



Sudan University of Science and Technology

College of Engineering

Biomedical Engineering Department



Production of Biogas From Biomedical Waste (Blood)

A Research Submitted In Partial fulfillment for the Requirements of the
Degree of B.Sc. (Honors) in Biomedical Engineering

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الآية

قال تعالى:

بسم الله الرحمن الرحيم

﴿ وَيَسْأَلُونَكَ عَنِ الرُّوحِ ^{صُلِّ} قُلِ الرُّوحُ مِنْ
أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا ﴾

صدق الله العظيم

سورة الإسراء الآية (85)

DEDICATION

We humbly dedicate this effort to:

Our parents for enriching our lives with wisdom, knowledge; care for others and passion to make change.

Our teachers those humble but graceful individuals that thankfully made us what we are now.

Our best friends for the support when things were up and mostly when there were down.

Finally, to everyone who stood by us; and helped by any means to bring this work to its final form.

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LIST OF ABBREVIATIONS

Abb.	Stands for
WHO	World Health Organization
SOPs	Standard Operation Procedure
Rh D	Rhesus D
PH	Potential of Hydrogen
CH₄	Methane
H₂O	Water
N₂	Nitrogen
H₂	Hydrogen
MNEs	Multinationa Enterprises
CaO	Milk of Lime
Na₂CO₃	Sodium Carbonate
C/N Ratio	Carbon to Nitrogen Ratio
CSTR	Continuous Stirred Tank Reactor
C/P Ratio	Carbon to Phosphate Ratio
FPU	Floating Point Unit
H₂SO₄	Sulfuric Acid
GC	Gas Chromatography
ASBR	Anaerobic Sequencing Batch Reactor
MOH	Ministry Of Health
BPL	Blood Plate Let
MOP	Ministry Of Petroleum
CHNS	Carbon, Hydrogen, Nitrogen and Sulphur
WR	Weight of Dry Sample
WD	Water Displacement
UMST	University of Medical Science and Technology
QAV	Quantity Average
BMW	Biomedical Waste

AIDS	Acquired Immune Deficiency Syndrome
NRMW	Nonregulated Medical Waste
ABO	Blood Group System
PPE	Personal Protective Equipment
pKa	Professional Karate Association
CO₂	Carbon Dioxide
H₂S	Sophie
NH₃	Ammoniac
HCO₃	Bicarbonate
KVIC	Khadi and Village Industries Commission
Ca(OH)₂	Milk of Lime
NaOH	Caustic Soda
ADL	Acid Detergent Lignin
BM	Basal Medium
DM	Dry Matter
CO	Carbon Oxide
qPCR	Quantitative Polymerase Chain Reaction
O₂	Oxygen
UAF	Up flow Anaerobic Filter
RBCs	Red Blood Cells
NCER	National Center for Energy Research
PLRS	Petroleum Laboratories for Research and Studies
WW	Weight of Sample (Wet)
FFP	Fresh Frozen Plasma
GCMS	Gas Chromatograph Mass Spectrometer
CH₃NO	Methane, Nitroso-
PAV	Production Average
HIV	Human Immunodeficiency Virus

ABSTRACT

Present study investigates and helps in future managing of biomedical waste in a safe disposal and eco-friendly way, produce renewable energy recourse, from biomedical waste (blood bank waste), producing biogas (methan-nitroso). Present methods were the disposal of biomedical waste through incineration and landfill are environmentally hazard and polluted with viruses and bacterial infections that endanger the health of human animal and plant. This investigation helps reducing the cost of the disposal and gas in Sudan market were the prices of biogas are 10% to 12% lower price than diesel and Up to 30% cheaper than petrol.

المستخلص

الدراسة الحالية تتحرى وتساعد في الإدارة المستقبلية للتخلص من النفايات الطبية الحيوية بإيجاد طرق امنة وصديقة للبيئة، وإنتاج مصدر طاقة متجدد (غاز حيوي) من مخلفات ونفايات بنك الدم حيث تنتج غاز حيوي (نيرو-ميثان).

الطرق الحالية للتخلص من النفايات الطبية عن طريق ارسالها الى المحرقة او دفنها في مكب النفايات وهذه الطرق تمثل خطر بيئي حيث انها ملوثة بالفيروسات والعدوى البكتيرية والتي تمثل خطر على حياة الانسان والحيوان والنبات. كما يساعد هذا البحث أيضا في تخفيض تكلفة التخلص وسعر المحروقات من الديزل وبنزين في السوق السوداني، حيث ان الغاز الحيوي اقل تكلفة من الديزل بحوالي 10% الى 12%.

CHAPTER ONE
INTRODUCTION

1.1 General View:

People have known of the existence of naturally produced biogas since the 17th century and experiments with the construction of actual biogas systems and plants started as early as the mid-19th century. ^[1]

Biogas is produced by anaerobic digestion, a process in which organic matter is broken down in an oxygen-free environment producing significant amounts of methane. The organic matter for anaerobic digestion can originate from a wide range of waste streams, such as municipal solid waste, discards from food processing, animal manure, sewage, stillage and glycerin from biofuels production, as well as energy crops and agricultural residues. The use of these waste streams for methane production by anaerobic digestion helps to reduce the amount of waste that must be disposed of using other methods that generally do not have environmental benefits.

Biogas resources present an opportunity to address several key energy and environmental issues because it can be used as a fuel for a variety of electricity and transportation applications. Reducing waste through the use of anaerobic digestion can help decrease greenhouse gas emissions and other environmental pollution to the air and water.

Biogas production and subsequent purification to more readily useful products, such as bio methane, can incur significant costs, which is often due to the dispersed nature of biogas sources and economies of scale. This analysis begins to identify potential production system sizes and the geographic element of aggregating dispersed sources into larger, economically viable systems. ^[1]

This study examined the medical waste management practices of a hospital in Southern Africa. The results revealed that the hospital does not quantify medical waste.

Medical wastes are defined to include all types of wastes produced by health facilities such as general hospitals, medical centers and dispensaries. Medical wastes represent a small amount of total residues generated in a community.

According to Biomedical Waste (Management and Handling) Rules, 1998 of India, “Biomedical waste” means any waste, which is generated during the diagnosis, treatment or immunization of human beings or animals or in research activities pertaining thereto or in the production or testing of biological.^[2]

According to a World Health Organization (WHO) report, around 85% of the hospital wastes are actually nonhazardous, 10% are infective (hence hazardous), and the remaining 5% are non-infectious but hazardous (chemical, pharmaceutical and radioactive).^[3]

Biogas is a combustible mixture of gases. It consists mainly of methane (CH₄) and carbon dioxide (CO₂) and is formed from the anaerobic bacterial decomposition of organic compounds, i.e. without oxygen. The gases formed are the waste products of the respiration of these decomposer microorganisms and the composition of the gases depends on the substance that is being decomposed. If the material consists of mainly carbohydrates, such as glucose and other simple sugars and high molecular compounds (polymers) such as cellulose and hemicellulose, the methane production is low. However, if the fat content is high, the methane production is likewise high.

Methane and whatever additional hydrogen there may be makes up the combustible part of biogas. Methane is a colorless and odorless gas with a boiling

point of 162°C and it burns with a blue flame. Methane is also the main constituent (77-90%) of natural gas as showing in Table (1.1).

Chemically, methane belongs to the alkanes and is the simplest possible form of these. At normal temperature and pressure, methane has a density of approximately 0.75 kg/m³. Due to carbon dioxide being somewhat heavier, biogas has a slightly higher density of 1.15 kg/m³.

Table 1.1: Biogas content

Component	Formula	Concentration
Methane	CH ₄	50-75 vol.-%
Carbon Dioxide	CO ₂	25-45 vol.-%
Water Vapor	H ₂ O	2-7 vol.-%
Sophie	H ₂ S	0.002 -2 vol.-%
Nitrogen	N ₂	< 2 vol.-%
Ammoniac	NH ₃	<1 vol.-%
Hydrogen	H ₂	<1 vol.-%
Trace Gases		<2 vol.-%

1.2 Problem Statement:

- Price of gas is increasing due to the energy consumption worldwide is spontaneously increasing due to industrialization, population growth and state of development in both developing and developed countries.
- The quantity of Bio-Medical Waste generated is growing up depending on The data available from developed countries indicate a range from 1-5 Kg/bed/day, with substantial inter country and inter specialty differences. Meagre data from developing counties indicates that the range is essentially similar but the figures are lower i.e. 1-2 Kg/day/patient.

- Medical waste management has become a critical issue as it poses potential health risks and damage to the environment. In developing countries, medical wastes have not received sufficient attention. In many countries, hazardous and medical wastes are still handled and disposed together with domestic wastes, thus creating a great health risk to municipal workers, the public and the environment.

1.3 Solution:

- Minimizing the price of gas due its production from medical waste.
- Minimizing medical waste and maintain healthy environment due the production of biogas from medical waste.

1.4 Objective:

1.4.1 General:

The main objective of this study is to investigate biogas production from biomedical waste (Blood Bank waste) and Help maintaining clean environment benefiting from biomedical waste.

1.4.2 Specific:

1. Testing the effectiveness of the method that has been chosen to product large amount of gas (market volume).
2. Lowering the cost of gas by providing biogas.
3. Collecting statistic data about bio medical waste that have been used in the process of production.
4. Modifying bio gas plant method to fit biomedical waste.

5. Building safe unit to produce bio gas from bio medical waste.

1.5 Methodology:

- Collecting specific information and data base about biogas and bio medical wastes from web, books.
- Visiting hospitals and see the amount of waste produced and the views of specialist of such waste and recycling.
- Visit places of biogas production (the Central Veterinary Research Laboratories).
- Make interviews with chemists, engineers and health officers' in hospitals.
- Questionnaire about medical waste for data collection.
- Data analysis for information that has been collected from questionnaire.
- Experimental trail for the method (plane) chosen to product biogas from medical waste (blood bank waste).
- Start the Production of biogas from (blood bank waste).
- Testing the effectiveness of the method that has been chosen to product large amount of gas (market volume).

CHAPTER TWO
BACKGROUND

2.1 Medical Waste:

Medical waste is defined as all waste generated from health care or health related facilities. The types of waste classified as medical waste vary according to the institution or department in which they are generated. [4]

The term “medical waste” covers all wastes produced in health-care or diagnostic activities 75 % to 90 % of hospital wastes are similar to household refuse or municipal waste and do not entail any particular hazard. Refuse similar to household waste can be put through the same collection, recycling and processing procedure as the community’s municipal waste. The other 10% to 25% is called hazardous medical waste or special waste. This type of waste entails health risks. It can be divided into five categories according to the risks involved. [3]

2.2 Types of medical waste:

Medical waste can be classified into two categories: general waste and special waste. These two waste types are distinct in character and require specific waste treatment and disposal programs because they may contain infectious matter shown in Figure (2.1).

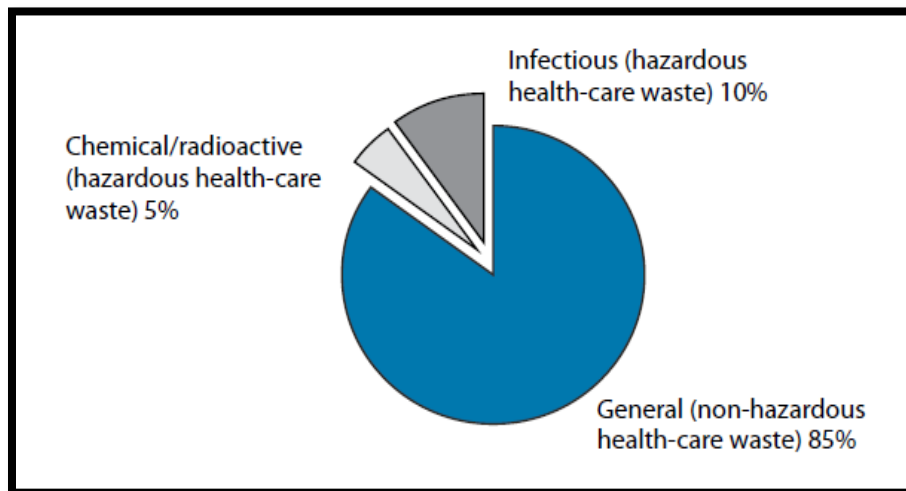


Figure (2.1): Types of medical waste

2.2.1 General Waste:

General waste consists of all waste materials that are not regulated or defined as hazardous, special, or potentially dangerous and do not require special handling and disposal. These wastes are sometimes referred to as Nonregulated Medical Waste (NRMW).^[4]

2.2.1.1 Sharps waste:

Sharps are items that could cause cuts or puncture wounds, including needles, hypodermic needles, scalpels and other blades, knives, infusion sets, saws, broken glass and pipettes. Whether or not they are infected, such items are usually considered highly hazardous health-care waste and should be treated as if they were potentially infected.

2.2.1.2 Pathological waste:

Pathological waste could be considered a subcategory of infectious waste, but is often classified separately especially when special methods of handling, treatment and disposal are used. Pathological waste consists of tissues, organs, body parts, blood, body fluids and other waste from surgery and autopsies on patients with infectious diseases. It also includes human fetuses and infected animal carcasses. Recognizable human or animal body parts are sometimes called anatomical waste. Pathological waste may include healthy body parts that have been removed during a medical procedure or produced during medical research.

2.2.1.3 Pharmaceutical waste including genotoxic waste:

Pharmaceutical waste includes expired, unused, spilt and contaminated pharmaceutical products, prescribed and proprietary drugs, vaccines and sera that are no longer required, and, due to their chemical or biological nature, need to be disposed of carefully. The category also includes discarded items heavily

contaminated during the handling of pharmaceuticals, such as bottles, vials and boxes containing pharmaceutical residues, gloves, masks and connecting tubing.

Genotoxic waste is highly hazardous and may have mutagenic (capable of inducing a genetic mutation), teratogenic (capable of causing defects in an embryo or fetus) or carcinogenic (cancer-causing) properties. The disposal of genotoxic waste raises serious safety problems, both inside hospitals and after disposal, and should be given special attention. Genotoxic waste may include certain cytostatic drugs, vomit, urine or faeces from patients treated with cytostatic drugs, chemicals and radioactive material.^[5]

2.2.2 Special Wastes:

The types of waste classified as special waste require special handling, treatment, and disposal, usually according to specific regulations and guidelines. Such waste may pose potential health, safety, or environmental hazards or may simply be objectionable for disposal with general waste because of appearance or aesthetics.

There are three categories of special waste: chemical waste, infectious waste, and radioactive waste. These may be in solid, biological, or liquid forms.^[4]

2.2.2.1 Non-hazardous general waste:

Non-hazardous or general waste is waste that has not been in contact with infectious agents, hazardous chemicals or radioactive substances and does not pose a sharps hazard. A significant proportion (about 85%) of all waste from health-care facilities is non-hazardous waste and is usually similar in characteristics to municipal solid waste.

More than half of all non-hazardous waste from hospitals is paper, cardboard and plastics, while the rest comprises discarded food, metal, glass, textiles, plastics and wood.

2.2.2.2 Infectious waste:

Infectious waste is material suspected to contain pathogens (bacteria, viruses, parasites or fungi) in sufficient concentration or quantity to cause disease in susceptible hosts. This category includes:

- Waste contaminated with blood or other body fluids.
- Cultures and stocks of infectious agents from laboratory work.
- Waste from infected patients in isolation wards.

Waste contaminated with blood or other body fluids include free-flowing blood, blood components and other body fluids; dressings, bandages, swabs, gloves, masks, gowns, drapes and other material contaminated with blood or other body fluids; and waste that has been in contact with the blood of patients undergoing haemodialysis (e.g. dialysis equipment such as tubing and filters, disposable towels, gowns, aprons, gloves and laboratory coats).

2.2.2.3 Chemical waste:

Chemical waste consists of discarded solid, liquid and gaseous chemicals; for example, from diagnostic and experimental work and from cleaning and disinfecting procedures. Chemical waste from health care is considered to be hazardous if it has at least one of the following properties:

- Toxic (harmful).
- Corrosive (e.g. acids of Ph <2 and bases of Ph >12).
- Flammable.
- Reactive (explosive, water reactive, shock sensitive).
- Oxidizing.

2.2.2.4 Radioactive waste:

Radioactive wastes are materials contaminated with radionuclides. They are produced as a result of procedures such as in vitro analysis of body tissue and fluid, in vivo organ imaging and tumor localization, and various investigative and therapeutic practices.

Radionuclides used in health care are in either unsealed (or open) sources or sealed sources. Unsealed sources are usually liquids that are applied directly, while sealed sources are radioactive substances contained in parts of equipment or encapsulated in unbreakable or impervious objects, such as pins, “seeds” or needles. ^[5]

2.3 Management of medical waste:

State and local governments can choose from a limited suite of technologies and programs to deal with waste, from simply collecting trash and dumping it to highly technical processes that are being used in pilot studies to determine their effectiveness.

This section identifies the options that are available, in alphabetical order like shown in Table (2.1).

2.3.1 Collection:

Most communities rely on compaction trucks or other types of vehicles to bring waste from the location where it is created or produced to another site for sorting and treatment. Collection is the most expensive element of waste management, accounting for between 50 and 70 percent of the costs for operations.

2.3.2 Composting:

Composting is a method of dealing with organic waste and can be done by both individuals and municipalities. Composting is considered a valuable strategy

because the volume of waste can be reduced by as much as half by breaking down easily degradable plant and animal tissue, such as yard waste and food scraps.

2.3.3 Incineration:

One of the common ways in which the volume of waste is reduced is through combustion or incineration, a controlled burning process used by both governments and private companies around the world. ^[6]

2.3.4 Autoclaving:

The autoclave should be dedicated for the purposes of disinfecting and treating bio-medical waste.

2.3.5 Microwaving:

Most microorganisms are destroyed by the action of microwave at a frequency of about 2450 MHz and a wavelength of 12.24 cm. The water contained within the waste is rapidly heated by the microwaves and the infectious components are destroyed by heat conduction.

2.3.6 Inertization:

The process of inertization involves mixing waste with cement and other substances before disposal, in order to minimize the risk of toxic substances contained in the wastes migrating into the surface water or ground water.

Table 2.1: color code for medical waste and treatment options.

Color	Waste category	Treatment options
Yellow	infectious Clinical Waste Hazardous	Incineration or burning
Orange	Waste which may be “treated”	Incineration or burning
Purple	Cytotoxic and cytostatic waste	landfill
Yellow/black	Offensive/hygiene waste*Offensive/noninfectious waste Non Hazardous	landfill
Red	Anatomical waste for incineration1Body parts ;Organs ;Blood bags; Blood preserves	Shredding after autoclaving
Black	Domestic (municipal) waste	Autoclaving/Destruction
Blue	Pharmaceutical waste ;Non Hazardous	Autoclaving/Destruction
White	Amalgam waste For recovery	landfill

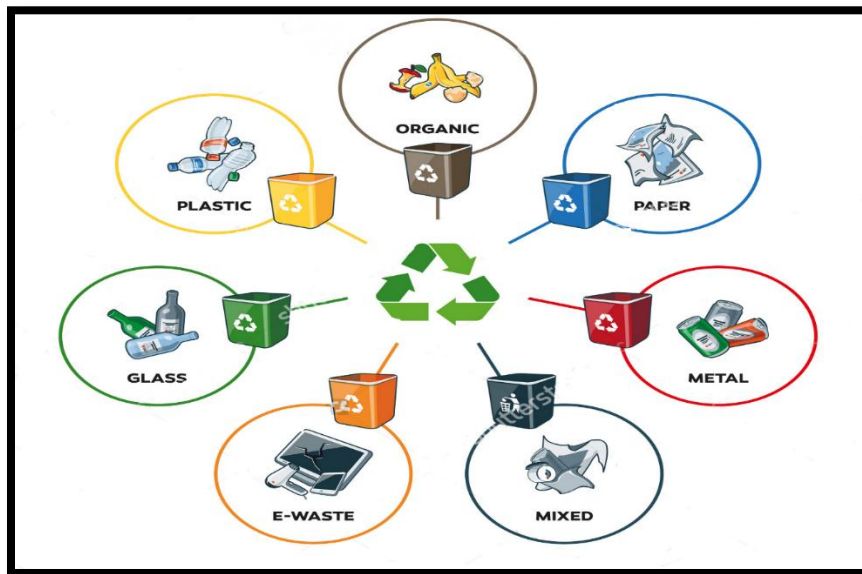


Figure (2.2): Medical Waste color code.^[7]

2.4 Blood Bank:

Hospital Blood Bank: is a laboratory, or part of a laboratory within a hospital which receives and stores supplies of tested whole blood and blood components from a blood center. The hospital blood bank performs compatibility testing and issues blood and blood components for clinical use within the hospital^[8]

2.4.1 Management of blood in blood bank:

In order to provide the required level of safety in blood donation and transfusion service, the principles of quality, good manufacturing and laboratory practice shall be implemented through a quality management system^[9].

- **Pre-Transfusion testing:**

Each hospital transfusion laboratory should follow standard operating procedures (SOPs). All laboratories cross matching blood should participate in external quality control. The hospital transfusion laboratory should verify the patient's ABO and Rhesus D (Rh D) group against previous records for the patient; any discrepancies should be Resolved before blood components are issued. Where there is an urgent requirement for transfusion, group O Rh D Negative blood should be issued until the discrepancy is resolved. Hospital transfusion laboratory practices should comply with the guidelines for blood bank computing.

- **Issuing of blood:**

Computer generated self-adhesive compatibility labels are recommended. Efforts to standardize these labels nationally should continue for consistency between hospitals and to minimize checking errors. The hospital transfusion laboratory should also provide a blood compatibility report form with the blood component issued. The compatibility label on each unit should show:

- Surname and first name of patient.
- Date of birth.
- Unique identification number.
- Gender.
- ABO group and Rh D group of the patient.
- ABO group and Rh D group of the unit.
- Donation number.
- Expiry date of unit.
- Time when blood is required.
- Location of patient.

The ABO group, Rh D group and unit number must be identical on the Irish Blood Transfusion Service Label, Hospital Compatibility Report Form and the Compatibility Label on the blood pack. Occasionally the ABO group and Rh D group issued for a patient may be of a different group, usually due to a shortage of a particular group. In these circumstances, the hospital transfusion laboratory should inform the patient's doctor and include the information on the compatibility report form.

- **Storage of blood component:**

Blood transfusion is an essential therapeutic intervention. We all may need blood in an emergency, and some of us need regular transfusions. The purpose of a transfusion is to provide the blood component(s) that will improve the physiological status of the patient. Various blood components can be harvested from a single donation of whole blood. Most blood banks are able to separate red cells and plasma components. Others are able to prepare components such as platelet concentrates and cryoprecipitate. All these components, prepared by centrifugation, are often referred to as 'wet or labile products'. Other plasma

products, generally referred to as plasma derivatives, can be harvested from plasma by a pharmaceutical process called plasma fractionation, which renders their properties stable. The collection of blood from donors may take place within the blood transfusion center or hospital blood bank. It is also often collected from donors during mobile blood collection sessions. The blood is then taken to a laboratory for testing and processing into components and for storage and distribution as the need arises. Blood is collected at body temperature, i.e. +37 °C. But in order to maintain its vital properties, it must be cooled to below +10 °C to be transported, and stored at refrigeration temperatures of around +4 °C until use. Hence the term, blood cold chain, which begins the moment the blood is collected and continues until it is transfused. If blood is stored or transported outside of these temperatures for long, it loses its ability to transport oxygen or carbon dioxide to and from tissues respectively upon transfusion. Other factors of serious concern are the risk of bacterial contamination if blood is exposed to warm temperatures. Conversely, blood exposed to temperatures below freezing may be damaged, and the transfusion of such blood can be fatal.^[9]

- Red blood cells and whole blood should only be stored in a designated controlled blood refrigerator.
- Plasma is stored frozen and thawed in the laboratory immediately before use.
- Platelets are stored at room temperature on a controlled agitator to avoid clumping, and should never be

Stored in a refrigerator.

- The time of removal of all components from the controlled storage should be logged – ideally electronically, or failing that, manually.
- Once a unit of blood has been removed from controlled storage the transfusion should be commenced immediately on delivery to the clinical area. If the transfusion cannot be initiated promptly, the blood should Be returned to the

hospital transfusion laboratory for storage, unless the transfusion to the intended Recipient can be completed within 4 hours. Blood should be returned to the hospital transfusion laboratory for documented disposal if out of controlled storage for more than 30 minutes.

- The transfusion of plasma and platelets should be commenced as soon as possible following issue from the laboratory and must not be stored outside the laboratory.
- The use of validated blood transport containers is recommended.

- **Blood warmers:**

Routine warming of blood is not indicated. Patients who will benefit from warmed blood include adults and children receiving massive transfusion, infants requiring exchange transfusion and patients with clinically significant high-titre cold agglutinins active in vitro at 37°C. Blood warmers must be subject to regular servicing and used in accordance with the manufacturer's instructions. Red blood cells and plasma exposed to temperatures over 40°C may cause severe transfusion reactions. Blood components must NOT be warmed by improvisations such as putting the pack into hot water, in a microwave, or on a radiator, as uncontrolled heating can damage the contents of the pack.

- **Handling and disposal of blood packs:**

Hospital policy should cover the use of gloves, trays, bags, accidental spills/damage and disposal. Unless the patient has an acute transfusion reaction, used blood packs should be disposed of after the transfusion in an appropriate designated container i.e. a rigid spill proof bin with yellow lid. If more than 100mls remains in the pack, disposal should be in a spill proof container in accordance with local hospital policy.^[10]

2.4.2 Safety in collection of blood samples:

- **Hand Hygiene:**

- Use Alcohol-based hand rub OR Clean, running water Soap Disposable (paper) towel shown in Figure (2.3).

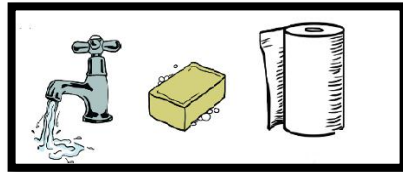


Figure (2.3): Hand Hygiene.

- **Assemble materials for packaging of samples:**

- Plastic leak-proof packaging container
- Disposable (paper) towels Cooler or cold box, if sample requires refrigeration shown in Figure (2.4).^[11]

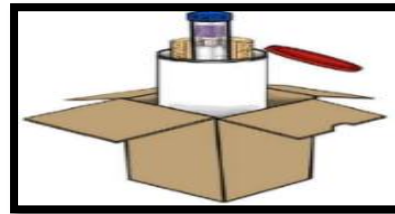


Figure (2.4): packaging of samples

- **Personal Protective Equipment (PPE):**

- Several pairs of disposable gloves (non-sterile, ambidextrous, single layer) one pair of gloves for blood collection one additional pair as a replacement if they become damaged or contaminate.
- Long-sleeved, cuffed gowns (if in hospital) or disposable coverall suit (if in rural area) Note: Tasks where contact with blood or body fluid could happen, Impermeable gown or a plastic apron over the non-impermeable gown are recommended.
- Face protection: Face shield or "goggles and mask" shown in Figure (2.5).

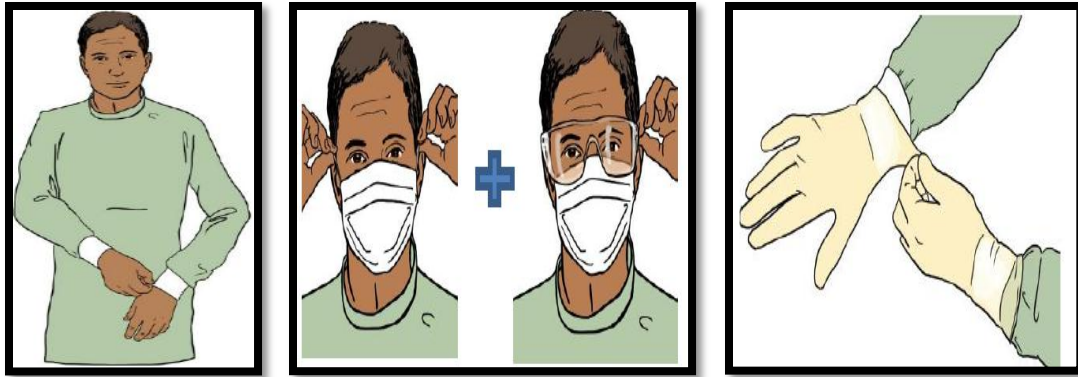


Figure (2.5): Personal Protective Equipment (PPE)

Table 2.2: Standard requirements for storage, transport, expiry of blood and blood components:

Components	Properties	Storage	Transport	Expiry
Whole blood	Vol: 350 ml +/-10% Hct: 35-50% Dosage:10ml/kg body wt Increases Hb by 1-1.5gm%	In a blood bank refrigerator at +2°C to +6°C	Can be transported For next 24hrs if maintained at +1°C to +10°C	35 days in a closed system 24 hours if open system
Packed Red Cells	Vol:250ml± 30ml Hct: 60 to 70% Dosage:5ml/kg body wt Increases Hb by 1-1.5gm%	In a blood bank refrigerator at +2°C to +6°C	Can be transported For next 24hrs. if maintained at +1°C to +10°C	35 days in a closed system 24 hours if open system
Fresh Frozen Plasma (FFP)	Vol:200-220ml Contains stable & 70% unstable clotting factors Dosage:15ml/kgbody wt	In a plasma freezer at below -30°C	Transported in frozen state	1 year
FFP Thawed	Vol:200-220ml Contains stable & 70% unstable clotting factors Dosage:15ml/kg body wt	+2°C to +6°C in a blood bank refrigerator	Maintain between +1oC to +10°C	4 -6 hours
Liquid Plasma	Vol:200-220ml Contains onl stable clotting factors	+2°C to +6°C in a blood bank refrigerator	Maintain between +1oC to +10°C	40 days
Platelet concentrate	Vol:50 to 70ml Contains 3.5- 4.5x10 ¹⁰ platelets/unit Dosage:1unit/10kg body wt Increases the platelet count by 10,000 to 20,0000/ul blood	In a platelet incubator with agitator at +20oC to +24°C with continuous gentle Agitation	+20oC to +24°C	5 days if closed system. 6 hours if open

- **Centrifugation of blood components:**

The sedimentation behavior of blood cells is determined primarily by their size as well as the difference of their density from that of the surrounding fluid (see Table 4-1 below). Other factors are the viscosity of the medium and flexibility of the cells (which is temperature dependent). The optimal temperature for centrifugation with respect to these factors is + 20 °C or higher.

Table 2.3: Volume and density of principal blood constituents:

Blood components	Mean density (g/mL)	Mean corpuscle volume (10-15 L)
Plasma	1.026	-
Platelets	1.058	9
Monocytes	1.062	470
Lymphocytes	1.070	230
Neutrophils	1.082	450
Red cells	1.100	87

Table 2.4: Five different methods of initial separation of whole blood and the approximate at composition of the fractions obtained (figures refer to a standard donation of 450 mL ± 10 per cent, with 60-70 mL of anti-coagulant):

Method	1	2	3	4	5
Initial filtration	No	No	No	No	Yes
Centrifugation speed	Low	Low	High	High	High
Separation into	plasma + buffy coat + red cells	plasma + red cells	plasma + buffy coat + red cells	plasma + red cells	plasma + red cells, leucocyte depleted

- **Bacterial safety of blood components:**

Although blood collection and processing procedures are intended to produce non-infectious blood components, bacterial contamination may still occur. Bacterial quality control testing in all blood components may be appropriate. However, for collection of whole blood, bacterial cultures of platelet components provide the best indication of the overall rate of contamination provided that the sample for culture is obtained in a suitable sample volume and at a suitable time after collection. Surveillance studies have found rates of contamination as high as 0.4 per cent in single donor platelets, although rates at or below 0.2 per cent are more often reported. The causes of bacterial contamination include occult bacteremia in the donor, inadequate or contaminated skin preparation at the phlebotomy site, coring of a skin plug by the phlebotomy needle and breaches of the closed system from equipment defects or mishandling. Platelet components are more likely than other blood components to be associated with sepsis due to their storage at room temperature, which facilitates bacterial growth. A variety of procedures may be used to obtain a valid platelet sample for bacterial culture. Large volume samples removed from a multiple-unit pooled platelet component or single donor apheresis platelets can be cultured any time post-collection. However, small volume samples (e.g. 2-5 mL removed from a single whole blood unit) should be taken for culture after a 24 to 48 hour delay post-collection. Delayed sampling of a small volume permits bacterial growth to a level that subsequent assays can detect reliably, thereby overcoming sampling errors at low contamination levels. PRT may offer an alternative approach to assuring the bacterial safety of blood components. Currently, systems are available for platelets, but not for red cells.^[12]

- **PH in blood:**

There are three major contributors to regulating the pH of blood. Bicarbonate, phosphate and proteins Blood pH Must be Kept Close to 7.4 – Hydrogen ion is extremely reactive and effects many molecules which regulate physiological processes – Blood pH is set at a slightly alkaline level of 7.4 (pH 7.0 is neutral) – A change of pH of 0.2 units in either direction is considered serious – Blood PHs below 6.9 or above 7.9 are usually fatal if they last for more than a short time Blood Buffers The bicarbonate system is the most important and is controlled by the rate of respiration - Dissolved carbon dioxide in water reacts to form carbonic acid - The pKa of carbonic acid is 6.35. the pH of blood is 7.4 so the acid is greater than 1 pH away from the pKa and it is primarily dissociated - Under physiological conditions the equilibrium for the first reaction is far to the left, and the combined pKa for the two reactions is 6.4 CO₂ At first glance this does not look like a good buffer for blood. The buffering capacity is poor. To maintain a pH of 7.4 there would have to be a ratio of 11 to 1 of bicarbonate to carbon dioxide. $pH = 6.4 + \text{Log} [HCO_3^-] / [CO_2]$ Because this is an open system, the CO₂ dissolved and the bicarbonate can rapidly change Changes resulting in loss of carbonic acid are replaced by CO₂ dissolving - This is an open system Normal concentration of carbon dioxide is 1.2 mM and bicarbonate is 15 Mm.

Acidosis and Alkalosis A decrease in arterial carbon dioxide partial pressure causes the bicarbonate/carbon dioxide ratio to exceed 20 and the pH to rise above 7.45 – Alkalosis Increases in partial pressure of CO₂ have the opposite affect and decrease the pH below 7.2 – Acidosis shown in Figure (2.6).

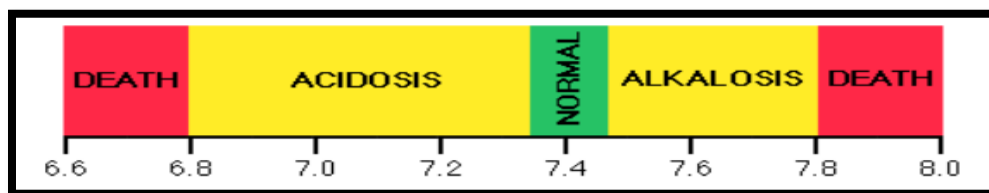


Figure (2.6): PH in blood

2.5 Biogas:

Biogas is a combustible gas which is produced with microorganism assistance during the decay of organic materials like manure, agricultural feedstock or bio waste in an anaerobic, or oxygen free, environment. Oxygen free conditions occur in natural systems such as the bottom layers of wetlands and bogs, but also in artificial systems like landfills, lagoons, and specially designed tanks in biogas plants, then called anaerobic digesters. In a biogas plant, biomass is fed into a digester for a period of several days, in which archaeobacteria under exclusion of light and oxygen decompose the material, with biogas as product. It is a gas mixture, consisting of mainly methane (CH_4 , 40 to 75 %), carbon dioxide (CO_2 , 25 to 60 %), and other gases (hydrogen, hydrogen sulphide and carbon monoxide) as shown in Figure (2.7). As methane is also the main component of natural gas, the composition of biogas resembles the characteristics of natural gas and therewith provides manifold ways of application.^[13]

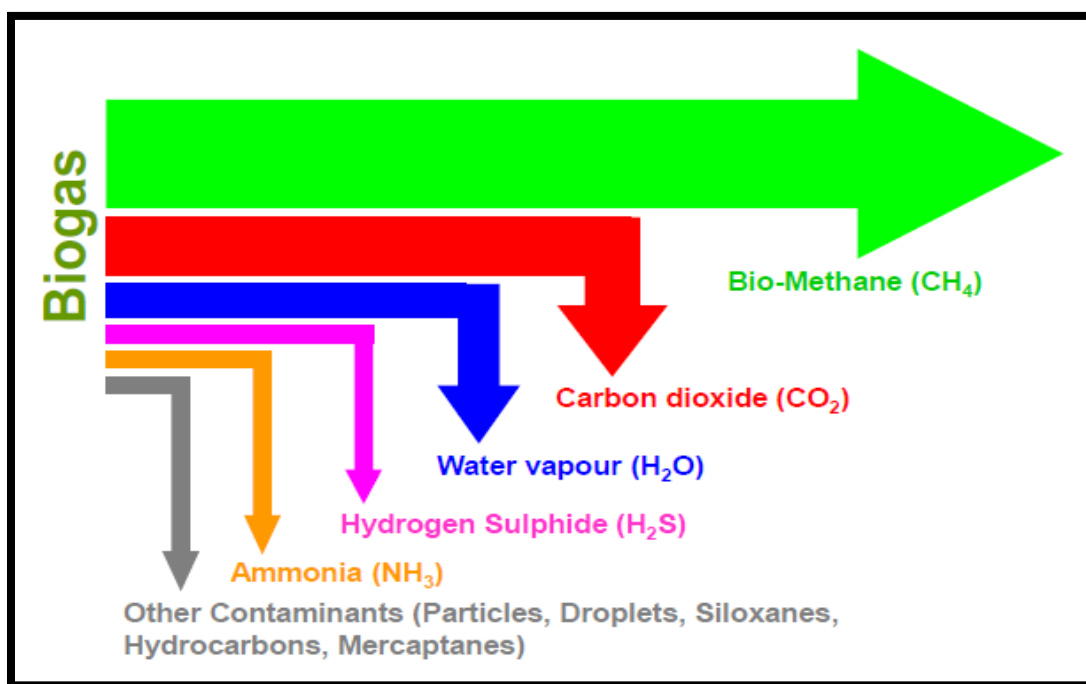


Figure (2.7): Biogas content^[14]

2.5.1 Biogas Plants types:

A total of seven different types of biogas plant have been officially recognized by the MNES.

1. The floating-drum plant with a cylindrical digester (KVIC model).
2. The fixed-dome plant with a brick reinforced, molded dome (Janata model):

A fixed-dome plant consists of a digester with a fixed, non-movable gas holder, which sits on top of the digester. When gas production starts, the slurry is displaced into the compensation tank. Gas pressure increases with the volume of gas stored and the height difference between the slurry level in the digester and the slurry level in the compensation tank. The costs of a fixed-dome biogas plant are relatively low. It is simple as no moving parts exist. There are also no rusting steel parts and hence a long life of the plant (20 years or more) can be expected.

The plant is constructed underground, protecting it from physical damage and saving space. While the underground digester is protected from low temperatures at night and during cold seasons, sunshine and warm seasons take longer to heat up the digester. No day/night fluctuations of temperature in the digester positively influence the bacteriological processes.

The construction of fixed dome plants is labor-intensive, thus creating local employment. Fixed-dome plants are not easy to build. They should only be built where construction can be supervised by experienced biogas technicians. Otherwise plants may not be gas-tight (porosity and cracks).

The basic elements of a fixed dome plant (here the Nicarao Design) are shown in the Figure (2.8).

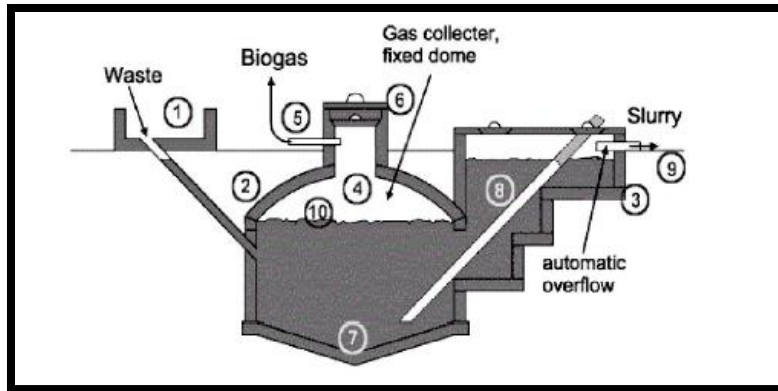


Figure (2.8): Fixed dome plant Nicarao design:

1. Mixing tank with inlet pipe and sand trap.
2. Digester. 3. Compensation and removal tank. 4. Gasholder.
5. Gas pipe. 6. Entry hatch, with gastight seal.
7. Accumulation of thick sludge. 8. Outlet pipe.
9. Reference level. 10. Supernatant scum, broken up by varying level.

Digester - The digesters of fixed-dome plants are usually masonry structures, structures of cement and ferrocement exist. Main parameters for the choice of material are:

- Technical suitability (stability, gas- and liquid tightness);
- Cost-effectiveness;
- Availability in the region and transport costs;
- Availability of local skills for working with the particular building material.

Fixed dome plants produce just as much gas as floating-drum plants, if they are gas-tight. However, utilization of the gas is less effective as the gas pressure fluctuates substantially. Burners and other simple appliances cannot be set in an optimal way. If the gas is required at constant pressure (e.g., for engines), a gas pressure regulator or a floating gas-holder is necessary.

3. The floating-drum plant with a hemisphere digester (Pragati model):

Floating-drum plants consist of an underground digester and a moving gas-holder. The gas-holder floats either directly on the fermentation slurry or in a water jacket of its own. The gas is collected in the gas drum, which rises or moves down, according to the amount of gas stored. The gas drum is prevented from tilting by a guiding frame. If the drum floats in a water jacket, it cannot get stuck, even in substrate with high solid content.

4. The fixed-dome plant with a hemisphere digester (Deenbandhu model).
5. The floating-drum plant made of angular steel and plastic foil (Ganesh model).
6. The floating-drum plant made of pre-fabricated reinforced concrete compound units.
7. The floating-drum plant made of fiber-glass reinforced polyester.

2.5.2 Biochemical process and mechanism of Biomethanation:

- **Mechanism of Biomethanation process:**

The anaerobic biological conversion of organic matter occurs in three steps. The first step involves the enzyme-mediated transformation of insoluble organic material and higher molecular mass compounds such as lipids, polysaccharides, proteins, fats, nucleic acids, etc. into soluble organic materials, i.e. to compounds suitable for the use as source of energy and cell carbon such as monosaccharide's, amino acids and other simple organic compounds. This step is called the hydrolysis and is carried out by strict anaerobes such as Bactericides, Clostridia and facultative bacteria such as Streptococci, etc.

In the second step, abiogenesis, another group of microorganisms ferments the break-down products to acetic acid, hydrogen, carbon dioxide and other lower weight simple volatile organic acids like propionic acid and butyric acid which are in turn converted to acetic acid. In the third step, these acetic acid, hydrogen and carbon dioxide are converted into a mixture of methane and carbon dioxide by the

methanogen bacteria (acetateutilizers like *Methanosarcina* spp.and *Methanotherix* spp.and hydrogen and format utilizing species like *Methanobacterium*, *Methanococcus*, etc.).The three stages of methane fermentation are shown in Figure (2.9).^[15]

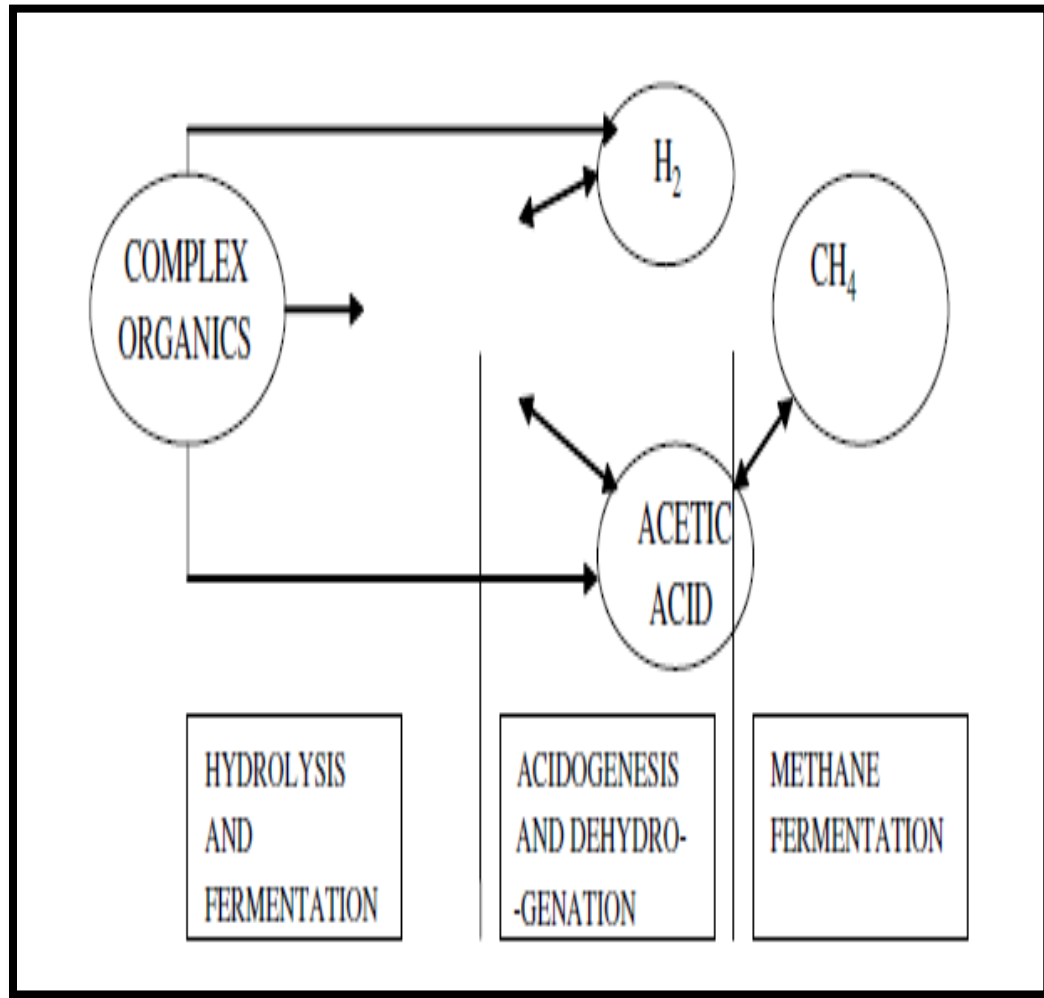


Figure (2.9): Different stages of methane fermentation

- **Biochemical process:**

Methane fermentation is a complex process, which can be divided up into four phases: hydrolysis, acidogenesis, acetogenesis/dehydrogenation, and methanation shown in Figure (2.10).

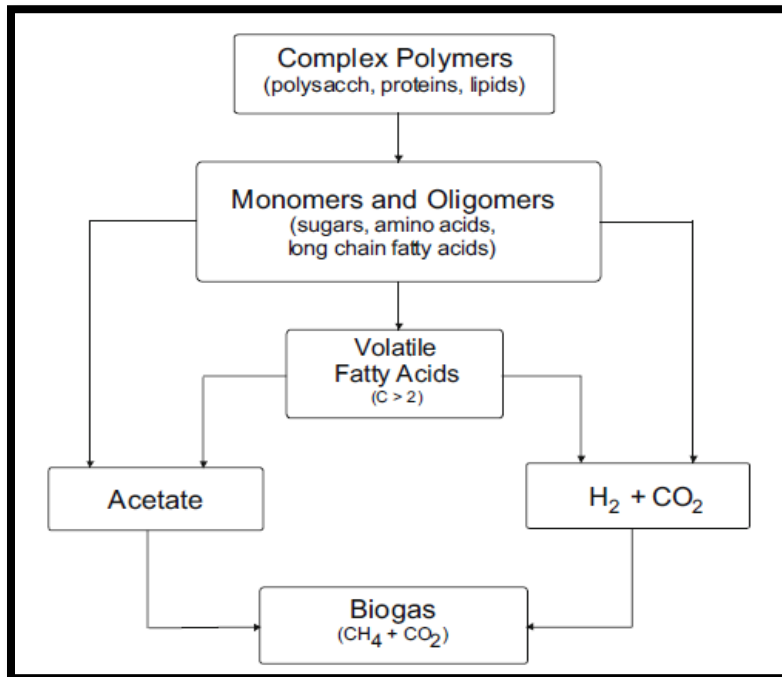


Figure (2.10): The stages of the methane fermentation process (according Gujer W and Zehnder AJB 1983)

The individual degradation steps are carried out by different consortia of microorganisms, which partly stand in syntrophic interrelation and place different requirements on the environment. Hydrolyzing and fermenting microorganisms are responsible for the initial attack on polymers and monomers and produce mainly acetate and hydrogen and varying amounts of volatile fatty

Acids such as propionate and butyrate. Hydrolytic microorganisms excrete hydrolytic enzymes, e.g., cellulose, Celosias, xylenes, amylase, lipase, and protease. A complex consortium of microorganisms participates in the Hydrolysis and fermentation of organic material. Most of the bacteria are strict anaerobes such as Bactericides', Clostridia, and Bifid bacteria. Furthermore, some facultative

anaerobes such as Streptococci and Enterobacteriaceae Take part. The higher volatile fatty acids are converted into acetate and hydrogen by obligate hydrogen-producing acetogenic bacteria. The hydrogen-producing acetogenic bacteria are not well characterized. Typical homoacetogenic bacteria are *Acetobacterium woodii* and *Clostridium aceticum*. The accumulation of hydrogen can inhibit the metabolism of the acetogenic bacteria. The maintenance of an extremely low partial pressure of hydrogen is, therefore, essential for the acetogenic and H₂-producing bacteria. Although many microbial details of metabolic networks in a methanogenic consortium are not clear, present knowledge suggests that hydrogen may be a Limiting substrate for methanogens .This assumption is based on the fact that addition of H₂- producing bacteria to the natural biogas-producing consortium increases the daily biogas production. At the end of the degradation chain, two groups of methanogenic bacteria produce methane from acetate or hydrogen and carbon dioxide. These bacteria are strict anaerobes and require a lower redox potential for growth than most other anaerobic bacteria.^[16]

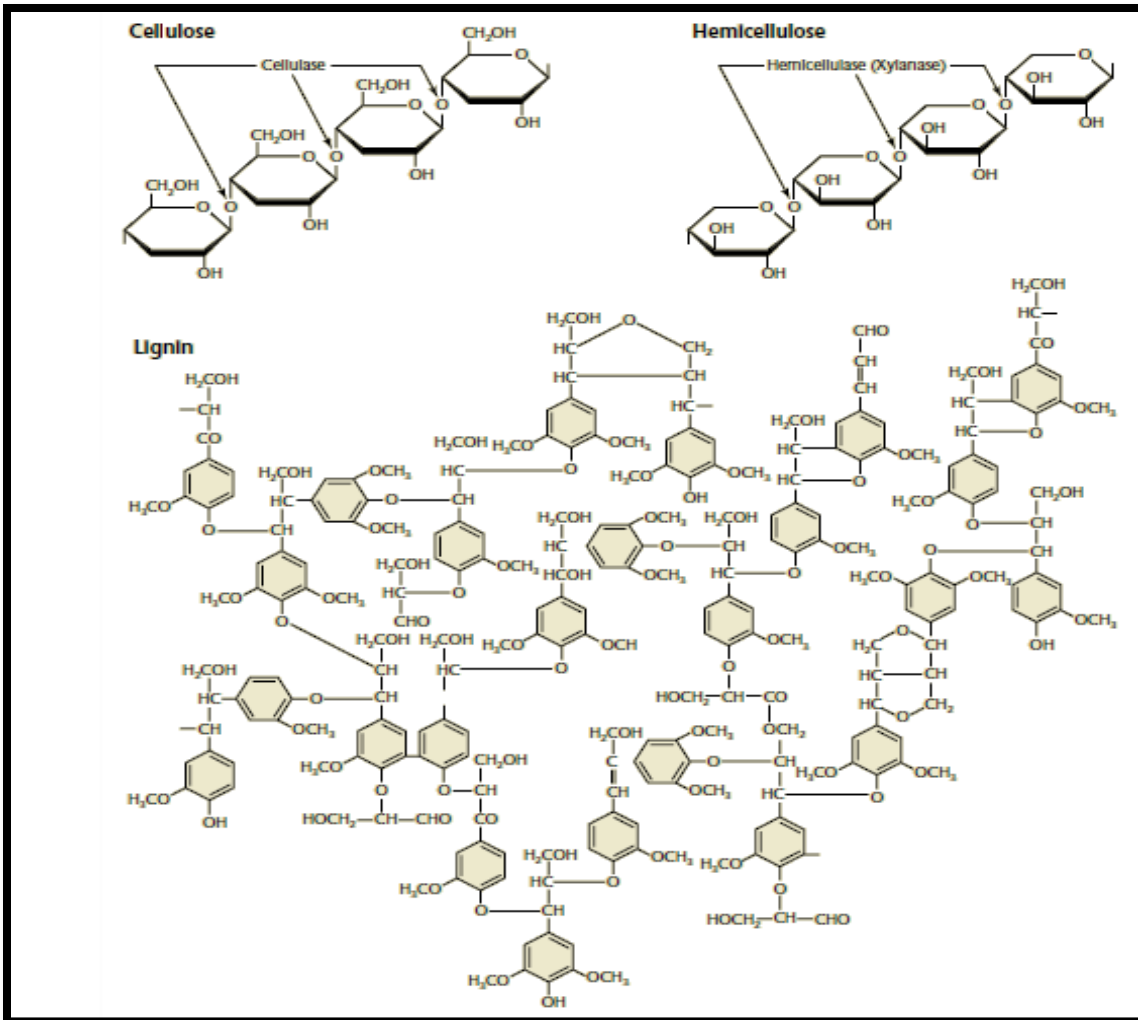


Figure (2.11): Cellulose and hemicellulose are long-chain polysaccharides that can be broken down by specific enzymes present in certain bacteria, but not in animals. Lignin has a compact structure and is practically biologically inert.

Table 2.5: Energy yield of methanogens from decomposition of different sources.

Source	Process	Energy yield kJ/mol methane
Hydrogen	$4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$	131
Formic acid	$4\text{HCOOH} \rightarrow \text{CH}_4 + 3\text{CO}_2 + 2\text{H}_2\text{O}$	145
Methanol	$4\text{CH}_3\text{OH} \rightarrow 3\text{CH}_4 + \text{CO}_2 + \text{H}_2\text{O}$	105
Acetic acid	$\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$	36

Table (2.6): Biogas and methane yield at a complete digestion of carbohydrate (cellulose), protein and fat.1) STP = Standard Temperature and Pressure (0°C and 1 atm.). 2) Glycerol trioleic acid.

Organic substance	Process	Gas yiled (STP)		CH ₄ %
		MI biogas/g	MI CH ₄ /g	
Cellulose	$(C_6H_{10}O_5)_n + nH_2O \rightarrow 3nCH_4 + 3nCO_2$	830	415	50.0
Protein	$2C_5H_7NO_2 + 8H_2O \rightarrow 5CH_4 + 3CO_2 + 2(NH_4)(HCO_3)$	793	504	63.6
Fat	$C_{57}H_{104}O_6 + 28H_2O \rightarrow 40CH_4 + 17CO_2$	1,444	1,014	70.2

2.5.3 Process parameters for a biogas plant:

In order for a biogas process to be effective and productive, there are a number of parameters that have to be optimized.

- **Anaerobic environment:**

As mentioned earlier, the methanogens need an oxygen-free environment – they are obligatory anaerobic. A biogas reactor therefore has to be airtight. The small amount of oxygen dissolved in the liquid/biomass fed to the plant is quickly used up by, for example, aerobic bacteria that must have oxygen, or by facultative anaerobic bacteria that can use oxygen for their respiration, if it is present.

- **Temperature:**

The rate of biochemical processes generally increases with temperature. As a rule of thumb, the rate is doubled for every 10-degree rise in temperature within certain limits ($Q_{10} = 2$). This is also the case with the biogas process. In this situation there are, however, several types or strains of bacteria involved that have adapted to the different temperatures:

psychrophiles 0 – 20°C

mesophiles 15 – 45°C

Thermophiles 40 – 65°C

Common to the bacteria is that they are very sensitive to changes in temperature. This sensitivity increases with temperature. In practice, biogas plants are run at either a mesophilic level of around 37°C, where fluctuations of approx. $\pm 2^\circ\text{C}$ are tolerated, or at a thermophilic level of around 52°C, where fluctuations of only approx. $\pm 0.5^\circ\text{C}$ are tolerated.[1]

Common to all growth intervals is that the temperature that allows the highest rate is close to the so-called maximum temperature, which results in cell death. If the temperature increases above this maximum temperature, the cell's proteins and other components are quickly inactivated, causing the organism to die. The maximum temperature varies depending on which temperature range the microorganism is adapted to shown in Figure (2.12).[17]

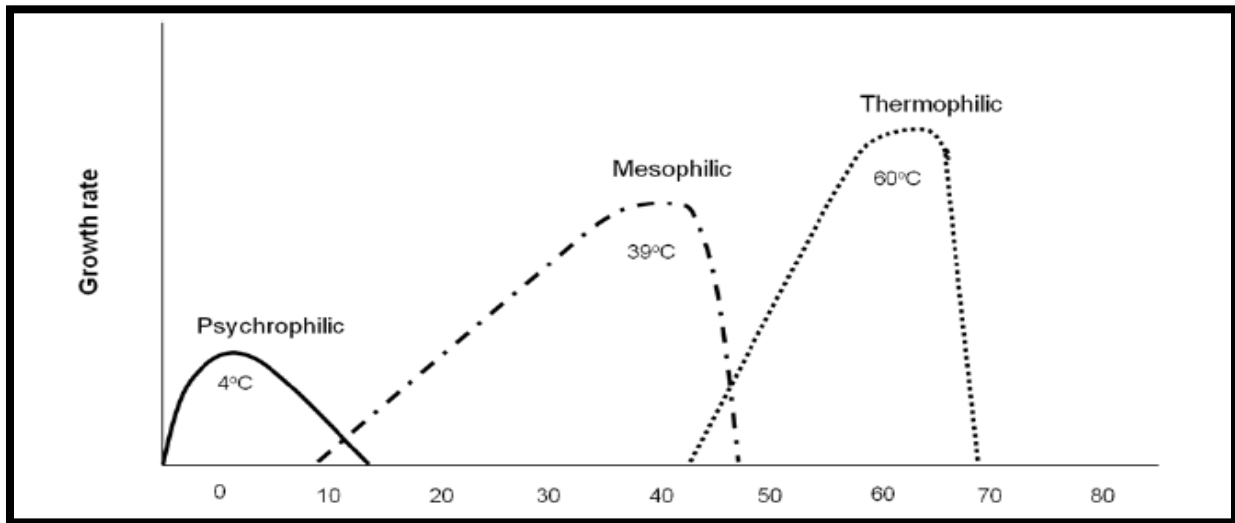


Figure (2.12): Growth of microorganisms at different temperatures (Modified based on Madigan and Martinko 2006)

- **pH:**

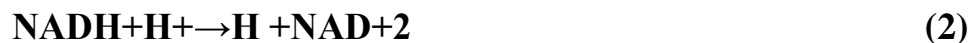
The equation below gives the relationship between pH and hydrogen ion concentration

in mol ml⁻¹ :

$$[H^+] = 10^{-pH} \quad (1)$$

Water with a hydrogen ion concentration of 10⁻⁶ mol l⁻¹ or 10⁻⁴ g l⁻¹ has, for instance,

a pH value of 6. Because of the hydrogen transportation by NAD, different products of fermentation are developed: the H⁺ ions isolated from the substrate are carried over to the uncharged NAD. The NAD molecules so charged (NADH + H⁺) regenerate (oxidize), by forming H₂ molecules:



$$\Delta G_f^\circ = +18.07 \text{ kJ mol}^{-1} \quad (3)$$

This reaction occurs independently of the hydrolysis and acidification of hydrocarbon and proteins. Hydrocarbons are easier to acidify, and no pH-buffering ions are released as with the degradation of proteins. Therefore, the pH decreases more easily. With the degradation of carbohydrates, the partial pressure of hydrogen increases more easily, as with other substances. This happens in combination with the formation of reduced acidic intermediate products. Even when the hydrolysis and the acidification occur in different apparatuses and are separated from

the methanation, complete suppression of the methanation is almost impossible.

The pH optimum of the methane-forming microorganism is 6.7 – 7.5. In a two-stage biogas plant, it is important, therefore, to adjust the pH value in the second stage to be higher than in the first stage. Only Methanosarcina is able to withstand lower pH values (pH = 6.5 and below). With the other bacteria, the metabolism is considerably suppressed at pH < 6.7. If the pH value falls below 6.5, then the

production of organic acids leads to a further decrease in pH by the hydrolytic bacteria and possibly to cessation of the fermentation. In reality, the pH value is held within the neutral range by natural

Procedures in the fermenter. Two buffering systems ensure this. A too strong acidify caption is avoided by the carbon dioxide – hydrogen carbonate – carbonate buffer system. During the fermentation, CO₂ is continuously evolved and escapes into the air. With falling pH value, more CO₂ is dissolved in the substrate as uncharged molecules. With rising pH value, the dissolved CO₂ forms carbonic acid, which ionizes. Thus, hydrogen ions are liberated shown in Figure (2.13).

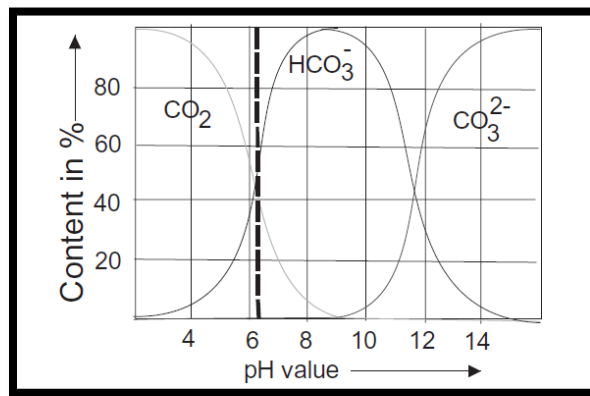
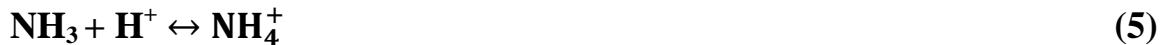


Figure (2.13): PH.

At pH 4, all CO₂ is present as free molecules; at pH 13, all CO₂ is dissolved in the form of carbonate in the substrate. The center around which the pH value swings with this system is at pH 6.52. A rising pH value results in more CO₂ dissociating out of the gas, whereas a decreasing pH value means that more CO₂ is released into the environment. At a concentration of 2.

5 – 5 g l⁻¹, hydrogen carbonate gives particularly strong buffering. A too weak acidify caption is avoided by the ammonia – ammonium buffer system. With falling pH value, ammonium ions (NH₄⁺) are formed with release of hydroxyl ions.



With rising pH value, more free ammonia molecules (NH_3) are formed. The center around which the pH value swings with this system is at pH 9.25. Fatty acids also work as a weak buffer system with a pH value around 4.5, depending on the specific fatty acid. Since the solubility of gases is dependent on temperature, the effect of the buffer system is smaller if the digester is operated thermo physically compared with a mesospheric operational mode. Both buffering systems can be overloaded by a feed of particularly rapidly acidifying wastewater or organic material, by toxic substances, by a decrease in temperature, or by a too high volume load in the bioreactor; for example, by feeding Wastewater out of a starch processing plant, which incurs the possibility of acetic acid toxification. Consequences are as follows:

- Increase in the amount of uncharged fatty acid molecules this sometimes leads to an increase in the hydrogen content in the substrate and CH_4 production, sometimes to the detriment of the methanation.
- Inhibition of the methanation by an increase in the proportion of un hydrolyzed inhibitors, for example, sulfa de.
- A rise in the pH value due to degradation of sulfate to H_2S .
- Inhibition of reactions by a rise in the proportion of free ammonia.

A fall in the pH value and a rise in the CO_2 in the biogas are an indication of a disturbance of the fermentation process. A first sign of the acidify caption is an increasing propionic acid concentration. Measures for the prevention of excessive acidify caption are as follows:

- Termination of the substrate supply, so that the methanogen bacteria are able to degrade the acid.

- Reduction of the organic space load (increase in the residence time).
- Increase in the buffering potential of the substrate by addition of selected cosubstrates, in particular if the buffering potential of the substrate is low. It must be taken into consideration that the buffering potential changes because of the removal of the CO₂.
- Continuous removal of the acids.
- Addition of neutralizing substances: milk of lime [CaO, Ca(OH)₂], sodium carbonate (Na₂CO₃), caustic soda solution (NaOH).
- Addition of diluting water.
- Emptying and restarting the fermenter. As a result of the feed of special caustic solutions for adjusting the pH value or of the addition of cleaning and disinfecting agents, values of pH > 10 can arise in the reactor, which will lead to an irreversible loss of the activity of the bacteria. Cleaning and disinfecting agents should therefore be tested for their inhibiting potential before their first application in the plant.

- **Carbon to nitrogen ratio C\N:**

The cell contains the 10 macro - elements carbon dioxide, oxygen, hydrogen, nitrogen, phosphate, sulphate, potassium, calcium, magnesium, and iron, and the main trace minerals manganese, molybdenum, zinc, copper, cobalt, nickel, vanadium, boron, chlorine, sodium, selenium, silicon, and tungsten. The essential growth factors are amino acids, purines/pyrimidines, and vitamins. The optimum ratio of all nutrients and trace minerals results from the elemental composition of the cell biology. The C : N ratio of the substrate should be in the range 16 : 1 – 25 : 1. However, this is only an indication, because nitrogen can also be bound in lignin structures. If the lignin content (ADL) in the biomass is higher than 7%, the methane output is lower than in a biomass containing less than 6.5% of lignin with

a further comparable composition. The need for nutrients is very low because with the anaerobic process not much biomass is developed, so that for methane formation even a nutrient ratio

C : N : P : S of 500 – 1000 : 15 – 20 : 5 : 3

and/or an organic matter ratio of

COD : N : P : S = 800 : 5 : 1 : 0.5

is sufficient. In general, intensive frothing is caused by a high content of nitrogen resulting from a high amount of wheat. Substrates with a too low C : N ratio lead to increased ammonia production and inhibition of methane production. A too high C : N ratio means a lack of nitrogen, having negative consequences for protein formation and thus the energy and structural material metabolism of the microorganisms. A balanced composition is essential, for example, the mixture of rice straw and latrine waste that is common practice in China and the co-fermentation of elephant dung with human waste as applied in Nepal.^[18]

- **Nitrogen Inhibition:**

One of the most significant endogenous inhibitors is ammonia (NH₃). Ammonia is created during the bacterial degradation of nitrogen-containing substances such as proteins. Nitrogen is essential for bacterial growth and ammonia is an important source of nitrogen. But ammonia at high concentrations is highly toxic to the bacteria. In an aqueous solution ammonia is always found in an equilibrium with ammonium (NH₄⁺). This equilibrium is determined by the acidity, pH and temperature of the environment and, as ammonium is not as toxic as ammonia, this equilibrium is important:



- **Substrate (feedstock):**

Nearly all organic matter can be decomposed anaerobically, but the degree of decomposition can be increased in various ways. Lignin is, however, indigestible.

- **Comminution:**

The finer the material, the larger the relative surface and the easier it is for the bacteria to Attack the material.

- **Dry matter content:**

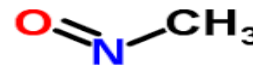
For bacteria to be able to degrade the material, the dry matter content must not be higher than around 50%. In a biogas plant, however, it should only be around 8-10%, if it is to remain liquid enough to be pumped. A slightly higher level can be tolerated in special reactor Types with a direct feed line.

- **Organic load:**

The rate at which biomass is added to the reactor has to be adjusted to the growth rate of the Methanogens and organic acids have to be removed at the rate at which they are produced. The normal load for a CSTR reactor is 1-6 kg COD/m³ reactor volume/day. If more biomass is added than the bacteria are able to degrade, the process will become Acidic. The biomass also has to be fed to the reactor at an even rate and volume, preferably as a continuous feed. If the substrate has to be changed, this must be done gradually, so that Bacteria can adapt to the new conditions.

2.5.4 Methane, nitroso-

- Formula: CH₃NO
- Molecular weight: 45.0406 Da
- Average mass : 45.041 Da



The monomers of nitrosomethane (CH₃NO) are blue in color in solvents of a low dielectric constant, while in aqueous solutions, they are colorless.

Methanol is an alternative fuel for internal combustion and other engines, either in combination with gasoline or directly ("neat"). It is used in racing cars in many countries. In the U.S., methanol fuel has received less attention than ethanol fuel as an alternative to petroleum-based fuels. In general, ethanol is less toxic and has higher energy density, although methanol is less expensive to produce sustainably and is a less expensive way to reduce the carbon footprint. However, for optimizing engine performance, fuel availability, toxicity and political advantage, a blend of ethanol, methanol and petroleum is likely to be preferable to using any of these individual substances alone[citation needed].

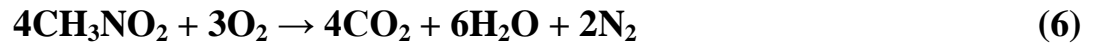
Methanol may be made from hydrocarbon or renewable resources, in particular natural gas and biomass respectively. It can also be synthesized from CO₂ (carbon dioxide) and hydrogen.^[19]

The use of biogas in engine lead to a decrease in periodic maintenance periods in the engine compared to any other type of gas, were biogas combustion products do not corrode the metal parts of the engine unlike fuel combustion products that is full of sulfur oxides and lead compounds and less heat generated via bio gas during combustion and more safe if any accident happened where gas is lighter though less able to start fires and prolonged its life time, the safety of gas cylinder bear high pressure up to 200 bar. Care works on bio gas changes oil after 10 to 15 thousand km compared to cars work on fuel were it change the oil every 2 to 5 thousand km, the energy content per cubic meter of biogas is 6.5kwh.

- **As fuel for engines:**

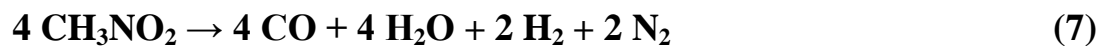
Nitro-methane is sometimes used as fuel for rockets, some aircraft and some racing cars.

The nitrogen content of the nitro-methane compound can burn with the lowest amount of air oxygen relative to other hydrocarbons according to the combustion equation:



Where 1.7 kg of air per kg of nitro methane is needed, compared with 14.6 kg for gasoline. However, the energy density produced by gasoline when burned is four times greater than the combustion of nitro methane.

Nitro-methane can be used as a propellant fuel without the need for oxygen as it disintegrates thermally in isolation from the air as follows:



The nitro-methane complex has a regular laminar combustion velocity of 0.5 m / s and is higher than that of gasoline, so nitro-methane is used for high-speed motors.

Nitro-methane can be mixed with hydrazine or methanol in some special fuel mixtures.^[20]

CHAPTER THREE
BACKGROUND STUDIES

Iscia, G.N. Demirerb (2007), The objective purpose of this study was to investigate the anaerobic treatability and methane generation potential of cotton stalks, cotton seed hull and cotton oil cake by performing a biochemical methane potential test. In addition, the effects of nutrient and trace metal supplementation on the biogas yield were also examined. Most of the gas production in the bottles was completed within **23** days at **32.2 °C**. Since Basal Medium (BM) provided all the necessary micro and macro nutrients required for optimum anaerobic microbial growth, the bottles with (BM) generally had higher biogas production rates than the ones without (BM). The addition of (BM) positively affected gas production amount and rate for cotton stalks, the increase was not as significant as the other cotton wastes. The average amount of biogas generated from three different cotton wastes was **76 ml** in the presence of (BM), which indicates that **13 kg** of cotton waste is needed to produce **1m³** of pure methane. ^[21]

V. Singhal, J.P.N. Rai (2008), The paper reports on the biogas production from water hyacinth (*Eichhornia crassipes*) and channel grass (*Vallisneria spiralis*) employed separately for phytoremediation of lignin and metal-rich pulp and paper mill and highly acidic distillery effluent. Biogas production from channel grass was relatively greater and quicker (maximum in **6–9 days**) than that from water hyacinth (**in 9–12 days**). The plants grown in different effluent treatments for **45-days** were chopped separately to about 20-mm pieces, sun dried, finally oven dried at **60 °C** and ground to fine powder as substrate for laboratory scale digesters. Actively digested dairy cattle manure slurry was collected from a 6-m³ size biogas plant at Pantnagar, filtered and used as inoculum. Several factors are known to influence anaerobic digestion and biogas production including **C/N ratio C/P ratio**. ^[22]

J. Hoeks (1983), This paper describes the significance of biogas production in a waste tip with respect to leaching of contaminants into the groundwater, the rate of gas production, injury to vegetation, explosion hazards and recovery and utilization of the biogas produced, very high gas production rate in the early years after dumping of the waste is caused by the degradation of readily degradable food wastes. In this period the gas production rate can be 25-50 m³ ton⁻¹ year⁻¹ or even higher when only municipal refuse has been dumped, Complex organic materials are broken down by hydrolysis to smaller and more soluble degradation products like simple sugars, amino acids, alcohols, fatty acids and other low molecular compounds and CO₂. This stage in the fermentation process is called the "acidification stage". The next stage is the "methane fermentation stage". The hydrolysis products are transformed to fatty acids, which are fermented by methane-producing bacteria yielding biogas consisting of CO₂ and methane as end products. The methane fermentation process is rather sensitive to the environmental conditions, e.g. Temperature, acidity, redox potential and availability of nutrients. Biogas can cause injury to vegetation or create explosion hazards in buildings on or in the near vicinity of the waste disposal site. Another interesting aspect is that the biogas produced can be recovered and utilized as an energy source. Like pH value and redox potential and processes like dissolution and precipitation of insoluble compounds dominate the leaching process^[23]

Prema Viswanath (1991), In this paper observations made on the use of various fruit wastes in succession or mixed as feedstock for biogas production are presented. The mango, orange, pineapple and tomato processing wastes were collected from fruit processing, jackfruit and tomato can be used as feedstock for biogas production by using them in succession for short periods of time (5 days) without any supplementation with nitrogen under mesophilic conditions and

banana wastes the samples were sun-dried, ground and stored at 12° c and used throughout these studies.^[24]

Prasad Kaparaju (2009), In this study the straw was cut into 1–5cm pieces on the field by forage harvester and stored in containers at ambient temperature until further use. For chemical analysis and biogas potential assays, wheat straw was milled to 1mm. The dry matter (DM) content was 90–91% .The first step being operated at temperature of 80C and residence time around 6 min. The presoaking wheat straw was then heated up in stage two to approx.180C for 15min. followed by heating at 190C for 3min. in a third stage Eight grams of the solid fiber fraction were mixed with 60ml of a 0.2M acetate buffer (pH 4.8). Prehydrolysis of the solid fraction was performed at 50C for 24h at an enzyme loading of 15 FPU/g DM filter cake using Cellubrix L. After liquefaction, the flasks were supplemented with a second dose of Cellubrix L enzyme at a loading of 20 FPU/g DM and 0.2ml of urea (24%). The suspensions were then inoculated with 0.2g yeast after cooling down to room temperature. The flasks were sealed with a loop trap filled with glycerol and incubated at 32C for 6–8h. The CO₂-production was followed by measuring the weight loss indicating the ethanol yield (=0.51g,CO₂). The final ethanol was measured by HPLC, Ethanol production started immediately without any lag phase. Was achieved within the first 50h and further increased after 6 days, accounting for 80% of ethanol. No inhibition was found during the fermentation.^[25]

Vladimir Strezov (2008), This work investigates thermal conversion of elephant grass to bio-gas, bio-oil and charcoal under two heating rates of 10 and 50C/min. The energy required to pyrolysis elephant grass was evaluated using computer aided thermal analysis technique, while composition of the resultant bio-gas and

bio-oil products were monitored with gas chromatographic and mass spectroscopic techniques. At **500C**, the bio-gas compounds consisted primarily of CO₂ and CO with small amounts of methane and higher hydrocarbon compounds. The heat of combustion of the bio-gas compounds was estimated to be 3.7–7.4 times higher than the heat required to pyrolysis elephant grass under both heating rates, which confirms that the pyrolysis process can be self-maintained. Faster heating rate was found to increase the amount of liquid products by 10%, while charcoal yields remained almost the same at 30%. The bio-oil mainly consisted of organic acids, phthalate esters, benzene compounds and amides. The amount of organic acids and benzene compounds were significantly reduced at 50C/min, while the yields of phthalate esters and naphthalene compounds increased. The difference in bio-oil composition with increased heating rate is believed to be associated with the reduction of the secondary reactions of pyrolysis, which are more pronounced under lower heating rate.^[26]

Anthony Njuguna Matheri (2016), Biogas production follows four fundamentals processes. These processes include; hydrolysis, acidogenesis, acetogenesis and methanol genesis In the present study, anaerobic digestion of pig waste and grass clippings were studied in laboratory experiments in a 10 liters digester under constant temperature of 37 C°. To determine biogas production rate, a batch digester was fed with the co-digested substrates and inoculum under pre-set conditions of 37 C° and pH of 7pH was neutralized by a solution of 8g NaOH in 100ml and H₂SO₄. The digester was flushed with nitrogen to expel the oxygen and make the process anaerobic. It was then immersed in the water bath and kept under constant temperature. The gas produced was measured using downwards displacement method on a daily basis until the end of retention time. The elemental

analysis of pig waste indicated low C/N ratio compared to grass clippings. Through co-digestion, the C/N ratio increased to 17.28 .^[27]

Etelka Kovács Roland Wirth (2014), This study demonstrates that appropriate adaptation of the microbial community to protein-rich biomass can lead to sustainable biogas production. The process of acclimation to these unusual mono-substrates was controlled by the protease activity of the microbial community. Meat extract (C/N = 3.32) and kitchen waste (C/N = 12.43) were used as biogas substrates. Metagenome analysis highlighted several mesophilic strains that displayed a preference for protein degradation. *Bacillus coagulans*, *Bacillus subtilis* and *Pseudomonas fluorescens* were chosen for detailed investigation. Pure cultures were added to biogas reactors fed solely with protein-rich substrates. The bio-augmentation resulted in a 50% increase in CH₄ production even without any acclimation. The survival and biological activity of the added bacteria were followed in fed-batch fermenters by qPCR. Stable biogas production was observed for an extended period of time in laboratory CSTR reactors fed with biomass of low C/N.^[28]

Slawomir Jan Jablonski (2016), Drought and pest resistance, together with high oil content in its seeds, make *Jatropha curcas* a good oil source for biodiesel. Oil cake from *J. curcas* is not suitable for animal feeding and thus may be profitably used for additional energy production by conversion into biogas; however, the anaerobic digestion process must be optimized to obtain good efficiency. We subjected oil cake to thermal and acidic pretreatment to deactivate protease inhibitors and partially hydrolyze phytate. We then digested the samples in batch conditions to determine the effects of pretreatment on biogas production. Thermal

pretreatment changed the kinetics of anaerobic digestion and reduced protease inhibitor activity and the concentration of phytate; however, biogas production efficiency was not affected (0.281m³kg⁻¹). To evaluate the possibility of recirculating water for SSF hydrolysis, ammonium nitrogen recovery from effluent was evaluated by its precipitation in the form of struvite (magnesium ammonium phosphate). Concentration of ammonium ions was reduced by 53% (to 980 mg L⁻¹). We propose a water-saving concept based on percolation of *J. curcas* cake using anaerobic digestion effluent and feeding that percolate into a methanogenic bioreactor.^[29]

Fabiana Passos (2015), Biological methods operate with mild conditions, where microalgae cell wall is degraded enzymatically rather than disrupted as in mechanical techniques. Indeed, enzymatic pretreatment consists in converting molecules from the cell wall into more usable substrates for anaerobic microorganisms. Therefore, it is necessary to know the composition of microalgae cell wall in order to select the appropriate enzymes. For most species it is composed of cellulose, hemicellulose, pectin and glycoprotein. The hydrolysis of cellulose and hemicellulose is well studied for lignocellulosic biomass biodegradation. The enzymes investigated in this study were cellulase for enhancing cellulose hydrolysis, along with glucohydrolase and an enzyme mix composed of cellulase, glucohydrolase and xylanase for enhancing hemicellulose hydrolysis. The goal was to evaluate organic matter solubilisation and methane yield increase after enzymatic pretreatment of microalgal biomass grown in open ponds for wastewater treatment, Biogas production was determined periodically by measuring the pressure increase with an electronic manometer. After each measurement gas was released until atmospheric pressure. Samples from the gas headspace volume were taken every 2-3 days to determine biogas composition

(CH₄/CO₂) by gas chromatography (GC). Results were expressed as methane yield calculated by subtracting the blank results to each trial, divided by the amount of microalgal biomass (g VS) added to each bottle.^[30]

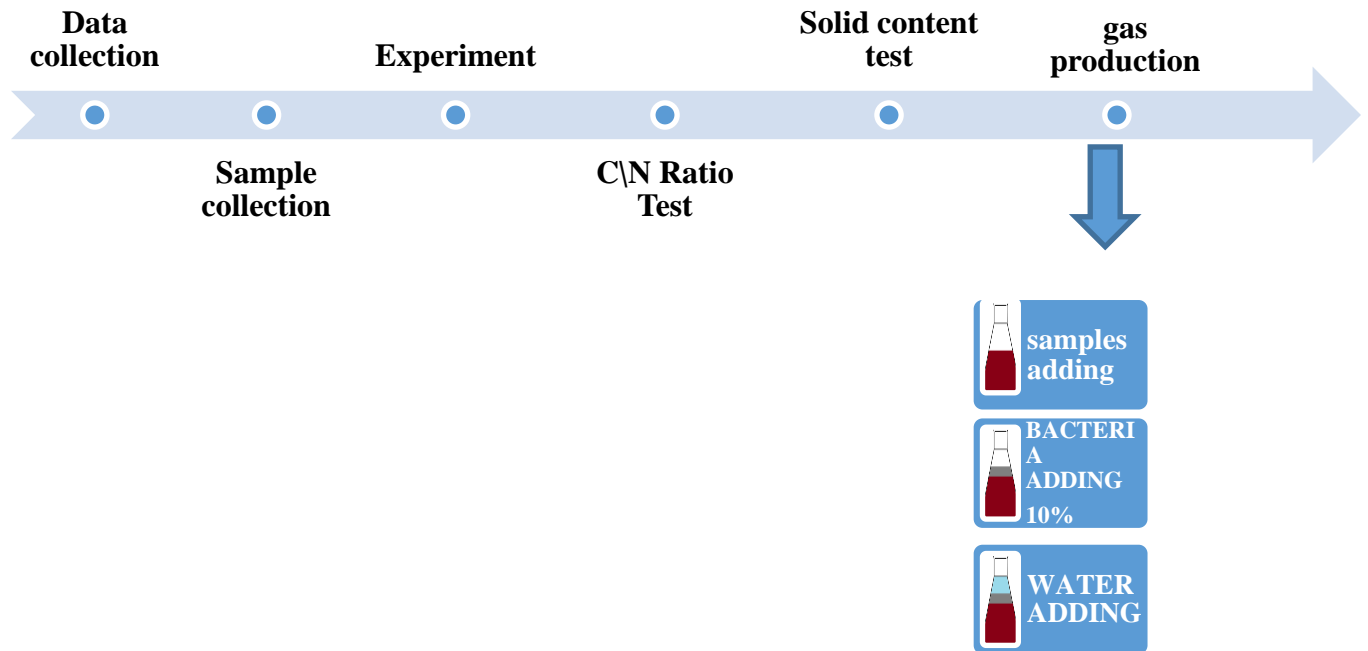
RABAH (2010), The biogas production potential of abattoir waste at different retention time was investigated and the bacteria associated with the production as well as the pH of the slurry before and after the biogas production was determined. It also indicated a slight shift from a neutral medium to a slightly acidic environment in all the digesters. The plates were replicated three times. Modified Mackintosh and Fields pattern of anaerobic jar was used to incubate the plates. The residual oxygen (O₂) in the anaerobic jar was evacuated by placing a kindled match stick, which quenched immediately the left-over oxygen was exhausted. The jar was incubated for a period of 72 hours at 37C°. The highest volume of biogas (2240cm³) was obtained in week 2 while the least volume (1820cm³) was obtained in week 4. Significant difference (p < 0.05) was observed in the volume of biogas produced in the first and second week as well as to that of third and fourth week. However, no such difference (p <0.05) was observed in the volume of biogas produced in the third and fourth weeks.^[31]

Alberto Vergara-Ferna' ndeza (2007), The marine algae are considered an important biomass source; however, their utilization as energy source is still low around the world. The technical feasibility of marine algae utilization as a source of renewable energy was studied to laboratory scale. The anaerobic digestion of *Macrocystis pyrifera*, *Durvillea antarctica* and their blend 1:1(w/w) was evaluated in a two-phase anaerobic digestion system, which consisted of an anaerobic sequencing batch reactor (ASBR) and an upflow anaerobic filter (UAF). The results show that 70% of the total biogas produced in the system was generated in

the UAF, and both algae species have similar biogas productions of 180.4(71.5)mLg₋₁ dry algae d₋₁, with a methane concentration around 65%. The same methane content was observed in biogas yield of algae blend; however, a lower biogas yield was obtained. In conclusion, either algae species or their blend can be utilized to produce methane gas in a two-phase digestion system.^[32]

Yan Li (2011), The objective of this work was to examine the feasibility of biogas production from the anaerobic co-digestion of herbal extraction residues with swine manure. Batch and semi-continuous experiments were carried out under mesophilic anaerobic conditions. Batch experiments revealed that the highest specific biogas yield was 294 mL CH₄ g₋₁ volatile solids added, obtained at 50% of herbal-extraction residues and 3.50 g volatile solids g₋₁ mixed liquor suspended solids. Specific methane yield from swine manure alone was 207 mL CH₄ g₋₁ volatile solid added d₋₁ at 3.50 g volatile solids g₋₁ mixed liquor suspended solids. Furthermore, specific methane yields were 162, 180 and 220 mL CH₄ g₋₁ volatile solids added d₋₁ for the reactors co-digesting mixtures with 10%, 25% and 50% herbal-extraction residues, respectively. These results suggested that biogas production could be enhanced efficiently by the anaerobic co-digestion of herbal-extraction residues with swine manure.^[33]

CHAPTER FOUR
METHODOLOGY AND RESULTS



4.1 Data collection:

Data collection from scientific papers and references about biogas and medical waste and but the theoretical steps to start a plane to produce biogas from medical waste. Collection of general information about blood banks and their waste in Sudan via Ministry of Health in (MOH) Khartoum the data has been collected to start a tour in Khartoum hospitals (71.8%) as shown in Figure (4.1) below, Percentage of public hospitals (70.4%) and private (29.6%) as shown in Figure (4.2) below.

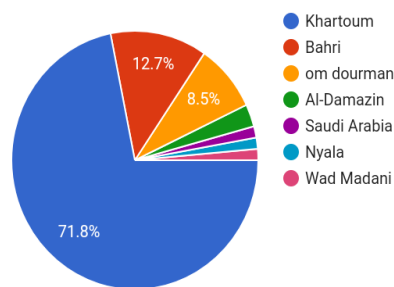


Figure (4. 1): location of hospital

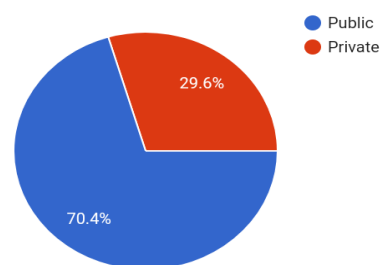


Figure (4.2): hospital type

The Amount of medical waste produced by hospital, 100 bags a day (23.9%), &More than 100 bags a day (32.4%), Less than 100 bags a day (35.2%) as shown in Figure (4.3) below, the bag weight is usually around , 2 k(12.7%),5kg(40.8%),8kg(26.8%),10kg(14.1%)shown in Figure (4.4) below, the Collection of medical waste in hospital happens every Day(67.6%), 2Day(12.7%), 3 Day(8.5%) shown in Figure (4.5) below.



Figure (4.3): Amount of medical waste

Figure (4.4): the bag weight

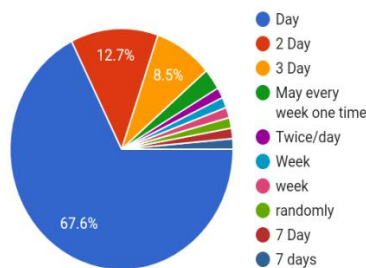


Figure (4.5): Collection of medical waste

The Storing of medical waste in Hallway(5.6%) ,Special warehouse (60.6%), There is no storing area(33.8%)shown in Figure (4.6) below ,and if this storage of medical waste is save yes(59.2%), no(40.8%)shown in Figure (4.7) below, and the Disposal of medical waste from hospital storage happens every Day(47.9%), 3 times a Week(33.8%), 4 times a weak (14.1%),shown in Figure (4.8) below.

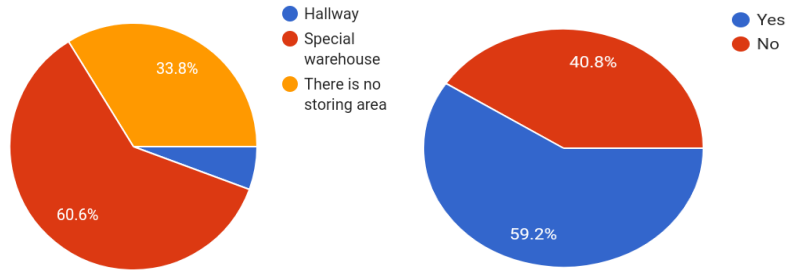


Figure (4.6): Storing of medical waste Figure (4.7): safety of storage of medical waste

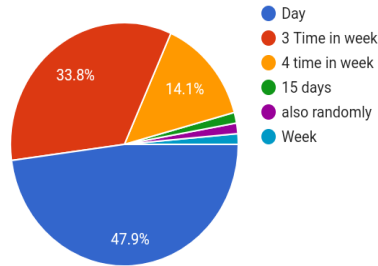


Figure (4.8): Disposal of medical waste

Waste containers what kind of containers do you use No specific container (7%), plastic (63.4%) Metallic (9.9%), Cardboard (8.5%), Bag (21.1%), Box (50.7%) - shown in Figure (4.9) below, and if they have a specific color coding system yes (70.4%), no (29.6%) shown in Figure (4.10) below.

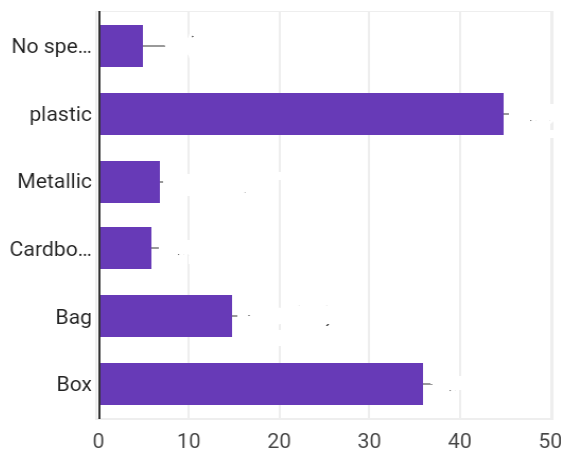


Figure (4.9): Waste containers

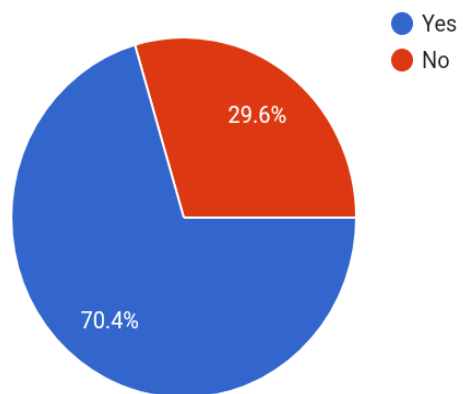


Figure (4.10): specific color coding

Table 4.2 medical waste color code:

Do you use this color coding shown in table (4.14) yes (59.2%), no (40.8%) shown on Figure (4.11), and does the hospital have an environmental policy that includes managing medical waste prevention procedures yes (60.6%) , no (39.4%) shown on Figure (4.12) below.

Yellow	infectious Clinical Waste Hazardous
Orange	Waste which may be “treated”
Purple	Cytotoxic and cytostatic waste
Yellow/black	Offensive/hygiene waste*Offensive/noninfectious wasteNon Hazardous
Red	Anatomical waste for incineration1Body parts ;Organs ;Blood bags; Blood preserves
Black	Domestic (municipal) waste
Blue	Pharmaceutical waste ;Non Hazardous
white	Amalgam waste For recovery

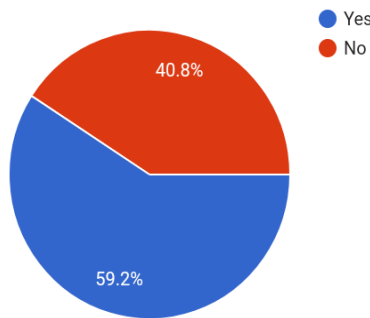


Figure (4.11): use this color coding

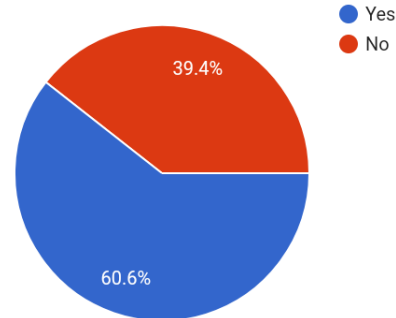


Figure (4.32): environmental policy

hospital department produce the largest amount of medical waste the ER (25.4%), Dialysis unit (16.9%), Laboratories(50.7%), all (31%) , shown on Figure (4.13) below, manage all medical waste in the same way yes (39.4%), no(60.6%), shown on Figure (4.14) below ,kind of medical waste is being treated differently the Dialysis unit waste(8.5%), Laboratories waste(26.8%), Blood bank(29.6%), All (60.6%) shown on Figure (4.15) below.

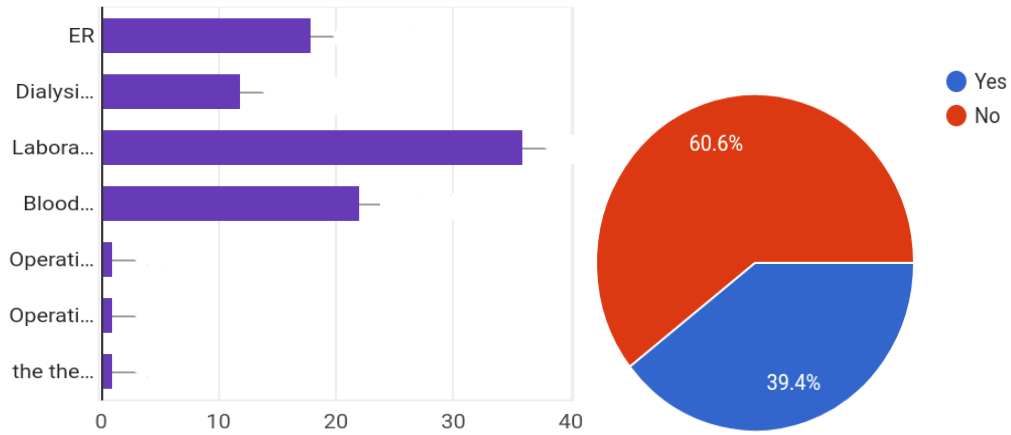


Figure (4.13): largest amount of medical waste Figure (4.14): manage medical waste in ways

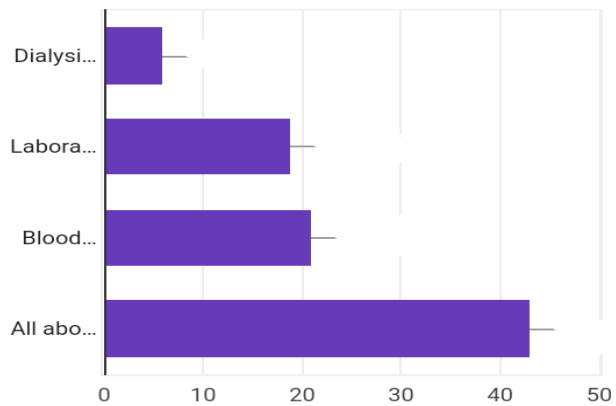


Figure (4.15): medical waste is being treated differently.

Kinds of medical waste is dangerous to deal with Sharps(36.6%), Chemical(18.3%), Pathological(12,7%), Bio medical waste (tissue, blood)(59.2%), shown on Figure (4.16) below, The largest amount of expired blood in hospital comes from the Dialysis unit waste(7%), Laboratories waste(21.1%), Blood bank(54.9%),shown on Figure (4.17) below.

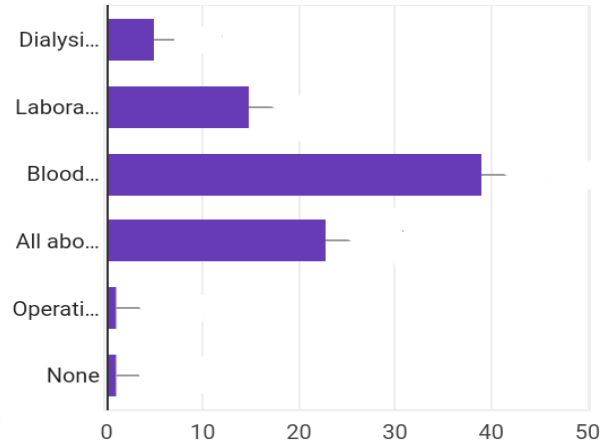
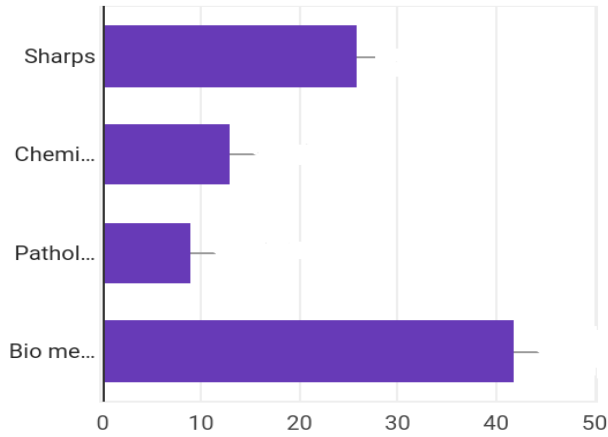


Figure (4.16): dangerous medical waste

Figure (4.17): largest amount of expired blood

The largest amount of waste in Laboratories Blood (77.5%), Solutions (22.5%), shown on Figure (4.18) below. The largest amount of waste in blood bank are Blood (77.5%), Plasma (11.3%), and Blood platelet (4.2%), RBCs (31%), shown on Figure (4.19) below.

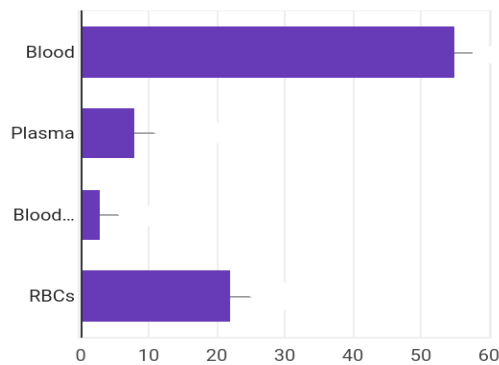
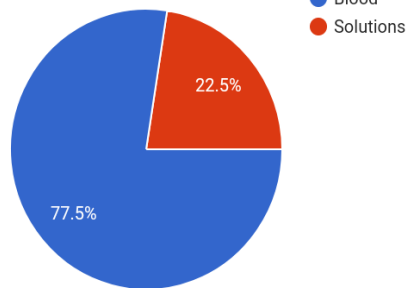


Figure (4.18): waste in Laboratories

Figure (4.19): waste in blood bank

The expired blood bags produced by blood bank weekly are 100 bag (22.5%), More than 100 bag (12.7%), Less than 100 bag (53.3%), shown on Figure (4.20) below, and the expired blood platelet bags produced by blood bank weekly 100 bag (7%), Less than 100 bag (70.4%), shown on Figure (4.21) below, and expired (blood platelet; blood) bags is being managed by send to incinerator (70.4%), landfill(26.8%) shown on Figure (4.22) below.

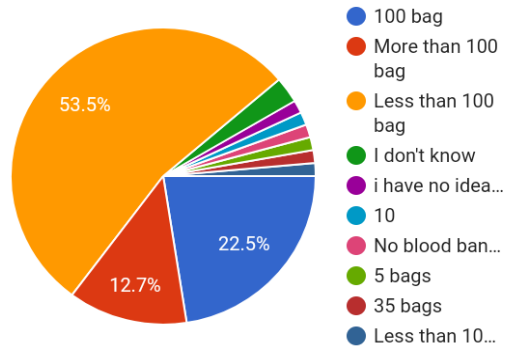


Figure (4.20): The expired blood bags

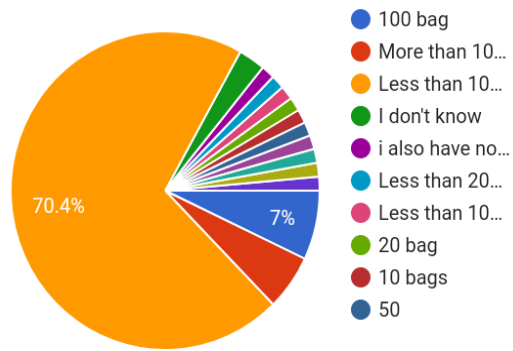


Figure (4.21): expired blood platelet bags

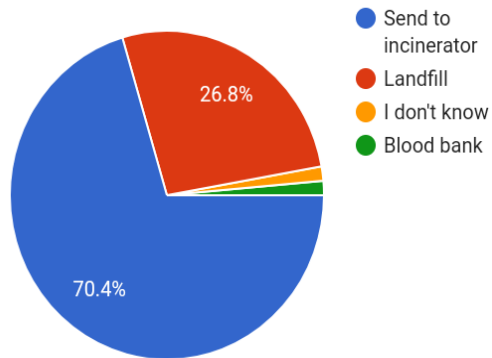


Figure (4.42): bags is being managed via

4.2 Samples collection:

Blood or RBCs or Blood plate let (BPL) Samples have been collected from:

- Blood bank in Stack Lab.
- Blood bank in Alsilah ALtibi Hospital.
- Blood bank in Jafie Ibn Auf Hospital.
- Blood bank in Altamaize Hospital.
- Blood bank in Academic Hospital.
- Academic Turkic Hospital.
- Ibn Sina Hospital.

All authorization were given by Ministry of Health (MOH) to visit and collect samples for the project.

4.3 Experiment:

The experiment toke place in the National Center for Energy Research (NCER) in Khartoum- Soba.

4.3.1 Tools:

- **Balance:**
Determines the mass of something, such as a dry chemical. While balances once used two flat trays one to hold the material and the other to hold weights electronic balances represent the norm in most laboratories shown in Figure (4.23)(4.24).



Figure (4.24): Balance types A.

Figure (4.24): Balance types B.

- **Erlenmeyer flask:**

Used to hold liquids, has narrow neck to prevent splashes shown in Figure (4.25).

- **Beaker:**

Used to hold liquids shown in Figure (4.25).



Figure (4.25): flask and Beaker

- **Crucible and cover :**

Used to hold small amounts of chemicals during heating at high temperatures shown in Figure (4.26).

- **Crucible tongs:**

To hold hot crucibles shown in Figure (4.26).

- **Graduated cylinder:**

Accurately measures liquid volumes shown in Figure (4.26).

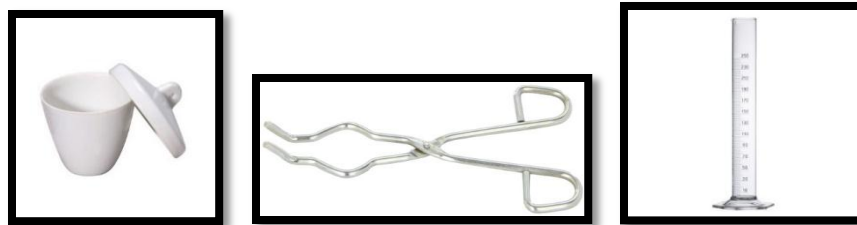


Figure (4.26): Crucible, cover, Crucible tongs and • Graduated cylinder

- **Volumetric pipet :**

Measures small amounts of liquids accurately shown in Figure (4.27).

- **Wash bottle:**

Used to rinse various pieces of laboratory glassware shown in Figure (4.27).

- **Rubber stopper:**

Used to cover ends of test tubes and flasks shown in Figure (4.27).



Figure (4.27): Volumetric pipet, Wash bottle and Rubber stopper

- **Thermometer:**

Measures temperature (science uses degrees in Celsius) shown in Figure (4.28).

- **Dropper pipet or disposable pipet:**

For drawing in a liquid and expelling it in drops shown in Figure (4.28).

- **Safety Goggles:**

Provide the extra protection you need for certain jobs, hobbies, and environments. If you need protection from liquid splash, acid vapors,

airborne dust and impact hazards, Safety Goggles are your best choice shown in Figure (4.28).



Figure (4.28): Thermometer, Dropper pipet and Safety Goggles

- **Medical gloves** shown in Figure (4.29).
- **Lab coat** shown in Figure (4.29).
- **Medical mask** shown in Figure (4.29).



Figure (4.29): Medical gloves, Lab coat and Medical mask

- **Blood bag** shown in Figure (4.30).
- **Blood platelet** shown in Figure (4.30).



Figure (4.30): Blood bag and Blood platelet

4.3.2 Carbon to nitrogen ratio C\N test:

All samples have been collected were tested in Ministry of Petroleum and Gas (MOP) Petroleum Laboratories, Research and Studies (PLRS), in Khartoum Elamarat Street 61, By device called CHNS Elemental Analyzers shown in Figure (4.31) below, and the results of the test are shown in Table (4.2).



Figure (4.31): CHNS

CHNS elemental analyzers provide a means for the rapid determination of carbon, hydrogen, nitrogen and sulphur in organic matrices and other types of materials. They are capable of handling a wide variety of sample types, including solids, liquids, volatile and viscous samples, in the fields of pharmaceuticals, polymers, chemicals, environment, food and energy.

Table (4.2): carbon \ nitrogen ratio in blood component:

Samples	Carbon	Nitrogen
Blood	13.91	4.079
RBCs	13.38	4.007
Blood2	9.119	1.431
Blood platelet	5.1059	1.323

4.3.3 Total solid test:

What is total solid: The term “solids” is generally used when referring to any material suspended or dissolved in water or wastewater or liquid bio waste (blood) that can be physically isolated either through filtration or through evaporation. Solids can be classified as either filterable or non-filterable. Filterable solids may either be settleable or non settleable. Solids can also be classified as organic or inorganic. Total Solids is the term applied to the material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Measurement of Solids can be made in different liquid bio waste or blood samples (howl blood, RBCs and blood platelet) and it is defined as residue upon evaporation of free water. Thus, Total solids are nothing but summation of total dissolved solids and total suspended solids.

- **Principle:**

The sample is evaporated in a weighed dish on a steam bath and is dried to a constant mass in an oven either at 103-105°C or 179-181°C. Total solids/residue is calculated from increase in mass.

- **Tools required :**

1. Crucible.
2. Oven.
3. Desiccators.
4. Analytical Balance.
5. Dish Tongs.
6. Magnetic Stirrer.
7. Wash Bottle. All shown in Figure (4.23) below.

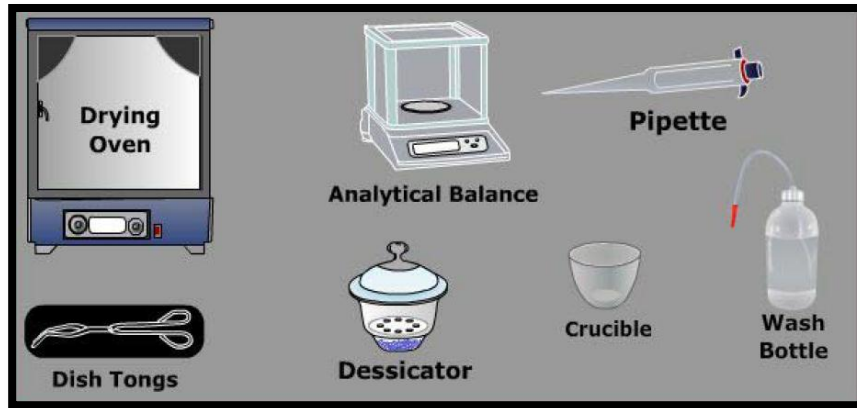


Figure (4.32): Total solid test Materials

- **Procedure:**

1. To measure total solids, take a clean porcelain dish which has been washed and dried in a hot air oven at 105°C for one hour. Now weigh the empty evaporating dish in analytical balance. Let's denote the weight measured as (W1).
2. Now should have to decide what should be the volume of sample to be taken for analysis. Volume may be estimated either from values of specific conductance or general thumb rule.
3. In general, select a sample volume that will yield residue between 100 and 200 mg after drying.
4. Using pipette transfer 75mL of unfiltered sample in the porcelain dish. Samples need to be measured accurately, weighed carefully Note the weight with residue as (W2).
5. Switch on the oven and allowed to reach 105°C . Check and regulate oven and furnace temperatures frequently to maintain the desired temperature range.
6. Place it in the hot air oven and care should be taken to prevent splattering of sample during evaporation or boiling. Dry the sample to get constant mass.

Drying for long duration usually 6 to 12 up to 24 hours is done to eliminate necessity of checking for constant mass.

7. Cool the container in a desiccator. Desiccators are designed to provide an environment of standard dryness. This is maintained by the desiccant found inside. Don't leave the lid off for prolonged periods or the desiccant will soon be exhausted shown in Figure (4.33) below.
8. Keep desiccator cover greased with the appropriate type of lubricant in order to seal the desiccator and prevent moisture from entering the desiccator as the test glassware cools.
9. Should weight the dish as soon as it has cooled to avoid absorption of moisture due to its hygroscopic nature. Samples need to be measured accurately, weighed carefully, and dried and cooled completely. Note the weight with residue as (W3).

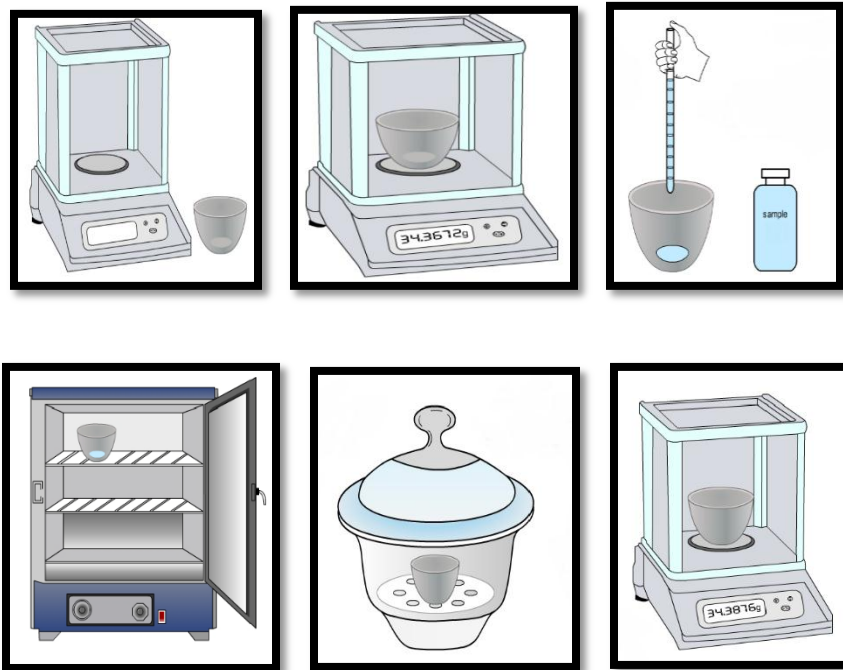


Figure (4.33): Total solid test Procedure

- **Calculation:**

1. Initial weight of the Crucible (W1).
2. Final weight of the Crucible + sample (W2).
3. Final weight of the Crucible + dry sample (W3).
4. Weight of sample (WW) = W2 - W1 g
5. Weight of dry sample (WR) = W3 - W1 g
6. Amount of total solids present in the sample is given by the equation :

$$\text{Total solid \%} = \frac{WR}{WW} \times 100 \quad (8)$$

W = weight of total residue in (mg).

(Therefore multiply W with 1000) V = Volume of the sample (mL)

(To convert mL to L) =mg/L the readings are required to be tabulated.

7. Total solid stander should be in the range of (8% to 10%)
For better production of bio gas.
8. If total solid is larger than this range then the sample will be diluted till it reaches the standard range.
9. Sample dilution is established using water via this equations:

$$\text{Sample weight g} = \frac{10\%}{\text{total solid \%}} \quad (9)$$

$$\text{Water value} = 1000 \text{ g} - \text{sample weight} + (100\text{g}) \quad (10)$$

Note:

1000g = flask capacity

100g = bacteria weight

Table 4-3: Total Solid:

SAMPLE no	WW g	WR g	TOTALSOLID %
1. Whole blood	1.002	0.20	19.9%
2. Whole blood2	0.620	0.21	33.8%
3. Whole blood3	1.000	0.12	12%
4. Whole blood4	1.010	0.13	12.9%
5. Whole blood5	1.017	0.37	36.5%
6. Whole blood6	1.017	0.37	36.5%
7. Whole blood7	1.040	0.31	29.8%
8. RBCs	0.980	0.22	22.4%
9. RBCs 1	1.010	0.29	28.7%
10. RBCs 2	1.010	0.29	28.7%
11. Blood platelet	1.000	0.12	12.0%
12. Blood platelet1	1.020	0.09	8.8%
13. FFP (Fresh Frozen Plasma)	1.020	0.09	8.8%

4.3.4 Bio gas plant:

The plant used in this Experiment called Water Displacement (WD) method used in laboratories shown in Figure (4.34), for chemical reactions involving gases, gas volume measurements provide a convenient means of determining stoichiometric relationships. A gaseous product is collected in a long, glass flask, by displacement of a liquid, usually water. Magnesium reacts with hydrochloric acid.

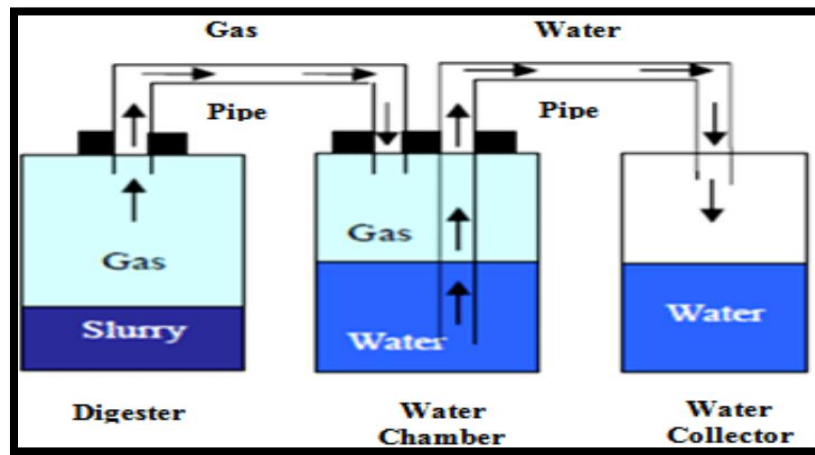


Figure (4.34): Water Displacement (WD)

The collection of the gas is usually done with the use of vessels containing a suitable liquid which is displaced as the gas gets collected. Conversion procedures of biogas from Normal conditions to Standard conditions are presented below. Fluctuation of room temperature and atmospheric pressure during the measurement of gas can contribute errors in volume calculations. Therefore, to apply corrections, the record of change of atmospheric pressure and temperature is important. The gas pressure inside the tube collected over the liquid solution is the sum of the biogas pressure and the vapor pressure.

- **Experimental set up :**

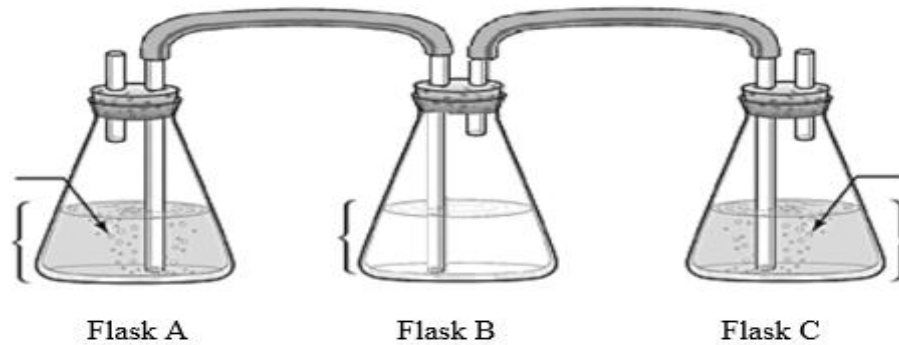


Figure (4.35): Experimental set up A

- **Flask A (The Digesters):**

First adding Blood or RBCs or Blood plate let (BPL), then Bacteria ,then water using the equation (7,8) above as shown in figure (4.36)below :

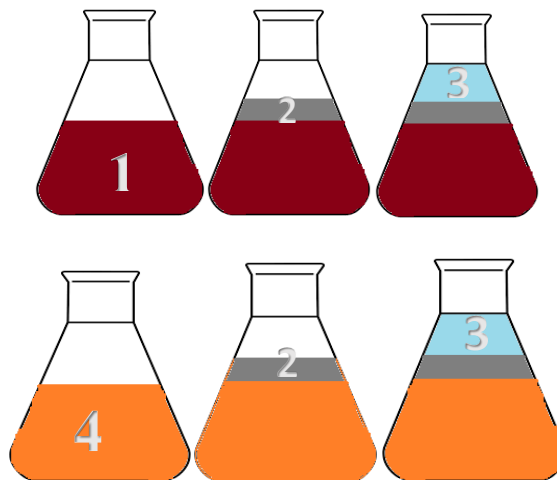


Figure (4.36): Flask A (The Digesters)

1. Blood or RBCs.
2. Bacteria.
3. Water.
4. Blood plate let (BPL).

Note:

1000g = flask capacity

100g = bacteria weight

- **Flask B (Water+ Methyl red):**

First filling the flask with water, then drops of Methyl red (red color), water color becomes pink as shown in figure (4.37)below :

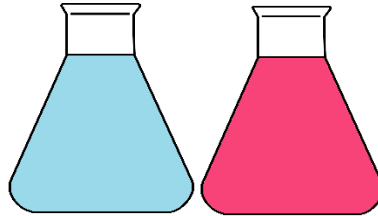


Figure (4.37): Flask B (Water+ Methyl red)

- **Flask C (Empty):**

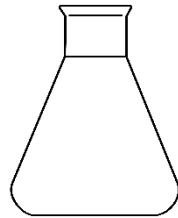


Figure (4.38): Flask C (Empty)

At the beginning of the experiment this flask C will be empty, then after the bacteria start digesting the biomaterial (Blood , RBCs , Blood plate let) in flask A and producing gas the water is displaced in to flask C from flask B by the end of the experiment flask B is full of gases (Methane, CO₂), flask C is full of water as shown in Figure below (4.39).

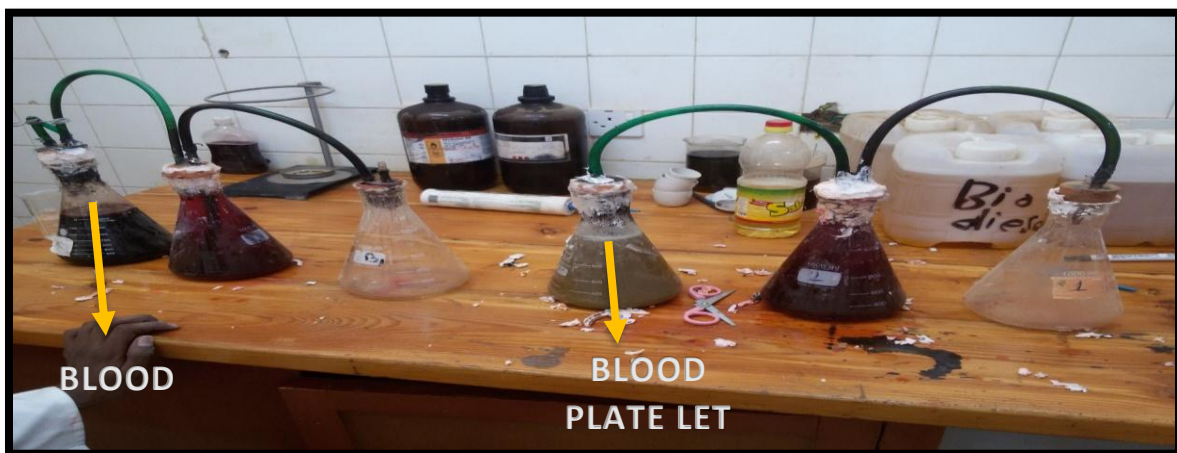




Figure (4.39): Experimental set up B

4.4 Gas testing:

The gas test is carried out Using Gas Chromatograph Mass Spectrometer (GCMS) shown in figure below (4.40)



Figure (4.40): GCMS

4.4.1 Procedure:

- The gas carried out by a syringe by injecting the tube carrying the gas out of flask A (The Digesters) to flask B which is full of gas shown in Figure (4.41).
- And then put the gas taken by the syringe in small glass tube closed with rubber that's prevent leakage of gas sample shown in Figure (4.42).
- And then glass tube put into the machine for analysis.

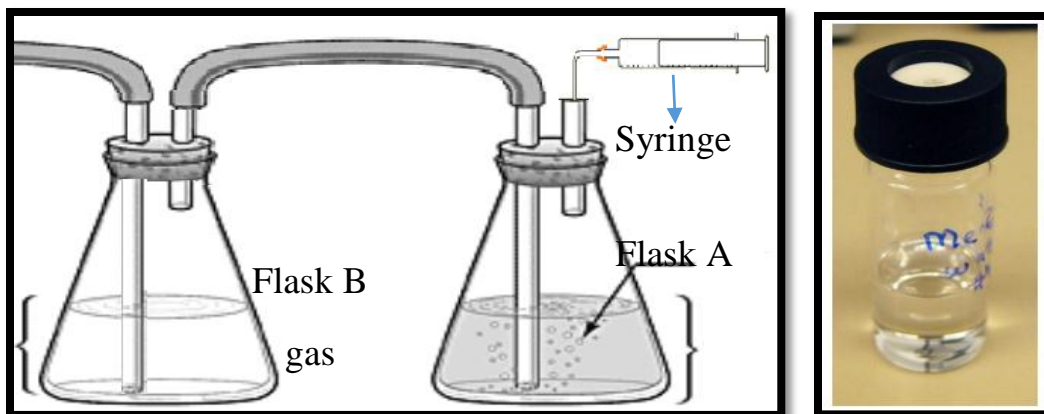


Figure (4.41): carrying the gas out Figure (4.42): glass tube closed with rubber

4.4.2 Stander Device Parameters:

Table 4-4: Stander Device Parameters.

Analytical line 1	
# of Rinses with presolvent	2
# of Rinses with solvent (post)	2
# of Rinses with sample	2
Plunger speed (suction)	High
Viscosity comp. Time	0.2 sec
Plunger speed (Injection)	High
Syringe insertion Speed	High
Injection Mode	Normal
Pumping Times	5
Inj. Port Dwell Time	0.3 sec
Terminal Air Gap	No
Plunger Washing Speed	High
Washing Volume	8 ul
Syringe Suction Position	0.0 mm
Syringe injection position	0.0 mm
Use 3 Solvent Vial	1 vial

4.4.3 Test parameters:

Table 4-5: Device Test parameters.

Analytical line 1	
Column oven temp	30.0 °C
Injection temp	200.00 °C
Injection Mode	Splitless
Sampling Time	0.00 min
Flow control Mode	Linear velocity
Pressure	85.1 kpa
Total Flow	50.0 ml/min
Column Flow	1.61 ml/min
Linear Velocity	45.5 cm/sec
Purge Flow	3.0 ml/min
Split Ratio	-1.0
High Pressure Injection	OFF
Carrier Gas Saver	OFF

- **Oven Temp. Program:**

Table 4-6: Oven Temp. Program.

Rate	Temperature (°C)	Hold Time (min)
-	30.0	0.00
3.00	90.0	0.00

4.5 Results:

4.5.1 First Trails:

- **Whole blood:**
 - The experiment didn't produce any flammable gas, because of error in the calculations of the carbon nitrogen C/N ratio.
 - cotton should have been added to increases C/N ratio.
- **Whole blood 2:**
 - The experiment didn't produce Methane CH_4 which has blue color flame, but it did produce carbon dioxide CO_2 which has red/yellow color flame.
 - The experiment environment was cold, the bacteria didn't work efficiently because of temperature degree.
- **Whole blood 3:**
 - The experiment didn't produce any flammable gas.
 - Technical error were the blood leakage from Flask A to Flask B were that led to fungal growth inside the flask and rots, the experiment were disposed of.
- **Whole blood 4:**
 - The experiment didn't produce any flammable gas, because of error in the calculations of the carbon nitrogen C/N ratio the sample didn't take enough time in the oven under 150 0C degree for 24 hours .
- **Whole blood 7:**
 - The experiment didn't produce Methane CH_4 which has blue color flame yet, but it did produce carbon dioxide CO_2 which has red/yellow color flame.

- The experiment still working at the National Center for Energy Research (NCER) in Khartoum- Soba.

- **Blood platelets 1&2:**

- The experiment didn't produce any flammable gas.

- Carbon nitrogen C/N ratio is low 1.3 to 5.1 compared to blood.

- Blood platelets are non-cellular bodies that do not contain nuclei or cell membranes, they are made up of cytoplasm, that's mean bacteria can't feed of it.

- **FFP:**

- Plasma content are (92% water and, 7% proteins, 1% enzymes and hormones) the carbon presence's is very low for the bacteria to feed of it.

4.5.2 Second Trails:

- **RBCs:**

The experiment started to produce gas from the digester at day twenty fifth 25 shown in Figure (4.43) below, and it continued to producing gas for 150 days, the full quantity of produced gas were 1470 ml from 446.4 g of RBCs with temperature value of 31.57 °C .

- **RBCs 1:**

The experiment started to produce gas from the digester at day fifteen 15 shown in Figure (4.44) below, and it continued to producing gas for 110 days, the full quantity of produced gas were 2625 ml from 348.4 g of RBCs with temperature value of 29.44 °C .

- **RBCs 2:**

The experiment started to produce gas from the digester at day 12 shown in Figure (4.45) below, and it continued to producing gas for 90 days, the full quantity of produced gas were 304 ml from 348.4 g of RBCs with temperature value of 29.25 °C .

- **Whole Blood 5:**

The experiment started to produce gas from the digester at day 5 shown in Figure (4.46) below, and it continued to producing gas for 20 days, the full quantity of produced gas were 3075 ml from 274 g of whole blood with temperature value of 29.25 °C .

- **Whole Blood 6:**

The experiment started to produce gas from the digester at day 6 shown in Figure (4.47) below, and it continued to producing gas for 20 days, the full quantity of produced gas were 2570 ml from 274 g of whole blood with temperature value of 29.89 °C .

NOTE: Velocity of production and length of time period is determined by the concentration of bacteria.

- **Concentrated bacteria** $\xrightarrow{\text{digestion ratio}}$ **Fast** $\xrightarrow{\text{time period}}$ **less time**
- **Un Concentrated bacteria** $\xrightarrow{\text{digestion ratio}}$ **low** $\xrightarrow{\text{time period}}$ **more time**

Table 4-7: summary of second Trails outcome.

Experiment	Quantity	Production
RBCs	446.4 g	1470 ml
RBCs 1	348.4 g	2625 ml
RBCs 2	348.4 g	304 ml
Whole Blood 5	274 g	3075 ml
Whole Blood 6	274 g	2570 ml

4.5.3 Experiment reading Graphs:

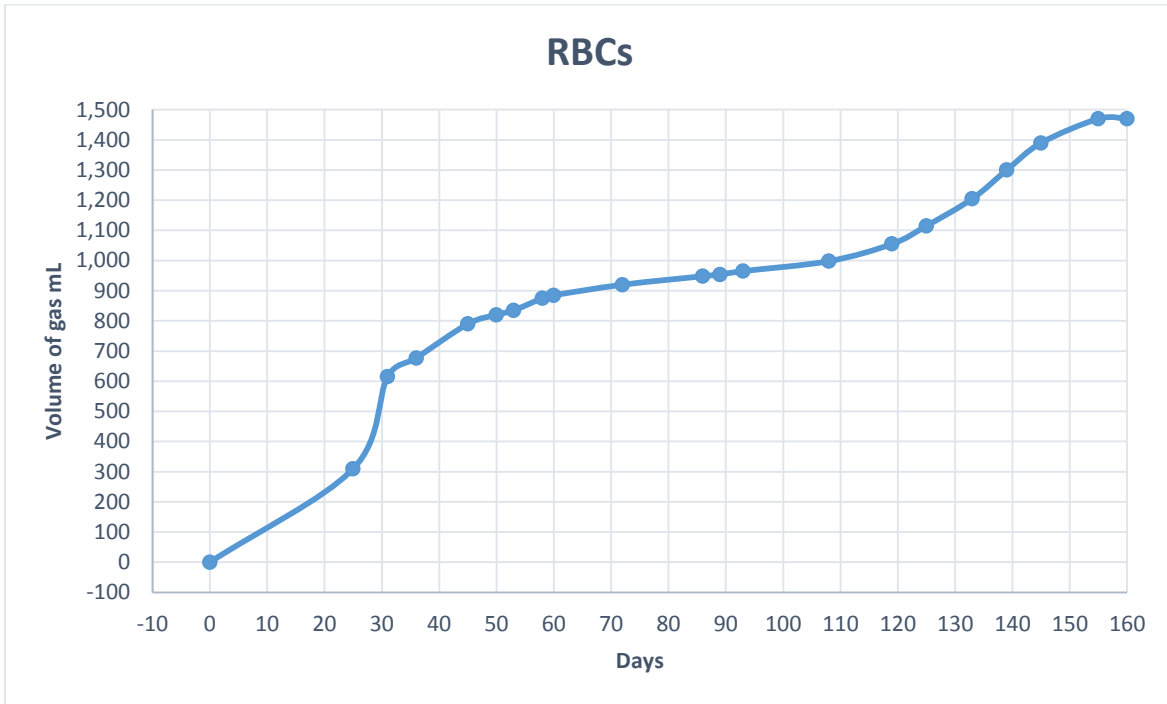


Figure (4.43): RBCs

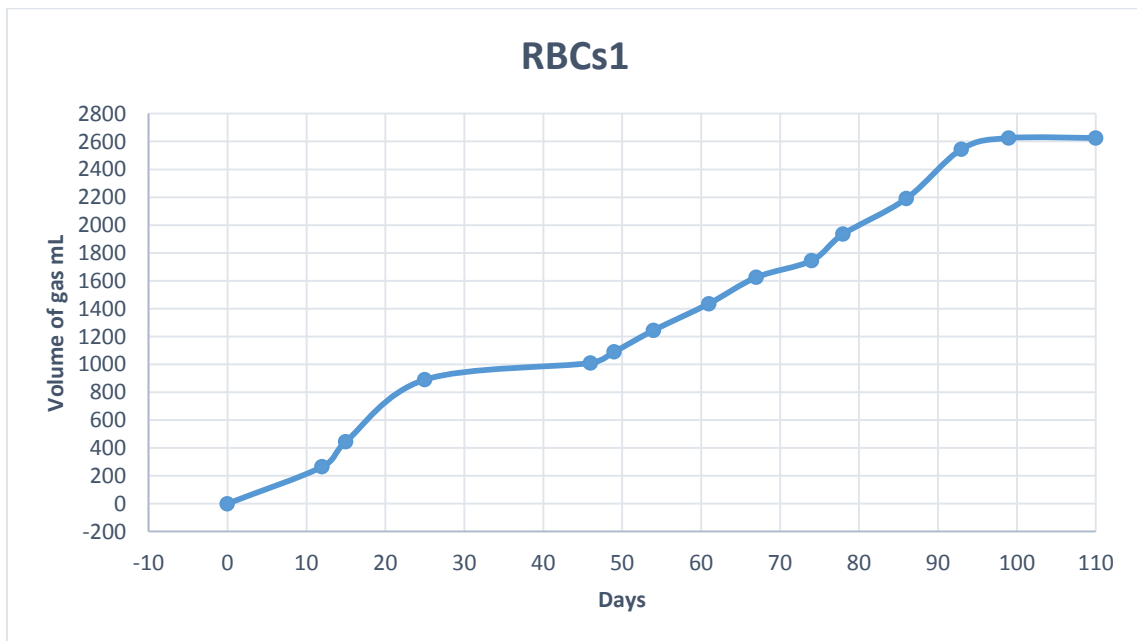


Figure (4.44): RBCs 1

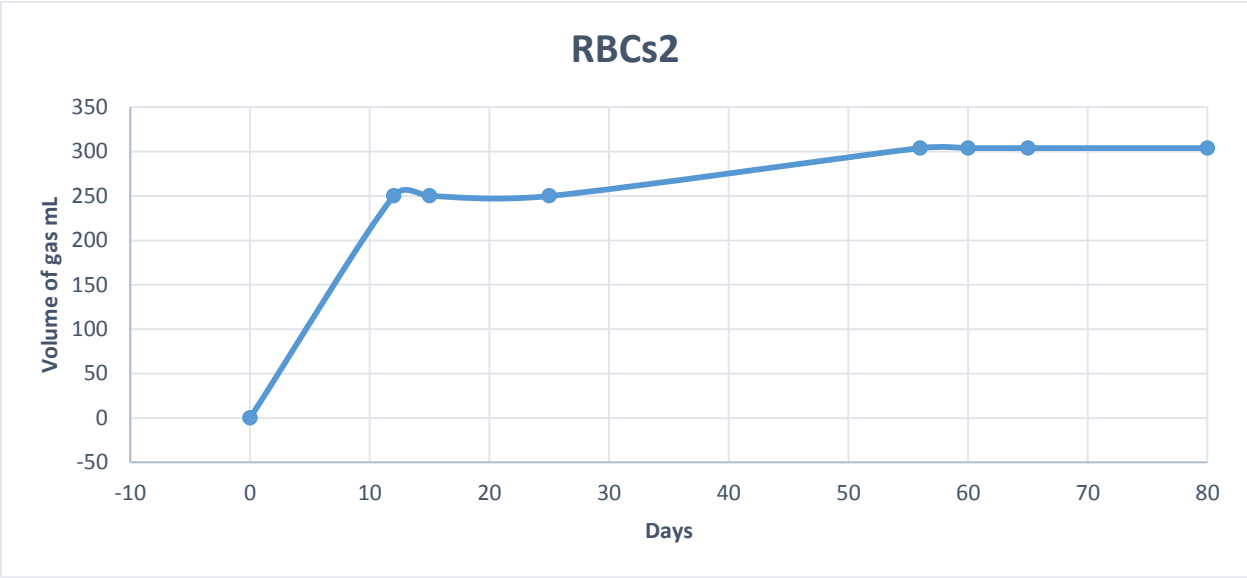


Figure (4.45): RBCs2

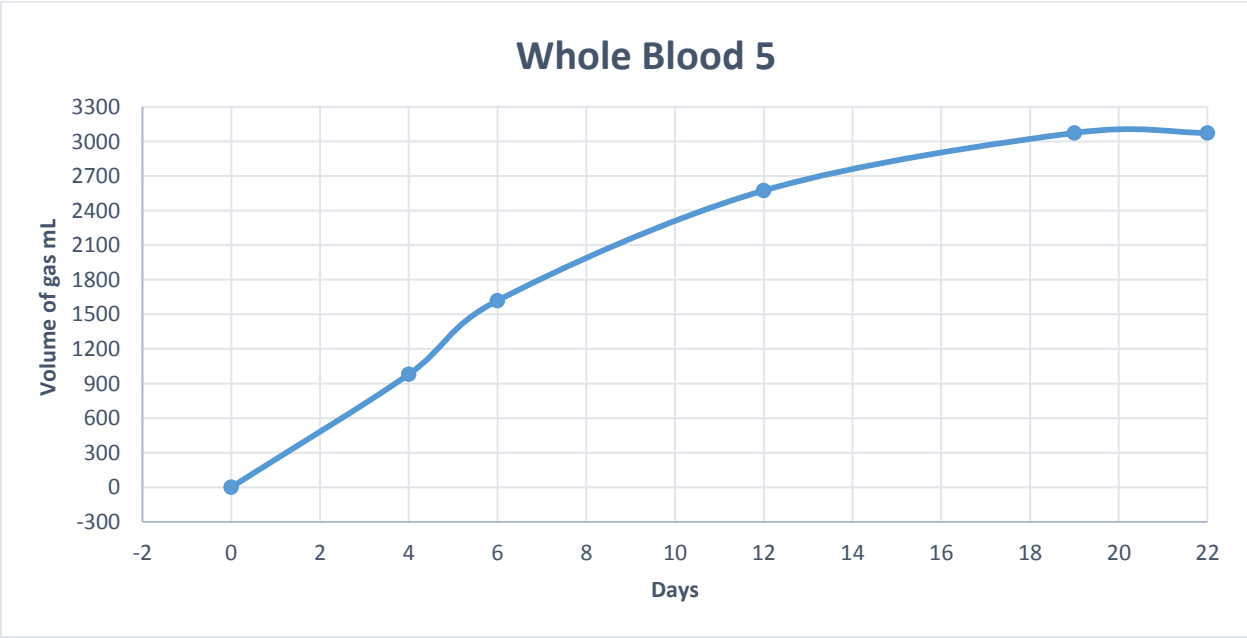


Figure (4.46): whole blood 5

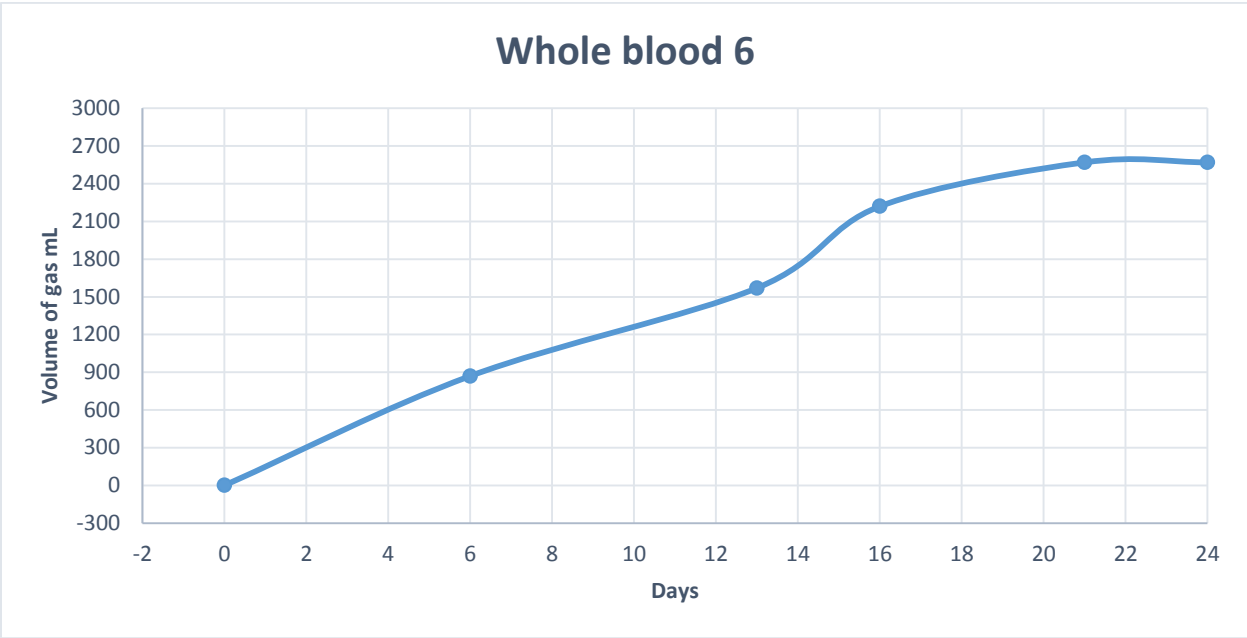
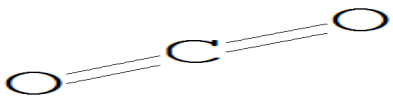
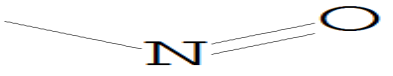


Figure (4.47): whole blood 6

4.5.2 Final Results:

The result test were performed in University of Medical Sciences and Technology (UMST) via mass spectrometer.

Table 4-8: Final Results.

Name	Ret-Time	Area%	Formula	Structure
Carbon dioxide	1.195	0.55	CO ₂	
Methane, nitroso-	1.375	99.45	CH ₃ NO	

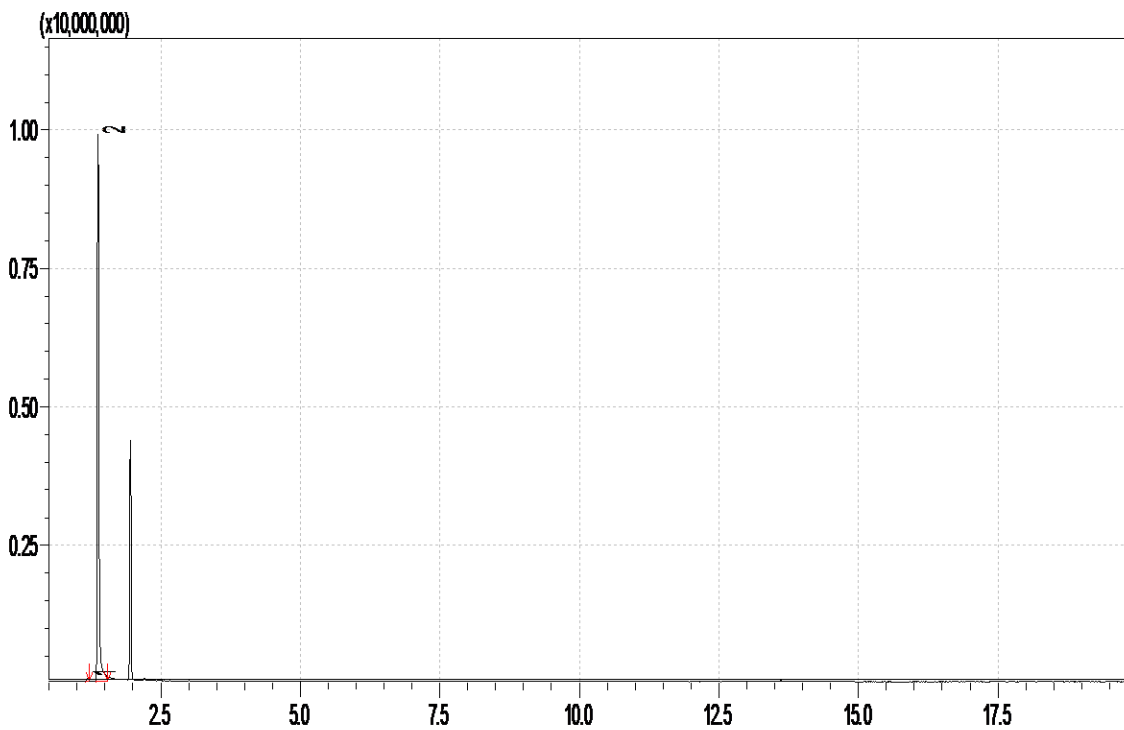


Figure (4.48): Final Results

1/ Carbon dioxide:

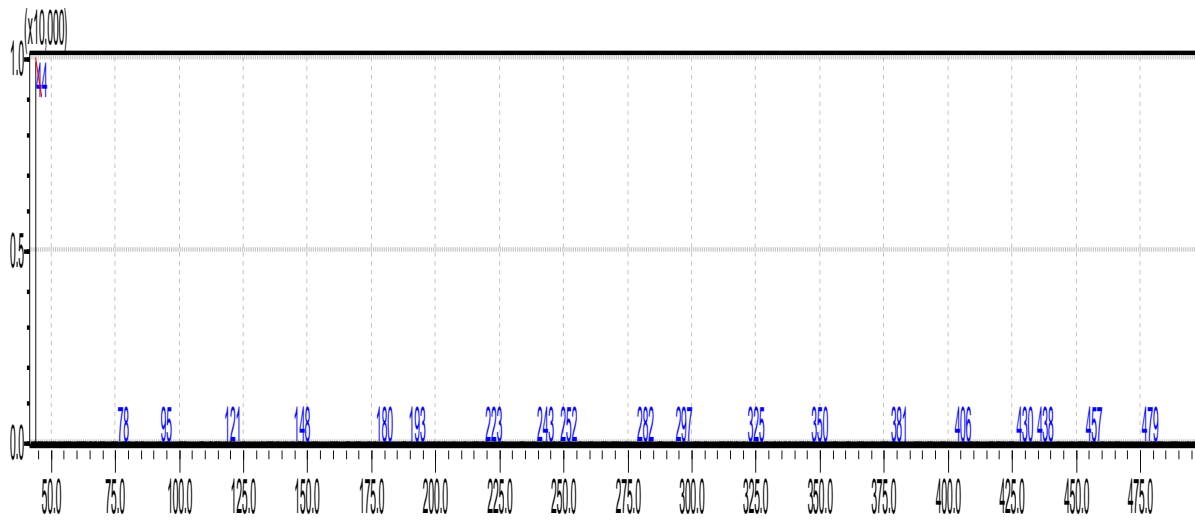


Figure (4.49): Carbon dioxide A

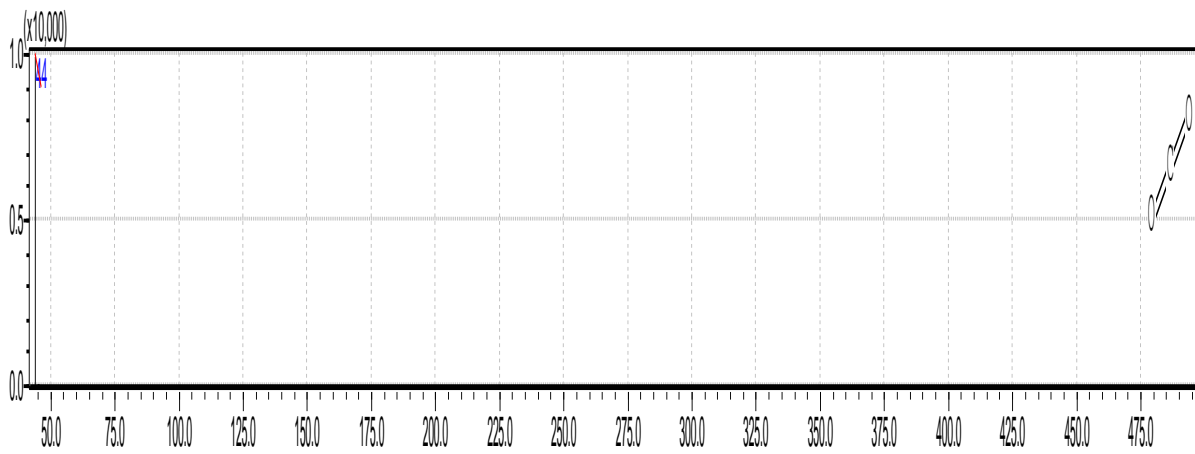
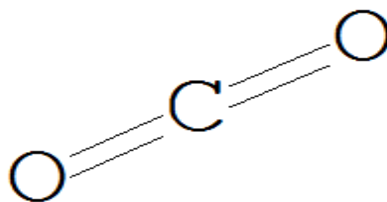


Figure (4.50): Carbon dioxide B



2/ Methane, nitroso-

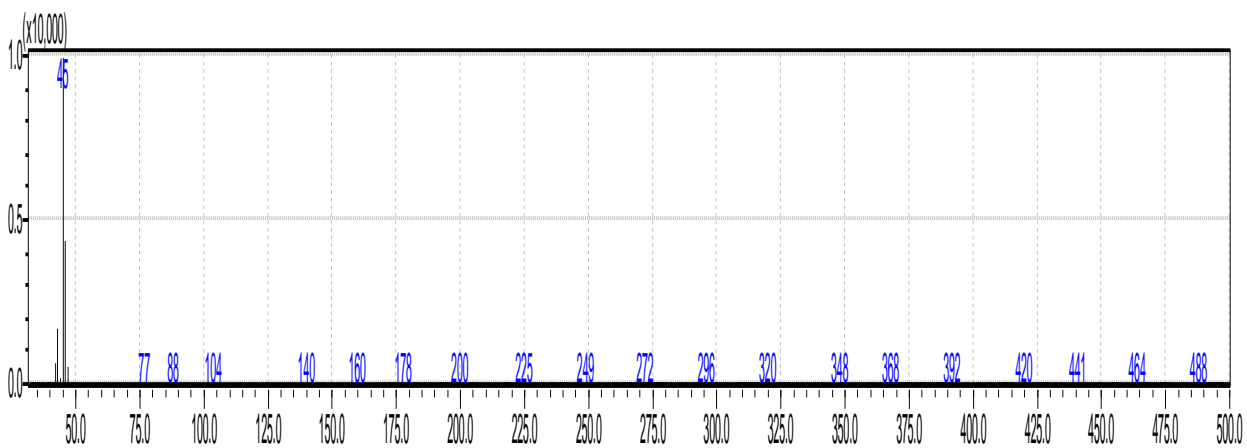


Figure (4.51): Methane ,nitroso A

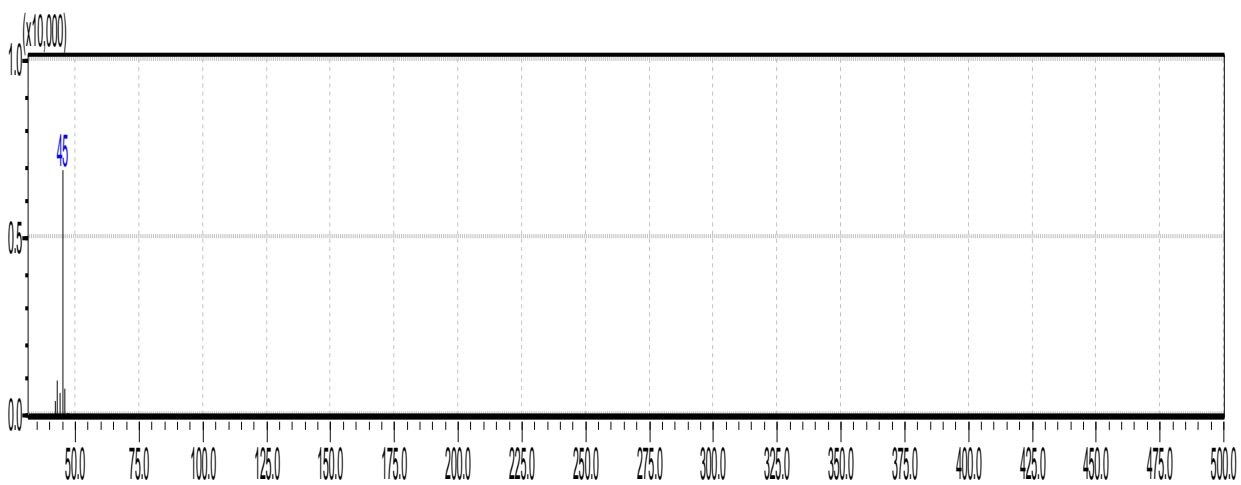
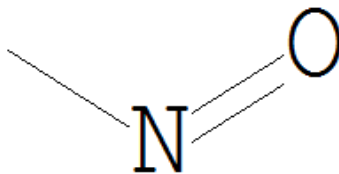


Figure (4.52): Methane, nitroso B



4.6 Economic Evaluation:

The economic analysis of the examined biogas plants as a time factor of future payments and dynamics business ratio aimed at illustrating the profitability of a specific investment for the entire time-frame of production to draw the most realistic picture of its economic performance.

- 10% to 12% lower price than diesel.
- Up to 30% cheaper than petrol.

One liter of biogas moves car in the same distance that flue do, to calculate how much the biogas made of blood can produce per ton see the following steps.

4.6.1 RBCs:

Quantity average (QAV) = 381g

Produce average (PAV) = 1466.3 ml

$$1 \text{ g} \rightarrow 3.84855643 \text{ ml}$$

$$1 \text{ g} \rightarrow \approx 3.848 \text{ ml}$$

$$1 \text{ ton} = 10^6 \text{ gram} = 3.848 \times 10^3 \text{ L}$$

So this means 1 ton of RBCs products 3848 L of biogas

$$1 \text{ L} = 0.001 \text{ m}^3 \rightarrow 3848 \text{ L} = 3.848 \text{ m}^3 \text{ of flue}$$

4.6.2 Whole Blood:

Quantity average (QAV) = 274 g

Produce average (PAV) = 2822.5 ml

$$1 \text{ g} \rightarrow 10.30109489 \text{ ml}$$

$$1 \text{ g} \rightarrow \approx 10.301 \text{ ml}$$

$$1 \text{ ton} = 10^6 \text{ gram} = 10.301 \times 10^3 \text{ L}$$

So this means 1 ton of whole blood produces 10301 L

$$1 \text{ L} = 0.001 \text{ m}^3 \rightarrow 10301 \text{ L} = 10.301 \text{ m}^3 \text{ of flue}$$

CHAPTER FIVE
DISCUSSION

As known medical waste are dangerous to handle and get rid of it, the methods used to manage medical waste unsafe (send to landfill or to incinerator) and both of previous way are environmentally hazardous and threaten human's health, animal and plant.

Here comes the purpose of our project via handling medical waste in an environmentally friendly manner.

After visiting (MOH) and hospitals in Sudan and collection data via survey made by an questionnaire about medical waste and after the data analysis statistic showed that there is a huge problem with blood bank waste (blood, platelet, RBCs and FFP) where large quantities accumulate in hospitals for month due to its cost where it is found expensive, blood bank waste managing or handled by private companies, the cost of 1 expired blood bag is 12.4 bound.

Given an authorization from MOH for the benefit of this research to find new way to handle biomedical waste that is more safe and environmentally friendly, expired (blood, platelet, RBCs and FFP) samples were collected from public hospitals .

Trails experiment took place in the National Center for Energy Research (NCER) in Khartoum-Soba to produce biogas from blood waste all preparations and tools used are mentioned in details in the previous chapter.

The results of trails experiment show that's the platelet and FFP failed to produce flammable gas but via gas analyser it showed the presence of the following gas in table (5-1) and table (5-2).

Table 5-1: gas analyser reading for platelet

GAS	VOLUME vol%
CO	0.01
HC	2226
CO ₂	64.9
NOX	5894

Table 5-2: gas analyser reading for FFP

GAS	VOLUME vol%
CO	0.01
HC	1954
CO ₂	35.4
NOX	108.39

Not: x unknown gas

Otherwise the blood and RBCs were it did produce flammable gases, Methanenitroso- CH_3NO which has blue color flame, and carbon dioxide CO_2 which has red/yellow color flame shown in table below, Nitro-methane is sometimes used as fuel for rockets, some aircraft and some racing cars

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

In the present investigation it has been found that biomedical waste is a lethal, highly polluting and cannot be disposed through landfill or incineration until and unless a proper measure is taken. However, this waste can be anaerobically digested and that will not only be eco-friendly but also it will generate biogas (methane-nitroso) as high quality manure. So, the investigation will also help to all municipal corporations, academicians related to waste management system, energy and environmental issues in disposing such wastes to worthy resources.

The result of this project:

- Manage blood bank waste in a safe way and in a more environment friendly.
- Reducing environmental hazard of medical waste.
- Producing biogas renewable energy.
- Lowering the cost of gas

6.2 RECOMMENDATION

Regarding to the achieved results recommended that:

- Pay attention to recycling the BMW specially blood.
- Developing this biogas plant via design an incubator to maintain temperature and the trial becomes more safety.
- Increasing carbon ratio via adding organic substance.
- Mixing the blood bank wastes (whole blood, RBCs, platelet and plasma) and do the experiment for it.
- Doing this experiment to an infection blood (HIV, AIDS and hepatitis).
- Developing research centers.

- Building advanced research center in any biomedical engineering (college, facility or company) in Sudan to develop such a research.
- Building a biogas units in hospitals.
- Test the remaining of the digester by the end of the experiment.

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APPENDICES

Appendix A: Equations

Equations	Number	Page No
$H^+ = 10^{-pH}$	1	32
$NADH + H^+ \rightarrow H^+ + NAD + 2$	2	32
$\Delta G_f^\circ = +18.07 \text{ kJ mol}^{-1}$	3	32
$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$	4	34
$NH_3 + H^+ \leftrightarrow NH_4^+$	5	34
$4 CH_3NO_2 + 3 O_2 \rightarrow 4 CO_2 + 6 H_2O + 2 N_2$	6	38
$4 CH_3NO_2 \rightarrow 4 CO + 4 H_2O + 2 H_2 + 2 N_2$	7	39
Total solid % = $\frac{WR}{WW} \times 100$	8	64
Sample weight g = $\frac{10\%}{\text{total solid \%}}$	9	64
Water value = $1000 \text{ g} - \text{sample weight} + (100\text{g})$	10	64

Appendix B: Questionnaire

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
Sudan University of Science & Technology
COLLEGE OF ENGINEERING
BIOMEDICAL ENGINEERING DEPARTMENT

QUESTIONNAIRE

Health care facility: _____

Address:

- Khartoum
- Bahri
- Om dorman

Function:

- Doctor
- Engineer
- Nurse
- Worker

Type of hospital (pick one):

- Specialist
- General
- University (training/provincial)
- Regional
- District
- Sub-district

Health care facility services type (pick one):

- Public
- Private

No. of inpatients:

- 20
- 50
- 80
- 100
- Others _____

No. of outpatients:

- 20
- 50
- 80
- 100
- Others _____

No. of beds (total):

- 20
- 50
- 80
- 100
- Others _____

Objectives:

1• to characterize the health-care waste management in this kind of health-facility.

2• to quantify the amount of health-care waste generated in this kind of health facility, when they are not segregated and to provide an estimation in kg/day.

1. Amount of medical waste produced by hospital :

- 100 bags a day
- More than 100 bags a day
- Less than 100 bags a day
- Others _____

2. Bags Weight is usually around :

- 2 kg
- 5 kg
- 8 kg
- 10 kg
- Others _____

3. Collection of medical waste in hospital happens every :

- Day
- 2 Day
- 3 Day
- Others _____

4. Storing of medical waste in :

- Hallway
- Special warehouse
- There is no storing area
- Others _____

5. Is this storage of medical waste is save :

- YES
- NO

-If no why? _____

6. Disposal of medical waste from hospital storage happens every :

- Day
- Week 3 times
- Month 4 times
- Others _____

7. Waste containers what kind of containers do you use?

- No specific container
- plastic
- Metallic
- Cardboard
- Bag

- Box
- Other _____

8. Do you have a specific color coding system?

- NO
- YES

- If no why? _____

9. Do you use this color coding;

- YES
- NO

-If no what color code do you use: _____

Yellow	infectious Clinical Waste Hazardous
Orange	Waste which may be "treated"
Purple	Cytotoxic and cytostatic waste
Yellow/black	Offensive/hygiene waste*Offensive/noninfectious waste Non Hazardous
Red	Anatomical waste for incineration Body parts ; Organs ; Blood bags; Blood preserves
Black	Domestic (municipal) waste
Blue	Pharmaceutical waste ; Non Hazardous
white	Amalgam waste For recovery

10. Does the hospital have an environmental policy that includes managing medical waste prevention procedures?

- YES
- NO

11. Which department of hospital produce the largest amount of medical waste?

- ER
- Dialysis unit
- Laboratories
- Blood bank

12. Do you manage all medical waste in the same way :

- YES
- NO

13. Which kind of medical waste is being treated differently :

- Dialysis unit waste
- Laboratories waste
- Blood bank
- All above
- Others _____

-Why _____

14. Which kind of medical waste is dangerous to deal with :

- Sharps

- Chemical
- Pathological
- Bio medical waste (tissue , blood)
- Others _____

15. The largest amount of expired blood in hospital comes from :

- Dialysis unit waste
- Laboratories waste
- Blood bank
- All above
- Others _____

16. The largest amount of waste in Laboratories is :

- Blood
- Solutions
- Others _____

17. The largest amount of waste in blood bank is:

- Blood
- Plasma
- Blood platelet
- RBCs

18. How many expired blood bags produced by blood bank weekly:

- 100 bag
- More than 100 bag
- Less than 100 bag
- Others _____

19. How many expired blood platelet bags produced by blood bank weekly:

- 100 bag
- More than 100 bag
- Less than 100 bag
- Others _____

20. How expired(blood platelet; blood) bags is being managed :

- Send to incinerator
- Landfill
- Others _____

Appendix C: C/N Raito Test

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 Fax: +(249)-1-83 429641
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 E-mail:info.cpl@spc.sd

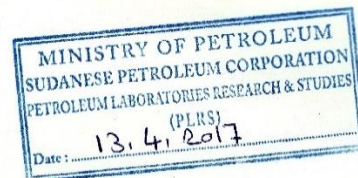


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TEST REPORT

Sample Type: Blood
 Sample Code: EA/0417/0004004 Report Number: 0020234
 Sample ID: 0020234 Date /Time Reported: 13/04/2017
 Customes Name: Student
 Customes Ref: _____
 Date / Time Receiver: 11/04/2017

Elements	Test Method	Unit	Result
Nitrogen	ASTM D 5291	Wt%	1.431
Carbon			9.119



<ul style="list-style-type: none"> • TEST (S) Has lhave been subcontracted ,please reter to PLRS for details • No part of this report can be reproduced except full in any form or by any electric or mechanical including photocopying and recording without awritten pemission of the issuing laboratry 	Reported by: <i>Taw/leg</i>
	Sig.: <i>new</i>

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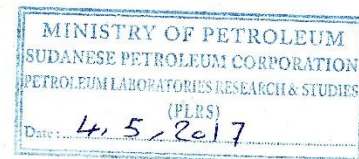


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TEST REPORT

Sample Type: Blood
Sample Code: EA/0417/000405 Report Number: 0020270
Sample ID: 0020270 Date /Time Reported: 04/05/2017
Customes Name: Student
Customes Ref: _____
Date / Time Received: 24/04/2017

Elements	Test Method	Unit	Result
Nitrogen	ASTM D 5291	Wt%	4.079
Carbon			13.91



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TEST REPORT

Sample Type: Blood let
Sample Code: EA/0417/000407 Report Number: 0020273
Sample ID: 0020273 Date /Time Reported: 04/05/2017
Customes Name: Student
Customes Ref: _____
Date / Time Received: 25/04/2017

Elements	Test Method	Unit	Result
Nitrogen	ASTM D 5291	Wt%	1.323
Carbon			5.1059

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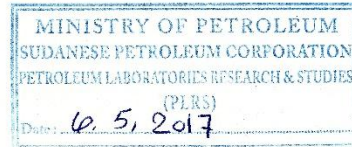


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TEST REPORT

Sample Type: Blood
Sample Code: EA/0417/000406 Report Number: 0020272
Sample ID: 0020272 Date /Time Reported: 04/05/2017
Customes Name: Student
Customes Ref: _____
Date / Time Received: 25/04/2017

Elements	Test Method	Unit	Result
Nitrogen	ASTM D 5291	Wt%	4.007
Carbon			13.38



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(F-04-22)

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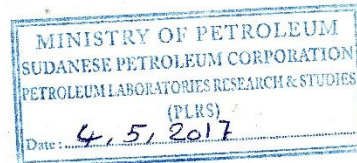
TEST REPORT

Sample Type: Blood let
Sample Code: EA/0417/000407
Sample ID: 0020273
Customes Name: Student
Customes Ref: _____
Date / Time Received: 25/04/2017

Report Number: 0020273

Date /Time Reported: 04/05/2017

Elements	Test Method	Unit	Result
Nitrogen	ASTM D 5291	Wt%	1.323
Carbon			5.1059



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Appendix D: Gas Test Results



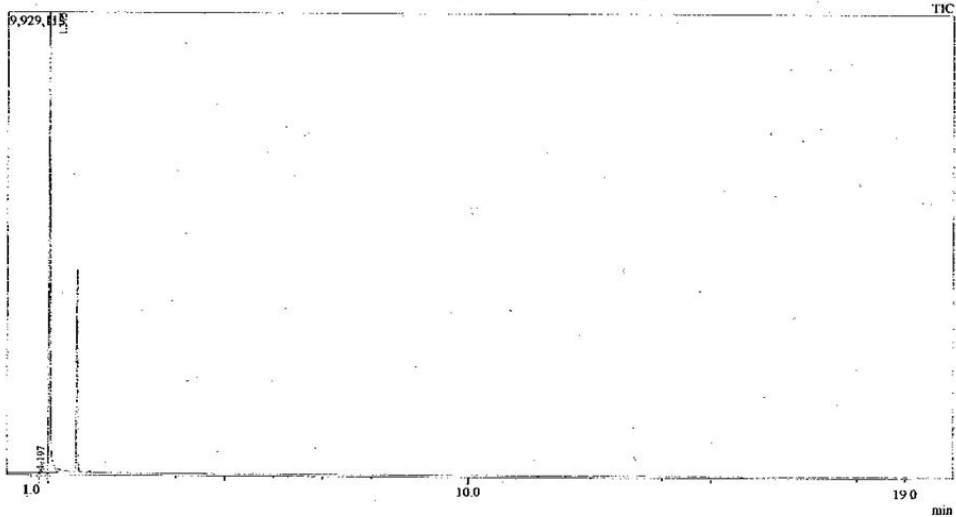
جامعة العلوم الطبية والتكنولوجيا
UMST University of Medical
Sciences & Technology
P. O. Box 12840,
Khartoum, Sudan

T. +249 183 228614
F. +249 183 224799
E. administration.office@umst-edu.sd
W. www.umst-edu.sd

Sample Information

Analyzed by : Babeker Siddig
Sample Type : Bio gas
Sample Name : Medical gas
Date : 16/10/2017
Customer Name : . Abd allah Mohammed
Sudan University
Tel 0922002462

Chromatogram Biogas C:\GCMSolution\Data\Project\NBiogas.QGD



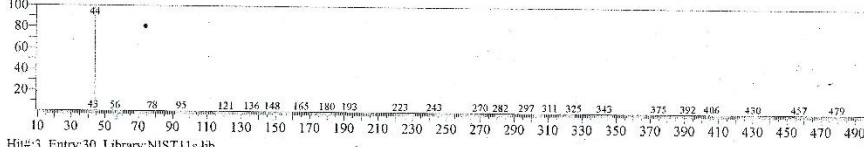
General manager ;.....
Assoc.Prof .Dhia Eldien A/Elmagied

Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	1.197	121805	0.55	Carbon dioxide
2	1.375	22096496	99.45	Methane, nitroso-
		22218301	100.00	

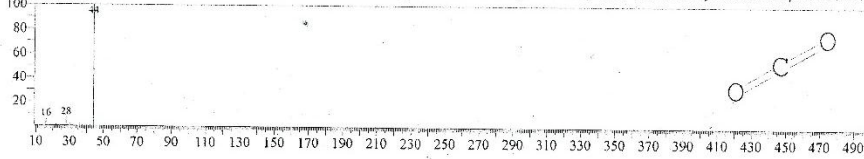
Library Search

<< Target >>

Line#:1 R. Time:1.195(Scan#:140) MassPeaks:182
RawMode:Averaged 1.190-1.200(139-141) BasePeak:43.95(10000)
BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:3 Entry:30 Library:NIST11s.bb
SI:97 Formula:CO2 CAS:124-38-9 MolWeight:44 RetIndex:0
CompName:Carbon dioxide SS Carbon oxide (CO2) SS Carbonic acid, gas SS Carbonic anhydride SS Dry ice SS CO2 SS Anhydride carbonique SS Carbonic



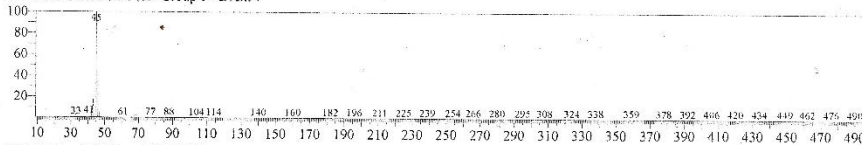
Library Search

<< Target >>

Line#:1 R.Time:1.375(Scan#:176) MassPeaks:221

RawMode:Averaged 1.370-1.380(175-177) BasePeak:45.00(10000)

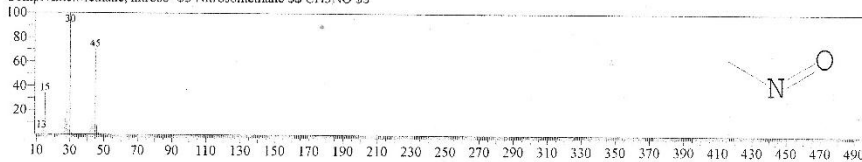
BG Mode:Calc. from Peak Group 1 - Event 1



Hit# 6 Entry:48 Library:NIST11.lib

SI:91 Formula:CH3NO CAS:865-40-7 MolWeight:45 RetIndex:0

CompName:Methane, nitroso- SS Nitrosomethane SS CH3NO SS



Appendix E: GCMS specifications

10/16/2017 14:34:30

```

Method

[Comment]
===== Analytical Line 1 =====

[AOC-201]
# of Rinses with Presolvent           :2
# of Rinses with Solvent(post)       :2
# of Rinses with Sample               :2
Plunger Speed(Suction)                :High
Viscosity Comp. Time                  :0.2 sec
Plunger Speed(Injection)              :High
Syringe Insertion Speed               :High
Injection Mode                        :Normal
Pumping Times                         :5
Inj. Port Dwell Time                  :0.3 sec
Terminal Air Gap                      :No
Plunger Washing Speed                 :High
Washing Volume                        :8uL
Syringe Suction Position              :0.0 mm
Syringe Injection Position            :0.0 mm
Use 3 Solvent Vial                    :1 vial

[GC-2010]
Column Oven Temp.                    :30.0 °C
Injection Temp.                      :200.00 °C
Injection Mode                        :Splitless
Sampling Time                         :0.00 min
Flow Control Mode                     :Linear Velocity
Pressure                              :85.1 kPa
Total Flow                            :50.0 mL/min
Column Flow                           :1.61 mL/min
Linear Velocity                       :45.5 cm/sec
Purge Flow                            :3.0 mL/min
Split Ratio                           :-1.0
High Pressure Injection               :OFF
Carrier Gas Saver                     :OFF
Oven Temp. Program
  Rate                               Temperature(°C)   Hold Time(min)
  -                               -                   -
  3.00                             30.0               0.00
                               90.0               0.00

< Ready Check Heat Unit >
  Column Oven                       : Yes
  SPL1                               : Yes
  MS                                 : Yes
< Ready Check Detector(FTD) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >
  SPL1 Carrier                       : Yes
  SPL1 Purge                         : Yes
< Ready Check APC Flow >
< Ready Check Detector APC Flow >
External Wait                        :No
Equilibrium Time                     :3.0 min

[GC Program]

[GCMS-QP2010 Ultra]
IonSource Temp                       :200.00 °C
Interface Temp.                      :220.00 °C
Solvent Cut Time                     :0.00 min
Detector Gain Mode                   :Relative
Detector Gain                        :0.89 kV +0.00 kV
Threshold                            :0

[MS Table]

```

Instrument Information's:-

- Name : GC.MS
- Detector : Mass spectrometer
- Model : GC.MS-QP2010 Ultra
- Company : Shimadzu
- Country : Japan
- Column : Rtx-5MS...Length (30 m)...Diameter (0.25 mm)...Thickness (0.25 μ l).
- Carrier gas : Helium
- Serial Number :020525101565SA

--Group 1 - Event 1--

Start Time :0.50min
End Time :20.00min
ACQ Mode :Scan
Event Time :0.30sec
Scan Speed :1666
Start m/z :33.00
End m/z :500.00

Sample Inlet Unit :GC

[MS Program]
Use MS Program :OFF