RECOVERY OF PROTEIN FROM SLAUGHTERHOUSE EFFLUENT

A CASE STUDY OF MINNA ABATTOIR.

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A RESEARCH PROJECT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF ENGINEERING (B.ENG) DEGREE IN CHEMICAL ENGINEERING.

NOVEMBER, 2004

DECLARATION

I, Muhammad Awwal Musa (98/7068EH) declares that this thesis is solely the result of my work and has never been submitted anywhere for any degree. All literature cited has been duly acknowledged in the reference.

Muhammad A. Musa

23/11/2004
Date:

CERTIFICATION

This research work is the original work undertaken by Muhammad A. Musa (98/7068EH), which has been examined and certified under the Supervision of Engr. D.O. Adeniyi to be adequate in scope and quality, following the requirement of the Department of Chemical Engineering, Federal University of Technology Minna, for the award of degree of Bachelor of (B. Eng), in Chemical Engineering

Project Supervisor
Engr. D.O. Adeniyi

23/11/2004
Date

Dr. Aberuagba
H.O.D Chemical

Date

External Examiner

Date

DEDICATION

This work is dedicated to the memory of my late father and brother who were around during the sunrise but could not witness the sunset. May Allah (SWT) grant you Jannatul Firdausi Amen.

ACKNOWLEDGEMENT

All praise goes to Allah (SWT), for giving me wisdom and guidance throughout my stay is school. May the peace and blessing of Allah (SWT) be with the noblest of men and His messenger, Muhammad (SAW), His family, companions and those who follow their footsteps until the end.

My sincere appreciation goes to Eng. Adeniyi my Supervisor for his constructive criticism that brought out the best in me. And to all my lecturers at the department of chemical engineering F.U.T. Minna, I say thank you Sirs.

I also have to acknowledge the members of my family; the whole of me would not be complete without you, my humble brother, Alh. Sani, his wife Asiya and their children for being patient with me. Hajiya Hassana, Amina, Gambo, coachy, ma'aji, you guys are all great, thank you for being there for me.

To my friends, Rooqaiyer, Yusuf, Abu, Musty, Daura, Haruna, Yahya and others, remember that there is a time to meet and there is a time to part, but our friendship shall forever remain for all to see. Merci Bokou.

ABSTRACT

Slaughterhouse produces large amount of liquid effluents that contain organic material that have become a source of pollution to the receiving environment. These have deleterious effects on humans, animals and aquatic life. However these effluents contain substances that could be recovered and used for industrial purposes.

This work was out carried at the Minna abattoir to recover protein as well as ascertain the level of pollution of the effluent if not purified. Physiochemical tests were carried out, and the level of organic material was seen to be high. The double stage pH adjustment method was used to recover protein. An amount of 26.5kg of protein was recovered per cubic meter of effluent. The solid protein has an average nitrogen and crude protein content of 6.64% and 41.4% respectively. The recovered solid could therefore be used as raw material for animal feed and for fertilizer production.

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CHAPTER ONE

1.0 Introduction

1.1 Background

The effluents from slaughterhouse have always been a source of pollution to the receiving environments. These effluents produced during the slaughter of animals and processing of meat and by products are characterized by high concentration of organic nitrogen and fat (Leonard et al 1979).

The provision of slaughtering facilities in the developing countries whether it being a small slab or a factory abattoir should be considered as an integral part of national livestock development plan, whose target is to establish an efficient livestock and meat industry that will consider the safety of animals as the environmental. (Williamson & Payne 1995). The trend in most African and developing countries is to site these abattoirs near water bodies into which their effluents can be discharged. To start with, these gives rise to rapid growth of bacteria and fungi that are often pathogenic causing diseases such as hepatitis, typhoid, dysentery, poliomyelitis etc (Wollman 1972).

Furthermore, there is rapid oxygen depletion in the affected streams as a result of high BOD of the effluent. This affects the fish and other respiring organisms in the water bodies. In addition some of the end products of biodegradation of this effluents such as carbonates, sulfates, nitrates and ammonia exhibit certain undesirable effects on the water bodies to which they are discharged. For instance, ammonia interferes with disinfecting of waters by

chlorination. Also high nitrate level in water causes certain infantile disorder and formation of carcinogenic nitrosamines in the stomach (Besselievre and Schwarz 1976).

The wastes associated with slaughter house are normally divided into two types, solid wastes which include bones, hooves, fur, offal, and horns which are in used industries for the production of glues, adhesives, cotton wool, feed supplement, fat and hides (grant 1980). The liquid wasters comprise of washed away blood, dissolved hydrophilic protein during the evisceration exercise and fat globules, consequently, slaughter house effluents are rich in organic nitrogen (Leonard et al 1970).

The process of upgrading these effluents by recovery of nutrient help to some extent in the reduction of these carcinogenic and harmful materials in the effluent, also animals, insects and rodents must be controlled. For example if a dog consumes the offal infected with hydatides it become a source of infection to man, and to break the cycle, all dogs must be excluded, and care must be taken that dogs have no access to condemned materials (Williamson & Payne 1995).

1.2 Nutrient Recovery

Some of the nutrients that can be recovered from slaughterhouse effluents include fats, and protein, which is due to high nitrogen content of the effluent. The biological oxygen demand (BOD) of effluent can also be removed to avoid rapid oxygen depletion of the effluent. High BOD and COD, decrease the chances of survival of aquatic live. It has been estimated that the total amount of

water on earth is 1.233×10^{18} m^3 (Wollman 1962) of which 3% is fresh portable water, of this fresh water, 0.06% is available for individuals and town supply. Therefore the conservation of water is of primary concern. The need to monitor the waste being dispose into these waterways is very important.

1.2.1 Fat Extraction

Fat extraction is not new and in particular, rendering have been with us for a very long time. However, the whole process of fat extraction including rendering, mechanical and solvent extraction to produce fats and protein rich meals for use in livestock and poultry feeding is under continue review. This is because the capital cost of equipment, the running cost, cost of energy, the availability of the materials and the value of the final product all have to balance (Leonard, Grant, Harold and John1979).

Recovery of fat from poultry to give a protein rich meal continue to expand and methods used to recover fat from their sources are discussed in this research.

However, inspite of the vast amounts of fats and oils now available experts predict a shortage in the future and in addition there will be shortage in the meat and dairy products. Although there are improved agricultural and farming methods, the fact is, the production must be stepped up to cope with the anticipated protein demand, particularly in developing countries where protein consumption averages will remain a problem. Some answers to these may to found in industrial food production techniques. Fermentation, which results in the

production of single cell protein (SCP) is an emerging industry which permits the growth of microorganism rich in protein. (DAVIE 1984).

Whether talking about fat extraction from oil seed, animals, or SCP, there is a basic similarity in the processes used. It is in the preparation of material prior to the extraction that the greatest differences in the unit operation are seen. In this research the method of extracting fat from poultry wastes shall be discussed.

1.2.2 Protein Recovery

Proteins are complex organic compounds of high molecular weight in common with carbohydrates and fats, they contain carbon, hydrogen and generally sulphur. (McDonald, Edwards, and Halgh 1995).

The slaughter house effluent contains large amount of colloidal nitrogen (Leonard et al 1979).

Some of the methods reported in literature for protein recovery include membrane separation, chemical treatment, acidification, precipitation by anions and cations and use of special precipitants (Bernard and Ferda 1991). Differences in the treatment efficiencies and the composition of the precipitated solids observed from different processes can be explained in terms of the mechanisms involve. The mechanisms are related to properties of protein in solution and their reactions with anions and cations. (Cooper and Russell 1982).

1.3 Scope

The scope of this work is to upgrade wastes from slaughterhouses and to purify the effluent of solids (Protein) to be used for other industries as raw material and as meal for animals and poultry.

1.4 Aims And Objectives

i. To recover nutrient from the effluent of slaughterhouses.

ii. To determine a suitable process for the recovery in terms of cost and efficiency amongst the various process alternatives.

iii. To purify the effluent before being allowed to enter the waters bodies so as to avoid the concentration of casinogenic and unwanted materials that can harm the aquatic life.

CHAPTER TWO

2.0. LITERATURE

The abattoir in Minna is a typical example of the types of abattoirs found in most African and developing countries. The effluents from this abattoir are mostly discharged into water bodies where they cause rapid oxygen depletion as a result of high biological Oxygen Demand (BOD) of the effluent. The effluents discharged into the water bodies have high concentration of organic nitrogen and fat (Leonard at al 1979). The removal of this material by any conventional means such as sedimentation and screening results in effluents with high soluble and colloidal organic nitrogen and fat together with a substantial amount of oxygen demand.

Table 2.1. Shows clearly the high proportion of soluble nitrogen typically found in slaughter house effluent:

Component	Range (g/m^3)
Biological Oxygen Demand (BOD)	98000 – 250000
Chemical Oxygen Demand (COD)	168000 – 420000
Ammonites – Nitrogen (NH^3-N)	700 – 7000
Total Fat	14000 – 126000

Aerobic and anaerobic lagoons commonly treat these effluents and oxidation ponds or sludge or trickling filters. The net results of these treatments in that the organic nitrogen is incorporated in the cell biomass or degraded to ammonia. (Denmead and Cooper 1975).

Some of the methods that can be used for nutrient recovery and concentration include membrane separation, chemical treatment, acidification etc. (Bernard and Ferda 1983).

2.1. **Proteins**

Proteins are the most abundant macromolecules found within living cells where they account for up to 50% of dry weight.

A substance resembling modern day protein was suspected to be a constituent of animal tissues and to a lesser extent of plant, and this dietary component was considered to be essential for the renewal of blood. (Monroe 1964). Chemically proteins are polymers of hundred or thousand of amino acid joined together by a peptide bond. (Bernard and Ferda 1983).

Proteins are found in all living cells, where they are intimately connected to all phases of activity that constitute the life of the cell. Each species has it's own specific proteins, and a single organism has many different proteins in its cell and tissue. It follows therefore that a large number of proteins occur in nature. (McDonalds, Edward and Halgh 1995).

2.1.1. **Structure of Proteins**

For convenience, the structure of proteins can be considered under four basic headings.

2.1.1.1. **Primary Structure**

Proteins are built up from amino acids by means of a linkage between the ∝ – carbonyl of one amino acid and the ∝ amino group of another acid. This type of linkage is known as the peptide linkage. Large numbers of amino acids can be found by this mean, with the elimination of one molecule of water at each linkage to produce polypeptides. The term primary structure refers to the sequence of amino acid along the polypeptide chains of protein.

2.1.1.2. **Secondary Structure**

The secondary structure of proteins refers to the conformation of the chain of amino acids resulting from the formation of hydrogen bonds between the amide (NH) and carboxyl groups of adjacent amino acids. The secondary structure may be regular in which case the polypeptide chains exist in the form of an ∝- helix or a β- pleated sheet or it may be irregular and exist as a random coil.

2.1.1.3. **Tertiary Structure**

The tertiary structures describe how the chains of the secondary structure further interact through the R- groups of the amino acid residue. This interaction causes folding, and bending of the polypeptide chain, the specific manner of the folding giving each protein its characteristics biological activity.

2.1.1.4 Quartenary Structure

Proteins possess quartenary structure if they contain more than one polypeptide chain. The forces that stabilize these aggregates are hydrogen bonds

and electrostatic or salt bonds formed between residues on the surface of the polypeptide chains. (McDonald, Edwards, and GreenHalgh 1979).

2.1.1 Classification of Proteins.

Proteins are classified into three major groups according to their shape, solubility and chemical composition.

2.1.2.1 Fibrous Proteins

These proteins are insoluble animal proteins that are very resistant to animal digestive enzymes. They are composed of elongated filamentous chains that are joined together by cross linkages. This group contains the collagen's that are the main proteins of connective tissues. Elastin is the protein found in elastic tissues such as tendons and arteries, while keratin are the proteins of hair, nail, wool and hooves.

2.1.2.2 Globular Proteins

This group includes all the enzymes, antigens and those hormones which are proteins they can be subdivided into albumin which are wastes soluble and heat coaguable and which occur in milk, egg, blood and many plants. The globulin are insoluble or sparingly soluble in water and are present in eggs, milk and blood, and are the main reserve protein in seeds.

2.1.2.3 Complex Proteins

The complex or compound proteins on hydrolysis yield non-protein groups, usually called 'prosthetic groups' as well as amino acids. The prosthetic group

may vary and may be phosphoric acid (phosphoproteins), a carbohydrate or carbohydrate derivative (glycoproteins), a lipid (lipoproteins), a pigment (chromoproteins) or nucleic acid (nucleoproteins). (McDonald, Edward and Halgh 1995).

2.1.3 **Properties of Proteins**

All proteins have colloidal properties, they differ in their solubility in water ranging from insoluble keratin to albumin's which are highly soluble. Soluble proteins can be precipitated from solution by addition of certain salts such as sodium chloride or ammonium sulphate. This is a physical effect and the properties are not altered. On dilution, the proteins can easily be redissolved (McDonalds, Edward and Halgh 1995).

Although the amino and carbonyl groups in the peptide linkage are non-functional to acid-base reactions, all proteins contain a number of free amino and carbonyl groups, either as terminal units in the side chain of amino and residues. Like amino acid, proteins are therefore amphoteric. They exhibit characteristic isoelectric points and have buffering properties.

All protein can be denatured or changed from their natural state. (Bernard and Ferda 1983). Denaturation can be defined as any non-proteolytic modification of the unique structure of a native protein rise to definite changes in chemical, physical or biological properties (Neurath et al 1985). Products of protein hydrolysis are not included in this term. Perhaps the best known example of denaturation is the coagulation of a protein solution, such as egg white upon

heating. Many proteins are heat coagulate. Apart from heat there are many other agents, which can bring about the denaturation of proteins, these include strong acid, alkali, alcohol, acetone, urea and salts of heavy metal.

(Plummer 1993). The most notable effects denaturation are the changes in the biological properties for example enzymes are usually inactivated, changes in solubility and optical activity may also occur, solution of protein are laevorotatory and denaturation increases the specific rotation. (McDonald, Edward and Halgh 1995).

2.1.3.1 **Acid – Base Reaction**

Proteins in solution carry a net charge, the nature of which may be positive or negative, depending on the pH of the solution (fig 2.1). At a particular pH value, the isoelectric point (PI), the net charge is zero. The net charge on the protein is positive at pH values below the isoelectric point and negative at pH values above. (Leonard et al 1979).

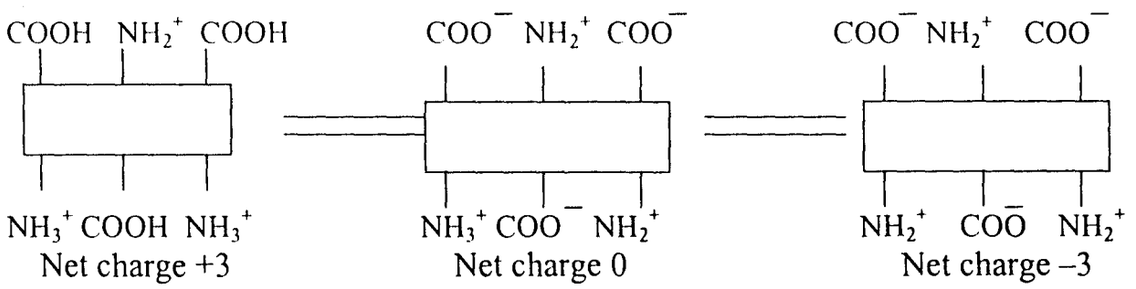


Fig 2.1 Schematic representation of effect of pH on protein charge.

Proteins also behave in solution as colloidal particles and are stabilized by their surface charge. At the isoelectric point, attractive forces (Van der Waal) may lead to agglomeration and flocculation. However, some proteins such as

An important disadvantage of the isoeletric pH adjustment processes is that blood proteins which can be major contributor to the soluble organic nitrogen of a slaughter house effluent are not precipitated.

2.1.3.2 **Protein Cation Reaction**

The charged surface of colloidal molecules attract ions of the opposite charge, resulting in the concentration of the counter ion being higher near the bulk solution. This phenomenon is known as the charged double layer. Adding an electrolyte to the solution reduces the range over which repulsive forces act by compressing the double layers and many promote destabilization and agglomeration of colloids. With negative changed protein colloids, cations are responsible for the compression of the double layer and solutions of ferric and aluminum salts are of used (Cooper and Russell 1982).

Treating a slaughter house effluent with $40g/m^3$ of aluminum ($Al_2 So_4$) and reducing the pH to 5.0 produce result similar to those obtained by acidification to the average isoelectric point. Increment of the aluminum dose increases the range of minimum solubility. The material produce by this process is unlikely to have any value due to high aluminum content of the precipitated solids. Besides this method is only effective in the removal of negatively charged proteins colloids. (Fair et al 1966). Cations can also form complexes with proteins by chemical reaction with the protein groups resulting in flocculation. They may also form bridges between protein colloids and ployelectrolytes resulting in enhanced agglomeration and flocculation.

2.1.3.4. **Protein Anions Reaction**

Anions interact with positively charged protein colloids, the interactions being of a similar type to those described for cation. Anions species reported include polyphosphates, lignosulphonates and glucose trisulphates (Cooper and Denmead 1979).

2.1.4 **Methods of Recovery**

2.1.4.1 **Membrane Separation**

Membrane separation include ultra filtration dialysis, and reverse osmosis which is good in concentrating protein in pure solution. The ease of separation depends on the membrane pore size, shape and geometry and the size of the protein colloids to be retained (Fair et al 1966).

It will not be a good process for recovery of protein in slaughterhouse effluent because certain undesired molecules such as fat globules undigested polysaccharides and rumen content will also be retained. This will require further expense in purification.

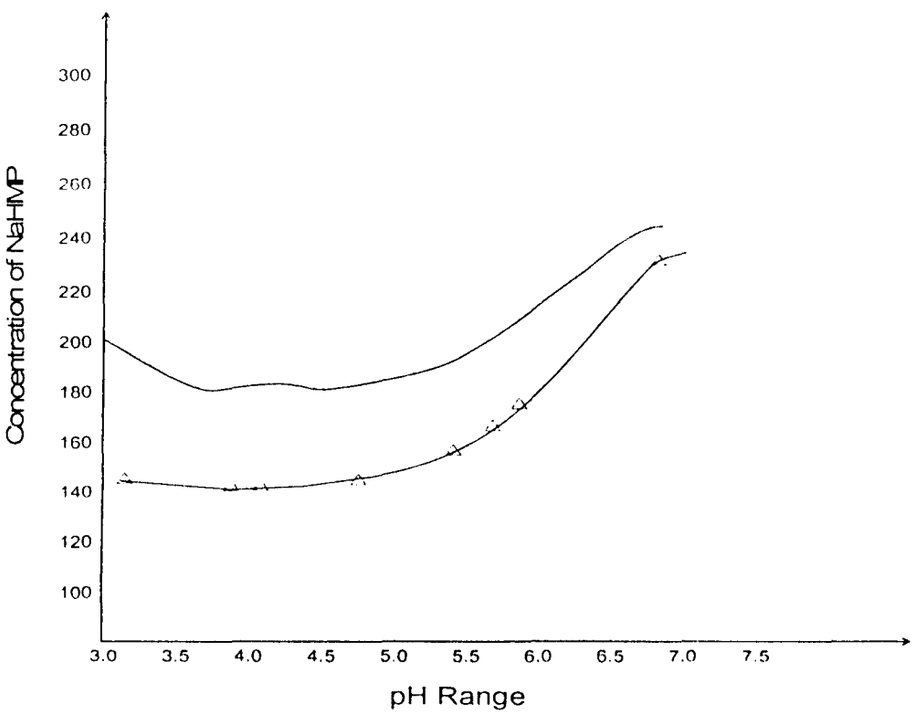
2.1.4.2 **Protein Precipitation**

Protein precipitation makes use of properties of charged proteins in solution and their reaction with cations and anions. It is achieved by adding a non-solvent or a salt to the solution or lowering the temperature of the solution containing the desired product (Bernard and Ferda 1991). It also allows the particles to remain in solution and to induce destablisation, which will in turn promote aggregation and precipitation (Cooper and Russell 1982). The method is

only effective in the removal of negatively charged protein colloids. Special precipitants used include sodium Hexametaphosphate (NaHMP), purified lignosulphonates (a by-product of wood pulping industry) and organic solvent such as ethanol, propanol, iso-propanol, acetone, trichloroacetic acid. NaHMP can precipitate both hemoglobin and fibrinogen at a pH of 3.5, the exact amount of NaHMP required to precipitate proteins at a stated pH will depend on the number and availability of positively charged groups in the protein colloids.

Fig 2.3 effect of sodium hexametaphosphate NaHMP on the soluble organic carbon of slaughter house effluent.

On the other hand, lignosulponates effect precipitation by charge neutralisation. When organic solvents are added to protein solution, they lower



the protein solubility. However, proteins are more easily denatured in organic solutes at temperatures above 100oc. So a refrigerated condition may be needed.

Ionic strength between 0.05m-0.2m is recommended. Precipitation by lignosulphonates shows many features in common with NaHMP for example they precipitate hemoglobin and the pH at which minimum protein solubility occurs and the degree of precipitation is dependent on the dose of lignosulphonate.

2.1.4.3 **Precipitation By Double Stage PH Adjustment Method**

This method produces an effluent superior to that produced by acidification alone but without the cost of specific precipitants such as NaHMP or lignosulphonates. The processes is useful and effective for effluents with high blood content (Cooper, Russell and Adam 1982)

2.1.4 **Recovered Solids**

The principal objective of the treatment processes outlined is to purify protein-containing effluents and at the same time recover solids whose composition makes it of value as a source of protein. There are two principal factors, which influence the composition of the solids, namely the characteristic of the influent to the process and recently, the process itself.

2.1.5 **Influence of Waste Water Characteristics**

Chemical treatment processes not only precipitate proteins and destablise colloidal material but also break fat emulsion. Thus the composition of an influent to such process will dictate the composition of the recovered solids. For

example an influent containing high level of fat will result in recovered solids which contain higher level of fat than would be the case for a low fat influent (Denmead and cooper 1975).

A similar relationship will exist relating the proportion of precipitable organic nitrogen to the percentage protein in the dry recovered solids.

2.1.6 **Influence of The Process**

The processes used for the purification of protein containing effluents will influence the composition of the recovered solids. Those processes, which remove organic nitrogen per unit volume of influent, will produce solid containing a higher percentage of protein.

2.1.7 **Utilisation of Recovered Solids**

Chemical treatment processes are expensive to build and operate. If the Recovered solids have no value the cost of disposal of the solids produced could be prohibitive and alternate treatment technologies more attractive.

2.2 **Fats**

Fats are constituents of both plant and animals and are important sources of stored energy. They have a general formula with oil, also structural and chemical properties but have different physical properties. The melting point of oils is that at room temperatures they are liquid. The term fat is frequently used in a general sense to include both groups. (McDonald, Edwards and GreenHalgh).

2.2.1 **Composition of Fats**

It is frequently important in nutritional investigations to assess the quality of fat being produced under a certain treatment. Where the effect of the diet is considerable the results may be obvious in a softening or hardening of the fat. Less obvious changes may occur, and for these a more objective assessment is necessary. Differences in fat are a result of the fatty acid composition, since glycerol is common to all fats. The logical method of following changes in fat is to measure their fatty acid composition. Analysis of fatty acid have present more problems in the past, but the introduction of techniques such as gas chromatography in recent years has allowed the determination to be made more easily and accurately. This means a more certain identification and characterisation of fats and provides a more accurate method of detecting and quantitatively estimating the adulteration of a given fat or oil than previously available (McDonald, Edward and GreenHalgh 1995)

2.2.2 **Properties of Fats**

2.2.2.1 **Hydrolysis**

Fats may be hydrolyzed by boiling with alkali to produce soap and glycerol. Such hydrolysis is known as saponification since it produces soap, which are sodium or potassium salts of fatty acid. The process may take place under the action of enzymes known as lipase.

2.2.2.2 Oxidation

The unsaturated fatty acids, readily undergo oxidation, the site of action being the carbon adjacent to the double bond, hydroperoxides are formed. These breakdown to give shorter chain products including free radicals which then attack other fatty acid much more readily than the original oxygen. More free radicals are produced with the result that the speed of the oxidation increases exponentially. Eventually, the concentration of free radicals becomes such that they react with each other and the reaction is terminated.

2.2.2.3 Antioxidants

Natural fats possess a certain degree of resistance to oxidation, owing to the presence of compounds termed antioxidants. These prevent the oxidation of unsaturated fats until they themselves have been transformed into products.

2.2.2.4 Hydrogenation

This is the addition of hydrogen to the double bonds of an unsaturated acids of fats, converting them to saturated analogues (Potter and Hotckiss 1989).

2.2.3 Fat Extraction

Stages Of Extraction

The raw material from which animal fat is extracted include fallen stock, butcher waste, reject carcasses, bones, slaughter house effluent, chicken and turkey waste. The finished products are fat, meat and bone meal.

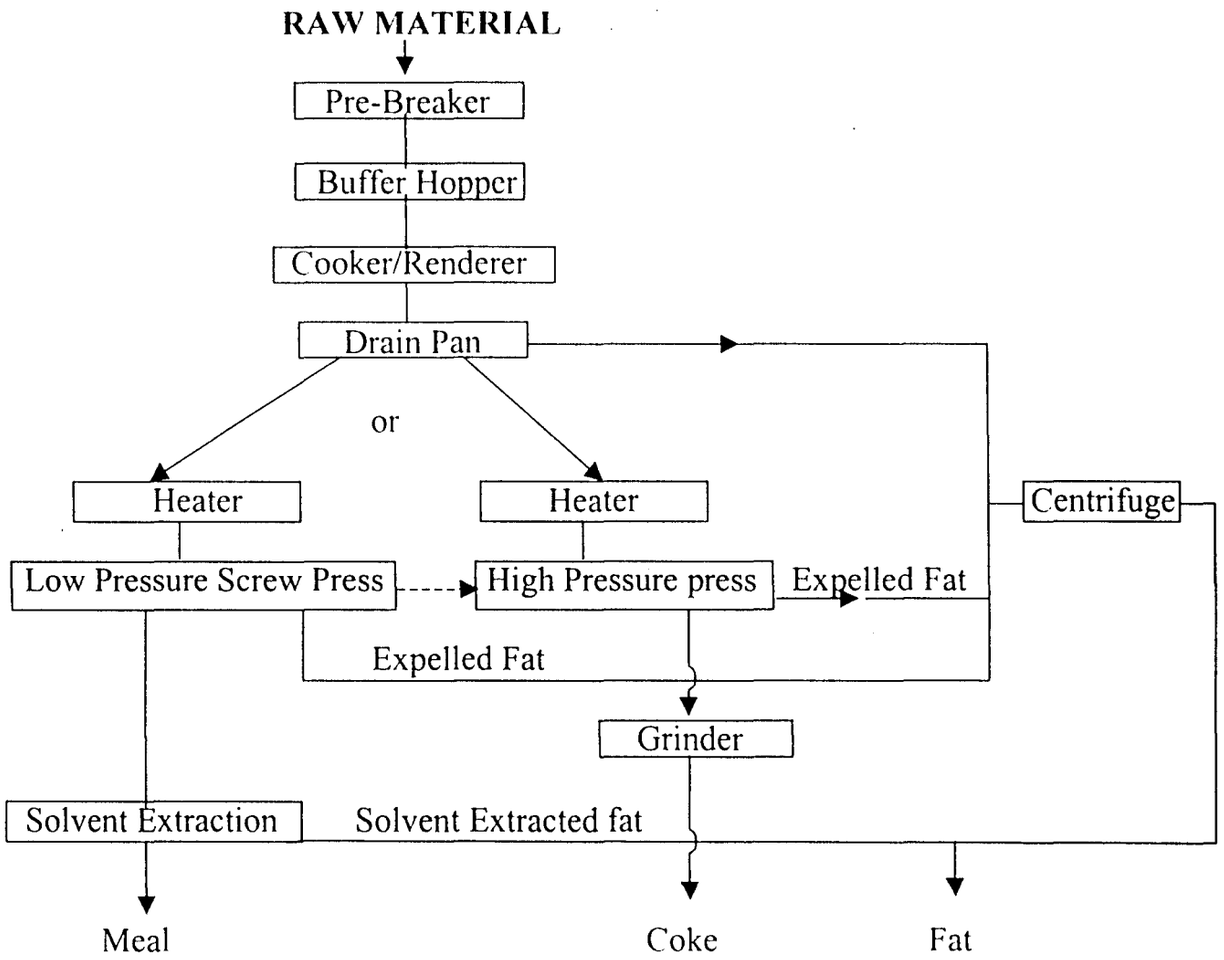


Fig 2.4 Flow diagram outlining the stages involved in the production of animal fat. (Davie 1983)

In order to reduce protein degradation, minimise the increase in fatty acids and limit the problem of smell as much as possible, the raw material should be received in as fresh a state as possible and processed rapidly. The various stages of the extraction are outlined in fig above, although for several of the stage shown alternatives may well exist and be used in certain circumstances. The detailed stages are outlined below: -

2.2.3.1 **Prebreaking**

Slaughterhouse effluent that has assumed a solid shape can be broken down into pieces less than $2cm^3$. The main factors to be considered in the design of prebreakers are safety and ruggedness. Irrespective of the design, of the prebreaker the products are sent to the next stage of the process which vary widely in consistency and the variation must be catered for by the machine which carry out these subsequent operations.

2.2.3.2 **Rendering**

This process is carried out to release fat from the materials and this produce a high protein meal of good storage stability. In addition it does serve to retain the protein quality and to sterilize the meat by destroying microorganisms whilst effectively removing moisture.

Where rendering is a batch operation it is necessary to have more than one unit and /or storage if continuous running of the downstream plant to be achieved. Other methods of cooking includes continuous rendering (Burton 1979), wet rendering (Blogg 1976) and a process where the effluent is mixed with liquid fat and then dried under vacuum to include rendering (Granofsky 1978).

2.2.3.3 **Fat Separation**

This step is achieved by percolation followed by mechanical extraction and where necessary solvent extraction.

2.2.3.4 Percolation Stage

When handling animal greaves, the free fat present can be run off into a tank fitted with perforated plate or wedge wire screen bottom or into a conveyor with a trough that has been similarly modified.

2.2.3.5 Mechanical Extraction

The percentage of fat which can be tolerated in the extracted cake or meal will determine whether this stage yields the final product or whether it is a preliminary operation to remove some easily won fat prior to a subsequent solvent extraction.

Solely, mechanical means can remove a significant amount of fat in the greaves but will invaluably leave between 8% and 10% remaining in the resulting cake.

2.2.3.6 Solvent Extraction

If required greaves from the low pressure screw press or from the basket centrifuge can be passed to a solvent extraction plant to further reduce the fat content to as low as 1% if desired.

Although, several solvent have been used in the past to defeat the stock, including trichloroethylene, hexane is now used almost exclusively to perform the task (Ledwards 1983)

2.4 BOD and COD

2.4.1 Biochemical Oxygen Demand (BOD)

High BOD (5 days biochemical oxygen demand) characterizes meat and poultry effluents, which are influenced by the blood content. Improved blood collection is the most effective way of reducing BOD load, while differences in discharge volume and strength exists. Abattoirs produce 1.6 –10.2kg BODS per head, and 0.4-3.4m^3 per head (Eckenfelder 1989).

2.4.2 Chemical Oxygen Damand

This is he measure of the total organic carbon with the exception of certain aromatics such as benzene which are not completely oxidized in a reaction. Raised temperature affect both the BOD and COD, as it accelerates the rate of organic decay, at the same time the water can carry less oxygen so that the more active animals are soon affected and possibly eliminated altogether (Eckenfelder 1989).

2.4.3 Treatment

Animal waste BOD can be treated readily aerobically, ammonia toxicity may be problem when dealing with fresh wastes that contain a large amount of urine. The anaerobic ponds are loaded to such an extent that anaerobic conditions exists throughout the liquid volume, being primarily organic acid formation followed by methane formation. The depth is selected to give a minimum surface area per volume ratio and thereby nitrides heat retention. (Eckenfelder 1989).

CHAPTER THREE

3.0 Materials And Experimental Procedure

3.1 Physiochemical Tests.

3.1.1 BOD Test

Biochemical oxygen demand is a measure of the amount of oxidizable organic matter present in water or waste.

Oxygen depletion occur when large amount of organic materials, which requires oxygen for their deposition are introduced into the stream. The biochemical oxygen demand of a sample is determined by an empirical, procedure. It is expressed as the amount of oxygen per unit volume consumed by microbes in the biological oxidation of temperature and time the biochemical oxygen demand was carried out on effluents collected from the slaughterhouse in Minna.

3.1.1.1 Materials

(a) Incubation bottles: - these are narrow mouthed glass stoppered bottles of nominal capacity of 250ml were used and essentially cleaned using 2.5mol/l of sulphuric acid and thoroughly rinsed.

(b) Incubator: - the temperature of incubation was $20^{o}c \pm 1$.

A water cold incubator was employed incubation was carried out in the dark.

(c) Magnetic Stirrer

(d) Sample from Minna slaughters house affluent.

3.1.1.2 Reagents:-

Sodium Hydroxide

3.1.1.3 Procedure

Inside two separate biochemical oxygen demand bottles, 40ml of the effluent sample was measured after which a magnetic strirrer was dropped into the bottles. Rubber quiver's having tweezers were inserted in the neck of each of the bottles.

Sodium hydroxide tablet was put in the rubber quiver. The tablet was not allowed to come in contact with the sample. The bottle was closed tightly with an oxitop. The m key on the oxitop was pressed until it displayed zero. The measuring bottle was then kept in the incubator for five days with the reading taken every twenty-four hours.

3.1.2. **COD Test.**

This is also known as the permanganate value because it is the amount of permanganate consumed by the microorganisms that in measured.

3.1.2.1 **Materials**

Beakers, 2000ml volumetric flask, volumetric flask pipette, burette, effluent samples.

3.1.2. 2 **Reagents**:-

Potassium iodide 10% w/v, potassium permanganate solution, sodium thiosulphate, concentrated tetraoxosulphate (vi) acid.

3.1.2.3 **Procedure:-**

10mls of the effluent sample were measured into a clean beaker, after which 10mls of potassium permanganate and 5mls of tetraoxosulphate (vi) acid

solution was added. The solution was shaken gently and examined at intervals. As the pink colour of the potassium permanganate tends to disappear, a further 10ml of potassium permanganate solution was added in order to maintain definite excess, this was then left for four (4) hours.

Similarly, a blank was prepared for the sample by adding the same volume of reagents used for the test to 10mls of distilled water. At the end of four hours1ml of potassium iodide solution was added to the beaker before the whole mixture was titrated against sodium thiosulphate in the burette, starch solution was added as an indicator. The titration continued until the entire blue colour disappeared.

The titration was repeated for blank.

3.1.2.3 Calculations

The chemical oxygen demand is calculated as follows: -

$$COD = \frac{D\text{-}S \times 1000}{\text{Vol. of Sample}}$$

Where

D = Vol. of potassium permanganate required for blank

S = Volume of potassium permanganate required for sample.

Volume of sample = 10mls

3.1.3 **Temperature and Ph.**T

The temperature and pH of the effluent were measured immediately after collecting the effluent. The mercury thermometer was used to measure the temperature while the pH meter was used to determine the pH.

The probes of the pH meters were dipped into the collected sample and the reading were taken when the values were stable.

3.1.4 **Determination of Crude Protein**: -

The Kjedahl method as described by James (1995) was used to determine the crude protein of the fresh effluent as well as the final supernatant.

A factor of 6.25 was used to convert the nitrogen to crude protein.

3.1.4.1 **Materials**

(a) Mixed catalyst: - 160g anhydrous $K_2 So_4$, 10g C_uS0_4. $5H_20$, 3g selenium power mixed well in a mortar and stored dry in a container.

(b). Sulphuric acid 98% concentration.

(b) . Sodium hydroxide 40% w/v.

(d). Boric acid AR 1% w/v.

(e). Hydrochloric acid, standard $\frac{N}{70}$.

(e) Mixed indicator; Bromo cresol green 99mg, methyl red 66mg, thymol blues 11mg mix and dissolve in 100ml ethanol.

3.1.4.2 **Procedure**

250mg of sample was weighed into a clean kjedahl flask then a catalyst was added. 6ml of conc. H_2SO_4 was added. Then digestion was carried out with low flame in the heater until frothing subsides and then at higher temperatures until content are clean greenish, digestion was continued for another 60 min. The

heat was allowed to cool off after switching off the heater, 15ml of water was added the content was then transferred to the 50ml volumetric flask.

10ml of the digest was transferred into Markham semi-nitrogen still, 10ml of 40% sodium hydroxide was added. Steam distilled ammonia, liberated into the 5ml boric acid solution containing 4 drops of mixed indicator taken in a conical flask. The indicator will turn green, distillation was continued for 2 more min.The distillate was removed thereafter and liberated against standard hydrochloric acid, the point being reached when the indicator change from green through gray to pink.

A blank was then run through the whole procedure to get the burette reading of standard HCl

3.1.4.3 **Calculations**

$$\% \text{ Nitrogen} = \frac{M \times V \times 14 \times 100}{\text{Wt of sample}}$$

Where M = molarity of acid
V = volume of acid

3.1.5 **Protein Recovery**

The two-stage pH adjustment method was used in the recovery of protein, this is because it is most effective for effluents with high concentration of blood (Cooper, Russell and Adam 1982). These observations can be explained in terms of the known properties of hemoglobin a major blood protein which does not precipitate at its isoelectric point (pH 6.5). If hemoglobin is acidified to a pH of 3.0 and then neutralized to between pH 6.5 and 7.0, flocculation and precipitation

of protein is observed. This is due to the splitting of hemoglobin into haem and globin units at pH 3.0 (Fanelli, Antonini, and Caputo 1963). The globin being precipitated at pH 6.5 –7.0 (Taylor, Dill and Landman 1973).

3.1.5.1 Materials

(a) Test tubes stand with a set of test tubes.

(b) 25ml pipette

(c) A screen with 200m mesh diameter.

(d) A centrifuge

(e) Filter papers

3.1.5.2 Procedure

The sample of effluent collected was screened to remove unwanted solids. The pH of the screened effluent was measured and it was about 8.0. The effluent was then acidified to a pH of 7.5; flocs, which formed, were allowed to agglomerate for 20mins. The flocs were removed as solid by centrifuging parts of the solids that stayed on the centrifuge cups were washed and the resulting solution filtered to remove unwanted solids. The clear liquid phase that is the decanted supernatant from centrifuging was acidified to pH of 5.5, 4.0 and 2.5. Floc recovery was done at each pH. The solution with pH of 2.5 was neutralized using sodium hydroxide to a pH of 4.0. This resulted in precipitation of protein colloids in the solution. This was then centrifuged and filtered to obtain the precipitated proteins.

The decanted supernatant (clear liquid phase) was further acidified to pH 5.5 and 7.5 thus protein recovery was done at each pH.

The solids recovered both by centrifuging and filtration were air-dried and weighed.

CHAPTER FOUR

4.0 Results and Discussion of Results.

4.1 Results of Physiochemical Test on Fresh Effluent

BOD TEST.

Fresh Effluent	Day 1	Day 2	Day 3	Day 4	Day 5
	9 x 50 = 450	9 x 50 = 450	11 x 50 = 550	17 x 50 = 850	15 x 50 =750

A factor of 50 was used to multiply each reading on the oxitop after every 24 hrs

Table 4.11

PARAMETER	RANGE
BOD (mg/l)	610
COD (mg/l)	880
Nitrogen (%)	0.10344
Crude protein (%)	0.6453
Temperature (^{O}C)	31
pH	7.2 –7.6
Colour	Reddish
Odour	Foul smelling

Table 4.12

RESULTS OF COD	EFFLUENT SAMPLE	BLANK OF SAMPLE
Final volume	22.30	54.6
Initial volume	2.00	24.4
Change in volume	20.30	30.2

Table 4.2 **weight of solids recovered using centrifuging and filtration**

Sample	Volume of effluent (x10-6m^3)	PH range & solid recovered	Acidification	Neutralization	Total solids recovered
		pH range	75-5.5 55-40. 40-2.5	25.40 40.7.0	
A	2800		0.01335 0.0312	0.0293	0.07385
		Solid recovered			
B		2400			
		Solid recovered	0.0089 0.0146	0.0312 -	0.0547
C	1800	Solid recovered	0.01836 0.0083	0.0198 -	0.04645
D	1208				
		Solid recovered	0.04324 0.0055	0.014 -	0.0308

TABLE 4.3 Weight of Solids Recovered Per m^3 of Effluent

Sample	Volume of effluent (x10-6m3)	Total solid recovered by centrifuging and filtration	Weight of solid recovered per volume of effluent
A	2800	0.07385	26.40
B	2400	0.0549	22.80
C	1000	0.04646	25.81
D	1200	0.03078	25.15

Table 4.4 **percentage nitrogen and crude protein content of recovered solids**

Sample	Weight (kg)	Molarity of acid (M)	Volume of acid (x10-6m3)	Nitrogen %	Crude protein (%)
A	0.002	0.055	192.60	6.62	41.4
B	0.00115	0.055	96.00	6.43	40.2
C	0.00105	0.055	87.00	6.38	39.9
D	0.001	0.055	79.00	6.08	38.0

Table 4.5 **efficiency of recovery method**

Sample	Mass (kg)	Morality of acid (m)	Vol. of acid (x10-6m3)	Crude protein
Fresh effluent	0.018	0.05	25.9	0.6295
Final supernatant	0.020	0.05	10.9	0.2385

CHAPTER FIVE

5.0 DISCUSSION OF RESULTS

The readings from the BOD shows that on the fourth day oxygen consumption reaches the maximum after which it starts to fall (from graph). The fall is due to the denitrification, so that from the fifth day the nitrate (No3) in the effluent will be forced to release oxygen by the bacteria in the effluent sample. This shows there is high BOD demand on the effluent sample.

There is also high COD in the effluent sample as can be seen from table 4.11. But the temperature and pH of the effluent have no effect on the environment since they are within the allowable limit. Thus the BOD and COD cause the deoxygenating of the stream to which the effluents are disposed. Pollution therefore results, when the oxygen demand exceeds the available oxygen. These causes annoying odour, taste and colour of the stream. Therefore there is need for purification of this waste. The method of recovery of protein was used for purification, the results are shown in table 4.2. The values are obtained directly from centrifuging and filtration processes. Table 4.3 gives the weight of solid recovered per m^3 of effluent. The nitrogen and crude protein content are shown in table 4.4, while the efficiency of the recovery is shown in table 4.5.

It was observed during the experiment that the colour of the sample changed from deep red at 7.5 to maroon at 5.0 and brown at 4.0. Also it was observed that there was no floc formation at pH below 4.0. It can therefore be concluded that pH range of 1.0 to 3.9 as not conducive to floc formation despite

the fact that protein colloids are present in the mixture. This may be due to induced protonation of the charged protein colloids, which may repel one another, the effluent was muddy brown within this pH.

From a pH of 2.5 to 4.0, large flocs where formed and the colour of the supernatant layer changed from muddy brown to light brown. Further addition of the base shows little or precipitation with no significant colour change. Thus, large flocs where obtained between pH of 4.0 to 5.0. Below this range no precipitation was observed as this agrees with that reported by Leonard.et al (1962).

Furthermore, hemoglobin and fibrinogen, the major constituents of blood proteins which do not precipitate under acidic conditions where precipitated with the addition of sodium hydroxide which resulted in the compression of the positively charge protein colloids. These colloids have hitherto repelled each other in the acidic range. Thus there is floc formation and agglomeration.

A table 4.4 show that the average nitrogen and crude protein content of the recovered solids which was 6.37% and 40.0% respectively. Grant (1980) reported that the crude or raw protein content of cotton cake, meat and bone meals and skimmed milk powder is 46,45-55 and 32-37% respectively. The value obtained in this work shows that the effluent contains appreciable amount of organic nitrogen. However, a higher value was expected, the lower value obtained may be due to: -

(1) There may have seen some loss of ammonia when using kjedahl method.

(2) Interrupted power supply.

(3) The mesh of the screen was 200m diameter. A mesh with a smaller size would have removed other contaminants.

CHAPTER SIX

6.0 CONCLUSION

From the foregoing, it has been observed that parameter such as BOD, COD and Ammonia have tremendous effect on the receiving water systems. These effects are due to deviation from acceptable national limits. Parameters such as temperature and pH fall along the acceptable limit whilst BOD, COD and ammonia are out of range. Therefore, the recovery of protein has helped to reduce the amount of these carcinogenic materials in the effluent.

The method employed is cost effective, as the reagents (H_2SO_4 and NaOH) are readily available and cheap. The effectiveness of the method is also observed as 26.4kg of solid protein was recovered from $1m^3$ of effluent, the nitrogen and crude protein content were 6.62% and 41.4% respectively. This protein could be used for animal feed as well as raw material for fertilizer production.

6.1 Recommendations

Chemical treatment processes are expensive to build and operate. If the recovered solid have no value the costs of disposal of the solids produced could be prohibitive and therefore alternative treatment procedure should be chosen. In order to establish the quality of this work a prototype plant should be built.

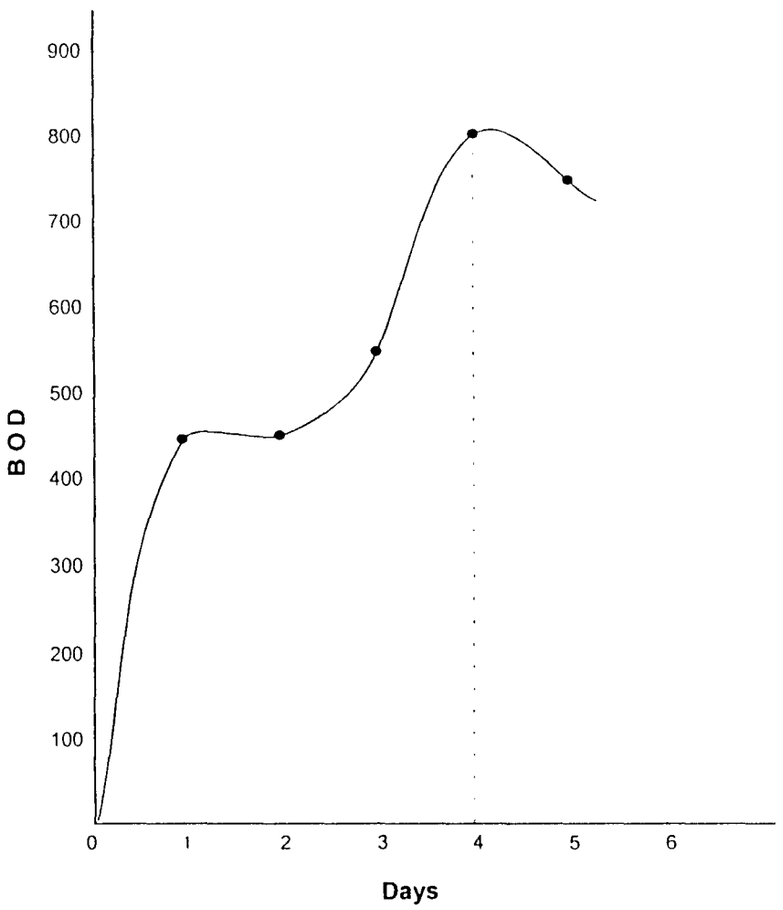
Animal feed industries and fertilizer production industries should be encourage to use the raw materials produced from this plants.

Students should be given more time to work on their research and government agencies and parastatals should support them.

APPENDIX

Graph of Biochemical Oxygen Demand

Day 1	Day 2	Day 3	Day 4	Day 5
9x50 = 450	9x50 = 450	11x50 = 550	16x50 = 800	15x50 = 750



Calculations of Physiochemical Properties

Average BOD

$$\frac{450 + 450 + 550 + 800 + 750}{5} = \frac{3050}{5} = 610\text{mg}$$

Calculations of COD

The chemical oxygen demand is calculated as follow:-

$$COD = \frac{D-S \times 1000}{\text{Vol. of sample}}$$

Where

D = vol. of potassium permanganate required for blank

S = volume of potassium permanganate required for sample

Volume of sample = 10mls

$$COD = \frac{(30.2-20.3) \times 1000}{10} = 990mg/l$$

Table 4.3: **Calculations for Weight of Solid Recovered Per m^3 of Effluent**

For sample A

$$A \quad \frac{\text{Solid recovered}}{\text{Vol. of effluent used}} = \frac{0.07385}{2800\times10^{-6}} = \frac{26.4kg}{m^3 \text{ of effluent}}.$$

For sample B

$$B \quad \frac{\text{solid recovered}}{\text{Vol. of effluent used}} = \frac{0.0547}{2400\times10^{-6}} = \frac{22.8kg}{m^3 \text{ of effluent}}.$$

For sample C

$$C \quad \frac{\text{Solid recovered}}{\text{Vol. of effluent used}} = \frac{0.04646}{1200\times10^{-6}} = \frac{25.81kg}{m^3 \text{ of effluent}}.$$

For sample D

$$D \quad \frac{\text{Solid recovered}}{\text{Vol. of effluent used}} = \frac{0.03018}{1200\times10^{-6}} = \frac{25.15kg}{m^3 \text{ of effluent}}.$$

Calculation of

Table 4.4. Percentage nitrogen and crude protein content of recovered solids.

For sample A.

$$\% \text{ Nitrogen A} = \frac{M \times v \times 14}{\text{Wt of sample}} \times 100 = \frac{0.055 \times 172.8\times10^{-6} \times 14 \times 100}{0.002}$$

$$= 6.622$$

% Crude protein = 6.622 x 6.25 = 41.4%

For sample B.

$$\% N = \frac{0.055 \times 96\times10^{-6} \times 14 \times 100}{0.0015} = 6.42$$

% Crude protein = 6.42x6.25 = 40.2%

For sample C

$$\% N = \frac{0.055 \times 87 \times 10^{-6} \times 14 \times 100}{0.00105} = 6.38$$

% Crude protein = 6.38 x 6.25 = 39.875

For sample D

$$\% N = \frac{0.055 \times 79\times10^{-6} \times 14 \times 100}{0.001} = 6.08$$

% Crude protein = 6.08 x 6.25 = 38%

For fresh effluent: -

$$\% N = \frac{0.05 \times 25.9 \times 10^{-6} \times 14 \times 100}{0.018} = 0.6295$$

% Crude protein = 0.1007 x 6.25 = 0.6295x 10.9

For final supernatant

$$\% N = \frac{0.05 \times 10.9 \times 10^{-6} \times 14}{0.02} \times 100 = 0.03815$$

% Crude protein = 0.03815 x 6.25 = 0.284

Calculation for percentage recovery:-

$$\% \text{ Recovery} = \frac{A - B}{A} \times 100$$

Where A = % crude protein of fresh efficient

B = % crude protein of final supernatant

$$\% \text{ Recovery} = \frac{0.6295 - 0.2384}{0.6295} \times 100$$

% Recovery = 62.12%

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