A

Minor Research Project

Under Research Initiation Scheme

Vivekanand College, Kolhapur (Autonomous)

Research Initiation Scheme for College Faculty

"Fertilizer and Biogas production from slaughter house waste of Sadar bazaar and local fish/Meat market of Kolhapur"

Principal Investigator-

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2018 - 2019

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To,

Co-ordinator

Internal Quality Assurance Cell

Vivekanand College, Kolhapur

Sub:- Submission of Project Thesis Copy

Respected Madam

With reference to subject cited above I am here by submitting the Project Thesis Copy My Minor research Project title **" Fertilizer and Biogas Production from Slaughter house waste of Sadar Bazar and local fish Market of Kolhapur"** under seed money sanctioned by research promotion cell Vivekanand College, Kolhapur(Autonomous). I submitted Utilization certificate audited by Auditor to Research promotion cell and all the original expenditure bills to Account section in college office.

With regards

S.G.Kulk 12/2/2021.

(Mr.S.G.Kulkarni)

Encl:- Project Thesis Copy

Coordinator - IQAC
Vivekanand College,
Kolhapur

DECLARATION

I the undersigned hereby declare that the project entitled **"Fertilizer and Biogas production from slaughter house waste of Sadar bazar and local fish/Meat market of Kolhapur"** is a original work done by me. The findings in this report are based on the data collected by me. The matter included in this report is not a reproduction from any other sources.

I also hereby declare that this project has not been submitted to any time to any other university or institution for the award of any degree or diploma.

Date: 30·12·2020

Place: Kolhapur.

Name and Signature

S. G. Kulkarni

Mr. S G. Kulkarni

ACKNOWLEDGEMENT

- At this juncture where the herculean task is nearing its pinnacle, researcher deems it a pleasure to look back and acknowledge efforts and support of all kith and kin that helped with zeal to turn a distant dream of a research in reality.
- I am extremely thankful to all members of Research promotion cell Vivekanand College, Kolhapur (Autonomous) for their valuable guidance and encouragement throughout this project work given to us.
- My Sincere thanks to former principal DR. S.Y. Hongekar, Vivekanand College, Kolhapur, (Autonomous).
- I am thankful to Principal Dr. R.R. Kumbhar, for his kind co-operation and valuable support.
- I am indeed grateful to Dr. K.P. Shinde Coordinator Research promotion cell for his kind co-operation and valuable support.
- I am thankful to Mr. A.L. Upadhye for his constant help and support in carrying out the research work during and throughout the project
- I am grateful all the staff members of our department for their direct and indirect support.
- Also, I sincerely thank my parents for helping us in all aspects to complete the project work. Finally we would like to appreciate my colleagues from other department for their direct and indirect contribution.

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Date:

Place: Kolhapur. **Name and Signature**

Mr.S.G.Kulkarni

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CHAPTER NO-1

INTRODUCTION

1.INTRODUCTION

1.1 GLOBAL SCENARIO

As per 1989 survey, India has the world's largest population of livestock, with nearly 191 million cattle. 70 million Buffaloes, 139 million Sheep and Goat, 10 million Pigs and over 200 million poultry. About 36.5% of Goat, 32.5% of Sheep, 28% of Pigs, 1.9% of Buffaloes and 0.9% cattle are slaughtered every year. The reported per capita availability of meat in India is about 1.4 kg per annum, which is rather low compared to 60-90 kg in European countries. As reported by the Ministry of Food Processing, as of 1989, a total of 3616 recognized slaughter houses slaughter over 2 million cattle and buffaloes, 50 million sheep and goat, 1.5 million pigs and 150 million poultry annually, for domestic consumption as well as for export purposes. While the slaughter houses come under the purview of the animal husbandry division of Ministry of Agriculture mainly for the purpose of funding towards expansion and modernization activities, the respective local bodies are mainly responsible for day-to-day operation/maintenance of the slaughter houses.

Most of the slaughter houses in the country are service-oriented and, as such, perform only the killing and dressing of animals without an onsite rendering operations. Most of the slaughter houses are more than 50 years old without adequate basic amenities viz. proper flooring, ventilation, water supply, lair age, transport etc. In addition to these deficiencies, slaughter houses suffer from very low hygiene standard posing a major public health and environmental hazards due to discrete disposal of waste and highly polluted effluent discharge. Un authorized and illicit slaughtering has also increased manifold and thus the related problems.

1.2 MAGNITUDE OF THE PROBLEM

With growing annual per capita meat consumption, high meat export potential, large non-utilization of potential meat animals, the development of meat industry in India is controlled not by the Government but the existing market forces. The unorganized nature of this trade is the main feature in this industry that has not been able to use state of the art of technology available in global meat market. This sector is facing many problems and constraints while going for modernization as under-mentioned: · Subjects of slaughtering of animals and related activities are governed as State subjects under the provisions of Article 48 of the Constitution of India. · There are religious and political controversies over the large animal slaughter particularly bullocks. ·

A vociferous pressure group emerging out of religious feelings does hinder the modernization of slaughter houses. · The Government's policies do not permit slaughtering of younger animals. Therefore, illegal slaughtering of calves is done in every city. · Moreover the introduction of humane slaughter methods has proved unsuccessful due to certain religion constraints, whereas existence of powerful religious concern over cruelty to animals cannot be ignored. · Due to Government control, religious beliefs and some of the constraints as explained above the ante-mortem and post-mortem inspections cannot be done at inadequately equipped slaughter houses and also it leads to illegal slaughtering of animals at a very high level. · Animals are often available for slaughter only when they are useless for any other purpose. · Lack of care during the transportation results into cruelty to animals, weight loss and high mortality. · Many of the animals are of poor breeds for meat production and suffer from malnutrition, endemic diseases and widespread parasitic infestation.

The meat industry is considered as unclean, unsocial and low caste occupation. · Comparatively small number of rich butchers who exploit the local labour force presently dominates the entire meat industry. · The long chain of middlemen results in high mark of prices between the farmers' gate and the terminal market. Because of the reasons stated above and the fact that most of the slaughter houses in the country are

more than 75 years old and also there is a noticeable increase in illegal activities of slaughtering animals, the meat industry does not meet the standards for discharge of effluents as laid down and notified under the Environment (Protection) Act1986.

1.3 OPERATIONS DURING SLAUGHTERING OF ANIMALS

1.3.1 Present Scenario

1.3.1.1 Slaughtering

In India mostly slaughtering of animals is done either by way of halal or jhatka method. Halal is the method preferred by Muslims and jhatka by the Hindus/Christians/Sikhs, etc. To slaughter the animals in a humane way stunning of the animals is prescribed, but in most of the cases stunning before slaughtering has yet not been adopted due to certain religious feelings.

1.3.1.2 Bleeding

In both the above methods of slaughtering, blood collection is not done immediately on slaughtering and most of the blood goes down into municipal drains causing pollution. Blood of the animals, which can be collected for making use in pharmaceutical industry, is thus by and large lost. Due to inadequate facilities at the slaughter houses and scattered illegal slaughtering of animals, a very few slaughter housed collect blood.

1.3.1.3 Dressing

Due to lack of means and tools, dehiding of the carcasses is done on the floor itself, which causes contamination of the meat. The hides and skins are spread on the floor of the slaughtering area. Similarly legs, bones, hooves etc. are not removed immediately from the slaughtering area.

1.3.1.4 Evisceration

This particular process during slaughtering generates maximum amount of waste. The butchers who carry out illegal slaughtering of animals generally throw visceral material at the community bins and wash the small intestines at their shops itself and thus create pollution problem.

1.4 MEASURES PROPOSED TO IMPROVE THE SLAUGHTER HOUSE WASTE MANAGEMENT

1.4.1 Liquid Waste/Effluent

During the above mentioned operations the waste generated is of liquid and solid nature. The liquid waste should be washed away by safe potable and constant supply of fresh water at adequate pressure throughout the premises of slaughtering. The waste water from slaughter house is heavy in pollution and, therefore, it should not be allowed to mix with the municipal drain system without pre-treatment meeting sewage standards as per the Bureau of Indian Standards (BIS).

The waste water treatment system should essentially comprise of:

(i) self cleaning type screening or two stage screening (Bar type);

(ii) anaerobic treatment;

(iii) aerobic treatment; and

(iv) filter press for dewatering of the sludge.

1.4.2 Collection of Blood

The blood available from the slaughter houses should be collected and made use of in pharmaceutical industry. Bleeding areas should be clearly identified in the slaughter houses and blood drains should be and collection should be done immediately so that its full potential could be utilized.

1.4.3 Improved Method of Dressing

At each slaughter house adequate tools should be provided for dehiding of the animals, hides and skins should be immediately transported out of the slaughtering area in a closed wheel-barrow or similar other devices. In no case the hides and skins should be spread on the floor of the slaughtering area for inspection. Legs, bones, hooves etc. should also be removed immediately from the slaughtering area through a spring load floor chute or closed wheel-barrow.

1.4.4 Evisceration

At slaughter houses adequate compartments for immediate separation and disposal of condemned material must be provided. The authority must take care that intestines are not punctured during evisceration to avoid contamination of carcasses.

1.5 SITUATION IN KOLHAPUR

1.5.1 NON- VEGETARIAN FOOD IN KOLHAPUR

Every Culture has a special food and cuisine attached to it. In case of Kolhapur it's the Non-Vegetarian that makes a mark as a special cuisine. Kolhapur has some good traditional dishes prepared from "Mutton" (goat meat) which are very tasty and delightful.

"Pandra Rassa" the white curry is a liked starter at Kolhapur. A Soup like dish of water used to boil the "Mutton" along with spices such as coriander, ginger & garlic etc. This is a very tasty pre-food item. Apart from taste it has certain medicinal use as well. This "rassa" is recommended for cough and throat related ailments. This is a part of a well-know duo curries. the other one is termed as "Tambda Rassa"

"Tambda Rassa" the second starter of the duo. This is a curry prepared using red chili powder to make it appear "Tambda" redish. Made in almost same ways as Pandra Rassa this type of curry is more famous within the rural area of Kolhapur. After these starters comes the special roasted mutton.

TAMBDA PANDRA RASSA' is identity of Kolhapur which indicates that Meat is an integral part of Non Vegetarian cuisine in the Kolhapur district. Due to high demand of meat, generation of slaughter house waste is also high. But there is no specific or proper disposal system or process for slaughter house waste in Kolhapur city.

1.5.2 PROBLEM OF WASTE DISPOSAL IN KOLHAPUR

Disposal of waste carried out on Dumping Ground or in Drains without any proper treatment. Due to this improper disposal of waste there is spreading of unpleasant odour and outbreak of endemic diseases responsible for soil and water pollution. Thus proper way of disposal and treatment of such kind of waste is required.

The Kolhapur city does not have any management system for treatment of slaughter house and fish/meat market. **The one located at Sadar bazaar was closed six years ago due to environmental concerns but slaughtering of animals at the most of the places are taking place illegally.**

The local fish/meat and slaughter house waste have been dumped directly into nullahs that further mixes in the Panchganga River. This is a major issue in Kolhapur city that has not been solved till this date.

1.5.3 PRODUCTION OF BIOFERTILIZER AND BIOMETHANE FROM TREATED WASTE

(A) BIOFERTILIZER PRODUCTION:-

In that process partial digestion of slaughter house work is carried to reduce complex biomolecules (proteins, lipids) to its simplest form.

For partial digestion of slaughter house waste Autoclaving, Partial Digestion with natural flora, Alkaline Hydrolysis carried out. Through these processes complex nature of biomolecules (proteins, lipids) to its simplest forms.

(B) BIOGAS PRODUCTION:-

Biogas production is the natural phenomenon carried out by Methonagens. Methanogens are organisms that have potential of producing Methane gas as metabolic byproduct in hypoxic conditions. Slaughter house waste contains such microorganisms.

Figure1 the improper disposal of slaughter house waste in Kolhapur

CHAPTER NO 2

REVIEWOFLITERATURE

2. REVIEW OF LITERATURE

About one third of the food produced globally is lost or wasted corresponding to an annual generation of roughly 1.3 billion ton of food waste (Gustavsson et al., 2011). In Europe this figure is estimated to about 88 Mt corresponding to ca. 173 kg per capita (Stenmarck et al., 2016; data for EU28 as for 2012); in economic terms, this incurs a loss of 143 billion$ each year. Food waste is often distinguished between unavoidable and avoidable, the latter intended as the food (and eventually drinks) which at some point, prior to being thrown out, was edible (Quested and Johnson, 2009). The avoidable portion represents a waste of resources, as food demands land-use, energy, chemicals and materials in order to be produced and delivered to the different actors involved in the food supply chain. Such a loss of resources inevitably translates into considerable environmental impacts that ideally may be avoided by prevention or mitigated by enforcing best waste management practices.

The unsanitary conditions in some slaughterhouses allow the proliferation of pathogens to the final meat product to be consumed. People from developing countries in Africa, Asia, and South America have experienced serious gastrointestinal diseases, bloody diarrhea, liver malfunctions, and, in some cases, death associated with the presence of viruses, protozoa, helminthes eggs, and bacteria in SWW. Furthermore, the presence of hepatitis A and E viruses has been reported in the sewage of animal origin in Spain. Therefore, SWW must be treated efficiently before discharge into water bodies to avoid environmental pollution and human health effects.

Another source of contamination of the meat processing industry is the addition of surfactants as a result of the cleaning process. Surfactants, major components in detergents, may enter the aquatic environment due to an inadequate SWW treatment, causing short-term and long-term changes in the ecosystem that affect humans, fish, and vegetation

According to the European legislation, thermal treatment of category 2 slaughterhouse by-products at 140 °C, 4–5 bar for 20 min is obligatory for their hygienization prior to disposal. This process is known as "rendering". The product of the rendering process is rich in lipids and proteins making it an appropriate feedstock for biogas plants. The mathematical modeling of biogas production from slaughterhouse wastes after the rendering process has been studied adjusting the anaerobic digestion model (ADM1). For this purpose, two mesophilic (38–39 °C) continuous stirred tank reactors (CSTRs) have been operated in parallel under a hydraulic retention time of 21.5 ± 2.14 d, while the organic load was increased from 50 to 149.6 g COD L^{-1}.

Recirculation of the mixed liquor suspended solids (MLSS) took place in one of the CSTRs, resulting in a different solids' concentration in it. The ADM1 was calibrated by estimating key kinetic parameters, such as the maximum specific consumption rate constant and the half-saturation constants of volatile fatty acids and verified. The degradation kinetics of this type of waste seemed to be faster, as a result of its emulsification through rendering, while the coefficient yields of the acidogens were lower than the default values of ADM1. The structure of the model was proven suitable for predicting the response of both bioreactors under small or medium step transitions, but not for abrupt impulse disturbances in the organic loading rate.

Slaughter house wastes are a potential reservoir of bacterial, viral, fungal and parasitic pathogens. These microbes have potential to cause disease to animals and humans. A quick, applicable, cost effective and safe disposable method is needed to solve this issue. Different have been utilized to dispose such waste, including composting, **Anaerobic Digestion (AD), Alkaline Hydrolysis (AH)**, rendering, incineration and burning.

For the management of slaughter house and fish/meat wastes various methods have been employed. Throughout history burning has been the most commonly applied method of disposal of waste (Gwyther et al. 2011). However the European Union animal by product regulation (EC) No. 1774/2002 (Anon, 2002) does not allow this practice and limits to the disposal routes to incineration and composting.

Rendering is another method used for disposal of animal waste production. It involves grinding, mixing, processing, decanting, and separating. Thermal processes involve cooking, evaporation and drying.

CHAPTER NO-3

AIMS & OBJECTIVES

3.1 AIMS

"Fertilizer and Biogas production from slaughter house waste of Sadar bazaar and local fish/Meat market of Kolhapur"

3.2 OBJECTIVES

- Collection of Waste from the Slaughter houses of Kolhapur
- Anaerobic Digestion (AD), Alkaline Hydrolysis (AH), rendering, incineration and burning
- Reduction of odor of waste.
- Production of biogas, especially Biomethane from Anaerobic digestion of waste.
- Agriculture Biofertilizer production from residues obtained from alkaline hydrolysis of waste.

CHAPTER NO-4
MATERIALS AND METHOD

4. MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 Chemicals: 1NaOH, KOH, Biurret reagent, DNSA reagent, DPA reagent, Resorcinol reagent, Liquid Nitrogen, Ninhydrin Reagent, NaCl, Fructose, HCl, Thiourea, Glacial Acetic Acid, Folin-Ciocalteau Reagent, Sodium Carbonate, Copper Sulphate

4.1.2 Glassware: Conical Flasks, Test tubes, Round Bottom Flask, Beakers, Measuring cylinders, Petri plates, Plastic Bottles, pipettes, Biurret, Slides, Saline tubes and all other glassware, glass capillary tube

4.1.3 Others Requirements: Hand Gloves, Sanitizers, Disinfectant, Whatmann Filter Papers, tissue papers, Room fresheners, Sample collection bags, masks.

4.1.4 Instruments: Colorimeter, Autoclave, Digital Autoclave, Pressure Cooker, Digital weighing balance, Incubator, Oven, Gas Collection tube, Micropipettes, Deep Fridge, Centrifuge, Cooling Centrifuge, pH meter, Shaker incubator, Incubatory stirrer, Mixer Grinder, Microscope, Mortar Pestle, Magnetic Stirrer, Anaerobic Incubator, Cryogenic container.

4.2 METHODS

4.2.1 TREATMENT OF SOLID WASTES FROM SLAUGHTER HOUSES

4.2.1.1 Procuring of Waste material from Slaughter house

The waste material that generated from the waste of the slaughter house was procured in sample collection bags which was pre sterilized and brought to the lab. While collection care was taken to avoid direct exposure to the waste.

4.2.1.2 Homogenization of waste using liquid nitrogen

The waste obtained from the slaughter house is broken down into small pieces and subjected with liquid nitrogen due to which the water present in the cells present in these waste is crystallizes and due to its sharp edges the cells are crushed when hammered using mortar pestle and mixer grinder.

4.2.1.3 Autoclaving

The slurry thus formed from the liquid nitrogen crushing is subjected for autoclaving for 121 degree Celsius for 20 min in traditional autoclave and 135 degree Celsius for 10 min in digital autoclave for 121 lbs pressure. As a result of this the contamination and all micro organism present in the sample was killed and its treatment of this waste was made easier.

4.2.1.4 Biochemical tests

The autoclaved sample was diluted and subjected for different Biochemical tests **to check** the chemical constituents present in the waste.

Figure 2 Dilution of the sample according to the biochemical test

4.2.1.5.1 Estimation of Fructose by Resorcinol Method

Fructose undergo oxidative dehydration in presence of conc.HCl to form furfural derivative which is an unstable compound which then reacts with resorcinol to form a pink-orange colored complex. The intensity of color is directly proportional to conc. of fructose present. O.D. is measured at 530 nm.

4.2.1.6.2 Estimation of DNA by Diphenylamine Method.

When DNA is treated with DPA reagent under acidic condition a blue colored compound is formed with sharp absorption maxima at 595 nm .this reaction is given by Deoxypentoses in general and it is not specific for DNA. In acid solution the straight chain form of Deoxypentose is converted into highly reactive β-hydroxylevulinaldehyde which reacts with DPA to give a blue color complex. In DNA only the Deoxypentose of Purine nucleotides reacts so that the value obtained represents half of the total deoxyribose present.

4.2.1.7.3 Separation of Amino acid by Thin layer Chromatography.

Thin layer Chromatography is based on adsorption as well as partition chromatography. It is performed on open layer of adsorbent material like silica gel which is coated on glass

plate by using binder $CaSO_4$.The separation is based on partition coefficient. Two phases of silica TLC stationary phase and Mobile phase, Mobile phase is organic solvent of following constituent Butanol: Acetic acid: Water in proportion 4:1:1. The movement of Solute is based on solubility of solute in stationary phase and mobile phase. The difference in solubility responsible for creation of partition coefficient which is determined by Rf value

a) Preparation of TLC plate-

1. Dissolve a pinch of $CaSO_4$ in approx 200 ml distilled water and add 10 gm of silica gel powder to prepare slurry.
2. The slurry is poured on glass plate to make a thin layer.
3. The plates are kept in oven at 110 C for 12 hrs to carry out activation of plate.

b) Application of Sample: -

1. Initially make a mark of 2 cm from the base of plate and at the center load the sample by using capillary tube.
2. The plate was kept as it is to allow drying of spot.
3. Keep the plate in TLC chamber to allow separation & precaution is taken that mixture spot does not mix with the solvent system/mobile phase directly.

c) Identification of Amino acid:-1. Amino acids are identified/detected by using ninhydrin reagent. The reagent is sprinkled over the plate and after drying the colored spots were observed for determination of Rf values which are matched with standard table to conclude the amino acid present in the mixture.

Formula for Rf Value: - $\frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$

4.2.1.8.4 Protein estimation by Lowry's method

The blue color developed by the reduction of the phosphomolybdic-phosphotungstic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein are measured in the Lowry's method.

4.2.1.7.5 Protein estimation by Biruet method

Biruet Test determines the presence of a peptide bond in a substance. It is based on the biuret reaction in which a peptide structure containing at least two peptide links produces a violet color when treated with alkaline copper sulfate. In presence of an alkaline solution, blue-colored copper II ion can form a complex with the peptide bonds since the peptide has unshared electron pairs in nitrogen and oxygen of water. The colored coordination complex is formed between Cu2+ ion and carbonyl oxygen (>C=O) and amide nitrogen (=NH) of the peptide bond.

Once this complex has been formed, the solution turns from blue to purple. The deeper the purple color, the higher is the number of peptide-copper complexes. The reaction occurs in any compound containing at least two H2N-C, H2N-CH2-, H2N-CS- or similar groups joined together directly or through a carbon or nitrogen atom. One copper ion is probably linked to 6 nearby peptide linkages by co-ordinate bonds. The intensity of the color is directly proportional to the number of the peptide bonds present in the protein molecule that is reacting and also the number of the protein molecules present in the reaction system.

4.2.1.7.6 Estimation of total free amino acids

The amino acids are colorless ionic compounds that form the basic building blocks of protein. Apart from being bound as proteins, amino acids also exist in the free form in many tissues and are known as free amino acids. They are mostly water soluble in nature. Very often in plants during disease conditions, the free amino acid composition exhibits a change hence, the measurement of the total free amino acids gives the physiological and health status of the plants. Ninhydrin, a powerful oxidizing agent, decarboxylates the alpha-amino acids and yields an intensely colored bluish purple product which is colorimetrically measured in 570nm.

4.2.1.7.7 Estimation of Reducing by DNSA Method.

The DNS method for estimating the concentration of reducing sugars in a sample Reducing sugars contain free carbonyl group, have the property to reduce many of the reagents. All monosaccaride and some disaccaride are reducing sugars.

When alkaline solution of 3,5-dinitrosalicylic acid reacts with reducing sugars(eg. Glucose, lactose). It is converted into 3-amino-5-nitrosalicylic acid with orange color.

4.2.2. DIGESTION USING NATURAL FLORA

4.2.2.1 Methods of handling viscera, paunch and intestines

Viscera can be recovered as edible products (e.g. heart, liver, and lung). They can also be separated for inedible rendering or processing. The paunch contents, 'paunch manure' (partially digested feed), is estimated to range from 27 to 40kg/animal. The paunch can be handled in four ways: Total dumping- all of the paunch contents are flushed away into the sewer, wet dumping the paunch contents are washed out and the wet slurry is screened on the presence of gross solids, which are subsequently removed, dry dumping. The paunch contents are dumped for subsequent rendering or for disposal as solid waste without needless water flushing, whole paunch handling- the entire paunch may be removed, intact, for rendering or for disposal as solid waste. Intestines may be rendered directly, or hashed and washed prior to rendering.

After digestion using natural micro flora the waste is again subjected for biochemical tests.

4.2.2.2 Bio gas production

Due to the abundant biomass wastes generated by slaughterhouse, these biomass resources potential for biogas production by the process of biomethenation, this process use slaughterhouse waste for production of biogas. The success of the process, especially the effective removal BOD has led biogas plant to be acceptable for slaughter house. Wastes consisting of rumen and paunch contents, dung, agriculture residue, fat and blood are processed in biomethenation plant. Power plants have been designed to produce biogas (60% methane, 30% carbon dioxide and traces of hydrogen, carbon monoxide etc.) by digestion of animal waste. The biogas can be used for boiler or power generation. Large slaughter house are mostly located around cities and congested areas. They generate substantial quality of solid wastes, which have to be processed in environmentally acceptable manner. For the large slaughter houses, bio-methanation of waste is suggested. Bio-methanation requires less space, which is advantageous for the slaughter houses with land constraints. Bio gas produced from this operation may fulfill the energy requirements of slaughter unit and adjoining areas. The bio gas produced here is detected by simple flaming test.

4.2.2.3 Composting and production of Biofertilizer

Composting involves the aerobic biological decomposition of organic materials to produce a stable humus-like product. The slaughter house waste can be used for compost

making. The left over feed, dung from the lair age, ruminal and intestinal contents, blood, meat trimmings, floor sweepings, hair, feathers, hide trimmings can be stabilized by composting. It will produce very good quality bio-manure which may be utilized as fertilizers for the agriculture land and gardens.

4.2.2.4 Rendering

This is a useful method for the physical separation of fats from solids and water. All the animal matter such as inedible offal, tissues, meat trimmings, waste and condemned meat, bones etc. can be processed in a rendering system as the main constituents of animal matter are fat, water and solids. Rendering is affected by heating and rupturing connective tissue of individual fat and muscle cells so that raw fat and other material bound within is freed. In rendering, fat recovered is used for industrial purposes, such as soap and greases. Fat recovered from flesh of healthy parts can also be used for edible purposes. Solid portion, which is known as meat meal or bone meal, is utilized for the manufacture of stock feed and fertilizers. Rendering is carried out in dry rendering or wet rendering plants.

4.2.2.5 Alkaline hydrolysis of the waste

During alkaline hydrolysis, slaughterhouse waste is mixed with alkali NaOH/KOH and boiled for 3-6 h at 150°C with a pressure of 4 Bars. The chemical bonds of the large protein molecules, nucleic acids (DNA and RNA), lipids, viruses and prions break into smaller molecules which react with NaOH/KOH to form Na/K-salts. Fatty acids react with alkalis to form soaps. The resulting chemical compound is a neutral or partially alkaline solution of organic substances suitable for anaerobe microbiological decomposition, or a valuable raw material for anaerobic digestion.

CHAPTER NO-5

RESULTS AND DISCUSSIONS

5. RESULTS AND DISCUSSIONS

5.1 Crushing of the big pieces of slaughter house waste using liquid Nitrogen

The large pieces of waste meat obtained from slaughter house is first subjected for manual churning and then they are crushed in liquid nitrogen to fine powder/paste which makes it easy for further treatment.

Figure 3 Crushing of large pieces of waste into fine paste

5.2 Preparation of meat for biochemical tests and Bio fertilizer Production after autoclaving

Figure 4 Mixing of digested sample with NaOH and KOH

5.3.1 Alkaline hydrolysis of slaughter house waste

Slaughter house waste is highly valuable raw material, as well as alkaline hydrolysis as a method for the remediation of slaughterhouse waste or meat bone meal. In the process of alkaline hydrolysis, slaughterhouse waste is mixed with alkali NaOH/KOH and boiled for 3-6 hours at temperature of 150 degrees Celsius and pressure of 4 bars.

5.3.2 Bio fertilizer production of treated slaughter house waste

The waste generated after the treatment of slaughter house waste with alkaline hydrolysis is used for Biofertilizer production. The resulting chemical compound is a neutralized and partially alkaline solution of organic substances suitable for anaerobic microbiological decomposition is done and valuable raw material obtained from anaerobic digestion is used as very good fertilizer.

5.3 Biochemical tests before and after the treatment of slaughter house waste.

5.3.1 Estimation of Fructose by Resorcinol Method

Observation Table: - Std. Fructose 100ug/ml

Sr .No	Std.Fructose	D/W	Resorcinol	5:1 HCl		O.D. at 530nm
1	0.0 ml	1.0 ml	1 ml	5 ml	Keep all tubes in boiling water bath for 8 min	0
2	0.2 ml	0.8 ml	1 ml	5 ml		0.06
3	0.4 ml	0.6 ml	1 ml	5 ml		0.12
4	0.6 ml	0.4 ml	1 ml	5 ml		0.17
5	0.8 ml	0.2 ml	1 ml	5 ml		0.21
6	1.0 ml	0.0	1 ml	5 ml		0.24
7	Before treatment 1.0 ml	0.0	1 ml	5 ml		0.10
8	After treatment 1.0 ml	0.0	1 ml	5 ml		0.15

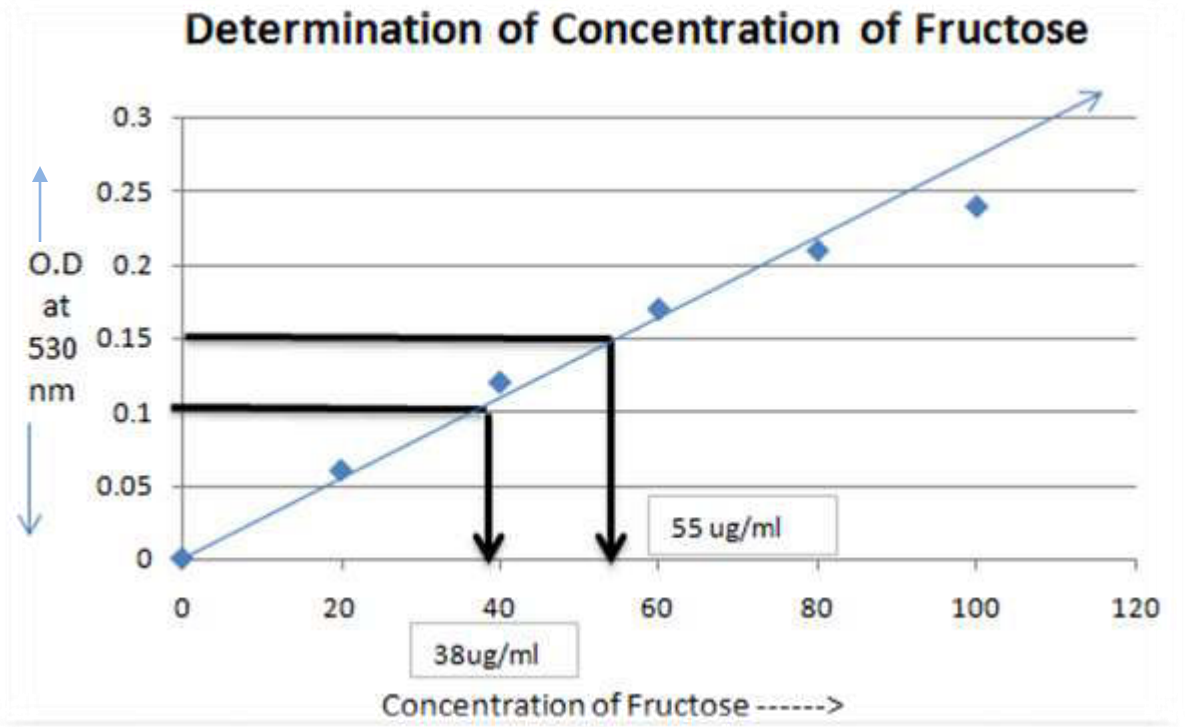


Table No 1 Estimation of Fructose by Resorcinol Method

5.3.2 Estimation of DNA by Diphenylamine Method.

Standard DNA (Deoxyribose sugar- 100 ug/ml)

Sr .No	Std. Deoxyribose sugar (ml)	D/W (ml)	Diphenylamine reagent		O.D. at 660 nm
1	0.0	1.0	1 ml	Keep all tubes in boiling water bath for 10 min	0
2	0.2	0.8	1 ml		0.04
3	0.4	0.6	1 ml		0.08
4	0.6	0.4	1 ml		0.12
5	0.8	0.2	1 ml		0.18
6	1.0	0.0	1 ml		0.23
7	Before treatment 1.0	0.0	1 ml		0.04
8	After Treatment 1.0 ml	0.0	1 ml		0.20

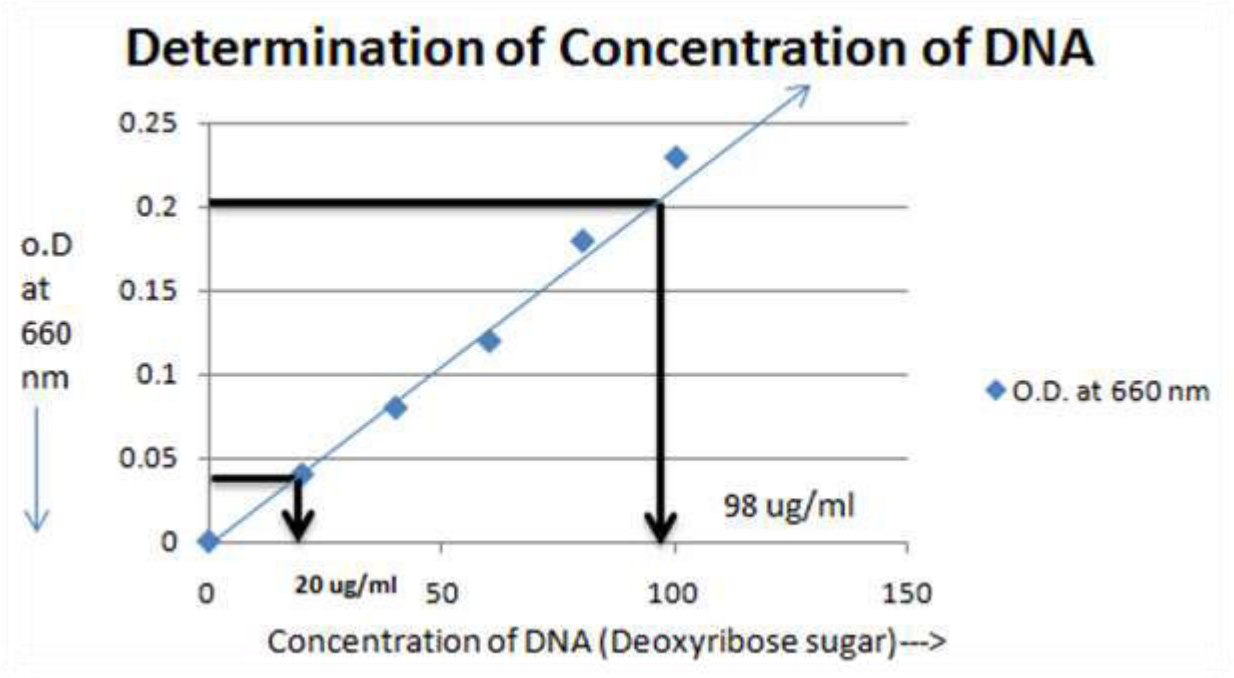


Table no 2 Estimation of DNA by Diphenylamine Method.

5.3.3 Estimation of Proteins by Lowry's Method.

Standard BSA= 100 u/ml

Sr .No	Std. BSA ml	D/W ml	Lowry's C		Folin's –C Reagent ml		O.D. at 620 nm
1	0.0	1.0	3ml	Keep all tubes in at RT for 10 min	0.5	Wait for 15 min at RT	0.0
2	0.2	0.8	3 ml		0.5		0.07
3	0.4	0.6	3 ml		0.5		0.14
4	0.6	0.4	3ml		0.5		0.21
5	0.8	0.2	3 ml		0.5		0.28
6	1.0	0.0	3 ml		0.5		0.35
7	Before Treatment-1.0	0.0	3ml		0.5		0.32
8	After Treatment 1.0	0.0	3 ml		0.5		0.10

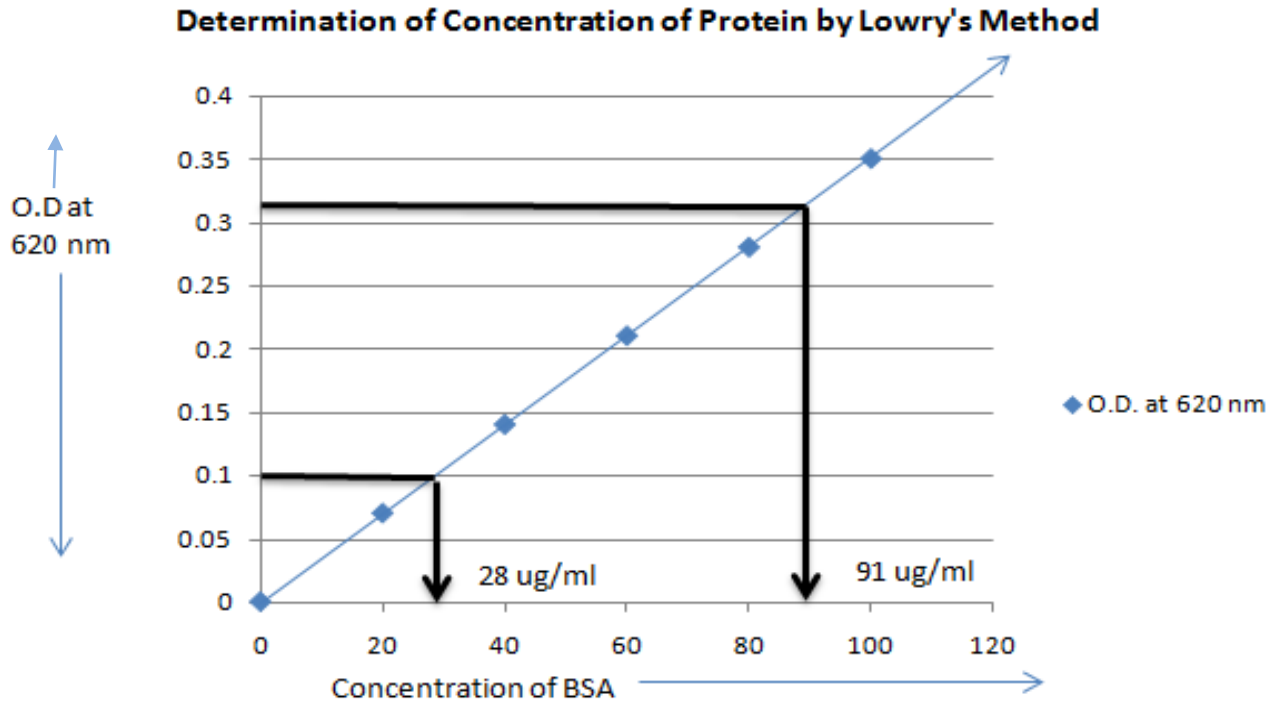


Table No 3 Estimation of Proteins by Lowry's Method

5.3.4 Estimation of Proteins by Biuret Method.

Standard casein = 5 mg/ml

Sr .No	Std. Casein ml	D/W ml	Biuret Reagent ml		O.D. at 530 nm
1	0.0	5	5 ml	Keep all tubes in boiling water bath for 10 min	0.0
2	1	4	5 ml		0.06
3	2	3	5 ml		0.12
4	3	2	5 ml		0.24
5	4	1	5 ml		0.34
6	5	0	5 ml		0.59
7	Before treatment 5ml	0.0	5 ml		0.45
8	After treatment 5 ml	0.0	5 ml		0.15

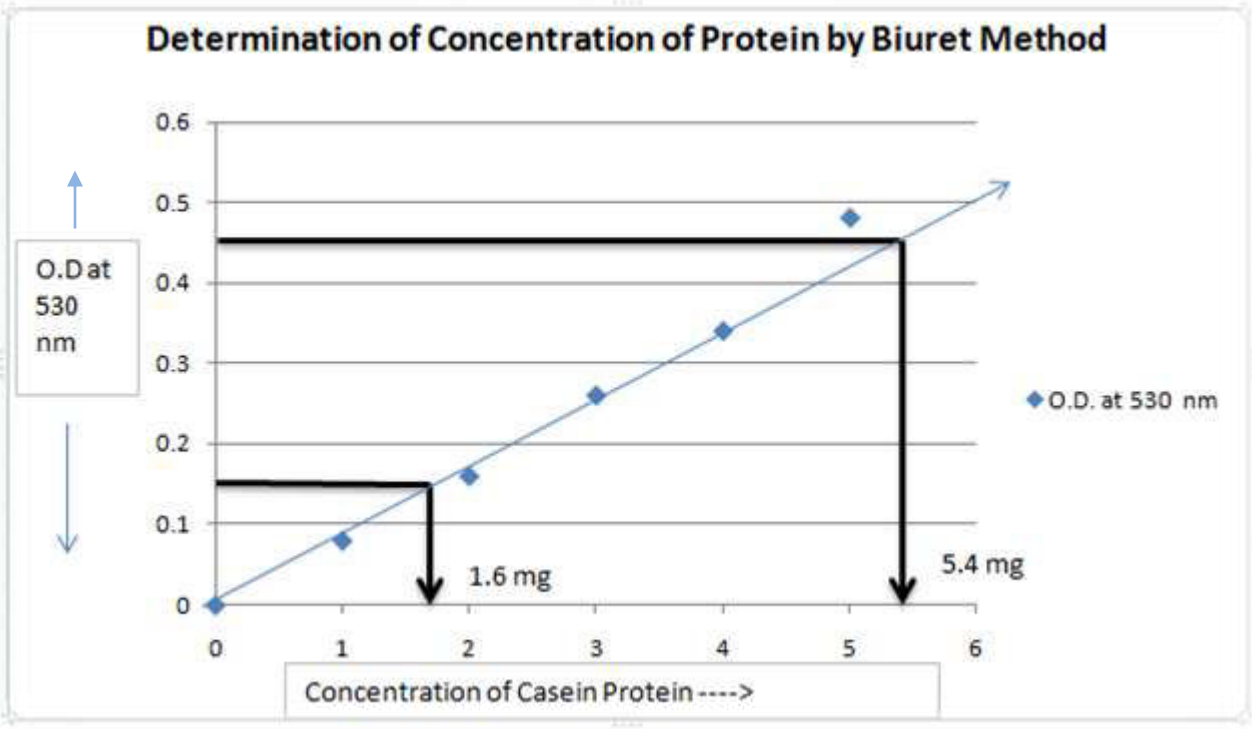


Table no 4 Estimation of Proteins by Biuret Method.

5.3.5 Estimation of Reducing by DNSA Method.

Standard Glucose= 500 microgram/ml

Sr .No	Std. Glucose (ml)	D/W (ml)	Biuret Reagent		O.D. at 530 nm
1	0	5	5 ml	Keep all tubes in boiling water bath for 10 min	0.0
2	1	4	5 ml		0.06
3	2	3	5 ml		0.12
4	3	2	5 ml		0.24
5	4	1	5 ml		0.34
6	5	0.0	5 ml		0.59
7	Before treatment 5	0.0	5 ml		0.07
8	After Treatment 5	0.0	5 ml		0.16

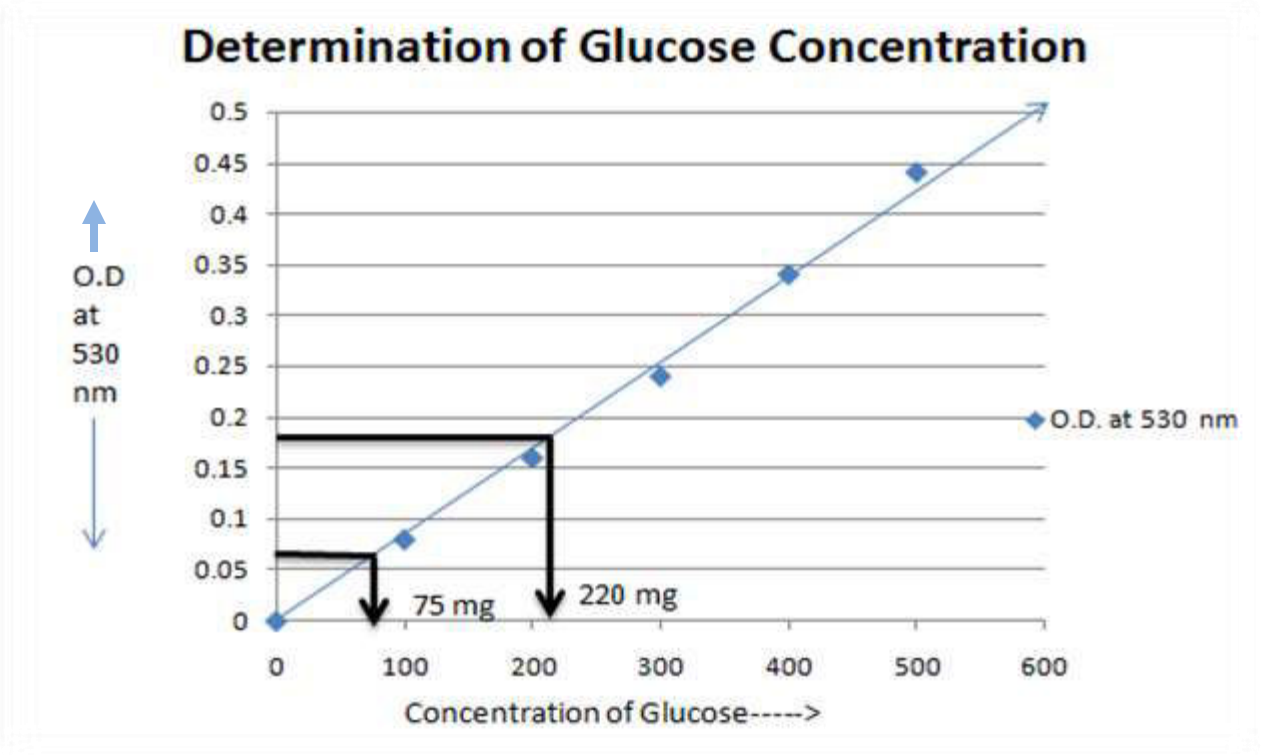


Table No 5 Estimation of Reducing by DNSA Method

5.3.5 Estimation of total free amino acids

Standard Leucine = 65 ug/ml

Sr .No	Std. leucine (ml)	D/W (ml)	Buffer (ml)	Ninhydrin Reagent (ml)		Alcohol ml	O.D. at 530 nm
1	0.0	1.0	0.5	0.5 ml	Keep all tubes in boiling water bath for 20 min and cool.	2	0.0
2	0.2	0.8	0.5	0.5 ml		2	0.23
3	0.4	0.6	0.5	0.5 ml		2	0.50
4	0.6	0.4	0.5	0.5 ml		2	0.95
5	0.8	0.2	0.5	0.5 ml		2	1.1
6	1.0	0.0	0.5	0.5 ml		2	1.3
7	1.0	0.0	0.5	0.5 ml		2	

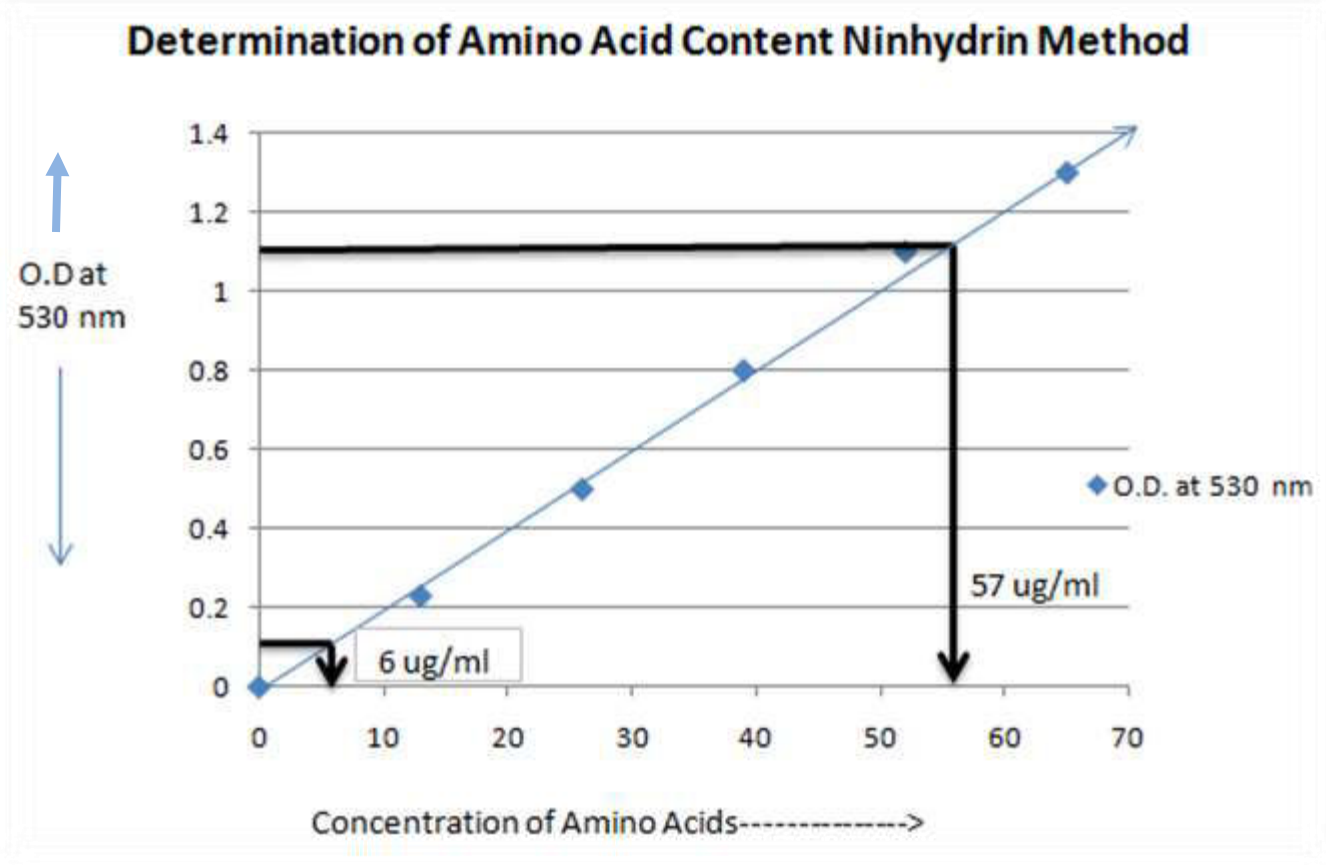
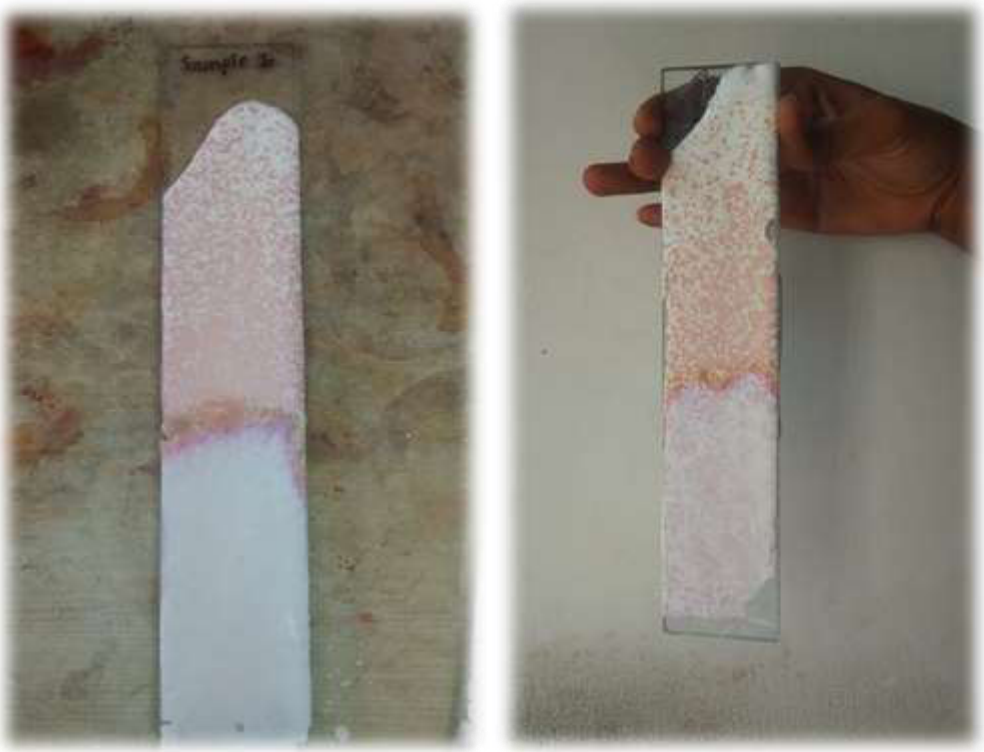


Table No 6 Estimation of total free amino acids

5.3.6 TLC of the digested sample for detection of digestion of proteins

The digested sample of the meat is decanted and the supernatant is subjected for TLC which shows that the protein is digested and its amino acids are separated and detected by using Ninhydrin reagent



Before **After**

Figure 5 Thin Layer Chromatography

5.3.6 Production of Biomethane

Anaerobic digestion can achieve a high degree of COD and BOD removal from slaughterhouse effluent at a significantly lower cost than comparable aerobic systems. The biogas potential of slaughterhouse waste is higher than animal manure, and reported to be in the range of 120-160 m3 biogases per ton of wastes. However the C:N ratio of slaughterhouse waste is quite low (4:1) which demands its co-digestion with high C:N substrates like animal manure, food waste, crop residues, poultry litter etc.

Slaughterhouse waste is a protein-rich substrate and may result in sulfide formation during anaerobic degradation. The increased concentration of sulfides in the digester can lead to higher concentrations of hydrogen sulfide in the biogas which may inhibit methanogens. In addition to sulfides, ammonia is also formed during the anaerobic digestion process which may increase the pH in the digester (>8.0) which can be growth limiting for some methanogens.

Figure 6 Production of Biomethane in Lab

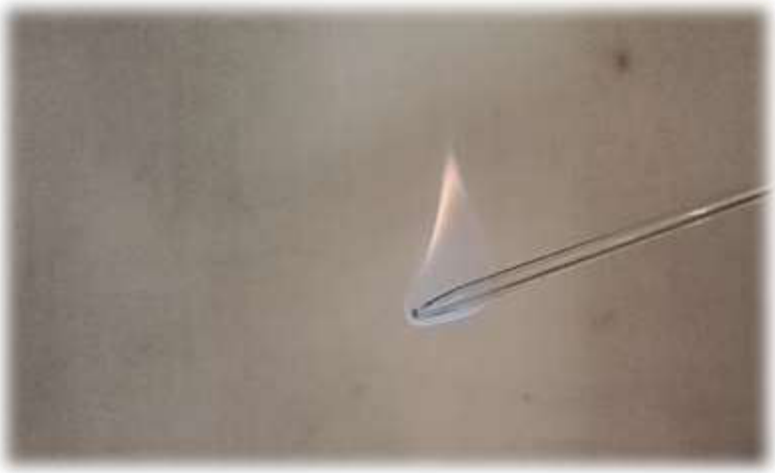


Figure 7 Flaming test for Bio Methane

Figure 8: Estimation of Fructose by Resorcinol Method

Figure 9: Estimation of Deoxyribose Sugar by Diphenlyamine Method

Figure 10: Estimation of Reducing Sugar by DNSA Method

Figure 11: Estimation of Amino Acid by Ninhydrin Method

Figure 12 Estimation of Protein by Lowry's Method

Figure 13 Estimation of Protein by Biuret Method

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6. BIBLIOGRAPHY

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APPENDIX

7. APPENDIX

1. **Resorcinol reagent**: Dissolve 1g resorcinol and 0.25g Thiourea in 100ml glacial acetic acid. This solution indefinitely...
2. **Dilute HCl:** Mix five parts of conc. HCl with one part of distilled water.
3. **Standard fructose solution**: Dissolve 10 mg (10,000ug) of fructose in 100ml water then it become 100 ug/ml working solution.
4. **Standard DNA**- Dissolve 10 mg (10000ug) DNA (Deoxyribose sugar) in 100 ml D/W to prepare 100ug/ml standard solution.
5. **Standard BSA**- Bovine Serum albumin Dissolve 10 mg (10000ug) BSA in 100 ml D/W to prepare 100ug/ml standard solution.
6. **Standard Protein Casein**-500 mg/ml –Dissolve Casein in 100 ml 0.1 N NaOH.
7. **Standard Glucose** – Dissolve 50 mg (50000ug) Glucose in 100 ml D/W to make 500ug/ml standard.
8. **Ninhydrin Reagent**: 0.1 g Ninhydrin in 100 ml Glacial Acetic Acid along with few drops of Acetone.
9. **Preparation of Biruet Reagent**: Dissolve 1.5 g Copper (II) Sulphate Pentahydrate and 6 gm Sodium Potassium Tartarate in 500 ml water. Add 300 ml 10 % (w/v) NaOH and make the volume to 1 liter with water. Add 1 gm Potassium iodide to inhibit the reduction of Copper. Store in a Plastic Container in dark. Discard if any black or Reddish Precipitate is observed.
10. **DNSA reagent**: 2 gm DNSA in 40 ml 2N NaOH+ 60 ml D/W+ 60gm Sodium Potassium Tartarate.
11. **Lowry's reagent- Reagent A:** consists of 2 gm Sodium Potassium Tartarate Tertahydrate+ 1 gm Sodium Carbonate+ 500ml 1 N NaOH+ D/W make volume up to 1 liter.
    Reagent B : Consists of 2 gm Sodium Potassium Tartarate Tertahydrate+ 1 gm Copper Sulphate+ 90 ml D/W+10 ml 1N NaOH.
    Reagent C: Consists of 1 volume of Folin-Ciocalteau reagent dilute with 15 volumes of D/W.
12. **Standard Leucine: (65ug/ml)** Dissolve 65 mg Leucine in 100 ml D/W it will be stock. Then take 1 ml from it and add 9 ml D/W to prepare standard 65ug/ml.