important a value of 1(+), to the most important a value of 5(+++++). If the varying degrees of importance are assigned to the different responses, the overall objective function is shown below Eq.3.20. Where n is the number of responses in the measure and T_i is the target value of i^{th} response [162].

- For a goal of maximum, the desirability will defined as:

$$d_i = \begin{cases} 0 & , \quad Y_i \le Low_i \\ \left(\dfrac{Y_i - Low_i}{High_i - Low_i} \right)^{wt_i} & , \quad Low_i \langle Y_i \langle High_i \\ 1 & , \quad Y_i \ge High_i \end{cases} \tag{3.16}$$

- For a goal of minimum, the desirability will define by:

$$d_i = \begin{cases} 1 & , \quad Y_i \le Low_i \\ \left(\dfrac{High_i - Y_i}{High_i - Low_i} \right)^{wt_i} & , \quad Low_i \langle Y_i \langle High_i \\ 0 & , \quad Y_i \ge High_i \end{cases} \tag{3.17}$$

- For a goal as a target, the desirability will defined by:

$$d_i = \begin{cases} \left(\dfrac{Y_i - Low_i}{T_i - Low_i} \right)^{wt_{1i}} & , \quad Low_i \langle Y_i \langle T_i \\ \left(\dfrac{Y_i - High_i}{T_i - High_i} \right)^{wt_{2i}} & , \quad T_i \langle Y_i \langle High_i \\ 0 & , \quad Otherwise \end{cases} \tag{3.18}$$

- For a goal within range, the desirability will defined by:

$$d_i = \begin{cases} 1 & , \quad Low_i \langle Y_i \langle High_i \\ 0 & , \quad Otherwise \end{cases} \tag{3.19}$$

$$D = \left(\prod_{i=1}^{n} d_i^{r_i} \right)^{\frac{1}{\sum r_i}} \tag{3.20}$$

3.7.2 Optimization approach in Design-Expert software.

The optimization part in Design-expert software V7 searches for a combination of factor levels that simultaneously satisfy the requirements placed (i.e. optimization criteria) on each one of the responses and process factors (i.e. multiple response optimization). Numerical and graphical optimization methods were used in this work by choosing the desired goals for each factor and response. Aforementioned, the numerical optimization process involves combining the goals into an overall desirability function (D). The numerical optimization feature in the design expert software package finds a point or more in the factors domain that would maximize this objective function. In the graphical optimization with multiple responses, the software defines regions where requirements simultaneously meet the proposed criteria. Superimposing or overlaying critical response contours on a contour plot. Then, a visual search for the best compromise becomes possible. In the case of dealing with many responses, it is recommended to do a numerical optimization step in the first instance; otherwise, it could be impossible to uncover a feasible region. The graphical optimization displays the area of feasible response values in the factor space. Regions that do not fit the optimization criteria are shaded [162]. Fig.3.2 shows flow chart of the optimization steps in the design-expert software.

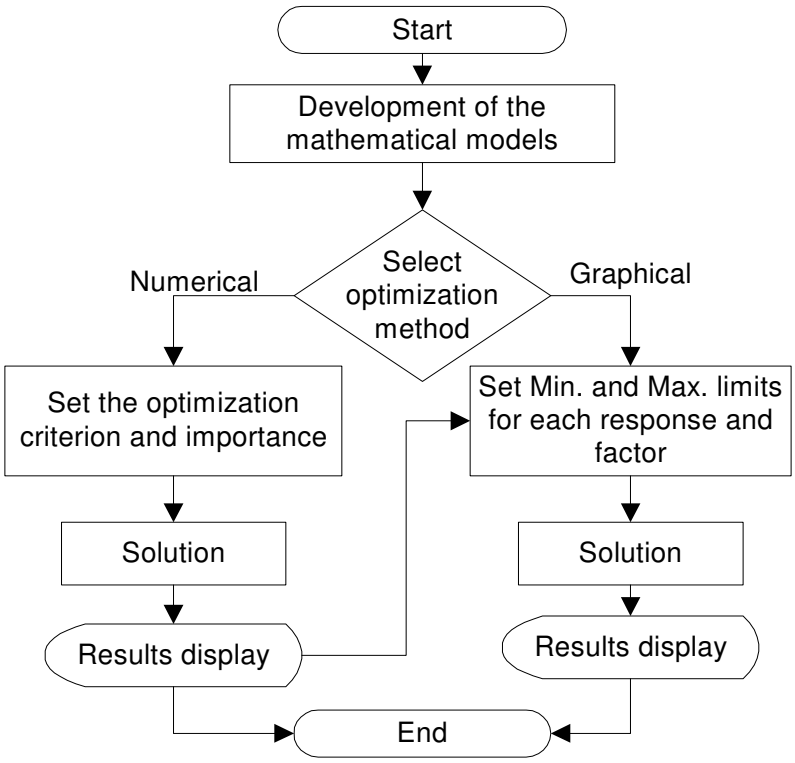


Fig. 3.2: Optimization steps.

CHAPTER 4

EXPERIMENTAL EQUIPMENT AND PROCEDURES

4 Experimental Equipment and Procedures.

The objective of the laboratory experiments was to quantify the volume of biogas yield released from the constituent grass silage before and after mechanical treatment and to optimize the factors that affect anaerobic digestion to enhance biogas yields.

Biogas research as a renewable energy source is currently carried out in DCU by different researchers; their research has been used as a guiding principle throughout this thesis. These documents, the citations and the findings by the researchers have contributed to the analysis of results and conclusions to be made regarding the concentration and the volume of the biogas produced before and after mechanical treatment of grass silage.

4.1 Materials.

4.1.1Grass silage.

Many different types of energy crops are suitable for anaerobic digestion. The evaluation recent of biogas plants in Germany and Austria show the grass silage is the second most frequent crop used as feedstock (50%) after maize silage (80%)[166,167]. The energy crops selected for these experiments were grass silage (see Fig. 4.1).

Fig. 4.1: Grass silage.

According to literature, these types of crops can generate massive amounts of energy through the AD process. Grass silage was obtained from UCD Lyons Research Farm. The farm consists of approximately 580 acres of land. It is used for teaching and research field activities by the School of Agriculture, Food Science and Veterinary Medicine in University College Dublin (UCD) [168]. Characteristics of the grass silage are reported in table 4.1[169].

Table 4.1: Characteristics of grass silage.

Parameters	**Unit**	**Values**
Lactic acid	g kg^{-1} DS	26.95
Ethanol	g kg^{-1} DS	11.54
Acetic acid	g kg^{-1} DS	3.93
Propionic acid	g kg^{-1} DS	0.25
Butyric acid	g kg^{-1} DS	1.43
Volatile fatty acids (VFA)	g kg^{-1} DS	5.61
Ammonia	g kg^{-1} DS	46.18
Water soluble carbohydrates (WSC)	g kg^{-1} DS	49.83
pH		4.3
Protein	% DS	9.5
Metabolizable energy (ME)	MJ kg^{-1} DS	10
Digestibility-value (DMD or D-value)	% DS or D-value	64
Silage intake or palatability	g kg^{-1} W0.75	89
Potential acid load (PAL)	meq kg^{-1} DS	821
Neutral detergent fibre (NDF)	% DS	59
Fermentable metabolizable energy (FME)	MJ kg^{-1} DS	8.2
FME/ME ratio		0.81
Oil	% DS	3.3
Carbon (C)	% DS	43.03
Hydrogen (H)	% DS	5.82
Nitrogen (N)	% DS	1.61
Dry Solid (DS)	%	30.66
Volatile solid (VS)	%	92.46

4.1.2 Sludge

The required live microbial culture for the experiments can be used with sewage sludge. The sewage sludge is a brown/dark, heavy, viscous fluid with an unpleasant odour (see Fig. 4.2). This type of residue contains numerous species of active micro-organisms which are required for anaerobic digestion as discussed in the Chapter 2. Fresh sludge was collected from the Dublin water sewage treatment plant located in Ringsend, Dublin, Ireland. The sludge composition is important for the determination of pathogenic organisms in a given culture. In general, the diversity of species of micro-organisms and the types of materials inside the sludge have the potential to release biogas. This will indirectly increase the production of biogas when combining sludge with maize during AD process. The sludge characteristics are listed in table 4.2.

Fig. 4.2: Sewage sludge.

Table 4.2: Characteristics of sludge.

Parameters	Value
Total Solids (TS)	5.60%
Volatile Solids	72%
COD	65.500 mg/L
Ammonia	2.770 mg/L
Alkalinity	12.135 mg/L
VFA's	42 mg/L

4.2 Equipment

The equipment used in the lab for this work includes:

4.2.1 Laboratory electronic component.

- **Hollander Beater**

The mechanical pre treatment of the substrate (grass silage) was done with Hollander Beater. The Hollander beater is shown in the Fig.4.3. More details about the beater have been given earlier in chapter 2. The main purpose of the mechanical beater was to break down the cellulose structure of the grass silage and to increase the surface area of the substrate. The samples after the beating process have less resistance and make good contact with the bacteria for digestion during fermentation process. Table 4.3 shows the technical specification for the Hollander Beater in the biofuel lab in DCU.

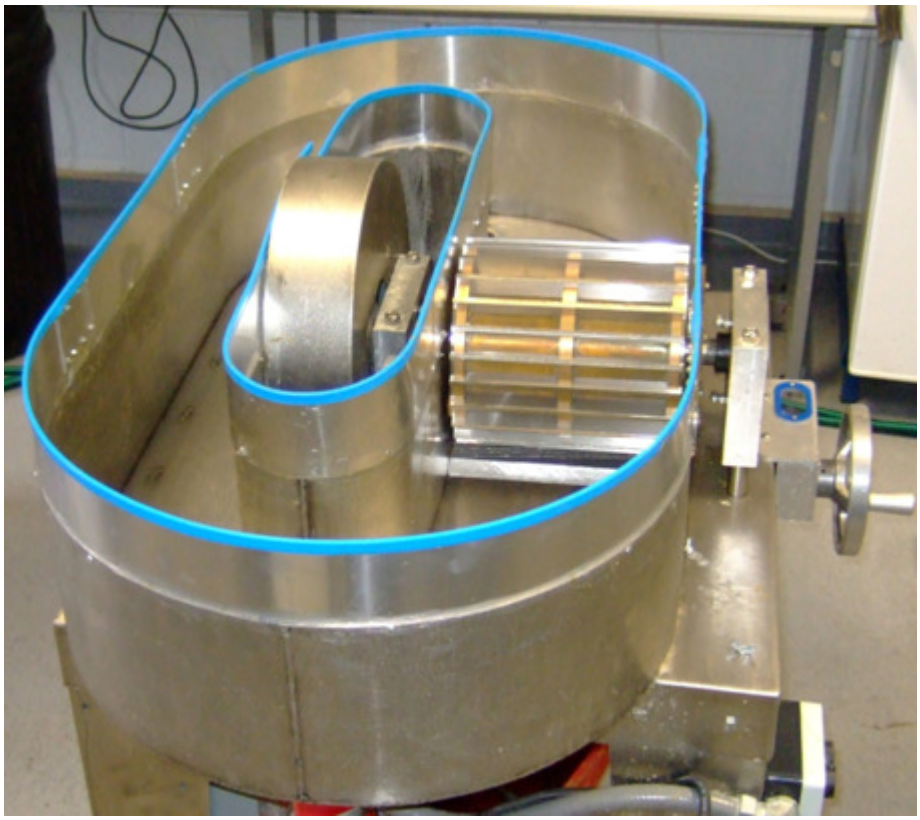


Fig. 4.3: The Hollander beater device used in the bio-fuels lab DCU.

Table 4.3: Technical specification of the Hollander Beater

Motor	1hp (746 watts)
	220v
	6.9 Amps
	1 Phase
	1450 rpm
V- Belt drive:	2.5: 1 Reduction
Drum Speed:	580 rpm
Tub Volume:	Maximum Capacity =90 litres
	working capacity = 40 litres
Drum diameter	200mm
Drum paddles	24 paddles

- **Water bath**

Water bath is shown in Fig. 4.4. The main function of this device is to maintain a uniform temperature throughout for all the samples that have been submerged in the water bath. The water bath device should operate throughout the duration of the experiment and it is designed to control the temperature levels of the experiment. The water in the bath can evaporate due to the heat, low water level can cause serious effects either on the samples, but it can also cause overheating in the tank if no water is present. For this reason small plastic balls were kept on the top area of the water to avoid vaporization. Also the irregular heat distribution in the tank can affect the accuracy of the experimental results; therefore routine daily check ups on the water level in the tank were required to ensure safety in the laboratory and to preserve the process of the experimentation.

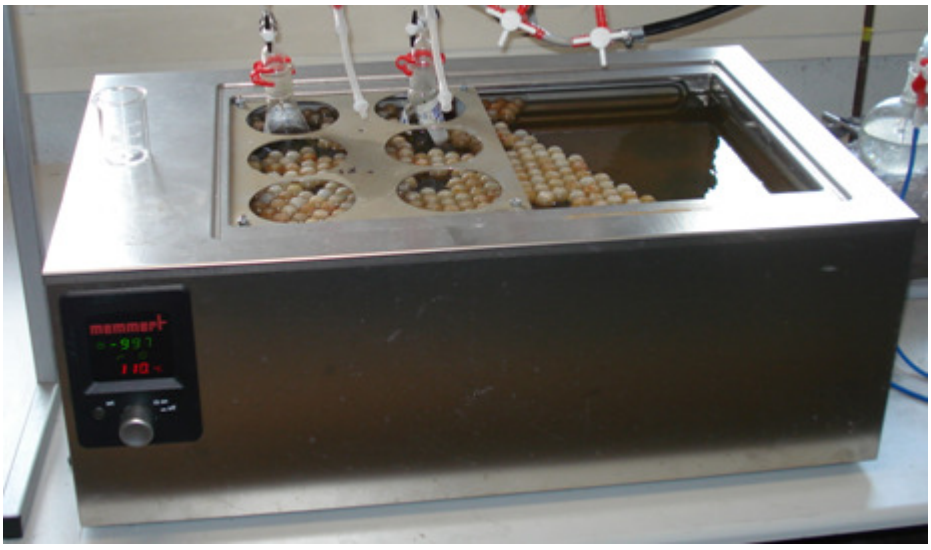


Fig. 4.4: Water bath.

- **Laboratory oven**

Laboratory oven is shown in Fig. 4.5. The utilization of the oven is to extract the moisture contents of the wet grass silage, without damaging the components of the samples. Temperature can be adjusted with range from 10-100 ^{0}C. The thermometer on top of the oven, tracks the temperature in the oven.

Fig. 4.5: Laboratory Oven.

- **The electric pump**

Electric pump is shown in Fig. 4.6. An electric pump was used to speed up gas removal. The extraction of gas by the pump can create an airtight environment and also monitor the pressure in the experiment. In order for AD to proceed, it is essential to have no air present in the experimentation, especially oxygen.

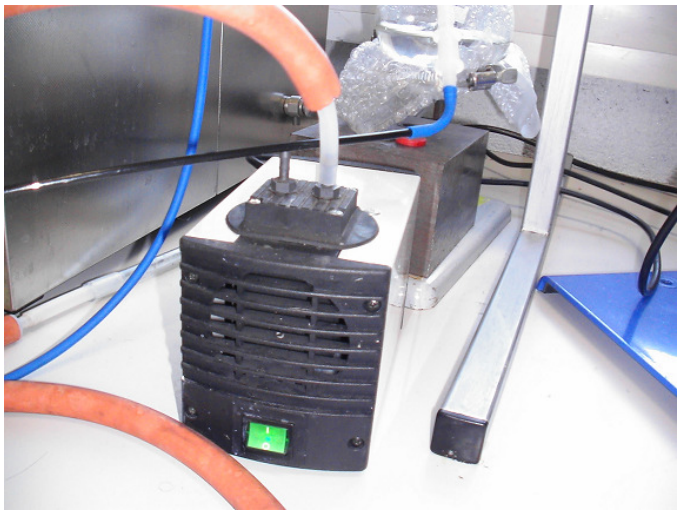


Fig. 4.6: Electronic Pump

- **The electronic weighting scale**

The electronic weighting scale is shown in Fig. 4.1. The function of the electronic scales used in this experiment is to measure the exact weight of the samples both in wet and dry form.

4.2.2 Laboratory glassware

- **The round bottom flask**, shown in Fig. 4.7. This equipment can be used to extract any air content in the experiment, by extracting the gas with the electric pump into the round bottom flask. The bubble present shows that air is being released and the water in the flask prevents air from flowing back into the system.
- **The volumetric flask** is shown in Fig. 4.8. The function of this equipment is to measure the volume of the gas. Water is filled in the flask and marks a suitable water level for comparison result. The inverted 250 mL cylinder is placed in the cylinder. When gas is pumped into the cylinder the water level rises. The gas level is determined by marking the water level and subtracting the initial selected water level.

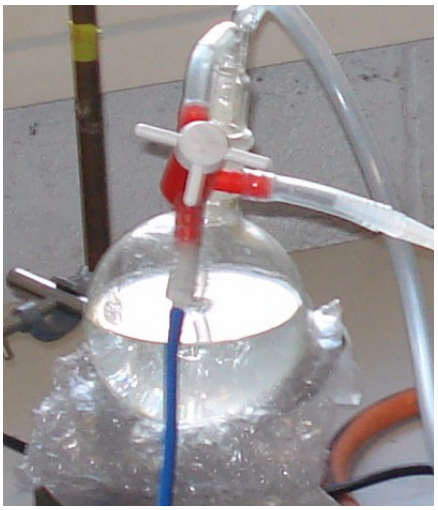


Fig. 4.7: Round bottom flask**.**

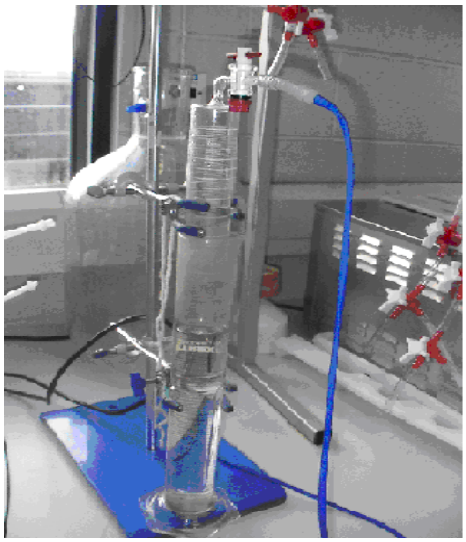


Fig 4.8:Volumetric flask.

Conical flasks shown in Fig. 4.9. Conical flasks used in this experiment act as a digester at a small scale (fermentation vessels). The vessels are filled with the samples mixture and placed into the water bath to maintain the required temperature for the AD process. The vessels are connected to the gas collecting system through taps with glass bores. The red colour clips ensure no gas leakage through the gaps of the vessels.

Fig. 4.9: Fermentation vessels.

All other glassware have been listed herein: dish plate, beaker, burette, pipette, connecting tube, stopper cap where all have been used for holding experimental samples and substances. The use of pipettes and burettes are required to extract accurate concentrations of solution content.

4.2.3 Miscellaneous material/component

- In Fig. 4.10, a three-way valve connecting tube is attached to an aluminium bag. The function of the three-way valve is to manipulate gas flow movement. By adjusting the tap, gas flow can be dictated or the tap can be closed to prevent any gas movement. For the purpose of the experiment, the biogas release from the experiment has to be stored overnight for a period of two weeks. Thus, it is mandatory to apply an aluminium bag as shown in Fig. 4.10 for the storage of such gases. The application of the aluminium bag is to prevent biogas leakage and to avoid fire hazards as described in the health and safety section contained in this thesis. The aluminium bag is sealed tightly, where atmospheric air can be prevented from diffusing through the bag, it can also retain accurate evaluation of the gases produced during the AD experiment. These types of aluminium storage bags were manufactured by *Linde* and were designed specifically for the application of gas storage.
- A Nitrogen gas cylinder. Nitrogen can be used to expel any gases present in the experimental fermentation vessels, aluminium gas collecting bags and connecting pipe lines supply gases such as O_2, H_2S, NH_3 and other traces of gases. For this experiment, nitrogen is used to flush out oxygen to achieve an anaerobic condition.

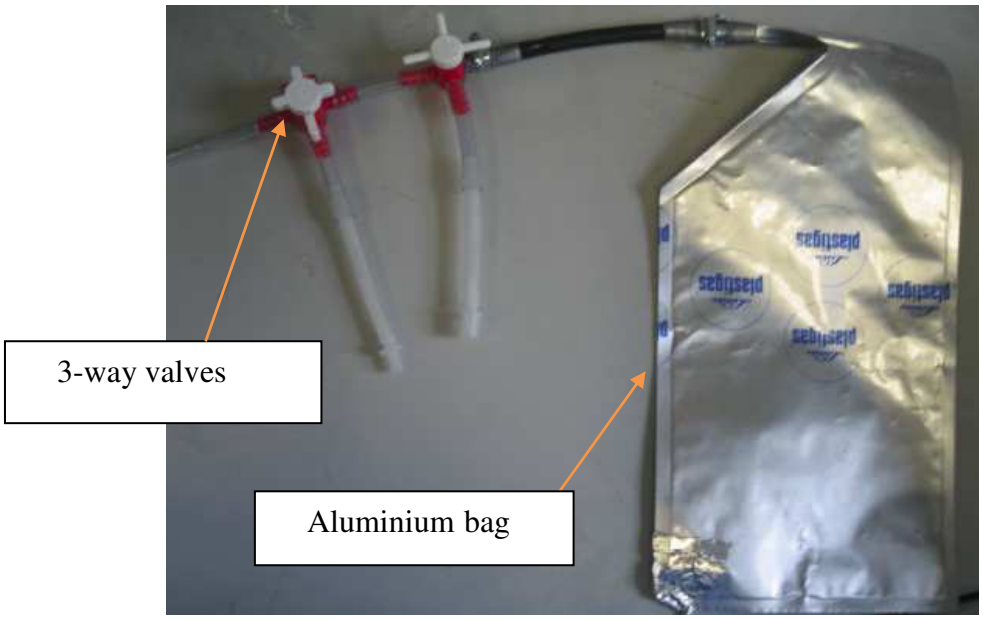


Fig. 4.10: Aluminium bag with 3 way connecting valves.

4.3 Experimental procedure guidelines

Previous DCU research teams have given precise experimental guidelines to follow when carrying out any fermentation experiments. This experimental procedure ensures the accurate comparison between the different results obtained using different organic materials during the fermentation process. Table 4.4 summarises the guidelines of the lap experimental procedure.

Table 4.4: Summary of the procedure guidelines.

Guide lines	Reasons and causes
Fermentation vessels used with volume capacity 500 mL	Working volume of the fermentation vessels at 200 mL
Temperature of the water baths set to 35^0C, 37^0C, 39^0C	Due to mesophilic condition and design matrix has been developed by design-expert 7 software
Continuous stir of fermentation vessels	Flasks shaking process will be done manually on daily basis to enhance the reaction between micro-organisms and substrate
Mechanical treatment of the substrate through beater	Beater will break down the cellulosic bonds of the organic material and surface area will increase of the substrate and also easy reaction between the substrate and micro-organisms
Plastic clips at the neck of the fermentation vessels	To prevent gas leakage
Pressure is kept at minimum above atmospheric pressure	The round bottom flask jar with confined water to pump out the excessive gas present in the fermentation vessels, aluminium bags and connection pipes between aluminium bags and the fermentation vessels.

4.4 Input factors of grass silage anaerobic digestion.

In this work, temperature and beating time were used as two main factors of grass silage anaerobic digestion shown herein. Table 4.5 shows the grass silage anaerobic digestion parameters and experimental design levels used. Experiments for the production of biogas from grass silage through to anaerobic digestion were carried out according to the design matrix illustrated in table 4.6.

Table 4.5: Process parameters and experimental design levels.

Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded	Mean	Std. Dev.
A	beating time	Min.	Numeric	0.00	10.00	-1.00	1.00	5.00	3.54
B	temp.	^{0}C	Numeric	35.00	39.00	-1.00	1.00	37.00	1.41

Table 4.6:Design matrix for grass silage AD.

		Factor 1	Factor 2
Std	Run	A: beating time (Min).	B: temperature (^{0}C)
1	9	0	35
2	6	10	35
3	5	0	39
4	7	10	39
5	12	0	37
6	2	10	37
7	11	5	35
8	10	5	39
9	4	5	37
10	8	5	37
11	3	5	37
12	1	5	37

4.5 Experimental procedures

4.5.1 Step 1: Grass silage treatment.

Aforementioned, the Hollander beater device, DOE and RSM have been employed in this work. Beating time with ranges of 0 -10 mins was one factor considered in this process. 0 min treatment means without treatment, while the 10 min beating time was considered as with treatment and on this basis of DOE taken in the order of; lowest being 0 and highest as 10 min while the middle was taken as 5 min.

30 L of water and 1 kg of grass silage were taken and added to the beater this was operated for 5 min according to the design matrix. The first sample was taken after 5 minutes of beating and was achieved by; (1) switching off the beater following a beating experiment, and (2) the mixed silage was mixed with water and then emptied into the drum. It was swirled around continuously to mix and then a sample was taken to the bucket of approximately 10 L, after that the 10 L bucket filled with mixed grass silage and water was separated into two fractions; liquid on and the suspension. This was performed to enable the prepared solution be of equal composition of designed matrices accordingly. The same processes were repeated with a 10 min beating time.

4.5.2 Step 2: determine the dry solid in each sample before beating treatment.

1. The weight of the three empty dish plates was measured.
2. The grass silage was selected randomly and placed into each dish plate.
3. Each dish plate with wet grass silage was placed on the weighing scales one by one where the total wet weight was measured.
4. Net wet weight of each sample was measured by subtracting the empty dish plate weight.
5. Three samples were placed in the oven which was set at 60 ^{0}C for a period of 24 h in order to extract all the moisture contents in each sample.

6. The weight of each dry sample after 24 h was measured and thus total dry weight of the sample was determined.

Simple calculations were made to estimate moisture content and dry solid for each sample as shown in Table 4.7.

Net dry sample weight = total dry sample weight - Empty dish plate weight.

Table 4.7: Total dry solid content for each sample without treatment.

Sample No.	Empty dish plate weight (g)	Total wet sample weight (g)	Net wet sample weight (g)	Total dry sample weight (g)	Net dry sample weight (g)
1	173.1	188.4	15.3	175.5	2.4
2	137.4	152.7	15.3	139.7	2.3
3	148.5	163.8	15.3	150.9	2.4
					2.37

Moisture Content is calculated by finding the difference in weight between the dry and wet samples, then dividing the result into the wet weight and changing the result to a percentage. The moisture content of grass silage before treatment = 84.5%

4.5.3 Step 3: determine the dry solid in each sample after beating treatment.

1. The weight of the six empty beakers was measured (three beakers for samples after 5 min treatment, three beakers for samples after 10 min treatment).
2. Three wet grass silage samples after 5 min treatment were selected randomly for the beating bath and collected into each beaker. Three wet grass silage samples after 10 min treatment were selected randomly and collected into each beaker.
3. Each beaker with wet grass silage samples where approximately 200 mL was placed on the weighing scale one by one and the total wet weight of each beaker with blended grass silage was measured.
4. Net wet weight of each sample was measured by subtracting the empty beaker weight.

5. Six samples were placed in the oven which was set at 60 ^{0}C for more a period of 24 hours in order to extract all the moisture contents in each sample.
6. The weight of each dry sample after more a period of 24 h was measured and this will be total dry sample weight.

Calculations were carried out to estimate dry solid and moisture content for each sample as shown in Table 4.8 and 4.9.

Table 4.8: Total dry solid contents for each sample after 5 minutes treatment.

Sample No.	Empty beaker weight (g)	Total wet sample weight (g)	Net wet sample weight (g)	Total dry sample weight (g)	Net dry sample weight (g)
4	172.5	380	207.5	174.5	2
5	230.9	437.3	206.4	232.9	2
6	231.4	439.8	208.4	233.5	2.1
			207.43		2.03

Moisture Content is calculated by finding the difference in weight between the dry and wet samples, then dividing the result into the wet weight and changing the result to a percentage. The moisture content of grass silage after 5 min treatment = 99.02%.

Table 4.9: Total dry solid contents for each sample after 10 minutes treatment.

Sample No.	Empty beaker weight (g)	Total wet sample weight (g)	Net wet sample weight (g)	Total dry sample weight (g)	Net dry sample weight (g)
7	233.1	443.9	210.8	236	2.9
8	233.1	443.6	210.5	236.2	3.1
9	231.7	442.3	210.6	234.3	2.6
			210.63		2.87

Moisture content is calculated by finding the difference in weight between the dry and wet samples, then dividing the result into the wet weight and changing the result to a percentage. The moisture content of grass silage after 10 min treatment = 98.64%.

4.5.4 Step 4: Anaerobic digestion of the grass silage

According to the design matrix (Table 4.6), 12 experiments with different conditions were carried out in triplicate, so the total was 36 experiments. Thus, 36 reactors were designed and built (see Fig. 4.11 and Fig. 4.12). Reactors were labelled with stickers; marking them as 1A, 1B and 1C for first condition and 2A, 2B and 2C for second condition ...etc. The following steps were followed to setup the experiments.

1. Place 13g of wet grass silage without treatment in the conical flask 1, 3 and 5 and add 200 mL water into each of them.
2. Place 13g of wet grass silage with 5 min treated (fibre), 200 mL of the filtered liquid and 200 mL of sludge into the conical flasks 7, 8, 9, 10, 11 and 12.
3. Place 13g of wet grass silage with 10 min treated (fibre), 200 mL of the filtered liquid and 200 mL of sludge into the conical flasks 2, 4 and 6.
4. Each conical flask was sealed with a stopper and the tap was closed to prevent the entry the air.
5. The conical flasks were connected to the aluminium bags.
6. Nitrogen gas was pumped into the aluminium gas collecting bags and all parts of the system (reactors) to clean the excessive air.
7. Extract the nitrogen gas (filled before in the gas collecting bags and fermentation conical flasks) and the atmospheric air contents by vacuum pump to prevent the air contamination and to prevent the inhabitation growth of the anaerobes present in the sludge in the presence of oxygen gas.
8. Step 6, 7 were repeated three times for each reactor.
9. Three water baths were filled with water up to the maximum fill level and were operated at temperature ranges from 35^0C, 37^0C and 39^0C according to the design matrix.
10. Each reactor was placed in the water bath according to the design matrix as shown in Fig 4.13.

11. Shaking the conical flasks once a day over the period of the AD process ensured a more complete biological reaction.
12. The water level in the baths was checked every day and more water was added to counteract the effects of evaporation.
13. The biogas was measured every 3 days for total period of 21 days for the AD process.

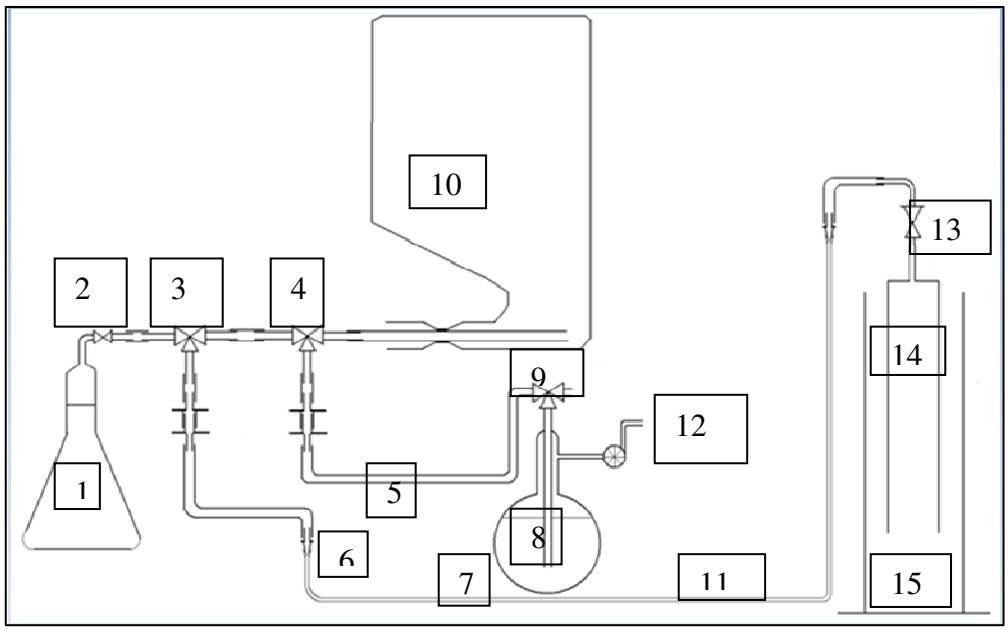


Fig.4.11: Anaerobic digestion Equipment set up.

1. Conical Flask
2. Tap above flask
3. Three way vent valve
4. Three way valve before gas bag
5. Quick connect/disconnect tube
6. Plastic tube reducer
7. Small diameter plastic tube
8. Bubbling round flask
9. Source of nitrogen gas
10. Aluminium gas bag
11. Gas pump
12. Gas analysis and vent
13. Tap above measuring cylinders
14. Inverted 250ml gas measuring cylinder
15. 500 ml gas measuring cylinder

Fig. 4.12: 36 reactors were designed and built according to design matrix.

4.5.5 Step 5: Biogas Measurement.

The volume of biogas was measured in the laboratory using volumetric flasks. Fig.4.11 shows the full apparatus set up to measure the volume of the biogas. The volumetric flask consists of two cylinders; one inverted inner 250 mL and other outer 500 mL. The water level was adjusted in a 500 mL cylinder. The next steps were followed to measure biogas yield:

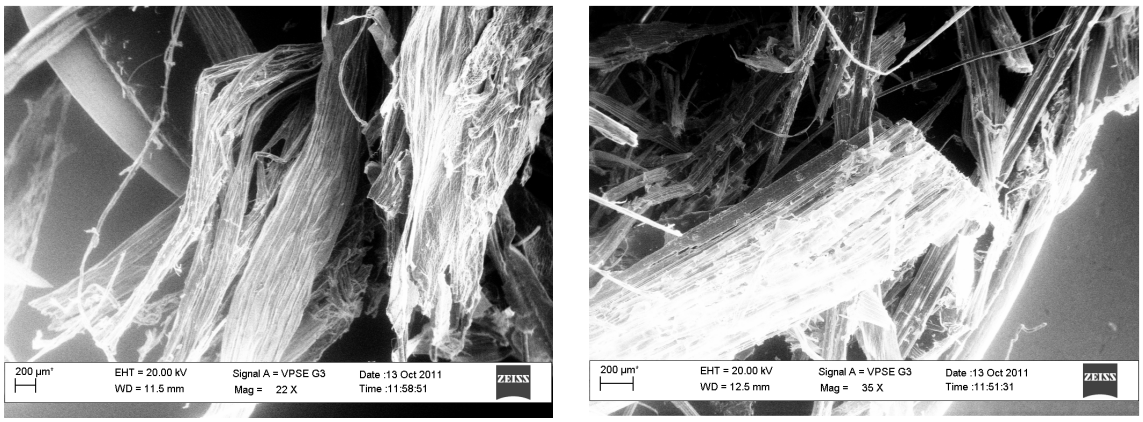
1. The reactors were attached to the gas measurement apparatus (volumetric flask, Round bottom flask and Electronic Pump) via 3 ways valves.
2. The conical flask valve, round bottom flask valve and the aluminium gas bag valve setting isolated. All air contaminations inside the apparatus were removed by circulating nitrogen gas for about 2 minutes. Filled Nitrogen gas inside the volumetric flask and bubbling round flask was removed by vacuum pump
3. Insert the inverted inner cylinder fully downwards in the outer cylinder. Flow of gas from the sample bag was opened by a valve.

4. The reading from the scale on the inverted cylinder where it crosses the water is recorded (initial reading).
5. A vacuum is created by lifting the inverted inner cylinder out of the outer cylinder and clamed; Record the value at which the water crosses the inverted cylinder (final reading).
6. The aluminium gas bag valve setting isolated and round bottom flask valve opened, then insert the inverted inner cylinder fully downwards in the outer cylinder to vent the measured gas to atmosphere.
7. The volume of biogas measured is found by subtracting the recorded reading in step 4 from recorded reading in step 5.
8. Repeat steps 3 to 6 until the gas bag is empty.

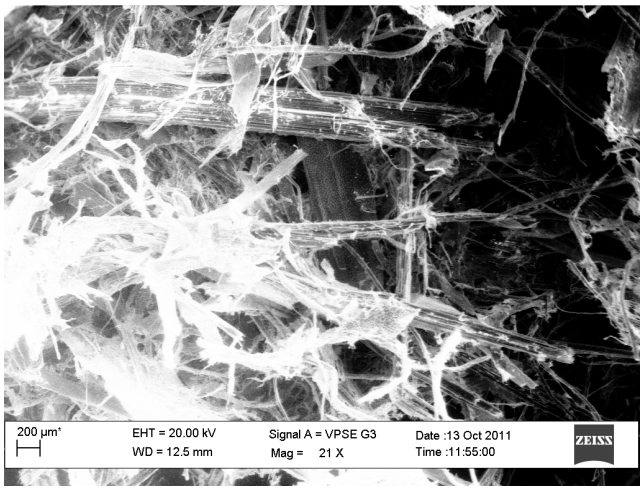
The recorded values of biogas refers to the mass of volatile solids content of grass silage in the vessel and have to adjusted to a constant value of 3 g of volatile solids content.

4.6 SEM examination of untreated and treated grass silage.

Previously discussed, the main goal of beating treatment is to decrease crystallinity, increase surface area and break down lignin. Fig. 4.13 illustrates the plant cell wall structure before and after treatment by using the spectrum electron microscope with magnification of 200 μm. The difference between image (a) where the grass silage without treatment, image (b) where grass silage was treated for 5 minutes beating and image (c) where grass silage was treated for 10 minutes beating is observed. It is clear that the crystalline structure of cellulose cells has been disrupted and the lignin component has been broken down.



(a) (b)



(c)

Fig. 4.13: SEM images (a) grass silage untreated, (b) treated 5 mints, (c) treated 10mints.

CHAPTER 5
RESULTS AND DISCUSSION

5 RESULTS AND DISCUSSION

In this chapter, the experimental design used, along with ranges of process parameters and experimental layouts are presented for this type of grass silage. This chapter also shows the results including statistical analysis using ANOVA for each response, along with validation experiments. The effects of process parameters on each response are described and discussed.

5.1 Grass Silage.

For this material, the experiment was designed based on face-centred composite design (FCCD) with full replication. Trial samples were performed by varying one of the process variables thus determining the working range of each variable (see chapter 3 for more details). Table 5.1 shows the process variables and experimental design levels used for this material. The experiment was carried out according to the design matrix shown in Table 5.2 in a random order to avoid any systematic error. For this material, seven mathematical models were developed successfully to predict the biogas yield every three days for 21 days, in a total seven responses. The procedures described earlier in chapter 4 were followed to determine and record these responses. The averages of at least three measurements for each response are presented in Table 5.3. Full experimental data measured for all responses can be found in Appendix B.

Table 5.1: Process variables and experimental design levels used.

Variables	Code	Unit	Limits coded/actual		
			-1	0	+1
Temperature	B	°C	35	37	39
Beating time	A	Min	0	5	10

Table 5.2: Design matrix in actual values.

Exp. No.	Run order	A, Min	B, °C
1	9	0	35
2	6	10	35
3	5	0	39
4	7	10	39
5	12	0	37
6	2	10	37
7	11	5	35
8	10	5	39
9	4	5	37
10	8	5	37
11	3	5	37
12	1	5	37

Table 5.3: Experimentally measured responses.

Exp. No.	First collection cc	Second collection cc	Third collection cc	Fourth collection cc	Fifth collection cc	Sixth collection cc	Seven collection Cc
1	1021	1769	2052	2225	2425	2560	2673
2	1049	1538	1807	2061	2272	2386	2477
3	1570	2128	2471	2694	2858	2950	3027
4	1409	1877	2252	2464	2576	2664	2728
5	1490	2079	2425	2674	2798	2926	3023
6	1252	1762	2149	2422	2521	2606	2675
7	1111	1651	1939	2160	2370	2497	2592
8	1418	1983	2400	2629	2788	2887	2973
9	1394	1918	2306	2551	2669	2768	2868
10	1491	1998	2368	2609	2707	2807	2901
11	1526	2072	2486	2723	2859	2969	3063
12	1582	2106	2518	2754	2887	2991	3088

5.2 Development of biogas models.

As a result of analysing the measured responses by the design expert software, the fit summary output indicates that, for all responses, the quadratic models are statistically recommended for further analysis as they have the maximum predicted and adjusted R^2

value [162]. The test for significance of the regression models, the test for significance on individual model coefficients and the lack of fit test were performed using the same statistical package for all responses. By selecting the step-wise regression method, the insignificant model terms can be automatically eliminated. The resulting ANOVA tables (Tables 5.4 to 5.10) for the reduced quadratic models outline the analysis of variance of each response and show the significant model terms. The same tables also show any other adequacy measurements of R^2, adjusted R^2 and predicted R^2. The entire adequacy measures are close to 1, which is in reasonable agreement and indicates adequate models [172-173]. The Adequate precision compares the range of the predicted value at the design points to the Average Prediction error. In all cases the value of Adequate Precision are dramatically greater than 4. An Adequate Precision Ratio above 4 indicates adequate model discrimination [160 and173].

For the six collections models, the analysis of variance indicates that the main effects are beating time (A), temperature (B), the second order effect of beating time (A^2) and the quadratic effect of temperature (B^2) are also significant model terms. For the seventh collection model the ANOVA analysis indicates that the main effects are beating time (A), temperature (B) and the second order effect of beating time (A^2) are significant model terms. The results indicate that the most significant model term associated with all collections models is temperature. The biogas model means a mathematical model developed using experimental data and the regression analysis. This model cannot be used unless it passes certain statistical tests. The adequate mathematical model can be used to predict the amount of biogas produced at a certain conditions or to optimize the process, which include the beating and AD.

The final mathematical models in terms of coded factors as determined by the design expert software are shown in Eqs. 5.1 to 5.7. The final empirical models in terms of actual factors are shown in Eqs. 5.8 to 5.14:

Table 5.4: ANOVA table for first collection reduced quadratic model.

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	462449	4	115612	21	0.0005	Significant
A-beating time	22918	1	22918	4	0.0808	
B-temp.	327844	1	327844	60	0.0001	
A^2	33147	1	33147	6	0.0439	
B^2	41427	1	41427	8	0.0288	
Residual	38567	7	5510			
Lack of Fit	19821	4	4955	1	0.6001	Not significant
Pure Error	18746	3	6249			
Cor Total	501016	11				
$R^2 = 0.9230$			Pred R^2 =0.7637			
Adj $R^2 = 0.8790$			Adeq Precision= 13.374			

Table 5.5: ANOVA table for second collection reduced quadratic model.

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	429484	4	107371	23	0.0004	Significant
A-beating time	106246	1	106246	23	0.0020	
B-temp.	246856	1	246856	53	0.0002	
A^2	22966	1	22966	5	0.0619	
B^2	28018	1	28018	6	0.0440	
Residual	32620	7	4660			
Lack of Fit	11815	4	2954	0	0.7861	Not Significant
Pure Error	20805	3	6935			
Cor Total	462104	11				
$R^2 = 0.9294$			Pred $R^2 = 0.8196$			
Adj $R^2 = 0.8891$			Adeq Precision= 15.246			

Table 5.6: ANOVA table for third collection reduced quadratic model.

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	623495	4	155874	24	0.0004	Significant
A-beating time	91000	1	91000	14	0.0075	
B-temp.	381488	1	381488	58	0.0001	
A^2	41961	1	41961	6	0.0395	
B^2	59076	1	59076	9	0.0201	
Residual	46068	7	6581			
Lack of Fit	16521	4	4130	0	0.7900	Not Significant
Pure Error	29547	3	9849			
Cor Total	669563	11				
R^2 =0.9312			Pred R^2 = 0.8246			
Adj R^2 = 0.8919			Adeq Precision=14.378			

Table 5.7: ANOVA table for fourth collection reduced quadratic model.

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	640685	4	160171	25	0.0003	Significant
A-beating time	69342	1	69342	11	0.0138	
B-temp.	389956	1	389956	60	0.0001	
A^2	37740	1	37740	6	0.0470	
B^2	85600	1	85600	13	0.0085	
Residual	45620	7	6517			
Lack of Fit	18275	4	4569	1	0.7429	Not Significant
Pure Error	27345	3	9115			
Cor Total	686305	11				
R^2 = 0.9335			Pred R^2 = 0.8225			
Adj R^2 = 0.8955			Adeq Precision= 14.131			

Table 5.8: ANOVA table for fifth collection reduced quadratic model.

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	508116	4	127029	15	0.0016	Significant
A-beating time	84709	1	84709	10	0.0161	
B-temp.	300483	1	300483	35	0.0006	
A^2	45190	1	45190	5	0.0547	
B^2	36865	1	36865	4	0.0761	
Residual	59631	7	8519			Not Significant
Lack of Fit	24356	4	6089	1	0.7337	
Pure Error	35275	3	11758			
$R^2 = 0.8950$			Pred $R^2 = 0.7149$			
Adj $R^2 = 0.8350$			Adeq Precision=11.692			

Table 5.9: ANOVA table for sixth collection reduced quadratic model.

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	479242	4	119811	13	0.0023	Significant
A-beating time	101067	1	101067	11	0.0127	
B-temp.	258803	1	258803	28	0.0011	
A^2	46158	1	46158	5	0.0596	
B^2	33669	1	33669	4	0.0967	
Residual	64107	7	9158			
Lack of Fit	26016	4	6504	1	0.7368	Not Significant
Pure Error	38091	3	12697			
Cor Total	543349	11				
$R^2 = 0.8820$			Pred $R^2 = 0.6820$			
Adj $R^2 = 0.8146$			Adeq Precision= 10.955			

Table 5.10: ANOVA table for seven collection reduced quadratic model.

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	442611	3	147537	12	0.0027	Significant
A-beating time	118194	1	118194	9	0.0156	
B-temp.	229290	1	229290	18	0.0028	
A^2	95127	1	95127	8	0.0253	
Residual	101146	8	12643			
Lack of Fit	63930	5	12786	1	0.5242	Not Significant
Pure Error	37216	3	12405			
Cor Total	543757	11				
$R^2 = 0.8140$			Pred $R^2 = 0.5851$			
Adj $R^2 = 0.7442$			Adeq Precision=10.927			

Coded mathematical modles:

first collection = $1493.11-61.80 * A + 233.75 * B -111.49 * A^2 -124.64 * B^2$ (5.1)

Second collection = $2019.90 -133.07 * A+202.84 * B -92.80 * A^2 -102.50 * B^2$ (5.2)

Third collection = $2417.11 -123.15 * A +252.15 * B -125.44 * A^2 -148.84 * B^2$ (5.3)

Fourth collection = $2661.70 -107.50 * A +254.94 * B -118.96 * A^2 -179.16 * B^2$ (5.4)

Fifth collection = $2783.68 -118.82 * A +223.79 * B -130.18 * A^2 -117.58 * B^2$ (5.5)

Sixth collection = $2888.49 -129.79 * A +207.69 * B -131.56 * A^2 -112.36 * B^2$ (5.6)

Seven collection = $2945.45 -140.3 * A +195.49 * B -178.07 * A^2$ (5.7)

Actual mathematical moles:

first collection = - 45539 +32.235* beating time + 2422.71667 * temp. -4.459 * beating time^2 -31.16 * temp.^{2} (5.8)

Second collection = -36773.79 +10.507 * beating time +1997.71458 * temp -3.712 * beating time^2 -25.62563 * temp.^{2} (5.9)

Third collection = -53190.5+25.545* beating time +2879.61667 * temp -5.0176 * beating time^2 -37.2 * temp.^{2} (5.10)

Fourth collection = -63385.306 +26.085 * beating time +3442.020* temp -4.758 * beating time^2 -44.79125 * temp.^{2} (5.11)

Fifth collection = -41608.634 +28.307 * beating time +2287.077* temp -5.207 * beating time^2 -29.39438 * temp.^{2} (5.12)

Sixth collection = -39412.414 +26.668* beating time +2182.595* temp -5.262 * beating time^2 -28.0912 * temp.^{2} (5.13)

Seven collection = -708.77 +43.157* beating time +97.743* temp -7.12280 * beating time^2 (5.14)

5.3 Validation of the models.

Figures 5.1 – 5.7 show the relationship between the actual and predicted values for all responses. These figures indicate that the developed models are adequate owing to the residuals in prediction of each response being small, as the residuals tend to be close to the diagonal line. To verify the adequacy of the developed models a further three confirmation experiments were carried out using new randomly selected test conditions, each within the experiment range defined previously. Using the point prediction option in the software, the seven responses of the validation experiment were predicted using the previous developed models and compared with the actual measured responses of this confirmation experiment. Table 5.11 to 5.13 summarises the experiments, conditions, actual experimental values, the predicted values and the percentages error in prediction. It is evident that the models can adequately describe the responses within the ranges considered as the error % in prediction is ranged between 1.2% and 11.1%, which is in agreement with the results reported in [172-173]. Full experimental data measured for all responses for confirmation experiments can be found in Appendix C.

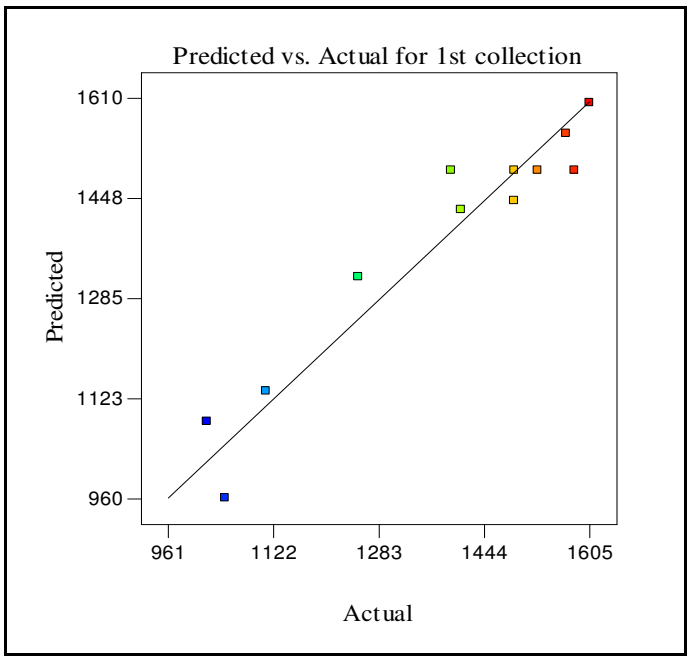


Fig. 5.1: Scatter diagram for the first collection model.

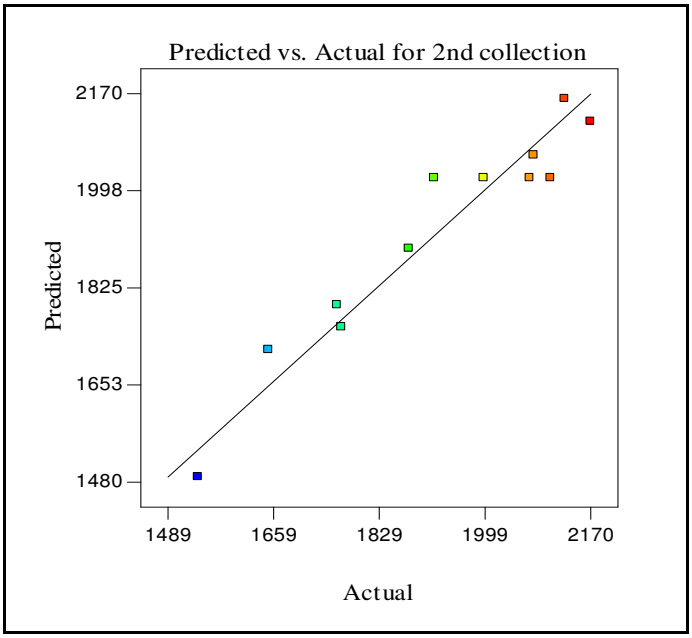


Fig. 5.2: Scatter diagram for the second collection model.

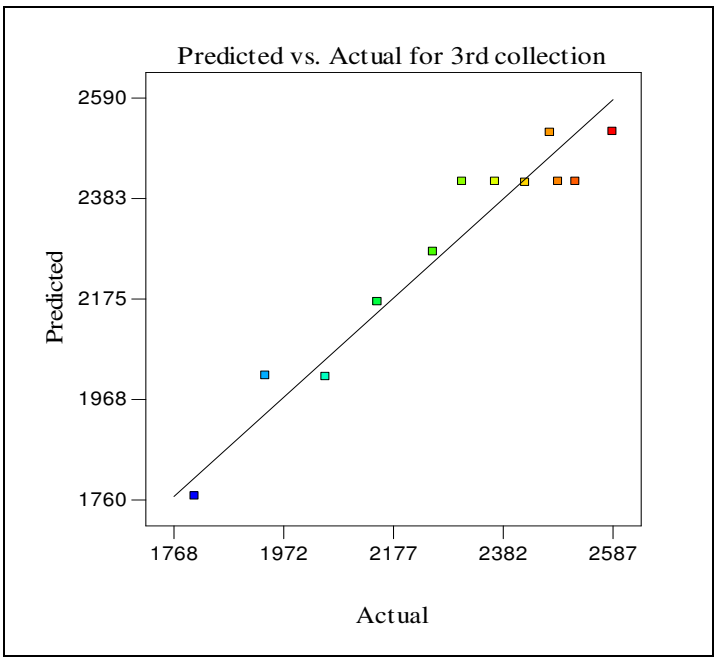


Fig. 5.3: Scatter diagram for the third collection model.

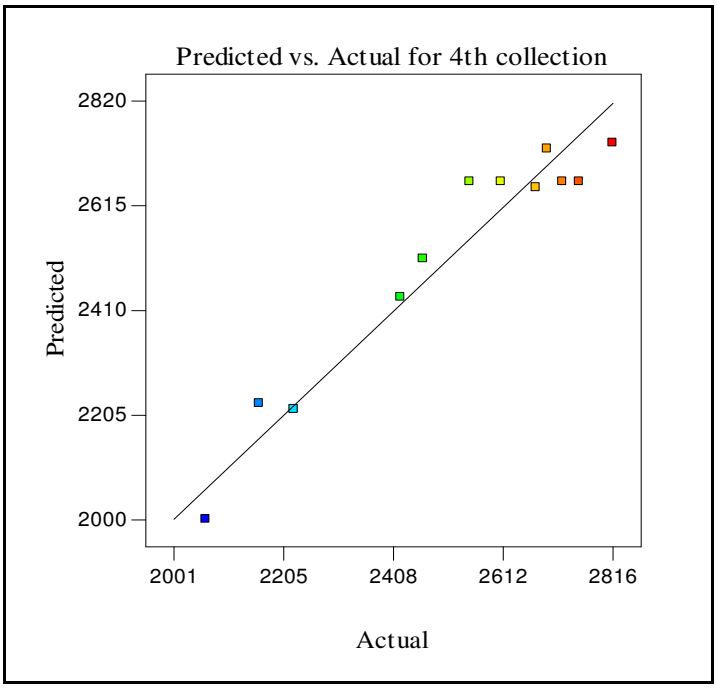


Fig. 5.4: Scatter diagram for the fourth collection model.

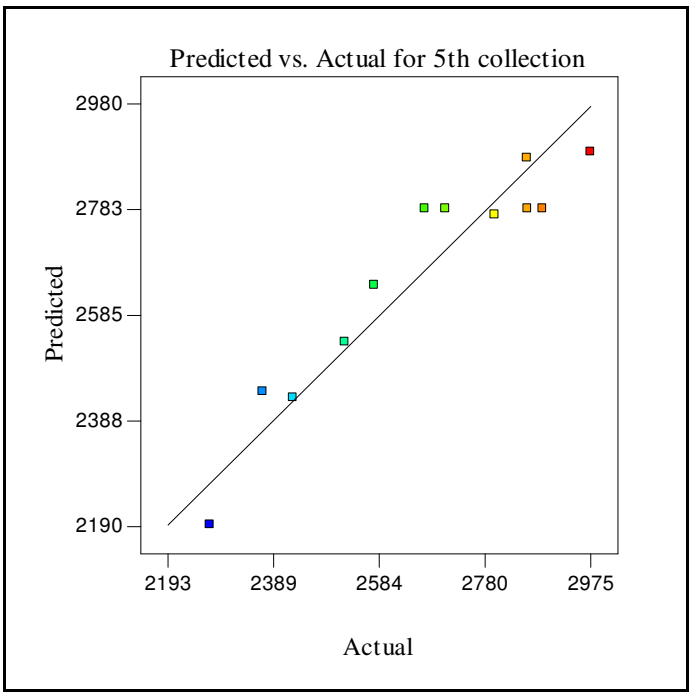


Fig. 5.5: Scatter diagram for the fifth collection model.

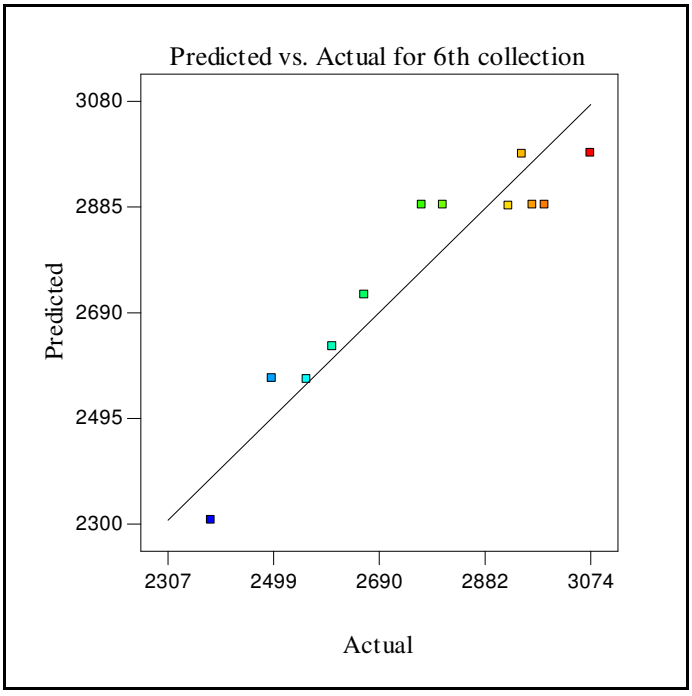


Fig. 5.6: Scatter diagram for the sixth collection model.

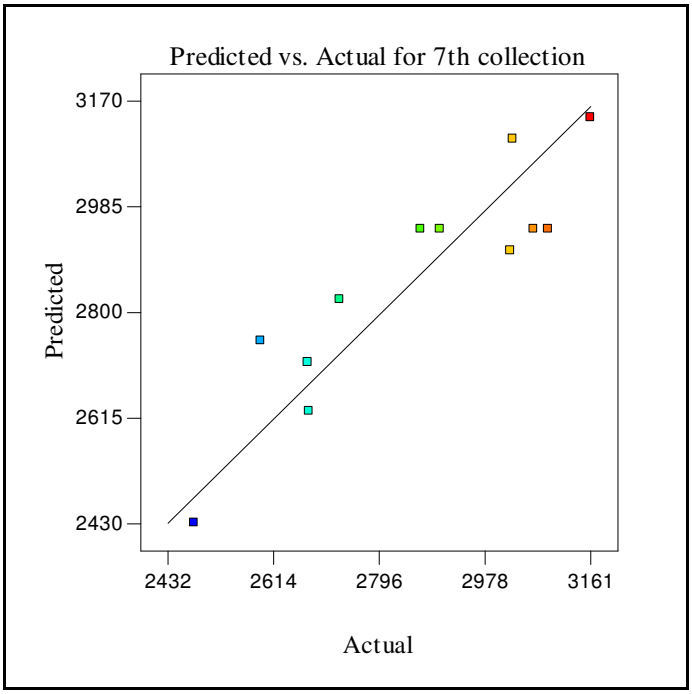


Fig. 5.7: Scatter diagram for the seventh collection model.

Table 5.11: Confirmation experiments.

beating time (min)	Temperature C	responses	prediction	actual	% of error
0.0	38.0	1st collection	1529.0	1380.8	-10.7
		2nd collection	2136.0	2044.0	-4.5
		3rd collection	2503.7	2372.8	-5.5
		4th collection	2732.9	2564.8	-6.6
		5th collection	2854.8	2712.0	-5.3
		6th collection	2962.5	2824.8	-4.9
		7th collection	3052.59	2926.44	-4.31

Table 5.12: Confirmation experiments.

beating time (min)	Temperature C	responses	Prediction	actual	% of error
1.6	37.3	1st collection	1518.1	1439.2	-5.5
		2nd collection	2097.3	2145.6	2.2
		3rd collection	2479.6	2509.6	1.2
		4th collection	2716.2	2752.0	1.3
		5th collection	2835.0	2910.4	2.6
		6th collection	2946.4	3011.2	2.1
		7th collection	3042.26	3097.16	1.77

Table 5.13: confirmation experiments.

beating time	temperature	responses	prediction	actual	% of error
2.7	37.3	1st collection	1530.5	1569	2.3
		2nd collection	2089.3	2352.6	11.1
		3rd collection	2481.6	2744.2	9.5
		4th collection	2720.2	3036.85	10.3
		5th collection	2841.7	3199.65	11.1
		6th collection	2948.9	3314.75	11.0
		7th collection	3044.77	3410.25	10.71

5.4 Effect of Anaerobic Digestion Factors on the Production of Biogas

It is necessary to indicate that all responses (collections of biogas) are related to biogas production and determined in a cumulative way. The analysis demonstrates that the effect of the beating time and the temperature on all responses all have the same trend as shown in Fig. 5.8 – 5.14. Aforementioned (in section 5.1.1) the temperature is the most significant factor associated with the collection models, further analysis can verify that temperature has highest influence on the process, this is in agreement with the findings reported by Vindis et al [121] and Yadvika et al [170].

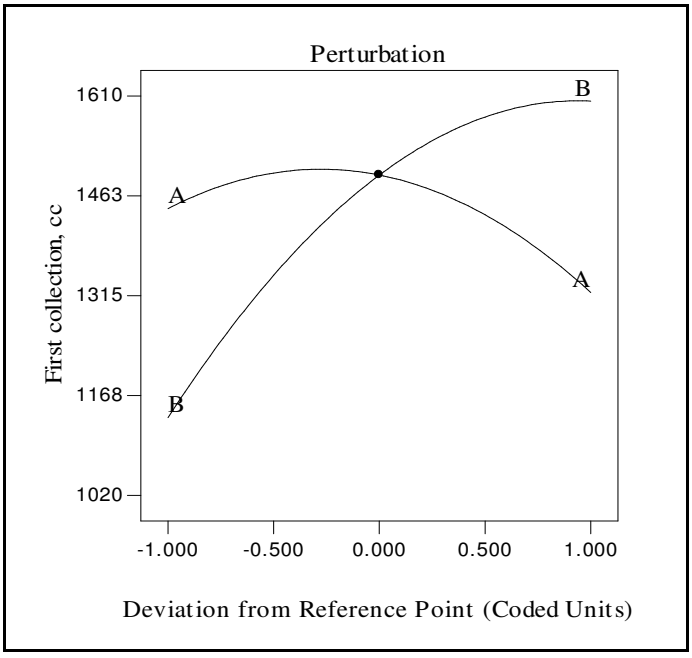


Fig. 5.8: Perturbation plot show the effect of the beating time (A) and temperature (B) on the first collection of biogas.

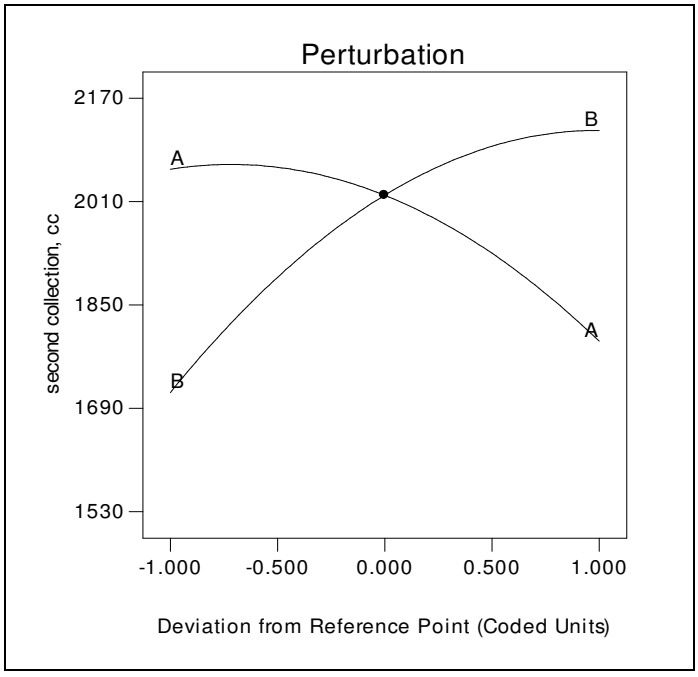


Fig. 5.9: Perturbation plot show the effect of the beating time (A) and temperature (B) on the second collection of biogas.

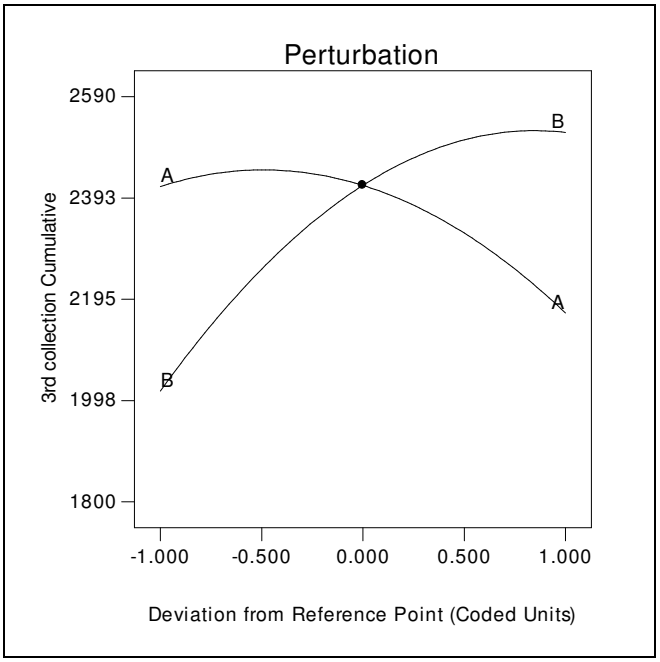


Fig. 5.10: Perturbation plot show the effect of the beating time (A) and temperature(B) on the third collection of biogas.

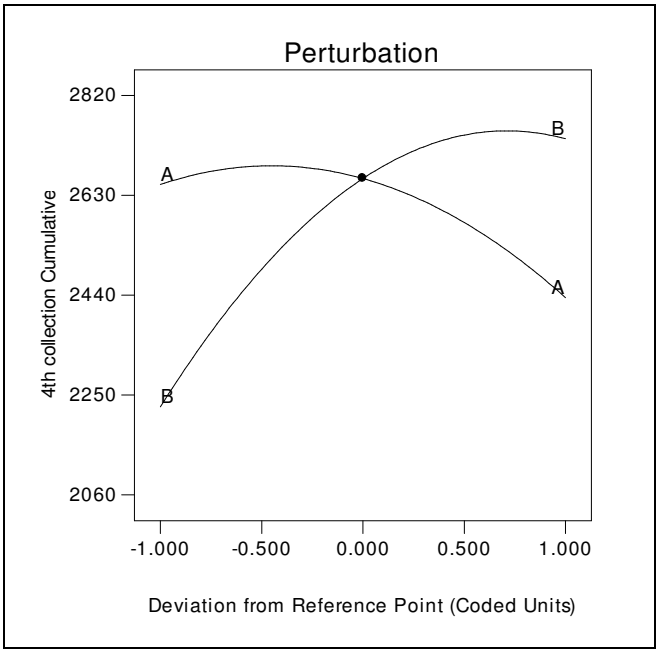


Fig. 5.11: Perturbation plot show the effect of the beating time (A) and temperature (B) on the forth collection of biogas.

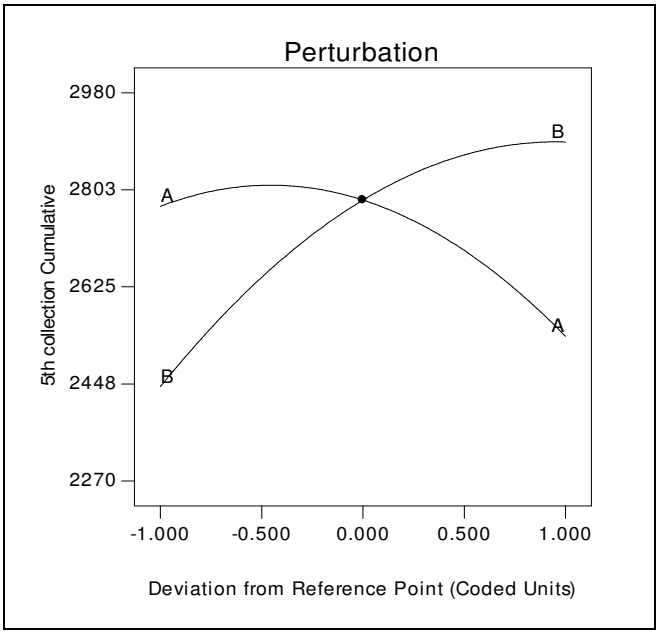


Fig. 5.12: Perturbation plot show the effect of the beating time (A) and temperature (B) on the fifth collection of biogas.

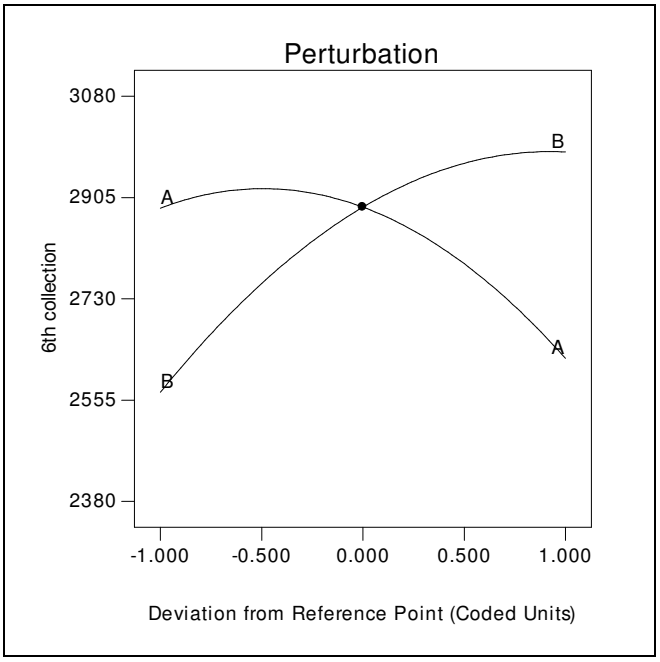


Fig. 5.13: Perturbation plot show the effect of the beating time (A) and temperature (B) on the sixth collection of biogas.

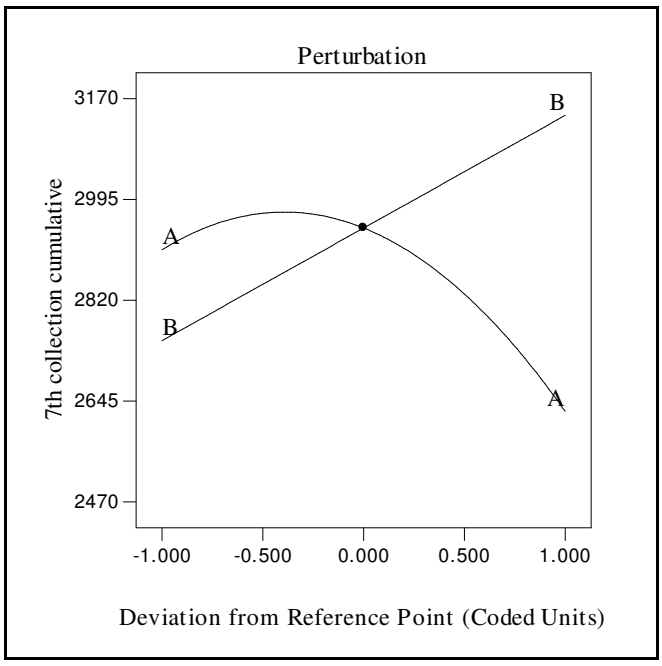


Fig. 5.14: Perturbation plot show the effect of the beating time (A) and temperature (B) on the seventh collection of biogas.

Furthermore, the results indicate that as temperature increases the biogas yield also increases with significant temperatures up to 38.3 °C. Above this temperature of 38.3 °C the yield of biogas will remain constant. The beating time also has a strong effect on the biogas production as indicated in Fig. 5.8 – 5.14. However, any increase in the beating time produces a general decrease in particle size. Smaller particle sizes produce a larger surface area of the substrate that is available to the microorganisms in the digestion period. This will accelerate the hydrolysis step and could enhance biogas production. The results obtained in this study indicate that any increase in the beating time results in an increased biogas production up to a beating time of about 3 min. Moreover, the results indicate that if beating were continued beyond 3 min this would result in a sharp decrease in the biogas production as presented in Figs. 5.8 – 5.14. This could be due to the effect of the particle size of the grass silage. The longer the beating times the smaller the particle size. Too small a particle size will dynamically accelerate

the rate of hydrolysis and acidogenisis reactions, and then VFA is produced rapidly, resulting in imbalance of production and consumption of VFA leading to accumulation of VFA, decreased pH and inhibition of biogas production. This is because the early stages of the anaerobic solubilisation process, especially the hydrolysis and acidogenesis steps, are significantly affected by physicochemical conditions such as temperature and pH rather than by the effects of biological factors [103, 171]. As a numerical example, the results indicate that the value of biogas produced was significantly reduced from 3022.9 cc to 2675.3 cc as the beating time increased from 0 min to 10 min respectively.

Figs. 5.15 – 5.21 illustrates contour graphs for the effect of beating time and temperature on biogas production for all responses. The contour plots provide a two-dimensional view where all points that have the same response are connected to produce contour lines of constant responses and illustrate the optimum level of each variable and the effect of their interactions on biogas production.

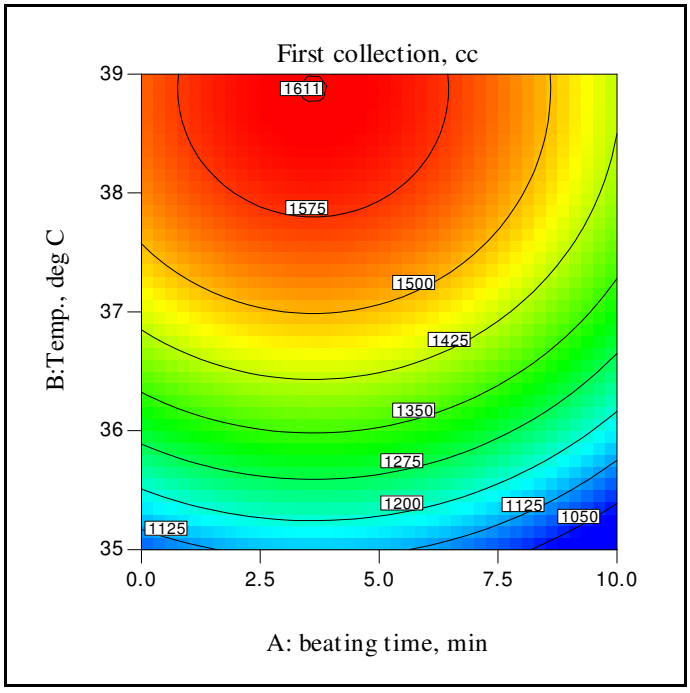


Fig. 5.15: Contour graph showing the effect of A and B on the first collection of biogas.

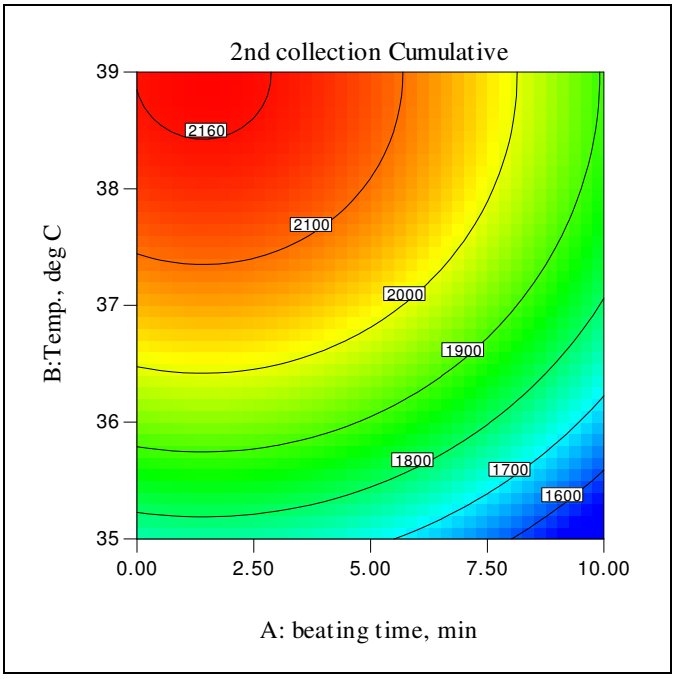


Fig. 5.16: Contour graph showing the effect of A and B on the second collection of biogas.

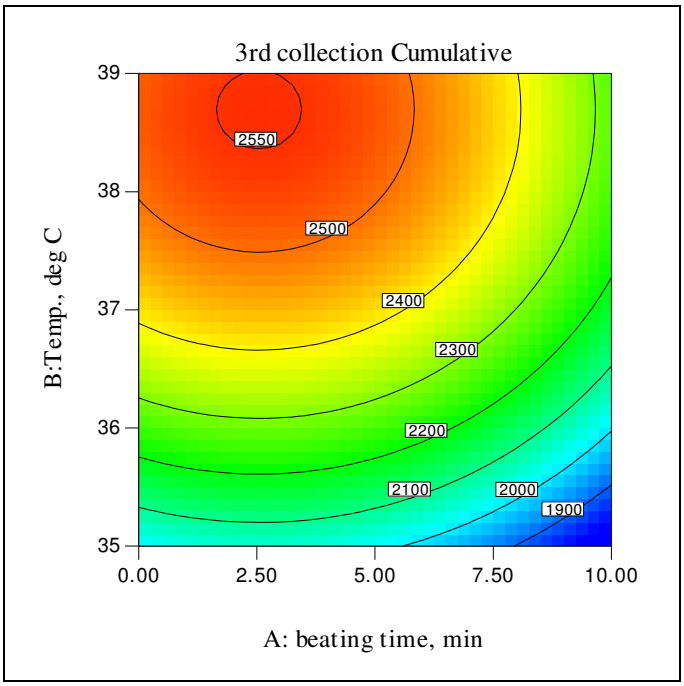


Fig. 5.17: Contour graph showing the effect of A and B on the third collection of biogas.

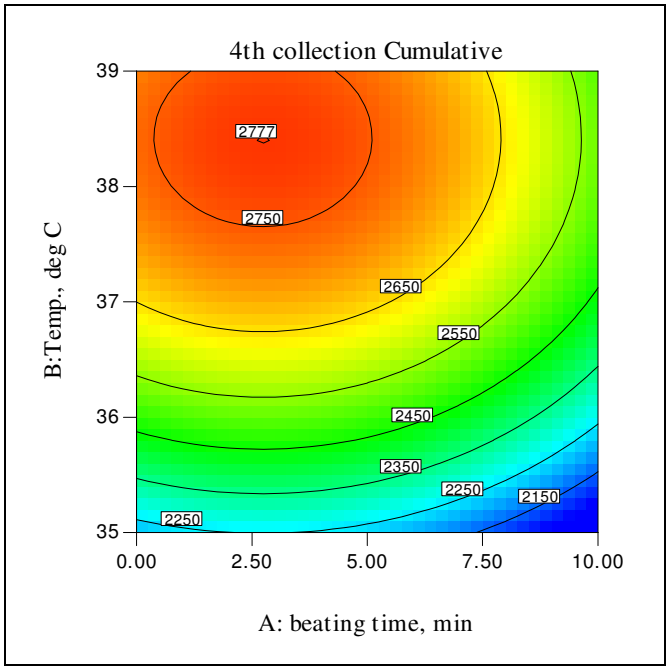


Fig. 5.18: Contour graph showing the effect of A and B on the fourth collection of biogas.

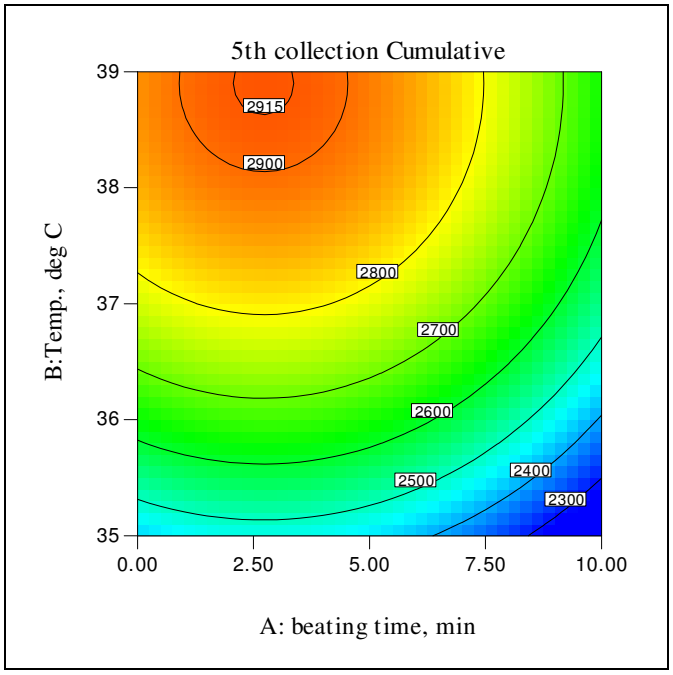


Fig. 5.19: Contour graph showing the effect of A and B on the fifth collection of biogas.

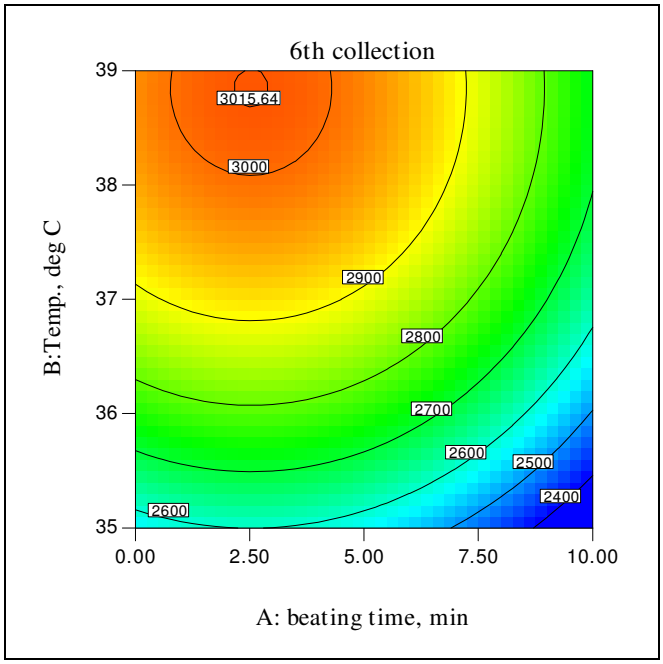


Fig. 5.20: Contour graph showing the effect of A and B on the sixth collection of biogas.

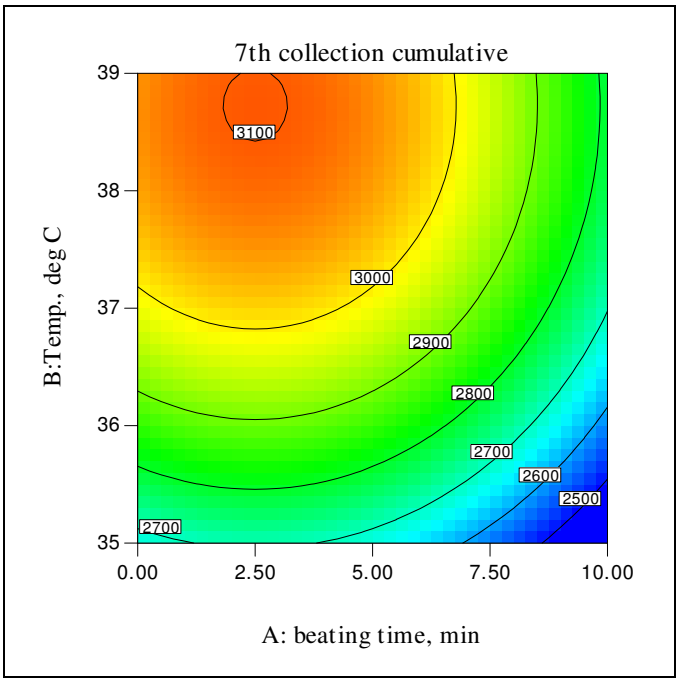


Fig. 5.21: Contour graph showing the effect of A and B on the seventh collection of biogas

5.5 Optimization.

Anaerobic digestion of grass silage is a complex biological process and has multi input and output parameters. Optimization of these kinds of processes can be achieved by using desirability approaches (explained earlier in chapter 3), which is built in the Design expert 7 software, by search for a combination of factor levels that simultaneously satisfy the requirements placed (i.e. optimization criteria) on each one of the input/output for anaerobic digestion of grass silage. Optimization can be performed either numerically or graphically.

5.5.1 Numerical optimization.

In this study, four optimization criteria have been set as shown in Tables 5.14 – 5.17. The differences between these four criteria are in the restriction sets for the factors and the importance level assigned for each factor or response. The results indicate that the optimal solutions for the first criterion were found to be: beating time of 2 min 29 s and AD temperature of 38.7 °C with a maximum biogas of 3103 cc. The results indicate that the optimum conditions for the second criterions were found to be: beating time of 2 min 42 s and AD temperature of 37.4 °C with a maximum biogas of 3052c c. While for the third optimization criterion the optimal conditions were found to be: beating time of 1 min 36 s and AD temperature of 37.95 °C with a maximum biogas of 3048 cc. Finally, the optimal conditions for the fourth criterion were found to be: beating time 2 min 42 s and AD temperature of 37.4 °C with a maximum biogas of 3085 cc. In comparison between the highest amounts of biogas (i.e. 3027 cc) produced during the practical experiments, see Table 5.3, with the amount of biogas of 3410 cc, see Table 5.13, obtained if the optimal condition of (beating time of 2 min 42 s and AD temperature of 37.4°C) is applied. It is clear that the percentage increase in biogas production for this optimal condition is 12.65%. The reason behind not selecting the biogas yields of 3068 and 3088 presented in Table 5.3 is that these values are obtained by applying the centre

point condition, which was repeated four times and the average of these values is 2980 cc, which is still below 3027 cc.

Table 5.14: First criterion for numerical optimization of AD of grass silage.

Name	Goal	Importance
beating time	is in range	3
temperature	is in range	5
first collection	Maximize	3
2nd collection Cumulative	Maximize	3
3rd collection Cumulative	Maximize	3
4th collection Cumulative	Maximize	3
5th collection Cumulative	Maximize	3
6th collection Cumulative	Maximize	3
7th collection cumulative	Maximize	3

Table 5.15: Second criterion for numerical optimization for AD of grass silage.

Name	Goal	Importance
beating time	is in range	3
temperature	minimize	5
first collection	maximize	3
2nd collection Cumulative	maximize	3
3rd collection Cumulative	maximize	3
4th collection Cumulative	maximize	3
5th collection Cumulative	maximize	3
6th collection Cumulative	maximize	3
7th collection cumulative	maximize	3

Table 5.16: Third criterion for numerical optimization for AD of grass silage.

Name	Goal	Importance
beating time	minimize	3
temperature	minimize	5
first collection	maximize	3
2nd collection Cumulative	maximize	3
3rd collection Cumulative	maximize	3
4th collection Cumulative	maximize	3
5th collection Cumulative	maximize	3
6th collection Cumulative	maximize	3
7th collection cumulative	maximize	3

Table 5.17: Fourth criterion for numerical optimization of AD of grass silage.

Name	Goal	Importance
beating time	minimize	1
temperature	minimize	3
first collection	maximize	5
2nd collection Cumulative	maximize	5
3rd collection Cumulative	maximize	5
4th collection Cumulative	maximize	5
5th collection Cumulative	maximize	5
6th collection Cumulative	maximize	5
7th collection cumulative	maximize	5

5.5.2 Graphical optimization.

Aforementioned in chapter 3, in a graphical optimization with multiple responses, the software defines regions where requirements simultaneously meet the proposed criteria. Also, superimposing or overlaying critical response contours can be defined on a

contour plot. Figs. 5.22 -5.25 are overlay plots where green highlighting areas are regions that meet the proposed criteria. These types of graphs are useful for quick visual searching tools for the optimal conditions, especially in laboratory studies.

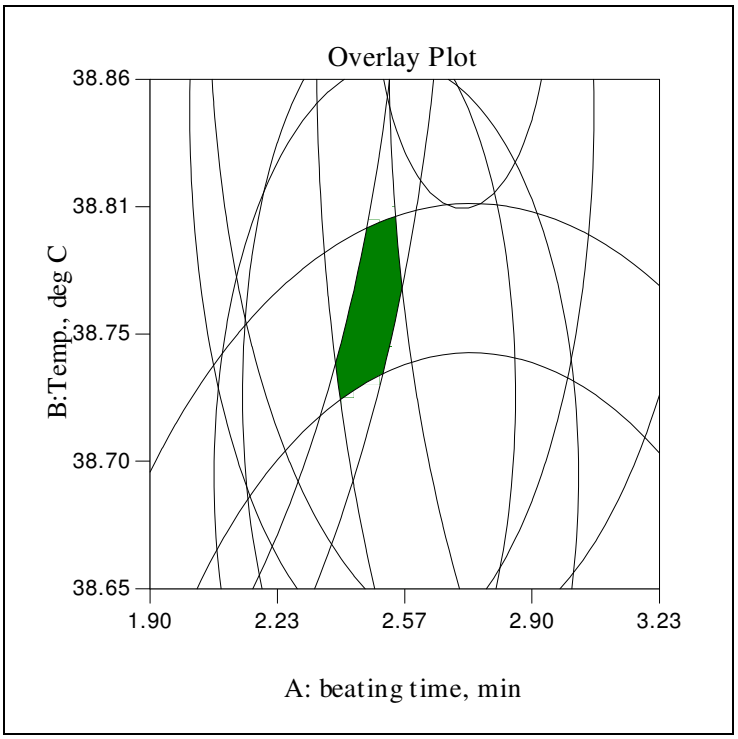


Fig. 5.22: Overlay plot showing the optimal conditions based on first criterion.

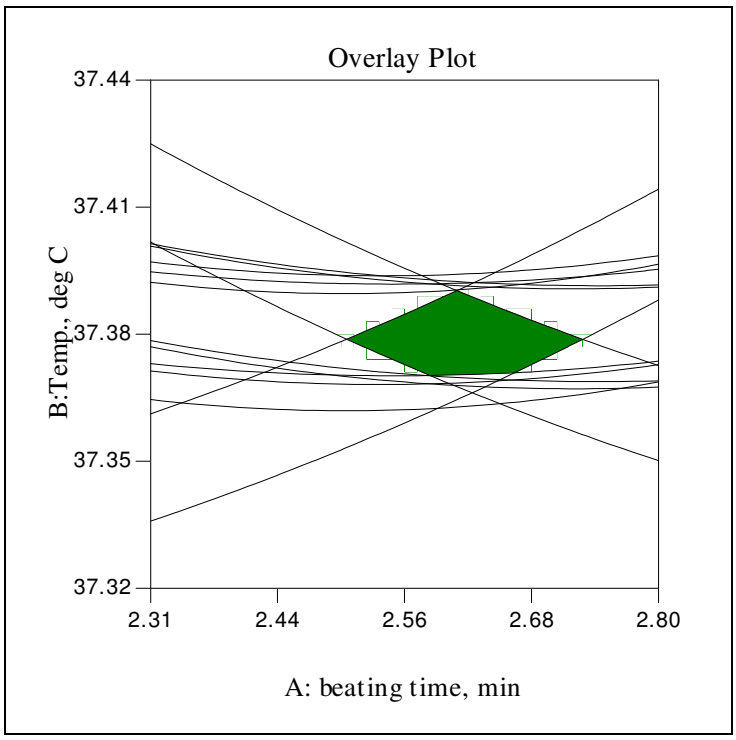


Fig. 5.23: Overlay plot showing the optimal conditions based on second criterion.

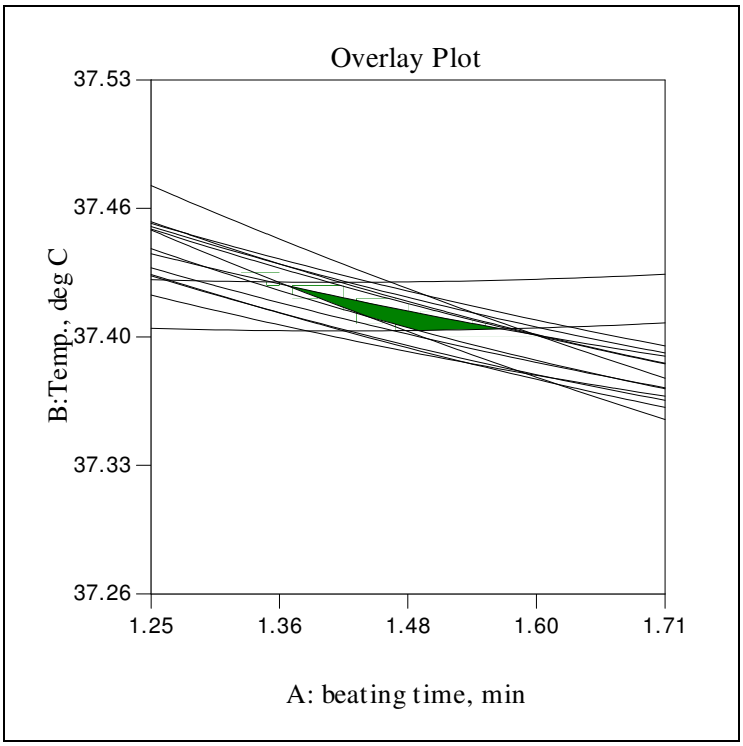


Fig. 5.24: Overlay plot showing the optimal conditions based on third criterion.

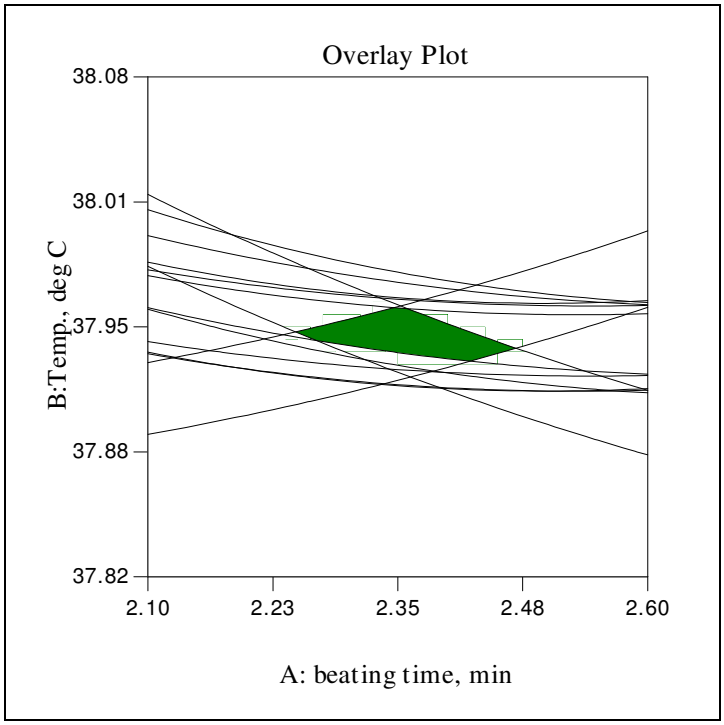


Fig. 5.25: Overlay plot showing the optimal conditions based on fourth criterion.

CHAPTER 6
CONCLUSIONS AND FUTURE WORK

6 Conclusions and Future work

6.1 Conclusions

In this study beating treatments as new methods of mechanical pre-treatment, which were introduced by the biomass research team in the school of mechanical and manufacturing engineering in DCU have been applied to enhance performance of the anaerobic digestion of grass silage. In addition, the design of experiment DOE and response surface methodology (RSM) have been employed to investigate the effects of the two most important factors (temperature and beating time) on the biogas production from anaerobic digestion of grass silage. The findings of these studies can be summarized in the following:

- Beating treatment as mechanical pre-treatment method is effective and accelerates the degradability for grass silage. However the prolongation of beating time does not always improve the biogas yield.
- Beating treatment achieves 12.65% increase in biogas production from grass silage.
- RSM is an effective tool to optimize anaerobic digestion of grass silage combined with beating treatment.
- Both factors (temperature and beating time) have a significant effect on the overall AD process.
- Fourteen adequate mathematical models have been developed for this process. These models can be used successfully for prediction or optimization analysis.
- The maximum biogas yield of 3410 cc can be achieved at the optimal condition of temperature 37.3 and beating time 2 min 42 s.

6.2 Main Contributions from this Work

- Verify that beating treatment does work with grass silage
- Verify that the DOE is successful tool to predict and optimize anaerobic digestion for grass silage process.

- The identification of the effect of each parameter (input factors) on each response (output of process).
- Sets of operating parameters (conditions) which lead to optimal quality were identified.

6.3 Future work

- Investigate the effect of other parameters, for example pH, N/C ratio , sludge quantity, VFA … etc.
- Analyze the chemical composition of biogas and investigate if it has any correlation with the input factors of process.
- Apply beating treatment to other legnocellulosic material.

References

[1] A. M. Mshandete, W. Parawira, Biogas technology research in selected sub-Saharan African countries – A review, 2009, African Journal of Biotechnology Vol. 8 (2), pp.116-125.

[2] T. Matsui, y. Koike, Methane fermentation of a mixture of seaweed and milk at a pilot- scale plant, 2010, Journal of Bioscience and Bioengineering, article in press.

[3] M. N. Sannaa , The Development of Biogas Technology in Denmark: Achievements & Obstacles, Department of environment, technology and social studies Roskilde University , denmark.

[4] U. Marchaim, Biogas processes for sustainable development, 1992, MIGAL Galilee Technological Centre Kiryat Shmona, Israel,

[5] http://www.renewables-made-in-germany.com/index.php?id=154 (accessed November2010)

[6] R. D. Perlack, L. L. Wright, A. F. Turhollow, R. L. Graham, B. J. Stokes, D. C. Erbach, Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility Of a billion-ton Annual supply, 2005, U.S. Department of energy

[7] T. Al Seadi, D. Rutz, H. Prassl, M. Köttner, T. Finsterwalder, S. Volk, R. Janssen, Biogas handbook, 2008, University of Southern Denmark Esbjerg.

[8] T. Amon, B. Amon, V. Kryvoruchko, A. Machmu¨ ller, K. Hopfner-Sixt, V. Bodiroza, R. Hrbek, J. Friedel, E. Po¨tsch, H. Wagentristl, M. Schreiner, W. Zollitsch, Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations, 2007, Bioresource Technology, vol. 98, p.p 3204–3212, Austria.

[9] K. Hopfner-Sixt, T. Amon, Monitoring of agricultural biogas plants in Austria – Mixing technology and specific values of essential process parameters, 2007, In: Proceedings of the 15th European Biomass Conference and Exhibition, Berlin, pp. 1718–1728.

[10] P. Weiland, Biomass digestion in agriculture: a successful pathway for the energy production and waste treatment in Germany, 2006, Engineering in Life Sciences, Vol 6, pp.302–309.

[11] T. Matsui, y. Koike, Methane fermentation of a mixture of seaweed and milk at a pilot-scale plant, 2010, Journal of Bioscience

[12] J. Raynal, J.P. Delgenes and R. Moletta, Two-phase anaerobic digestion of solid Waste by a multiple liquefaction reactors process, 1998, Bioresource Technology, Vol. 65, pp.97– 103

[13] B.K. Ahring, Perspectives for anaerobic digestion, Advances in Biochemistry, 2002, Engineering/Biotechnology, Vol. 81, pp. 1–30.

[14] S. Aldin, E. Elbeshbishy, G. Nakhla, M. B. Ray, Modeling the Effect of Sonication on the Anaerobic Digestion of Biosolids, 2001, Energy Fuels, Vol. 24, pp. 4703–4711, Canada

[15] A. Tiehm, k. Nickel and U. Neis, The use of ultrasound to accelerate the anaerobic Digestion of sewage sludge, technical university of hamburg-harburg, 1997, water science and technology, Vol. 36, No. 11, pp. 121-128, Germany

[16] J. Mata-Alvarez, S. Mace, P. Llabres, Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives, 2000, Bioresource Technology vol.74, p.p. 3±16, Spain

[17] E. Elbeshbishy, A. Nakevski, H. Hafez, M. Ray and G. Nakhla, Simulation of the Impact of SRT on Anaerobic Digestability of Ultrasonicated Hog Manure, 2010, Energies vol. 3, p.p. 974-988

[18] A . K. Mohamed, F. Alfarjani, K. Y. Benyounis, T. Prescott and A. G. Olabi, Application of Mechanical Pre-treatment to Produce Methane from Maize, 2011, ECOS 2011 conference, Nova Sad Serbia.

[19] C. Liu, X. Yuan, G. Zeng, W. Li, J. Li, Prediction of methane yield at optimum Ph for anaerobic digestion of organic fraction of municipal solid waste,2008, Bioresource Technology, Vol. 99, P.P 882–888

[20] A. J. Ward, P. J. Hobbs, P. J. Holliman, D. L. Jones, Optimisation of the anaerobic digestion of agricultural resources, 2008, Bioresource Technology, vol. 99, pp. 7928–7940

[21] http://physics.about.com/od/glossary/g/energy.htm. (accessed July 2010)

[22] http://www.eia.doe.gov/iea/overview.html, (accessed July 2010)

[23] IEA key world energy statistic, 2009, paris, cedex 15. www.iea.org (accessed July 2010)

[24] Connexions Project, University of California, non-renewable energy sources, 2009. http://cnx.org/content/m16730/1.2/ (accessed June 2010)

[25] http://www.sonic.net/~lilith/EnviraFuels/biokids/bk-energy.html, (accessed July 2010)

[26] http://www.pustakalaya.org/wiki/wp/o/Oil_refinery.htm , (accessed July 2010)

[27] D. Coley, Energy and Climate Change, 2008, centre for energy and the environment, University of Exeter, John Wiley & sons, Ltd

[28] Ristinen, R. A. and Kraushaar, J.J., Eneregy and the Environment, Wiley, 1999

[29] Bp plc, putting energy in the spotlight, BP, 2005.

[30] http://www.need.org/needpdf/infobook_activities/SecInfo/CoalS.pdf, (accessed July 2010)

[31] World Coal Institute, the coal resource: A Comprehensive Overview of Coal, 2009. http://www.worldcoal.org/search-results/index.php (accessed July 2010)

[32] BP, plc, BP statistical review of world energy, BP, 2004

[33] P. Davies, BP Statistical Review of World Energy, 2005, London

[34] http://www.darvill.clara.net/altenerg/nuclear.htm#intro (accessed August 2010)

[35] http://www.hko.gov.hk/education/dbcp/pow_stat/eng/r7.htm, (accessed August 2010)

[36] IEA renewable energy working party, Renewable Energy... into the Mainstream, 2002, Netherlands
http://www.iea.org/textbase/nppdf/free/2000/Renew_main2003.pdf

[37] World Energy Assessment (2001), energy and the challenge of sustainability,

http://www.undp.org/energy/activities/wea/drafts-frame.html

[38] http://greennature.com/article286.html (access August 2010).

[39] http://www.aglsolarenergy.com.au/what-is-solar/ (access August 2010).

[40] http://www.agl.com.au/home/solar-energy/Pages/default.aspx, (access August 2010)

[41] H. Stavroulia, socio-economic impacts of biomass deployment for the production of heat and electricity, 2003, thesis, University of Strathclyde.

[42] American Wind Energy Association, Wind Energy Teacher's Guide, 2003, Department of Energy, U.S.

[43] D. Dodge, illustrated history of wind power development, 2001, TellosNet Littleton, Colorado.

[44] G. Boyle, Renewable Energy- power for a sustainable future, 2004, Oxford, University Press, UK.

[45] S. Parravicini, World Wind Energy Report 2009, World Wind Energy Association WWEA, 2010, Istanbul, http://www.wwindea.org, (accessed November 2010)

[46] Z. Peker, integrating renewable energy technologies into cities through urban planning: in the case of geothermal and wind energy, 2005, thesis, Institute of Technology, izmir

[47] http://www.bwea.com/energy/myths.html (accessed November 2010)

[48] http://www.physics.uiowa.edu/~umallik/adventure/nov_06-04.htm

[49] H. Stavroulia. Pr.Umit Bititci, Socio-Economic Impacts of Biomass Deployment for the Production of Heat and Electricity, 2003**,** thesis, University of Strathclyde. http://www.esru.strath.ac.uk/Documents/MSc_2002/stavroulia.pdf

[50] http://www.cacefindiana.org/node/programs/cieep/global_warming/sound_solutions/geothermal_energy (accessed December 2010)

[51] F. O. Rourke, F. Boyle, A. Reynolds, Tidal energy update 2009, Applied Energy, 2010, Vol. 87, PP. 398–409

[52] V. N. gunaseelan, anaerobic digestion of biomass for methane production: a

review, 1997, Biomass and Biornergy, Vol. 13, pp. 83-114,

[53] M. R. Susta, P. Luby, S. B. Mat, Biomass Energy Utilization & Environment Protection - Commercial Reality and Outlook, 2003, POWER-CEN ASIA

[54] http://thoughtsonglobalwarming.blogspot.com/2007_02_01_archive.html (accessed November 2010)

[55] H. Stavroulia. Pr.Umit Bititci, Socio-Economic Impacts of Biomass Deployment for the Production of Heat and Electricity, 2003**,** thesis, University of Strathclyde. http://www.esru.strath.ac.uk/Documents/MSc_2002/stavroulia.pdf

[56] S. Siddharth, green energy-anaerobic digestion, 2006, heat transfer, thermal engineering and environment, conference, pp276- 280, Elounda, Greece.

[57] http://www.azocleantech.com/details.asp?ArticleID=34 (accessed November 2010)

[58] N. H. Ravindranath and D. O. Hall, Biomass Energy and Environment: a developing country perspective from India, 1995, Oxford University Press, Technology&Engineering. http://books.google.ie/books?id=ARms6L3xklEC&source=gbs_navlinks_s. .

[59] A. Gore, An Inconvenient Truth the crisis of global warming, (2007), published by (Viking).

[60] http://www.oregon.gov/energy/renew/Biomass/Environment.shtml (accessed September 2010)

[61] http://unfccc.int/kyoto_protocol/items/2830.php (accessed August 2010)

[62] http://www.esrl.noaa.gov/gmd/aggi/ (accessed November 2010)

[63] S. K. Soni, "Microbes: A Source of Energy for 21st Century", 2007, Department of Microbiology, Panjab University, published by New India Publishing Agency.

[64] Y. H. Hui, (2006), "Handbook of food science, technology, and engineering", 2006, Vol. 2, published by (Taylor and Francis).

[65] http://www.waste.nl/page/248 (accessed august 2010).

[66] The University of Adelaide Australia, [online], http://www.adelaide.edu.au/biogas, (accessed September 2010)

[67] Tietjen, C., (1975) "From Biodung to biogas-Historical Review of Eurpean

Experience", p- 274 in "Energy, Agriculture, and Waste Management"; Jewell W.J. editor, Ann Arbor Science Publishers

[68] Amon, T., (2007), "Biogas production from maize and dairy cattle manure – Influence of biomass composition on the methane yield, Agriculture, Ecosystems & Environment", Vol. 118, Issues 1-4, pp. 173-182.

[69] M. author, M. Gerber, R. Span, An Analysis of Available Mathematical Models for Anaerobic Digestion of Organic Substances for Production of Biogas, 2008, IGRC, Germany.
http://www.ruhr-uni-bochum.de/thermo/Forschung/pdf/IGRC_Full_Paper_Paris.pdf

[70] http://www.esru.strath.ac.uk/Documents/MSc_2002/stavroulia.pdf.(accessed November 2010)

[71] Epa, Strategic Policy Unit, Anaerobic Digestion: Benefits for Waste Management, Agriculture Energy and the Environment, 2005, Discussion Paper, http://www.epa.ie/downloads/consultation/epa_discussion_paper_anaerobic_ digestion.pdf

[72] C. Eliyan, anaerobic digestion of municipal solid waste in thermophilic continuous operation, 2007, thesis, Asian Institute of Technology, Thailand

[73] K. Ostrem. N. J. Themelis, Greening Waste: Anaerobic Digestion for Treating the Organic Fraction of Municipal Solid Wastes, 2004, thesis, Columbia University. http://www.seas.columbia.edu/earth/wtert/sofos/Ostrem_Thesis_final.pdf

[74] http://www.readigesters.com/digesterbasics.php (accessed August 2010)

[75] K. M. Ostrem, K. Millrath, and N. J. Themelis, Combining Anaerobic Digestion and Waste-To-Energy, 12th North American waste to energy conference, 2004, Columbia University. http://www.seas.columbia.edu/earth/wtert/sofos/ostrem-millrath- themelis_nawtec12_2004.pdf.

[76] P. Lens,P. Westermann, M. Haberbauer, and A. Moreno, "Biofuels for fuel cells renewable energy from biomass fermentation", 2007, IWA, London, Seattel.

[77] F. Monnet, an introduction to anaerobic digestion of organic wastes, 2003, http://www.biogasmax.co.uk/media/introanaerobicdigestion__073323000_1011_24042

07.pdf
[78] http://www.r-e-a.net/biofuels/biogas/anaerobic-digestion (accessed August 2010)
[79] H. Ge, P. D. Jensen, D. J. Batstone, Pre-treatment mechanisms during thermophilic–mesophilic temperature phased anaerobic digestion of primary sludge, 2010, water research, Vol. 44, pp.123 – 130.
[80] Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol. 96: 673-686. doi:10.1016/j.biortech.2004.06.025
[81] P. Kumar, D. M. Barrett, M. J. Delwiche, P. Stroeve, Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production, 2009, Ind. Eng. Chem. Res. , Vol(48), pp 3713–3729
[82] H. Carrère, C. Dumas, A. Battimelli, D.J. Batstone, J.P. Delgenès, J.P. Steyer, I. Ferrer, Pretreatment methods to improve sludge anaerobic degradability: A review, 2010, Journal of Hazardous Materials, Vol. 183, pp.1–15
[83] http://www.waste.nl/page/419 (accessed August 2010)
[84] S. K. Khanal, R. Y. Surampalli, T. C. Zhang, B. P. Lamsal, R. D. Tyagi, C. M. Kao, Bioenergy and Biofuel from Biowastes and Biomass, 2010, ASCE, USA
[85] G. Y. S. Mtui, Recent advances in pretreatment of lignocellulosic wastes and Production of value added products, 2009, African Journal of Biotechnology Vol. 8 ,No. 8, pp. 1398-1415, 20, Dar es Salaam.
[86] M. R. Salsabil, A. Prorot, M. Casellas, C. Dagot, Pre-treatment of activated sludge:Effect of sonication on aerobic and anaerobic digestibility, 2009, Chemical Engineering Journal, Vol. 148, pp. 327–335.
[87] S. Pilli, P. Bhunia, S. Yan, R.J. LeBlanc, R.D. Tyagi, R.Y. Surampalli, Ultrasonic pretreatment of sludge: A review, 2011, Ultrasonics Sonochemistry vol. 18, pp. 1–18
[88] Y. Yan, L. Feng, C. Zhang, H. Zhu, Q. Zhou, Effect of ultrasonic specific energy On waste activated sludge solubilization and enzyme activity, 2010, African

Journal of Biotechnology Vol. 9 (12), pp. 1776-1782, China.

[89] A. Elliott, T. Mahmood, Pretreatment technologies for advancing anaerobic digestion of pulp and paper biotreatment residues, 2007, water research vol. 41, pp. 4273–4286,Canada.

[90] K. S. B Kameswari, C. Kalyanaraman, K. Thanasekaran, 2010, Effect of ozonation and ultrasonication pretreatment processes on co-digestion of tannery solid wastes, Clean Technology Environment Policy, India.

[91] http://www.pcbdesign007.com/pages/zone.cgi?a=57265&_pf_=1

[92] M. J. Taherzadeh, K. Karimi, Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review, 2008, International Journal of Molecular Sciences, vol. 9, pp. 1621-1651

[93] Y. Zheng, Z. Pan, R. Zhang, Overview of biomass pretreatment for cellulosic Ethanol production, 2009, Agricultural and Biological Engineering, Vol. 2 No.3 pp.51-68, USA

[94] A.T.W.M. Hendriks, G. Zeeman, Pretreatments to enhance the digestibility of lignocellulosic biomass, 2009, Bioresource Technology, Vol. 100, pp. 10–18

[95] B. C. QI, C. Aldrich, L. Lorenzen, G. W. Wolfaardt, Acidogenic Fermentation of Lignocellulosic Substrate with Activated Sludge, 2005, Chemical Engineering Communications, vol. 192, pp. 1221–1242, South Africa

[96] C. L. Rai, J. Mueller, G. Struenkmann, P. G. Rao, Microbial growth reduction in sewage sludge by stirred ball mill disintegration and estimation by respirometry, 2008, Chemical Technology and Biotechnology, Vol. 83, pp. 269–278

[97] L. Kratky, T. Jirout, Biomass Size Reduction Machines for Enhancing Biogas Production, 2011, Chemical engineering technology, vol. 34, No. 3, p.p. 391–399

[98] H. J. Zhang, Sludge treatment to increase biogas production, 2010, Trita-LWR Degree Project 10-20.

[99] J. P. Delgenés, V. Penaud, R. Moletta, 2002. Pretreatments for the enhancement of anaerobic digestion of solid wastes Chapter 8. In: Biomethanization of the Organic Fraction of Municipal Solid Wastes. IWA Publishing, pp. 201–228.

[100] J. Geciova, D. Bury, P. Jelen, Methods for disruption of microbial cells for potential use in the dairy industryFa review, 2002, International Dairy Journal, Vol. 12, pp. 541–553, Canada.

[101] http://www.impact-crushers.com/Ball-Mill.html

[102] Y. Sun, J. Cheng, Hydrolysis of lignocellulosic materials for ethanol production: a review, 2002, Bioresource Technology, Vol. 83, pp. 1–11, USA

[103] K, Izumi, Y. Okishio, N. Nagao, C. Niwa, S. Yamamoto, T. Toda, Effects of Particle size on anaerobic digestion of food waste, 2010, International Biodeterioration & Biodegradation, Vol. 64, pp.601-608

[104] V.S. Chang, M.T. Holtzapple, Fundamental factors affecting enzymatic reactivity, 2000, Applied Biochemical and Biotechnology, Vol 84-86, pp 5–37.

[105] Z. Miaoa, T.E. Grift, A.C. Hansena, K.C. Tinga, Energy requirement for Comminution of biomass in relation to particle physical properties, 2011, Industrial Crops and Products, vol. 33, pp.504–513, USA

[106] U. Baier, P. Schmidheiny, Enhanced anaerobic degradation of mechanically disintegrated sludge, 1997, Water Sci. Technol, vol.36, no. 11,p.p. 137–143.

[107] L. Appels, J. Baeyens, J. Degre`ve, R. Dewil, Principles and potential of the Anaerobic digestion of waste-activated sludge, 2008, Progress in Energy and Combustion Science, Vol. 34, pp. 755–781

[108] T. I. Onyeche, Economic benefits of low pressure sludge homogenization for Wastewater treatment plants, CUTEC-Institut GmbH, Leibnizstr. 21+23, D-38678 Clausthal Zellerfeld, Germany.

[109] C. L. Rai, P. G. Rao, Influence of sludge disintegration by high pressure

Homogenizer on microbial growth in sewage sludge: an approach for excess sludge reduction, 2009, Clean Techn Environ Policy, vol. 11, p.p.437–446, India

[110] M. Engelhart, M. Kruger, J. Kopp, N. Dichtl, Effects of disintegration on Anaerobic degradation of sewage excess sludge in downflow stationary fixed film digesters, 2000, Water Science technology, Vol. 41, no(3), pp.171-9

[111] T. I. Onyeche, S. Schäfer, sludge homogenisation as a means to reduce sludge Volume and increase energy production, 2003, Electronic Journal of Environmental, Agricultural and Food Chemistry, Vol.2, no (2), pp. 291-296, Germany.

[112] I. W. Nah, Y. W. Kang, K. Y. Hwang, W. K. Song, Mechanical pretreatment of Waste activated sludge for anaerobic digestion process, 2000, Wat. Res. Vol. 34, No. 8, pp.2362±2368, South Korea

[113] J. Zabranska, M. Dohanyos, P. Jenicek, J. Kutil, Disintegration of excess Activated sludge – evaluation and experience of full-scale applications, 2006, Water Technology and Environmental Engineering, vol. 53, No (12), pp. 229–236.

[114] M. Dohanyos, J. Zabranska, P. Jenicek, Enhancement of sludge anaerobic Digestion by using of a special thickening centrifuge, 1997, Water Sci. Technol, Vol. 36, No (11), pp.145–153.

[115] J. Zabranska, M. Dohanyos, P. Jenicek, J. Kutil, 2000. Thermophilic process and enhancement of excess activated sludge degradability—two ways of intensification of sludge treatment in the Prague central wastewater treatment plant. Water Sci. Technol.Vol. 41, No (9), pp.265–272.

[116] A. K. Mohamed, F. Alfarjani, K. Y. Benyounis, T. Prescott and A. G. Olabi, Applicaton of Mechanical pre-treatment to produce Methane from Maize, 2011, ECOS conference, Nova Sad Serbia

[117] http://newsletter.handpapermaking.org/beginner/beg44.htm (access August 2011)

[118] http://www.forestprod.org/cdromdemo/pf/pf8.html (accessed August 2011).

[119] I. Siegert, C. Banks, The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors,2005, Process Biochemistry Vol, 40, pp. 3412–3418.

[120] D.A Stafford, B.I. Wheatley, "Anaerobic Digestion", 1979, published by Applied Science Publishers Ltd, London.

[121] P. Vindis, B. Mursec, M. Janzekovic, F. Cus, The impact of mesophilic and thermophilic anaerobic digestion on biogas production,2009, AMME journal of Achievements in Materials and Manufacturing Engineering, volume 36 issue 2.

[122] XU Da-yong, Effect of Temperature and Feedstock Size on Biogas Production of Water Hyacinth Used for Phytoremediation of Rural Domestic Wastewater in Shanghai, MACE international conference, 2010, Vol. 46, pp. 447-53, Shanghai.

[123] J. M. Alvarez, Biomethanization of the organic fraction of municipal solid wastes, 2003, IWA Publshing. ISBN: 1-900222-14-0.

[124] P. J. Meynell, Methane: Planning a digester, 1982, Prism Press. 2nd editon. ISBN: 0907061 14 1-X.

[125] H. Bouallagui, O. Haouari, Y. Touhami, R. Ben Cheikh, L. Marouani b, M. Hamdi, Effect of temperature on the performance of an anaerobic tubular reactor treating fruit and vegetable waste, 2003, Process Biochemistry, Vol. 39, pp. 2143–2148.

[126] D. Bolzonella, L. Innocenti, P. Pavan, P. Traverso, F. Cecchi, Semi-dry thermophilic anaerobic digestion of the organic fraction of municipal solid waste: focusing on the start-up phase, 2003, Bioresource technology, Vol. 86, pp. 123-129.

[127] Hobson, P.H & Wheatley, A.D., (1993), "Anaerobic digestion modern theory and practice", published by Elsevier Applied Science Publishers Ltd, London.

[128] S. Verma, N.J. Themelis, anaerobic digestion of biodegradable organics in municipal Solid wastes, 2002, thesis, Columbia University.

[129] A. J. Ward, P. J. Hobbs, P. J. Holliman, D. L. Jones, Optimisation of the anaerobic digestion of agricultural resources, 2008, Bioresource Technology, Vol. 99, pp. 7928–7940.

[130] J. Kim, C. Park, T. Kim, M. Lee, S. Kim, S. W. Kim, J. Lee, Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge, 2003, Journal of Bioscience and Bioengineering, Vol. 95, pp. 271–275.

[131] H.Q. Yu, H.H.P. Fang, Acidogenesis of dairy wastewater at various pH levels 2002, Water Science and Technology, Vol. 45, pp. 201–206.

[132] Q. Wang, M. Kuninobu, H. I. Ogawa, Y. Kato, Degradation of volatile fatty acids in highly efficient anaerobic digestion, 1999, Biomass and Bioenergy, Vol. 16, pp. 407±416.

[133] Y. Wang, Y. Zhang, J. Wang and L. Meng, Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria, 2009, Biomass and Biornergy Vol 33, pp. 848-853.

[134] A.C. Palmisano and M.A. Barlaz, Microbiology of solid waste. In: R.H. Vreeland, Editor, The microbiology of extreme and unusual environments, 1971, CRC Press, London, pp. 72–104

[135] University of Florida, [online],http://biogas.ifas.ufl.edu/uses.asp, (accessed September 2010).

[136] D.R. Boone and L.Y. Xun, Effects of pH, temperature, and nutrients on propionate degradation by a methanogenic enrichment culture, 1987, Applied and Environmental Microbiology, Vol. 53, pp. 1589–1592.

[137] P.C. Pullammanappallil, D.P. Chynoweth, G. Lyberatos and S.A. Svoronos, Stable performance of anaerobic digestion in the presence of a high concentration of propionic acid, 2001, Bioresource Technology, Vol. 78, pp. 165–169.

[138] N.Q. Ren, M. Liu, A.J. Wang, J. Ding and M. Hong, Organic acids conversion in methanogenic- phase reactor of the two phase anaerobic process, 2003, Environmental Science, Vol. 24, pp. 89–93.

[139] N.Q. Ren, A.J. Wang and F. Ma, Acid-producing fermentative microbe

physiological ecology, 2005, Science Press, Beijing.

[140] K. Fricke, H. Santen, R. Wallmann, A. Huttner, N. Dichtl, Operating problems in anaerobic digestion plants resulting from nitrogen in MSW, 2007, Waste Management, Vol. 27, pp. 30–43

[141] biogas process sustainable development, FAO Corporate Document Repository, http://www.fao.org/docrep/t0541e/t0541e06.htm

[142] United-Tech, Inc. Science and technology for a better environment, BZT Waste Digester, (2006) http://www.united-tech.com/wd- anaerobicdigestion.html (accessed 0ctober 2010).

[143] M. Ras, L. Lardon, S. Bruno, N. Bernet, J. Steyer, Experimental study on a coupled process of production and anaerobic digestion of Chlorella vulgaris, 2010, Bioresource Technology, in press.

[144] Y. Chen, J. J. Cheng, K. S. Creamer, Inhibition of anaerobic digestion process: A review,2008, Bioresource Technology, Vol. 99, pp.4044– 4064.

[145] A. Demirbas, Biofuels: securing the plant's future energy needs, 2009, springer-Verlag London Limited.

[146] K. Karim, R. Hoffmann, K. T. Klasson, M.H. Al-Dahhan, Anaerobic digestion of animal waste: Effect of mode of mixing, 2005, Water Research, Vol.39 , pp.3597–3606

[147] T. Al Seadi, D. Rutz, H. Prassl, M. Köttner, T. Finsterwalder, S. Volk, R. Janssen, Biogas handbook, 2008, University of Southern Denmark Esbjerg

[148] A. J. Ward, P. J. Hobbs, P. J. Holliman, D. L. Jones, Optimisation of the Anaerobic digestion of agricultural resources, 2008, Bioresource Technology, vol. 99, p.p. 7928–7940, UK.

[149] Mata-Alvarez, J., 2002. Biomethanization of the Organic Fraction of Municipal Solid Wastes. IWA Publishing.

[150] L. Sahlstrom, A review of survival of pathogenic bacteria in organic waste used in biogas plants, 2003, Bioresource Technology, vol, 87, p.p. 161–166.

[151] B. Demirel, P. Scherer, Production of methane from sugar beet silage without manure addition by a single-stage anaerobic digestion process, 2008, Biomass Bioenergy, Vol(32), pp. 203–209, Germany

[152] A. S. Nizami, J. D. Murphy, What type of digester configurations should be employed to produce biomethane from grass silage?, 2010, Renewable and Sustainable Energy Reviews, Vol(14), pp 1558–1568, Ireland

[153] S. Xie, J.P. Frost, P.G. Lawlor, G. Wu, X. Zhan, Effects of thermo-chemical pre-treatment of grass silage on methane production by anaerobic digestion, 2011, Bioresource Technology, Vol(102), pp. 8748-8755

[154] J.B. Holm-Nielsen, T. Al Seadi, P. Oleskowicz-Popiel, The future of anaerobic digestion and biogas utilization, 2009, Bioresource Technology vol 100, pp. 5478–5484

[155] http://www.renewables-made-in-germany.com/index.php?id=154 (accessed November 2010)

[156] http://www.iea- biogas.net/Dokumente/energycrop_def_Low_Res.pdf (accessed December 2010).

[157] European Commission, Eurostat, [online], http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home (accessed December 2010).

[158] C. Gatlin, Petroleum Engineering Drilling and well completions, 1960, Department of petroleum engineering, The University of Texas

[159] G. E. P. Box and K. B. Wilson, On the experimental attainment of optimum conditions, J. of the Royal Statistical Society B13, 1951, pp. 1- 45.

[160] D. C. Montgomery, Design and Analysis of Experiments. 2nd Edition, John Wiley & Sons, New York, 1984.

[161] A. I. Khuri and J. A. Cornell, Response Surfaces Design and Analysis. 2nd Edition, Marcel Dekker, New York, 1996.

[162] Design-Expert software, V7, user's guide, Technical manual, Stat-Ease Inc., Minneapolis, MN, 2005.

[163] http://www.itl.nist.gov/div898/handbook/pri/section3/pri3361.htm, (accessed July, 2010.

[164] S. Chatterjee and B. Price, Regression analysis by example, 2nd Ed., Wiley and Sons, Inc., 1977, pp. 200-202.

[165] R. H. MYERS AND D.C. Montgomery, Response surface methodology- process and product optimization using designed experiment, John Wiley & Sons, 1995.

[166] K. Hopfner-Sixt, T. Amon, Monitoring of agricultural biogas plants in Austria – Mixing technology and specific values of essential process parameters, 2007, In: Proceedings of the 15th European Biomass Conference and Exhibition, Berlin, pp. 1718–1728.

[167] P. Weiland, Biomass digestion in agriculture: a successful pathway for the energy production and waste treatment in Germany, 2006, Engineering in Life Sciences, Vol 6, pp.302–309.

[168]http://www.ucd.ie/campusdevelopment/developmentprojects/otherdevelopments/lyonsfarm/

[169] A.S. Nizami, A. Orozco, E. Groom, B. Dieterich, J.D. Murphy, How much gas can we get from grass?, 2011, Applied energy, in press, Ireland

[170] Yadvika, Santosh, T.R. Sreekrishnan, S. Kohli, V. Rana, Enhancement of biogas production from solid substrates using different techniques—a review, 2004, Bioresource Technology, in press, India

[171] Z –H. Hu, H-Q. Yu, R-F. Zhu, Influence of particle size and pH on anaerobic degradation of cellulose by ruminal microbes, 2005, International Biodeterioration &Biodegradation 55, 233–238, china.

[172] K. Y. Benyounis, A.G. Olabi, M. S. J. Hashmi, Multi-response optimization of CO_2 laser-welding process of austenitic stainless steel, J. of Optics & Laser Technology, Vol. 40, No. 1, 2008, pp. 76-87.

[173] A.G. Olabi, G. Casalino, K. Y. Benyounis and M. S. J. Hashmi, An ANN and Taguchi algorithms integrated approach to the optimization of CO_2 laser welding, Advances in Engineering Software, Vol. 37, No. 10, 2006, pp. 643-648.

LIST OF PUBLICATIONS

Conference's papers

1. F. A. Alfarjani, A. K. Mohamed, K. Y. Benyounis, A. G. Olabi, Mechanical Pre treatment to Enhance Anaerobic Digestion Process: Overview, 2011, ECOS conference, Nova Sad, Serbia.

2. A. K. Mohamed, F. Alfarjani, K. Y. Benyounis, T. Prescott and A. G. Olabi, Applicaton of Mechanical pre-treatment to produce Methane from Maize, 2011, ECOS conference, Nova Sad, Serbia.

3. F. A. Alfarjani, A. K. Mohamed, K. Y. Benyounis, A. G. Olabi, Modeling anaerobic process for grass silage after beating treatment using DOE, 2012, EPOL International Conference on Energy and Politics, Antalya, Turkey. (paper accepted to be publish on April 2012)

Paper under preparation

1. F. A. Alfarjani, A. K. Mohamed, K. Y. Benyounis and A. G. Olabi, Predication and optimization of biogas production from grass-silage, for publication in journal of Bioresource Technology Dec. 2011.

Appendixes

Appendix A

This Appendix contains the relevant equations which have been used to calculate volume of biogas

Volume of biogas for each sample (***Y***):

$$Y = \sum_{i=1}^{n} Xi$$

Where:

Xi= volume reading deference (VRD)

n = number of reading

VRD = Final Volume Reading (FVR) – Initial Volume Reading (IVR)

Volume of biogas for each condition:

$$\bar{Y} = \frac{\sum_{i=1}^{n} Yi}{n}$$

Where:

n = number of samples

Yi = volume of biogas for each sample

Appendix B

Volume of the biogas produced of first collection:

Reading No.	sample 1A			sample 1B			sample 1C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	252	162	92	250	158	
3	92	250	158	90	250	160	90	250	160	
4	90	250	160	90	250	160	90	250	160	
5	90	250	160	90	152	62	90	250	160	
6	90	134	44			0	92	168	76	
			0			0			0	
Sum			842			704			874	806.67

Reading No.	sample 2A			sample 2B			sample 2C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	250	160	90	250	160	90	250	160	
5	90	250	160	90	250	160	90	254	164	
6	90	250	160	90	250	160	90	250	160	
7	90	162	72	90	114	24	90	120	30	
Sum			1032			984			994	1003.3

Reading No.	sample 3A			sample 3B			sample 3C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	254	164	88	250	162	92	250	158	
2	90	252	162	90	250	160	90	254	164	
3	90	250	160	90	250	160	90	250	160	
4	90	250	160	90	250	160	90	252	162	
5	90	250	160	90	250	160	90	250	160	
6	90	252	162	90	250	160	90	252	162	
7	90	250	160	90	250	160	90	252	162	
8	90	200	110	90	194	104	90	218	128	
sum			1238			1226			1256	1240

Reading No.	sample 4A			sample 4B			sample 4C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	250	160	90	250	160	90	250	160	
5	90	250	160	90	250	160	90	250	160	
6	90	250	160	90	250	160	90	250	160	
7	90	250	160	90	250	160	90	250	160	
8	90	250	160	90	250	160	90	232	142	
9	90	200	110	90	202	112				
Sum			1390			1392			1262	1348

Reading No.	sample 5A			sample 5B			sample 5C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	252	162	92	250	158	94	250	156	
2	90	252	162	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	250	160	90	252	162	90	250	160	
5	90	250	160	90	252	162	90	250	160	
6	90	254	164	90	250	160	90	250	160	
7	90	244	154	92	250	158	90	250	160	
8				90	152	62	90	202	112	
Sum			1122			1182			1228	1177.33

Reading No.	sample 6A			sample 6B			sample 6C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	250	160	90	250	160	90	250	160	
5	90	250	160	90	250	160	90	250	160	
6	90	250	160	90	250	160	90	250	160	
7	90	262	172	90	250	160	90	250	160	
8	90	196	106	90	134	44	90	162	72	
Sum			1238			1164			1192	1198

Reading No.	sample 7A			sample 7B			sample 7C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	252	162	90	252	162	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	252	162	90	250	160	
4	90	250	160	92	250	158	90	250	160	
5	90	190	100	90	206	116	90	206	116	
Sum			742			758			756	752

Reading No.	sample 8A			sample 8B			sample 8C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	250	160	90	250	160	90	250	160	
5	90	250	160	90	250	160	90	250	160	
6	90	250	160	90	250	160	90	250	160	
Sum			960			958			962	960

Reading No.	sample 9A			sample 9B			sample 9C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	88	250	162	90	250	160	90	250	160	
4	88	250	162	90	250	160	90	250	160	
5	90	250	160	90	250	160	90	254	164	
6	90	250	160	90	250	160	90	260	170	
7	90	140	50	90	136	46	90	132	42	
Sum			1014			1006			1016	1012

Reading No.	sample 10A			sample 10B			sample 10C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	264	174	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	250	160	90	250	160	90	250	160	
5	90	250	160	90	250	160	90	250	160	
6	90	242	152	90	250	160	90	250	160	
7				90	166	76	90	154	64	
Sum			966			1036			1024	1008.66

Reading No.	sample 11A			sample 11B			sample 11C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	250	160	90	250	160	90	250	160	
5	90	250	160	90	250	160	90	250	160	
6	90	250	160	90	250	160	90	250	160	
7	90	166	76	90	162	72	90	160	70	
Sum			1036			1032			1030	1032.66

Reading No.	sample 12A			sample 12B			sample 12C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	250	160	90	250	160	90	250	160	
5	90	250	160	90	250	160	90	250	160	
6	90	250	160	90	250	160	90	250	160	
7	90	182	92	90	200	110	90	220	130	
Sum			1052			1070			1090	1070.66

Volume of the biogas produced of second collection:

Reading No.	sample 1A			sample 1B			sample 1C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	252	162	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	150	60	90	134	44	90	250	160	
5			0	90	100	10	90	146	56	
Sum			540			534			698	590.66

Reading No.	sample 2A			sample 2B			sample 2C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	96	250	154	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	244	154	90	242	152	90	210	120	
4			0			0	90	114	24	
Sum			468			472			464	468

Reading No.	sample 3A			sample 3B			sample 3C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	230	140	90	196	106	90	208	118	
Sum			460			426			438	441.3333

Reading No.	sample 4A			sample 4B			sample 4C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	252	162	90	250	160	90	250	160	
3	90	220	130	90	226	136	90	182	92	
4			0			0	90	114	24	
Sum			452			456			436	448

Reading No.	sample 5A			sample 5B			sample 5C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	236	146	90	228	138	90	240	150	
sum			466			458			470	464.66

Reading No.	sample 6A			sample 6B			sample 6C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	238	148	90	244	154	
4	90	130	40			0			0	
sum			520			468			474	487.33

Reading No.	sample 7A			sample 7B			sample 7C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	146	56	90	152	62	90	150	60	
sum			376			382			380	379.3333

Reading No.	sample 8A			sample 8B			sample 8C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	156	66	90	152	62	90	148	58	
sum			386			382			378	382

Reading No.	sample 9A			sample 9B			sample 9C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	244	154	90	246	156	
3	90	150	60	90	118	28	90	116	26	
sum			380			342			342	354.66

Reading No.	sample 10A			sample 10B			sample 10C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	244	154	90	250	160	
3	90	116	26	90	112	22	90	118	28	
sum			346			336			348	343.33

Reading No.	sample 11A			sample 11B			sample 11C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	144	54	90	134	44	90	140	50	
sum			374			364			370	369.3333

Reading No.	sample 12A			sample 12B			sample 12C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	124	34	90	128	38	90	120	30	
sum			354			358			350	354

Volume of the biogas produced of third collection:

Reading No.	sample 1A			sample 1B			sample 1C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	148	58	90	120	30	90	192	102	
sum			218			190			262	223.33

Reading No.	sample 2A			Sample 2B			sample 2C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	206	116	90	192	102	90	164	74	
sum			276			262			234	257.33

Reading No.	sample 3A			sample 3B			sample 3C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	204	114	90	196	106	90	202	112	
sum			274			266			272	270.66

Reading No.	sample 4A			sample 4B			sample 4C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	252	162	90	250	160	90	254	164	
2	90	250	160	90	250	160	90	242	152	
3	90	142	52	90	156	66			0	
sum			374			386			316	358.66

Reading No.	sample 5A			sample 5B			sample 5C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	220	130	90	196	106	90	194	104	
sum			290			266			264	273.33

Reading No.	sample 6A			sample 6B			sample 6C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	142	52	90	138	48	90	140	50	
sum			372			368			370	370

Reading No.	sample 7A			sample 7B			sample 7C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	252	162	90	250	160	
2	90	126	36	90	124	34	90	122	32	
sum			196			196			192	194.66

Reading No.	sample 8A			sample 8B			sample 8C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	204	114	90	202	112	90	232	142	
sum			274			272			302	282.66

Reading No.	sample 9A			sample 9B			sample 9C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	254	164	90	250	160	
2	90	204	114	90	182	92	90	188	98	
sum			274			256			258	262.66

Reading No.	sample 10A			sample10B			sample 10C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	180	90	90	180	90	90	182	92	
sum			250			250			252	250.66

Reading No.	sample 11A			sample 11B			sample 11C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	214	124	90	206	116	90	210	120	
sum			284			276			280	280

Reading No.	sample 12A			sample 12B			sample 12C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	212	122	90	208	118	90	208	118	
sum			282			278			278	279.33

Volume of the biogas produced of fourth collection:

Reading No.	sample 1A			sample 1B			sample 1C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	212	122	90	234	144	90	234	144	
sum			122			144			144	136.66

Reading No.	sample 2A			sample 2B			sample 2C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	254	164	90	250	160	90	250	160	
2	90	166	76	90	178	88	90	170	80	
sum			240			248			240	242.66

Reading No.	sample 3A			sample 3B			sample 3C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	240	150	90	250	160	90	240	150	
2	90	110	20	90	120	30	90	110	20	
sum			170			190			170	176.66

Reading No.	sample 4A			sample 4B			sample 4C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	134	44	90	134	44	90	130	40	
sum			204			204			200	202.66

Reading No.	sample 5A			sample 5B			sample 5C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	254	164	
2	90	120	30	90	120	30	90	136	46	
sum			190			190			210	196.66

Reading No.	sample 6A			sample 6B			sample 6C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	252	162	90	296	206	90	250	160	
2	90	204	114	90	134	44	90	190	100	
sum			276			250			260	262

Reading No.	sample 7A			sample 7B			sample 7C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	238	148	90	240	150	90	240	150	
sum			148			150			150	149.33

Reading No.	sample 8A			sample 8B			sample 8C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	240	150	90	244	154	90	250	160	
sum			150			154			160	154.66

Reading No.	sample 9A			sample 9B			sample 9C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	262	172	90	252	162	90	252	162	
sum			172			162			162	165.33

Reading No.	sample 10A			sample 10B			sample 10C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	254	164	90	252	162	90	252	162	
sum			164			162			162	162.66

Reading No.	sample 11A			sample 11B			sample 11C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	248	158	90	250	160	90	254	164	
sum			158			160			164	160.66

Reading No.	sample 12A			sample 12B			sample 12C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	252	162	90	246	156	
sum			160			162			156	159.33

Volume of the biogas produced of fifth collection:

Reading No.	sample 1A			sample 1B			sample 1C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	238	148	90	250	160	90	234	144	
2			0	90	114	24			0	
sum			148			184			144	158.66

Reading No.	sample 2A			sample 2B			sample 2C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	138	48	90	128	38	90	130	40	
sum			208			198			200	202

Reading No.	sample 3A			sample 3B			sample 3C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	212	122	90	232	142	90	214	124	
sum			122			142			124	129.33

Reading No.	sample 4A			sample 4B			sample 4C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	194	104	90	192	102	90	204	114	
sum			104			102			114	106.66

Reading No.	sample 5A			sample 5B			sample 5C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	194	104	90	184	94	90	188	98	
sum			104			94			98	98.66

Reading No.	sample 6A			sample 6B			sample 6C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	190	100	90	180	90	90	184	94	
sum			100			90			94	94.66

Reading No.	sample 7A			sample 7B			sample 7C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	230	140	90	234	144	90	232	142	
sum			140			144			142	142

Reading No.	sample 8A			sample 8B			sample 8C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	200	110	90	208	118	90	186	96	
sum			110			118			96	108

Reading No.	sample 9A			sample 9B			sample 9C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	178	88	90	164	74	90	168	78	
sum			88			74			78	80

Reading No.	sample10A			sample10B			sample10C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	160	70	90	152	62	90	158	68	
sum			70			62			68	66.66

Reading No.	sample 11A			sample 11B			sample 11C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	176	86	90	188	98	90	182	92	
sum			86			98			92	86

Reading No.	sample 12A			sample 12B			sample 12C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	180	90	90	178	88	90	182	92	
sum			90			88			92	90

Volume of the biogas produced of sixth collection:

Reading No.	sample 1A			sample 1B			sample 1C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	192	102	90	192	102	90	204	114	192	
sum			102			102			114	106

Reading No.	Sample 2A			sample 2B			sample 2C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	208	118	90	200	110	90	190	100	
sum			118			110			100	109.33

Reading No.	sample 3A			sample 3B			sample 3C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	162	72	90	170	80	90	156	66	
sum			72			80			66	72.66

Reading No.	sample 4A			sample 4B			sample 4C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	164	74	90	180	90	90	180	90	
sum			74			90			90	84.66

Reading No.	sample 5A			sample 5B			sample 5C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	190	100	90	200	110	90	182	92	
sum			100			110			92	100.66

Reading No.	sample 6A			sample 6B			sample 6C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	176	86	90	168	78	90	170	80	
sum			86			78			80	81.33

Reading No.	sample 7A			sample 7B			sample 7C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	176	86	90	172	82	90	180	90	
sum			86			82			90	86
Reading No.	sample 8A			sample 8B			sample 8C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	170	80	90	150	60	90	150	60	
sum			80			60			60	66.66

Reading No.	sample 9A			sample 9B			sample 9C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	170	80	90	156	66	90	146	56	
sum			80			66			56	67.33

Reading No.	sample 10A			sample 10B			sample 10C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	150	60	90	164	74	90	158	68	
sum			60			74			68	67.33

Reading No.	sample 11A			sample 11B			sample 11C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	166	76	90	164	74	90	164	74	
sum			76			74			74	74.66

Reading No.	sample 12A			sample 12B			sample 12C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	160	70	90	164	74	90	158	68	
sum			70			74			68	70.66

Volume of the biogas produced of seven collection:

Reading No.	sample 1A			sample 1B			sample 1C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	174	84	90	188	98	90	178	88	
sum			84			98			88	90

Reading No.	sample 2A			sample 2B			sample 2C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	88	176	88	90	190	100	90	164	74	
sum			88			100			74	87.33

Reading No.	sample 3A			sample 3B			sample 3C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	146	56	90	148	58	90	158	68	
sum			56			58			68	60.66

Reading No.	sample 4A			sample 4B			sample 4C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	156	66	90	148	58	90	150	60	
sum			66			58			60	61.33

Reading No.	sample 5A			sample 5B			sample 5C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	168	78	90	178	88	90	154	64	
sum			78			88			64	76.66

Reading No.	sample 6A			sample 6B			sample 6C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	156	66	90	158	68	90	154	64	
sum			66			68			64	66

Reading No.	sample 7A			sample 7B			sample 7C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	154	64	90	154	64	90	156	66	
sum			64			64			66	64.66

Reading No.	sample 8A			Sample8B			sample 8C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	154	64	90	150	60	90	142	52	
sum			64			60			52	58.66

Reading No.	sample 9A			sample 9B			sample 9C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	170	80	90	150	60	90	152	62	
sum			80			60			62	67.33

Reading No.	sample 10A			sample 10B			sample 10C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	154	64	90	150	60	90	158	68	
sum			64			60			68	64

Reading No.	sample 11A			sample 11B			sample 11C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	150	60	90	158	68	90	152	62	
sum			60			68			62	63.33

Reading No.	sample 12A			sample 12B			sample 12C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	160	70	90	154	64	90	152	62	
sum			70			64			62	65.33

Volume of biogas produced for seven collections:

sample No.	First collection	Second collection	Third collection	Fourth collection	Fifth collection	Sixth collection	Seven collection
1	806.67	590.67	223.33	136.67	158.67	106.00	90.00
2	1003.33	468.00	257.33	242.67	202.00	109.33	87.33
3	1240.00	441.33	270.67	176.67	129.33	72.67	60.67
4	1348.00	448.00	358.67	202.67	106.67	84.67	61.33
5	1177.33	464.67	273.33	196.67	98.67	100.67	76.67
6	1198.00	487.33	370.00	262.00	94.67	81.33	66.00
7	752.00	379.33	194.67	149.33	142.00	86.00	64.67
8	960.00	382.00	282.67	154.67	108.00	66.67	58.67
9	1012.00	354.67	262.67	165.33	80.00	67.33	67.33
10	1008.67	343.33	250.67	162.67	66.67	67.33	64.00
11	1032.67	369.33	280.00	160.67	92.00	74.67	63.33
12	1070.67	354.00	279.33	159.33	90.00	70.67	65.33

Volume of biogas adjusted for 3g dry solid.

sample No.	First collection	Second collection	Third collection	Fourth collection	Fifth collection	Sixth collection	Seven collection
1	1021.1	747.7	282.7	173	200.8	134.2	113.9
2	1048.78	489.2	269	253.7	211.1	114.3	91.3
3	1569.6	558.6	342.6	223.6	163.7	92	76.8
4	1409.1	468.3	375	211.9	111.5	88.5	64.1
5	1490.3	588.2	346	249	124.9	127.4	97.1
6	1252.3	509.4	386.8	273.9	98.9	85	69
7	1111.3	540	287.7	220.7	209.8	127.1	95.6
8	1418.7	564.5	417.7	228.6	159.6	98.5	86.7
9	1394.1	524.1	388.2	244.3	118.2	99.5	99.5
10	1490.6	507.4	370.4	240.4	98.5	99.5	94.6
11	1526.1	545.8	413.8	237.4	135.9	110.3	93.6
12	1582.3	523.2	412.8	235.5	133	104.4	96.5

Appendix C

The appendix C contents data of confirmation experiments.

First criteria (1.62 beating time and 37.3 °C).

Calculation for determining the volume of biogas produced from First collection.

Reading No.	sample A			sample B			sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	250	160	90	250	160	90	250	160	
5	90	250	160	90	250	160	90	250	160	
6	90	250	160	90	250	160	90	250	160	
7	90	250	160	90	250	160	90	250	160	
8	90	170	80	90	158	68	90	180	90	
sum			1200			1188			1210	1199.3

Calculation for determining the volume of biogas produced from second collection.

Reading No.	Sample A			Sample B			Sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	200	110	90	194	104	90	202	112	
sum			590			584			592	588.66

Calculation for determining the volume of biogas produced from third collection.

Reading No.	sample A			sample B			sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	230	140	90	250	160	90	220	130	
sum			300			320			290	303.33

Calculation for determining the volume of biogas produced from fourth collection.

Reading No.	sample A			sample B			sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	130	40	90	132	42	90	134	44	
sum			200			202			204	202

Calculation for determining the volume of biogas produced from fifth collection.

Reading No.	Sample A			Sample B			Sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	228	138	90	218	128	90	220	130	
sum			138			128			130	132

Calculation for determining the volume of biogas produced from sixth collection.

Reading No.	Sample A			Sample B			Sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	174	84	90	178	88	90	170	80	
sum			84			88			80	84

Calculation for determining the volume of biogas produced from seventh collection.

Reading No.	Sample A			Sample B			Sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	158	68	90	164	74	90	163	73	
sum			68			74			73	71.66

Volume of biogas produced for seven collections:

sample No.	First collection	Second collection	Third collection	Fourth collection	Fifth collection	Sixth collection	Seven collection
1	1199.3	588.66	303.33	202	132	84	71.66

Volume of biogas adjusted for 3g dry solid.

sample No.	First collection	Second collection	Third collection	Fourth collection	Fifth collection	Sixth collection	Seven collection
1	1439.16	706.4	364	242.4	158.4	100.8	86

Cumulative of volume of biogas produced according to first criteria.

sample No.	First collection	Second collection	Third collection	Fourth collection	Fifth collection	Sixth collection	Seven collection
1	1439.16	2145.56	2509.56	2751.96	2910.36	3011.16	3097.16

Second Criteria (0 beating time and 38 °C).

Calculation for determining the volume of biogas produced from First collection.

Reading No.	sample A			sample B			sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	252	162	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	250	160	90	250	160	90	250	160	
5	90	250	160	90	250	160	90	250	160	
6	90	250	160	90	250	160	90	250	160	
7	90	240	150	90	250	160	90	250	160	
8			0	90	114	24	90	166	76	
sum			1112			1144			1196	1150.66

Calculation for determining the volume of biogas produced from second collection.

Reading No.	Sample A			sample B			sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	252	162	90	250	160	90	250	160	
3	90	250	160	90	242	152	90	250	160	
4	90	178	88			0	90	226	136	
sum			570			472			616	552.66

Calculation for determining the volume of biogas produced from third collection.

Reading No.	sample A			sample B			sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	252	162	90	250	160	90	250	160	
2	90	210	120	90	196	106	90	204	114	
sum			282			266			274	274

Calculation for determining the volume of biogas produced from fourth collection.

Reading No.	Sample A			Sample B			Sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	252	162	90	252	162	90	246	156	
sum			162			162			156	160

Calculation for determining the volume of biogas produced from fifth collection.

Reading No.	Sample A			Sample B			Sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	226	136	90	204	114	90	208	118	
sum			136			114			118	122.66

Calculation for determining the volume of biogas produced from sixth collection.

Reading No.	Sample A			Sample B			Sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	180	90	90	184	94	90	188	98	
sum			90			94			98	94

Calculation for determining the volume of biogas produced from seventh collection.

Reading No.	Sample A			Sample B			Sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	180	90	90	180	90	90	164	74	
sum			90			90			74	84.66

Volume of biogas produced for seven collections:

sample No.	First collection	Second collection	Third collection	Fourth collection	Fifth collection	Sixth collection	Seven collection
1	1150.67	552.67	274	160	122.66	94	84.66

Volume of biogas adjusted for 3g dry solid.

sample No.	First collection	Second collection	Third collection	Fourth collection	Fifth collection	Sixth collection	Seven collection
1	1380.84	663.2	328.8	192	147.2	112.8	101.6

Cumulative of volume of biogas produced according to second criteria.

sample No.	First collection	Second collection	Third collection	Fourth collection	Fifth collection	Sixth collection	Seven collection
1	1380.84	2044.04	2372.84	2564.84	2712.04	2824.84	2926.44

Third Criteria (2.73 beating time and 37.3 °C).

Calculation for determining the volume of biogas produced from first collection.

Reading No.	sample A			sample B			sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	250	160	90	250	160	
4	90	250	160	90	252	162	90	250	160	
5	90	250	160	90	250	160	90	250	160	
6	90	250	160	90	250	160	90	250	160	
7	90	250	160	90	250	160	90	250	160	
8	90	250	160	90	248	158	90	250	160	
sum			1280			1280			1280	1280

Calculation for determining the volume of biogas produced from second collection.

Reading No.	Sample A			Sample B			Sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	250	160	90	250	160	90	250	160	
3	90	250	160	90	254	164	90	250	160	
4	90	252	162	90	246	156	90	248	158	
sum			642			640			638	640

Calculation for determining the volume of biogas produced from third collection.

Reading No.	sample A			sample B			sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	250	160	90	250	160	
2	90	252	162	90	248	158	90	250	160	
sum			322			318			320	320

Calculation for determining the volume of biogas produced from fourth collection.

Reading No.	sample A			sample B			sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	250	160	90	252	162	90	250	160	
2	90	170	80	90	166	76	90	170	80	
sum			240			238			240	239

Calculation for determining the volume of biogas produced from fifth collection.

Reading No.	Sample A			Sample B			Sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	226	136	90	220	130	90	223	133	
sum			136			130			133	133

Calculation for determining the volume of biogas produced from sixth collection.

Reading No.	Sample A			Sample B			Sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	184	94	90	184	94	90	186	96	
sum			94			94			96	94.66

Calculation for determining the volume of biogas produced from seventh collection.

Reading No.	Sample A			Sample B			Sample C			*Y*
	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	IVR (ml)	FVR (ml)	VRD (ml)	
1	90	168	78	90	168	78	90	168	78	
sum			78			78			78	78

Volume of biogas produced for seven collections:

sample No.	First collection	Second collection	Third collection	Fourth collection	Fifth collection	Sixth collection	Seven collection
1	1280	640	320	239	133	94.66	78

Volume of biogas adjusted for 3g dry solid.

sample No.	First collection	Second collection	Third collection	Fourth collection	Fifth collection	Sixth collection	Seven collection
1	1567.34	783.67	391.83	292.65	162.8	115.9	95.5

Cumulative of volume of biogas produced according to third criteria.

sample No.	First collection	Second collection	Third collection	Fourth collection	Fifth collection	Sixth collection	Seven collection
1	1567.34	2351	2742.83	3035.48	3198.28	3314.18	3409.68