PRODUCTION OF SINGLE-CELL PROTEIN (SCP) FROM LIQUID EFFLUENT OF A CASSAVA PROCESSING MILL

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IBRAHIM NURUDEEN GEGELE

97/6024EH

FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA

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OCTOBER, 2003

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DEPT. OF CHEMICAL ENGINEERING
SCHOOL OF ENGINEERING AND ENGINEERING TECHNOLOGY
FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA
NIGER STATE

A PROJECT REPORT SUBMITTED IN TO THE DEPARTMENT OF CHEMICAL ENGINEERING AS A PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF ENGINEERING IN CHEMICAL ENGINEERING.

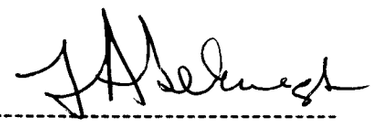
DECLARATION

I declare that the project report is entirely my efforts, and to the best of my knowledge, has never been submitted somewhere else before.

----------	28-10-13 ----------
Researcher	**Date**
IBRAHIM, N.G	

CERTIFICATON

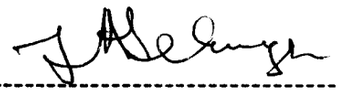
I certify that this project "Production of single-cell protein" was carried out by Mr. Ibrahim Nurudeen Gegele, of the Department of Chemical Engineering, School of Engineering and Engineering Technology Federal University of Technology, Minna, Niger State, Nigeria.



28/10/13

Dr. F. Aberuagba
(Supervisor)

Date



28/10/13

Dr. F. Aberuagba
(Head of Department)

Date

External Examiner

Date

DEDICATION

This project is dedicated to the Almighty God, Allah (S.W.T.). May His grace and mercies upon us never end. I also keep in me the memory of my late father Late (ALH) Ibrahim Babatunde Gegele, may his soul rest in peace Amin.

This is to all that contributed to the success of this research.

ACKNOWLEDGEMENT

Thanks goes to Allah (God Almighty), the Absolute and Arbitrator, with who anything is possible and upon whom all things depend. As long as I inhale air releases it, praises will be thee for all that you have done in my life. Light upon light, let them shine on me, verily you are my Lord and my all. I will never be able to thank you satisfactorily but please accept my gratitude.

To my parents: Alh. Babatunde Gegele (Late), may his gentle soul rest in peace and my mother Hajia Aisha .I. Babatunde, thanks a million times.

To Brother Shuaib .O. S. who has relentlessly supported me and my sisters financially, spiritually, and physically, I believe strongly that your effort. Will be crowned with multiples of reward. May you never lack in your life.

My entire family especially my elder ones has contributed immensely towards the success of my academic persuance. You have been my angles, may Allah reward you all for your generosity. Alh. Yusuf Babatunde, Lawyer Abdul Gegele, Bro. Abdulwaheed, Bro. Sakariyah, Engr. Sulayman Babatunde, Brother Abdulkareem and Brother Abdulmumeen – May you never know sorrow (amen).

Major Aduagba A. Edun and family have done much to be mentioned, you have shown me kindness and you have sacrificed a lot to see me through-may the grace and mercies of Allah be with you.

Thanks to the entire NASFAT congregations especially the youth wing for their endurance and understanding, Brother AbdulAzeez Y. Mustapha is a friend. Appreciation to Oyinlola's family – truly united we stand and divided we fall.

The rough road and sometimes heavy rain, she has stayed by me. Through her I saw confidence, joy, love and courage, Rukayah-Abeke, I love you. Thanks for being there for me through thick and thin.

Special appreciation goes to my close pals Bro. Kehinde A. Salako, Bro. Muritala Ozavinoyi Otaru, Brother Abdul Rasheed Mikhail T. Sis. Jemilah AbdulAzeez, Sis. Rukayah Yusuf, Sis. Khadijah AbdulRasak and all others whom I can not mention.

Thanks to all that contributed to the success of this research- !verily! Givers will never lack.

ABSTRACT

This project is aimed at producing single-cell protein (SCP) using crude liquid effluent of local cassava processor.

Single-cell protein production using the crude liquid effluent from cassava processing as substrate has been undertaken in a batch fermentation at temperature ranging between 25°C – 32°C and pH range of 4.6-4.8 at 0%, 50% and then 75% dilution level of the crude liquid effluent.

Fermentation runs A, B and C were carried out and kinetic parameters such as the specific growth μ, the generation time tg, and protein content of the SCP produced were determined.

The parameter obtained revealed that with variation in the concentration of the micronutrients in the medium preparation, there were differences in growth patterns and in the lag time of each fermentation run.

Line of best fit for the exponential growth phase was obtained using linear regression with minimum error.

The percentage protein obtained at 0% dilution for Run A, B and C were 48.5%, 58.5% and 60.0% respectively whilst at 50% dilution the percentage protein were 45.8%, 48.0% and 55.5% and the value at 75% dilution were 19.0%, 29.5% and 30.5%.

The result of this study have shown that SCP of high protein yield which is comparable to commercial SCP can be produced using the effluent from a cassava processing mill.

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CHAPTER ONE

1.0 INTRODUCTION

The need to find a remedy for the shortage of edible protein for human and animal consumption and the proposed use of single-cell protein as a supplement for animal feed to replace other major supplements like Soya beans and fish meal especially in the developing countries emanated in the last decades as a result of an alarming increase in population in the developing countries. It is therefore expediently necessary that serious attention be paid to the possibility of addition of new techniques of food production so as to supplement the customary food and feed sources with additional high quality edible protein food products. Since protein malnutrition is usually far more severe than that of other foods, the hope that micro organisms would help meet the world's protein deficiency; if produced in large quantity on an industrial scale.

1.1. THE DEFINITION OF SINGLE-CELL PROTEIN

Single-cell protein can be defined as a mass of non-viable dried cells of micro-organisms (Fungi, bacteria, yeast or algae) that are generally produced by cultivating such micro organisms on substrates which have source of carbon and energy can be used as a major source of protein in animal feed.

Single-cell protein through dried cell of micro-organisms, but still has a significant calorie and vitamin content in addition to protein content which has a higher nutritive value than any other components in it.

The production of single-cell protein of biomass as a source of protein was explored to a great depth in 1960 and the name "Single-cell protein" was coined by Professor Wilson of the Massachusetts Institute of Technology in 1966.

Single-cell protein can be produced on a variety of what man termed to be "waste materials" by growing microbes that have the ability to reproduce faster than either plants or animals using a large fermentation vessel with the inexpensive waste as source of carbon and energy.

1.2 PREVIOUS SOURCES OF SINGLE CELL PROTEIN

Sources of Single-cell protein previously depend, to a very large extend on local availability. The following are the previously sources of single-cell protein,

- Natural gas or methane (especially paraffins)
- Alcohols (derived from hydrocarbons via catalytic hydratin)
- Carbohydrates (such as sulphide waste liquor)
- Cellulose (available from waste paper, wood pulps)
- Starch (enhanced by using mixed culture for waste starch)
- Others include CO_2, chemical industry waste, Acid hydrolysate of wood.

The main criteria for the selection of substrates however depend on;

- Its availability
- Its chemical stability
- Its competitiveness.

1.3. CHRONOLOGICAL DEVELOPMENT

Microbial cells have been produced on a large scale e.g. Baker's yeast since early 1900s and the yeast was produced as a human food in Germany during the first world war. However, it was not until the 1960 that the single-cell protein as a source of food protein was explored to any great depth. A few very large-scale continuous processes have been established in recent years using different carbon source.

1.4 MODERN TECHNOLOGY

Several pilot plants and large scale industrial production of single-cell protein exists in Germany, Finland, South Africa, Europe, Japan and Russia.

A Number of research and development centres are concerned with the use of other mutticelular organisms and the use of new preparations in human diets and to reduce the dangers inherent in the process of production.

1.5 NEED FOR SINGLE-CELL PROTEIN

1. The need for single-cell protein as a supplement to the world protein was embraced to help improve the health of the people of the developing countries.
2. And to serve as food supplement for the less industrialized parts of the world especially as there was rapid and continuing population growth. However, single-cell protein is important because:
    i. Protein can be produced at a greater speed and efficient by single-cell protein than those produced by the plants and animals;

ii. Micro-organisms have a rapid mass due to their very short generation time;

iii. Microbes can be genetically manipulated to produce cells for specific purpose;

iv. Single-cell protein can be produced in a continuous culture independent of climate conditions;

v. Single-cell protein is nutritionally balanced, needed just a minimum landscape for cultivation, less time needed for harvesting and less capital cost of production.

1.6 OBJECTIVES

The objectives of this research study are;

i. To use the effluent of a local garri processor as a substrate for the production.

ii. To obtain kinetic parameter that will be suitable for the design of an industrial scale plant for the production of single cell protein using liquid effluent of cassava processing mill.

iii. To determine the protein content of the SCP produced and determine whether it is in quantity suitable for use as food supplement or for direct consumption.

iv. To determine the effect of percentage dilution level of the liquid effluent on the percentage protein produced.

CHAPTER TWO

2.0 LITERATURE REVIEW

The use of microbial fermentation and the development of a plant or an industry for the production of single-cell protein are possible solutions to shortage of proteins when the amount of protein produced from agricultural means and fishing is not sufficient. This is the believe of most scientists who have carried out research work on the single-cell protein.

2.1 DEFINITION OF SINGLE CELL PROTEIN

Single cell protein refers to a mass of non-viable, dried cells of micro-organisms (yeast, bacteria, fungi or algae), produced by cultivating such micro-organisms on substrates such as alkane, lower hydrocarbons (example methane), lower alcohols (methanol or ethanol) or industrial and agricultural waste products (sulphide waste liquor and destined to be used as major protein sources in animal feed.

Single cell protein was coined, by Professor Wilson of the Massachusetts Institute of Technology to replace the less inviting microbial or bacterial protein or petroprotein (for cells grown specifically on petroleum) in 1966. Single-cell protein processes are inherently closer to chemical than to micro-biological technology but it is strongly suggested to apply the traditional concept of good manufacturing practice in the sense that it is currently used in the food industry.

2.2. THE NEED FOR SINGLE CELL PROTEIN

The need for single cell protein as a supplement to world protein resources is well established and research and development on single cell protein production has been intense for over a decade. Among all other reasons, single cell protein advantages includes;

i. It may replace or help up world's protein deficiency

ii. Its easier to produce and at a cheaper rate

iii. Single-cell protein can be produced at a greater speed and efficiently, than those produced by other plant and animal

iv. It is nutritionally balanced, requiring just a minimum landscape for cultivation, shorter generation time, less time required for harvesting and less capital cost of production.

Also the problems of single cell protein were recognised, these includes;

a. Single cell protein may not be pleasing to taste and may cause gastro-intestinal upset; the intestinal response may relate to toxic properties of cell wall and o the high nucleic acid content of single- cell protein.

b. Its wide lack of acceptance especially in the presence of conventional modern existing food.

2.3 MATERIALS REQUIRED AS SUBSTATE FOR SINGLE-CELL PROTEIN PRODUCTION

Choice of materials for the production depend basically on local availability. Therefore, natural gas or methane would be of great importance in areas where it is available in abundance or where it may be discarded (flare gas). With regard to

petroleum fractions, local economic of the petroleum industry will determine, to some extent whether crude semi purified (gas oil) or refined fractions (n-paraffins) are the desirable substrates. Other substrates are categorized as;

ALCOHOLS: These are derived from hydrocarbons via catalytic hydration, particularly methanol and ethanol which can both be utilized by a wide variety of micro-organisms. They are prepared in very high states of purity, are totally water soluble and leave no residues in the cell mass leaving the fermentor. They are also intermediate in cell yield and oxygen yield between hydrocarbons and carbohydrates. Since lower alcohols maintain some of the advantages of both hydrocarbon and carbohydrate fermentations, they have significant potential for single-cell protein production processes.

CARBOHYDRATES: These are in different variety of sources, such as sulphite waste liquor, cheese whey, molasses and starch already operative is very special locales and may be used to produce cells by conventional fermentation technology.

CELLULOSE: This is the most abundant raw materials available from waste paper, baggages, wood pulp. It is of particular importance as a re-newable resource. In the U.S.S.R, hydrolysed wood pulp supports a feed yeast industry estimated to be approximately one million metric tons per year (Encyclopedia for Food and nutrition, second edition, J. N. Ferman 1973). From an economic point of view, the most crucial step in utilization of cellulose is convention to metabolizable sugar. In the case of agricultural by-product, materials such as solid cannery waste,

citrus waste, coconut wastes, and palm oil waste, problem exist because seasonal processing schedules, conversion to usable form, and the sometimes dilute of the fermentable materials. The possibility of simultaneously addressing the problems of waste material disposal and protein production makes these materials attractive.

STARCH: It is much more hydrolyzed than cellulose, and the potential exists for direct fermentation of starch by amylolytic fungi in a continuous process. An alternative approach is to use a mixed culture for waste starch. Starch may be of particular interest in tropical areas which may provide high yields of starchy root crops, such as cassava, provided that such subsistence crops can be accumulated economically for industrial processing of sugars such as the byproduct of sugar cane processing (molasses) which is similarly useful substrates.

OTHER SOURCES: Carbondioxide, waste from chemical industry, Acid hydrolyzed of wood, lipids and whey's. The main criteria for the selection of substrates however depend on;

(a) It's availability

(b) It's chemical suitability

(c) It's competitiveness

The substrates mentioned above can be categorized into three viz;

i) Carbondioxide (ii) Carbohydrates (iii) Hydrocarbons

Table 2.1 shows a summary of the substrates for single-cell protein production while;

Table 2.2 shows countries with plant or process for single-cell proteins production

TABLE 2.1

A	Carbondioxide
B.	Carbohydrates Sugars: Molasses, waste sulphur, liquor, whey Starches: grain, cassava, potato waste Cellulose: Wood, paper, baggage, husk manure
C.	Hydrocarbon n – alkanes Methanol, ethanol Methane

TABLE 2.2 Countries with plants for SCP production

Company	Plant Location	Substrate	Organism
British	U.K	N-Paraffin	Yeast
Chinese Petroleum	Taiwan	N-Paraffin	Yeast
UNIDO	Mexico	Carbondioxide	Algae
Dainippon	Japan	Methanol	Bacteria
IC.I	Japan	N-Paraffin	Yeast
Kohjin	Japan	N-Paraffin	Yeast
Kyowahakko	U.S.A.	Whey	Bacteria
Shell	Holland	Methane	Yeast
Svenska	Sweden	Potato	Yeast
Socker		Starch	
United Paper	Finland	Sulphite Waste	Yeast

From the process listed in Table 2.2, on extra credit can be realised for handling and the hydrocarbon into gasses, water soluble liquids and waster insoluble liquids. It is the abundant carbon component in all the above that is utilized for single-cell protein production.

Substrates containing cellulose require prior treatment due to its crystalline nature and assistance to fermentation, however there have been current attempts to convert cellulose to single cell protein by Jackman, 1976, Hammond, 1977, (13).

The major problem encountered in the use of hydrocarbon as substrate is the global shortage and uneven distribution thereby limiting single-cell protein production. It also determine in which area it's produced such in such cases, the product single-cell protein is of less importance than consumption of the substrate Ratledgem, 1975, (16). However, single-cell protein processes have contributed considerable spin-off benefits to the whole of microbial technology. For example, a reasonable part of the development processes of single-cell protein production has occurred in British Industries and much of what is known about the microbial metabolism of hydrocarbons is die to efforts of British scientists.

2.4 CASSAVA PROCESSING LIQUID EFFLUENT AS A SUBSTRATE

Few effluents couple with product formation have been successfully operated. These waste were ignored in the past but now have been accepted based on the fact that its also serve as carbon or energy source and it provides higher protein percentage compare to some other carbon source such as hydrocarbons (Aberuagba, 1998). Cassava liquid effluent as it name implies is the liquid pressed out of the grinned cassava. The liquid effluent of cassava contain starch, water, very low protein and insignificant minerals (17).

Micro-organisms require source of nitrogen, phosphorus and minerals salt in addition to the carbon and energy source to make them grow. These are added to the cassava liquid effluent in the form ammonium salts such as $(NH_4)_2SO_4$ and soluble phosphate salts such as KH_2PO_4. Other necessary minerals are available in water which forms a major component of the medium for the growth of yeast using spent grain extract as substrate.

Liquid effluent of cassava processing mill has been considered as a suitable substrate for this work based on the following reasons:

(a) Its availability

(b) It contains the required carbohydrate source to a reasonable amount

(c) It requires a simple pretreatment which is the sterilization.

(d) It can be fermented very easily.

(e) Alcohol is costly

(f) Gaseous hydrocarbon are highly inflammable while liquid ones leave residual hydrogen and carbon in single –cell protein

(g) Not all waste from chemical industries contain carbon, this is because they have treatment problems

(h) Miscellaneous substrate present pretreatment problems and problems involving the appropriate organism since these substrates are complex origin.

(i) Cellulose which is contained in plants and agricultural wastes products is crystalline and highly resistant to fermentation without prior treatment. This brings about high cost of production and large cost of accretion.

2.5 ATTRIBUTES OF MICROORGANISMS FOR SCP PRODUCTION

All classes of organism should be considered as potential sources of protein if they are capable of meeting the criteria of safety, nutritive value and economics. In General:

i. There should be absence of pathogenisity and toxicity

ii. High quality protein and amino acid required by man

iii. Must be digestible and have good organoleptic quantities, taste and aroma;

iv. Rapid growth rate

v. Adaptability (25)

Excretion of acetate by bacteria growing with glucose as the limiting nutrient has been observed by Chian and Mateles, 1968 – 1969, Wang and Mateles 1976 (13) The phenomenon is often seen in continuous culture, particularly when the dilution rate is relatively close to the maximum specific growth rate of the micro-organism.

Again, according to Ben – Bassat and Goldberg 1977 (16), There are several path ways for the oxidation of substrate which may operate simultaneously in some organism, this affect the growth rate of such organisms.

According to Abbort and Clamen, 1973 (16), the carbondixide content of the effluent gas released by organism is relatively high as a consequence of the highly acerbic nature of single-cell protein production together with the economic factors motivating growth at relatively high cell densities and good utilization of the oxygen supplied in the air stream. Although literature is replete with data concerning the effect of oxygen on metabolic activities of microorganism, the effect of carbondixide has been studied too. Production of fumaric acid by Rhizopus nigricna was not affected 5% $C0_2$ from studies of Foster and Dauis 1979 (13) While 4% CO_2 inhibited respiration of Pencillium Chrysogernum according to Nyiri and Lengyel 1965 (13). However, in connection with biomass production, the yield of Sacharomyces

Cerevisiae from molasses was not affected by up to 20% CO_2 according to Chen and Gutmanis in 1976 (13).

One main problem of utiizing micro-organisms as a sources of protein is the high nucleic acid content which must be reduced from a nutritional stand point Edozien et al 1970 (3). These substances should not be present in human diets in unlimited quantities, because nucleic acid purines are excreted as uric acid, which in susceptible individuals may increase the possibility of gout or kidney stones. The nucleic acid content in Single-cell protein can be lowered by several methods.

2.5.1 REMOVAL OF NUCLEIC ACIDS IN SINGLE-CELL PROTEIN

To use single-cell for human consumption, further processing may be required to remove nucleic acid. Cell biomass obtained from fermentation of organic waste can be processed further to obtain protein concentrate having no or low nucleic acid content. The nucleic acid content of single cell protein can be 8-25g/100g protein, with most of the nucleic acid present as ribonucleic acid (RNA).

The removal of nucleic acid from SCP is necessary because humans do not have the enzyme uricase, which oxidized uric acid to a soluble and excitable metabolite (e.g. allantoin). Dietary nucleic acid is converted to a uric acid as follows (Kihlberg 1972):

Dietary nucleic acid $\xrightarrow[\text{Pancreatic Juice}]{\text{nuclease in}}$ Depolymerized products

Depolymerized products $\xrightarrow[\text{enzymes}]{\text{intestinal}}$ nucleotides, tysamine and adenines

Adenine $\longrightarrow$ Uric acid

2.6 GROWTH CONDITIONS FOR SINGLE-CELL PROTEIN PRODUCTION (YEAST)

In single-cell protein production there are some conditions which are necessary for the growth of yeast. This conditions are being considered as optional and they vary with the yeast employed and the raw materials to be utilized. The following are the conditions;

i. Application of optimal aeration (the best oxygen supply)

ii. Feeding of carbohydrates and sources of nitrogen, phosphorus and potassium.

iii. Establishment of active yeast growth in fermentor.

It is advisable that the aeration should be considerable enough but at an optimal (or best) level because very scanty level encourages the production of alcohol and excess level favours increased respiration and heat production hence lowered use of the yeast cell. The optimal temperature depends on the yeast strain (most grow well between pH of 4.5 – 6.0). The concentration of fermentable sugar is maintained to a level no higher than necessary for good yields of cells. The amount and kinds of in-organic nutrients to be added depend on the substrate. For instance molasses from cane or beet sugar is usually high in potassium and fairly well supplied with available phosphorus and nitrogen while agricultural waste such as cassava liquid effluent is deficient in all three element or present in relatively low amounts.

2.7 SACCHAROMYCES CEREVISAE AS A MICRO-ORGANISM FOR SINGLE-CELL PROTEIN PRODUCTION

Factors stated below are considered for the choice of Saccharomyces cerevisiae;

(a) It has a high rate of fermentation

(b) It is able to utilize many types of fermentable sugar such as glucose, sucrose, galactose and fructose

(c) It reproduces well under normal conditions.

Saccharomyces cerevisae is employed in many food industries with special strains being used for the leavening of bread as to yeasts for ale, for wines and for the production of alcohol. Top yeast yeasts clump together during growth, collect CO_2, and are buoyed up to the surface of the fermenting liquid, from which they can be skimmed. Bottom yeasts do not clump but settle to the bottom of the liquid following the period of growth and activity.

Saccharomyces cerevisae is found in nature on ripe fruit thus, is a yeast of great importance. The cells of these yeast may be round, ovate, or elongated and may form pseudomycelium. Reproduction is by multipolar budding or by ascospore formation which may follow conjugation or may develop from diploid cells when thee represent the vegetative stage. Its cells are elliptical measuring about 6 to 8 NA by 5um.

The nutritional value of a protein is dependent on its amino acid pattern, it should be noted that amino acid would not be assimilated by Saccharomyces Cerevisiae unless magnesium ions are present in an appreciable concentration about 2.5% of

the total nitrogen. As with bacteria, S. cerevisiae a magnesium ion transport system for which cobalt, nickel, manganese and zinc ions are probably competitive substrate, this was reported in 1968 by Fuhman and Bothstein (13) literature has revealed that S. Cerevisae is also able to grow well with aspartic acid, asparagine and glutamic acid as alternative sources of nitrogen, these amino acid act as building substrate and are associated with the respiratory function of the cell. However, in 1977 Walter and Thieslton observed that yeast and S. cerevisiae will grow in a lysine medium.

2.8 GROWTH KINETICS OF MICRO-ORGANISMS

In any biological system, growth can be defined as the orderly increase of all chemical components in micro-organism. While growth kinetics of micro-organisms is the series of stages attained during growth. These occur in four main stages known as;

(a) The Lag phase

(b) The exponential or Logarithmic phase

(c) The death phase

2.8.1 THE LAG PHASE

Cells transferred from a culture in the stationary phase to fresh medium of the same composition undergo a change of chemical composition before they are capable of initiating growth. This period of adjustment is called the log phase. It's extremely variable in duration and its length is directly related to the duration of the preceding stationary phase.

The length of lag phase observed depend on both the changes in nutrient composition (if any) experienced by the ells and the age and size of the inoculum. The shock of rapid switch to a new environment has several effects on the living cells.

As a hypothesis for modeling, we shall suppose that the lag phase period is essentially ended when some biracial substances in the cell reaches a given value denoted b y C^1. For young as a function of time t ;

$$C' = a v + a' n_o t + a''t$$

Where

v ——→ Volume of old medium transferred

a ——→ Concentration of critical substance per unit volume

n_o ——→ Number of cells per unit new volume, which is assumed constant since grow-rate is negligible in lag phase

a" ——→ Increase in critical substance due to internal cell

a' ——→ Average increase in cell critical substance due to production by other cells, per time per cell

Then, if t_L ——→ Lag time

The equation becomes

$$t_L = \frac{C'/a' - av/a'}{n_o + a''/a'}$$

To minimize culture and process times normally, we minimize the lag time which can be achieved by;

- The inoculating culture should be as active as possible and the inoculation done is the exponential growth phase

- Use of reasonable large inoculi (order of 5% of the new medium volume) to avoid loss by diffusion of the required activator.
- Minimize the amount of activator
- The culture medium used to grow the inoculi should be as close as possible to the final full scale fermentation composition.

2.8.2 EXPONENTIAL OR LOGARITHMIC PHASE

In the exponential phase of growth, a bacterial culture undergoing balanced growth mimics a first order auto-catalytic chemical reaction that is the rate of increase to bacteria at any particular time is proportional to the number of mass or bacterial present at that time. At some time, this phase can be affected by the following factor: (a) Medium (b) Temperature (c) Aeration (d) The species themselves.

At the end of the lag phase, the microbes are well adjusted to the new environment and growth doubling with time. Thus;

$$\frac{dn}{dt} = un, \text{ with}$$

$n = n_o$ at $t = t_L$

$u \longrightarrow$ Specific growing rate

$n \longrightarrow$ number of cells per unit volume

Integrating the equation gives

$$\ln\left[\frac{n}{n_o}\right] = \mu (t - t_L)$$

$$\text{or } n = n_o e^{u(t - t_L)}$$

The time interval, t_d is readily deduced (time required to double the population) as

$$t_d = \frac{\ln 2}{\mu} = \frac{0.693}{\mu}$$

During exponential growth, only a simple parameter μ or td is required to characterize the population. However, the specific growth rate constant is widely used to describe the influence of the cell's environment on its performance .

2.8.2.1 MEDIUM CONSIDERATION

Medium making supports growth and/or high rate of production synthesis. Excessive concentration of medium nutrient can Poison or inhibit cell growth. In this case functional relationship between the specific growth rate constant, μ and the essential compound concentration was proposed by Mono in 1942.

$$\mu = \frac{\mu_{max} \cdot Ci}{Ki + Ci}$$

μ_{max} ——→maximum specific growth rate constant

Ki ——→Exact value of concentration of nutrient I

Where μ has half value its maximum value.

2.8.3 STATIONARY PHASE

Deviations form exponential growth eventually arise when some significant variable such a nutrient level or toxin concentration achieves a value which can no longer support maximum growth rate.

2.8.4 THE DEATH PHASE

Bacterial cells held is a non-growing state eventually die. Death results from a number of factors, and important one is the depletion of cellular reserves of energy. Like growth, death is an exponential function and hence is a logarithmic plot, the

death phase is a linear decrease in number of viable cells with time. The death rate of bacteria is highly variable, being dependent on the environment as well as on the particular organism.

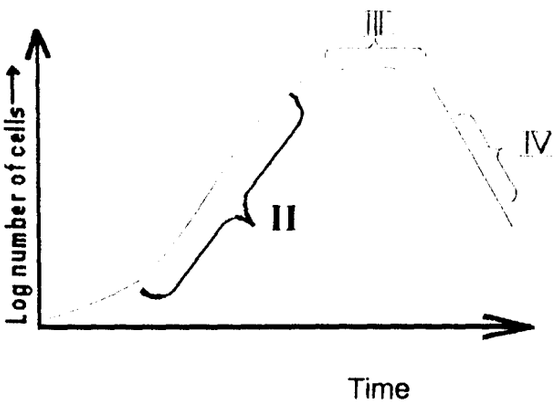


Fig. 2.1 Typical growth curve of a microbial culture

Where, I represents Log phase

II represents Exponential growth phase

III represents maximum stationary phase

IV represents Deaths phase.

2.8.5. GROWTH MEASUREMENT

To Follow the course of growth, it is necessary to make quantitative measurements. Exponential growth is usually balanced so any property of biomass can be measured to determine grow its rate. As a matter of convenience, the properties measured are usually cell mass or cell number.

2.8.6 MEASUREMENT OF CELL MASS

Three common techniques are available for measuring and monitoring growth processes, namely;

i) Hemacytometer ii) Colony count iii) Turbidimetric methods.

In Hemacytometer, a grid facilitate cell counting, while in colony-count method greatly diluted samples from the original culture are plated on nutrient agar in Petri dishes, where the number of colonies indicates the number of viable cells in the diluted sample. For turbidity measurement method, turbidity of a cell suspension provides a measure of population density.

2.8.6.1 DRYING METHOD

The dry weight of cell material in a fixed volume of culture by removing the cells from the medium, drying them, and then weighing them. This method is time consuming, relatively insensitive and prone to high inaccuracy.

2.8.6.2 OPTICAL METHOD

Determination of amount of light scattered by a suspension of cells. This technique is based on the fact that small particles scatter light proportionally to their concentration within certain limited. The consequence of scattering this is a measure of bacterial mass present. The measurement is successfully carried-out by spectrophotometer which reads Absorbency (A), this

$$A = \log \frac{Io}{I}$$

Io $\longrightarrow$ intensity of light striking the suspension

I ——→ Intensity of light transmitted by suspension.

The instrument are convenient for estimating cell concentration, and when calibrated against bacterial suspension of known concentration they provide an accurate and rapid way to estimate the dry weight of bacteria per unit volume of culture.

2.9 ACCURATE CELL NUMBER (TOTAL)

The number of unicellular organisms in a suspension can be determined microscopically by counting the individual cells in an accurately determined very small volume.

2.10 GROWTH YIELD

The net amount of a bacterial culture is the difference between the cell numbers used as an inoculum and the cell mass or number present in the culture when it enters the stationary phase. When growth is limited by a particular nutrient, there is a fixed linear relationship between the concentration of that limiting nutrient initially present in the medium and the net growth which results, the mass of cells produced per unit of limited nutrient is accordingly a constant. Thus;

$$Y = \frac{X - Xo}{C}$$

Y ——→ Yield of growth or growth yield

Where, X ——→ dry weight of cells present when the culture enters stationary phase, per ml

Xo ——→ Dry weight of cells immediately after inoculation per ml

C ——→ Concentration of limiting nutrient units?

2.11 SINGLE CELL PROTEIN PRODUCTION PROCESSES

The Pekilo process, operating in Finland, utilizes sulphite waste liquors, a by-product of the wood pulp and paper industry. The organism is Paecilomyles variotii, a filamentous mould, and the product is sold as pig, chicken and calf food stuff.

Figure 2.2 shows simplest flow sheet of production of single-cell protein

Figure 2.3 shows the shell type of process for single-cell protein production, microbial community for methane assimilation;

Figure 2.4 Production of single-cell protein from methanol

Figure 2.5 Production of single-cell protein from sulphate liquor.

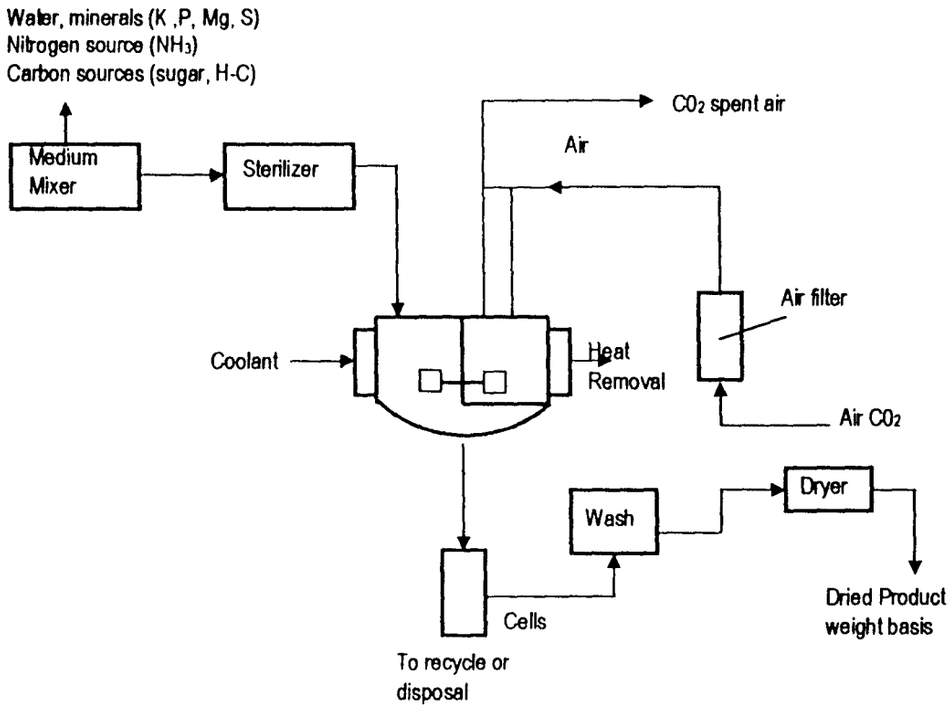


Fig: 2.2 Simplest flow sheet of production of single-cell protein (50% protein dry)

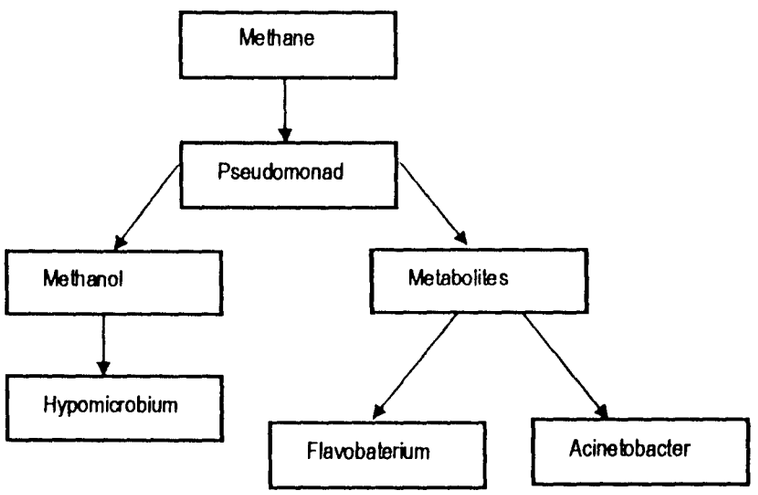


Fig. 2.3 The shell type of process for single-cell protein production, microbial community for methane assimilation

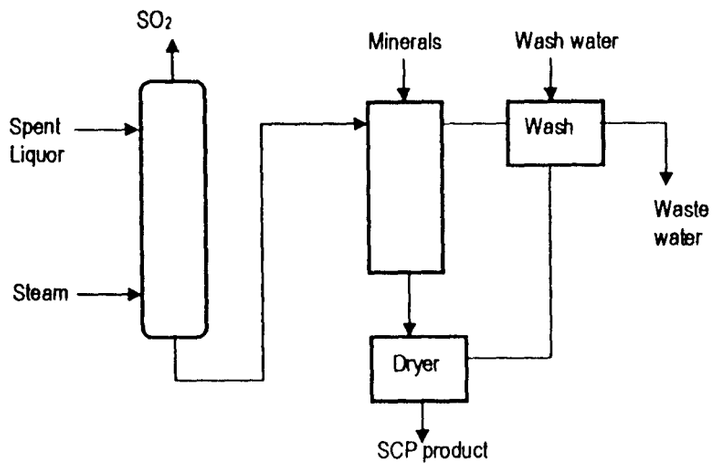


Fig: 2.4 Production of single cell form methanol

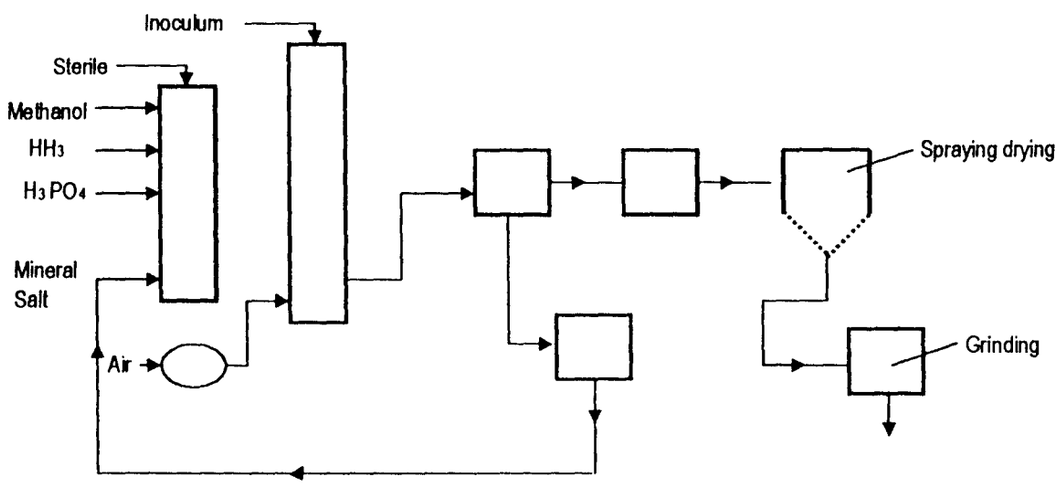


Fig . 2.5 Single-cell protein production from suphite waste liquor

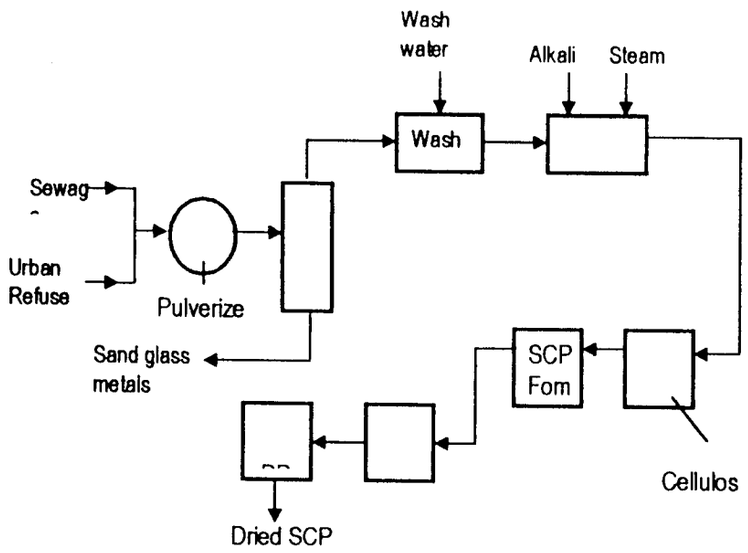


Fig. 2.6 SCP production from agricultural wastes

CHAPTER THREE

3.0 EXPERIMENTAL WORK

Three main stages are involved in the experimental work of this project for the production of a single cell protein using liquid effluent of a cassava processing mill as substrate. These stages are as follows;

i. To determine the specific growth rate and generation time for three fermentation runs

ii. To determine the effect of dilution of substrate on the single cell protein production and all other factors

iii. To determine the protein content of the yeast obtained per run.

3.1 MATERIALS AND EQUIPMENT

Organism: Yeast (Saccharomyces cerevisiae)

Substrate: Liquid effluent of cassava processing mill. It was obtained from Railway Gari Processing at Mobil Market, Minna.

Equipment: Filter paper, beakers, funnels,distilled water, thermometer Sampling bottles, measuring cylinders, glass rods, cotton wool, disinfectant, volumetric flasks, round bottom flask, pipettes, burettes, fermentor (Microfem fermentor, MF-114, New litres Brunswick scientific company, USA).

3.2 METHODS

3.2.1 MEDIUM PREPARATION:

The liquid effluent obtained from cassava processing mill was collected in 10 litres container, covered and then stored in a refrigerator at the temperature range of $\underline{4}^0C \pm 2^0C$ for subsequent experiments.

The liquid effluent served as a carbon and energy source for the growth of the micro-organism (yeast). Other growth requirements added to the medium per litre are 2.0g $(NH_4)_2SO_4$, 0.5g KH_2PO_2, 0.05g Na_2HPO_4, 0.2g $MgSO_4.7H_20$ and 0.1g $FeSO_4.7H_20$.

3.2.2 INOCULUM PREPARATION

The inoculum is defined as the substance inform of suspension containing microorganisms introduced to the culture.

First, 200ml of the medium was measured using a conical flask and sterilized at a pressure of 1.1 Kg/m^2, temperature of 121^0C and for 15 minutes after which it was cooled and inoculated with 40g of yeast. The flask was then attached to a Gallenkamp shaker operated at 200rpm to ensure proper mixing of the content. Here the yeast introduced was then allowed to grow for 18 hours at 32^0C (room temperature). Twenty milliliters of this inoculum were added to 200ml of the sterilzed medium in another flask and grown under the same conditions to raise a second generation. A third generation was subsequently raised following the same procedure.

3.2.3 FERMENTATION:

Four litres of the media was prepared and poured into a bench scale 14 liters fermentor (Microfem fermentor, MF-114, New Brunswick Scientific company, U.S.A.) The contents were then sterilized at 1210C under a pressure of 1.1 Kg/m^2 for 15 minutes and then cooled to room temperature. The inoculum prepared above was then asceptically transferred into the contents of the fermentor, the agitation and air flow rates were set at 200 rpm and 1.2scf per hour respectively whilst the temperature was maintained at 32^0C and pH at 4.6 – 4.8 range.

Sampling was done every 2 hours, period of 22 hours. Each sample was centrifuged and the supernatant separated whilst the biomass was washed with distilled water, filtered and dried to a constant weight at 80^0C in hot air oven. The same procedure was repeated for each of the second and third generations. The pH of the media was kept at 4.6 to 4.8 by the injection of either hydrochloric acid or sodium hydroxide solution.

The remaining content of the fermentor, after the sampling, was centrifuged, filtered and washed to obtain the residual yeast. The protein content of the yeast sample was determined by a method developed by Kjeldahl and studied by Broadstreet. The method involves determination of total nitrogen, the numerical value 6.25 being an empirical factor.

The whole processes of fermentation was repeated for different dilution of crude liquid effluent of cassava processing. Trials was done on 25% dilution, 50 dilution and 75% dilution of the effluent using distilled water as the diluent.

3.2.3.1 PROTEIN CONTENT DETERMINATION

The residual yeast obtained from the above stated procedures was carried for protein content analysis. This was done by weighing 0.5g of the yeast sample into a 100ml Kjeldah's flask. 15ml of 1M H_2SO_4, 1.176g of Na_2SO_4 and 0.15g of Hg_2O were then added (All thee available inform of a tablet called Kjeldah tablet). This content was then boiled by heating in a long necked flask (Kjeldah's flask) until the sample had digested (that is, when it turns colourless). The solution obtained was then made up to 100ml in a volumetric flask.

Five milliliters of this was measured into a round bottom flask and 7ml of 1M NaOH added. By means of steam distillation, steam was passed into the solution to evolve all NH_3 present, the NH_3 was absorbed into a boric acid solution (10ml) until 50ml of boric acid – ammonia solution was obtained. The H_3BO_3 – NH_3 solution was then distillated with standard 1M H_2SO_4 using methyl –red as indicator.

The percentage protein was then obtained by multiplying the percentage of nitrogen in the sample by 6.25.

Kjeldah's method is less general than Duma's. (Fawole M.O. and Oso B.A, 1988). It is satisfactory for compounds having nitrogen attached to hydrogen compounds with - N = N - or - N = O bonds may have to be reduced. Catalysts (mercuric compounds or selenium) may be added to the sulphuric acid to promote decomposition of organic substances to ammonia.

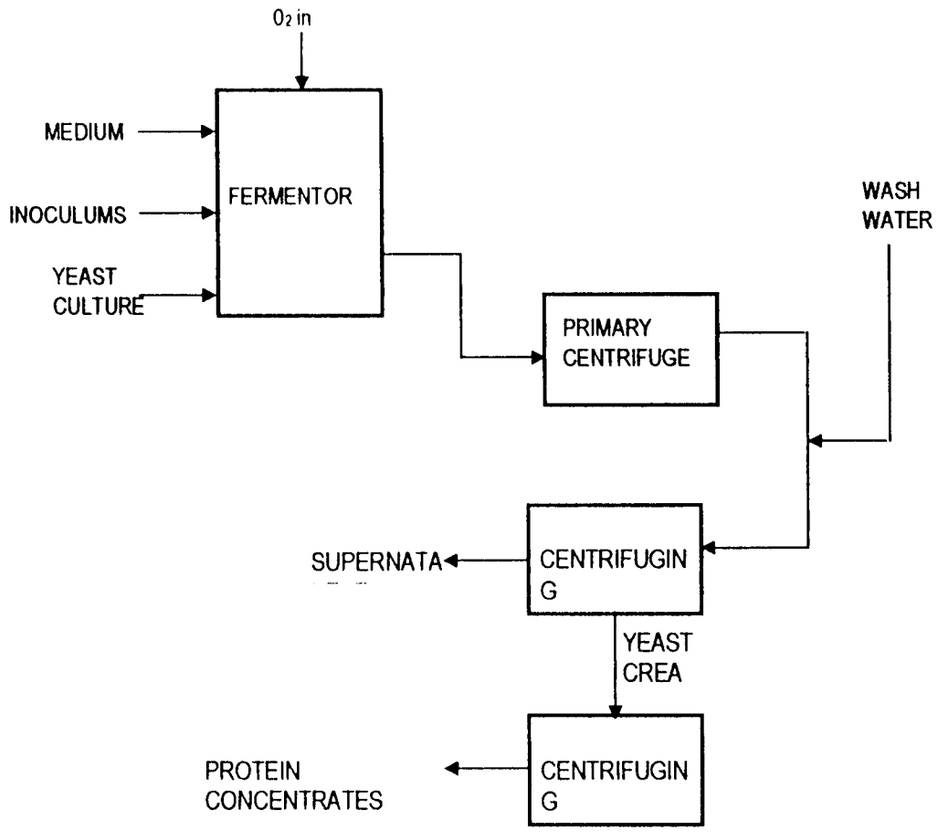


Fig: 3.1 schematic diagram for single cell protein production from liquid effluent of cassava processing

3.3 GENERATION OR DOUBLING TIME DETERMINATION

The doubling time is the period at which one cell divide or double in population, td. This can be determined from exponential or lag phase since cell division is approximately constant during the phase over a particular time interval.

Provided we start with a single micro-organism, the increase in population is given by geometric progression:

$1 - a - a^2 - a^3 - a^4 \ldots\ldots\ldots\ldots - a^n$

where $a = 2$

n = number of generations

Assuming no cell death, and N is the total population at the end of the given period;

$$N = 1 \times a^n$$

$$N = 2^n$$

Where as No is the number of organisms inoculated at time zero is not equal to I, but more likely several thousands, so the formula now becomes:

$$N = No \times 2^n$$

Take the logarithm of both sides

$$\log \{N\} = \log \{No\} + n \log 2$$

$$n = \frac{\log \{N\} - \log [No]}{\log 2}$$

$$n = 3.3 (\log_{10}[N] - \log_{10}[No])$$

But Generation time $= \frac{\text{time internal}}{\text{number of generation}}$

$$= \frac{t}{n}$$

$$t_d = \frac{t}{3.3\,(\log [N] - \log [No])}$$

The value of generation time could also be obtained from

$$t_g = \frac{\ln 2}{\mu}$$

Where μ ⟶ specific growth rate (hr^{-1})

3.4 TO OBTAIN SPECIFIC GROWTH RATE OF THE FERMENTATION RUN

The specific growth rate was obtained by taking the slope of the straight line from the graph of natural logarithm of concentration versus time;

$$\mu = \frac{\text{change in Y - axis}}{\text{change in X - axis}} \qquad (hr^{-1})$$

Since

$\ln C = \ln Co + \mu t$ represent a straight line equation .

3.5 LINEAR REGRESSION ANALYSIS

$$Y = A + BX \quad \text{——} \aleph$$

$$Lu\,C = lu\,Co + \mu t \quad \text{——} \beth$$

Compare $\aleph$ and $\beth$ to compute for the line of best fit which are linear model to the observed value.

$$A = \frac{\Sigma y - B\Sigma x}{n} \; ; \qquad B = \frac{n\Sigma xy - \Sigma x\Sigma y}{n\Sigma x^2 - (\Sigma x)^2}$$

And the repression, r is obtained from

$$r = \frac{n\Sigma xy - \Sigma x\Sigma y}{\sqrt{\left\{n\Sigma x^2 - (\Sigma x)^2\right\}\left\{n\Sigma y^2 - (\Sigma y)^2\right\}}}$$

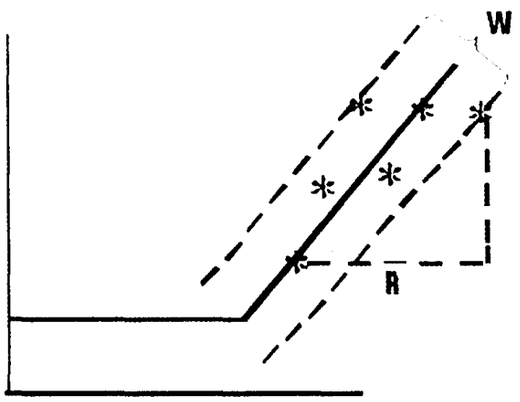
3.6 VARIANCE DETERMINATION

The derivation, w is obtained by subtracting the observed value from evaluated value. Then the variance, δ is calculated by

$$\delta = \frac{\sum w^2}{n}$$

where n ⟶number of observations

3.7 ERROR COMPUTATION



$S = \frac{4w}{nR}$ where R is the horizontal separation between

These two points (Called the range of the abscissae). Then if there are n points, the range estimator for the standard error is given by the expression above. W is the width between the highest point and the lowest point on the graph.

CHAPTER FOUR

4.0 RESULT

4.1 CRUDE LIQUID EFFLEUENT COMPOSITION

The crude liquid effluent contains 1.5% (W/W) of Protein

4.2 FERMENTATION RUNS

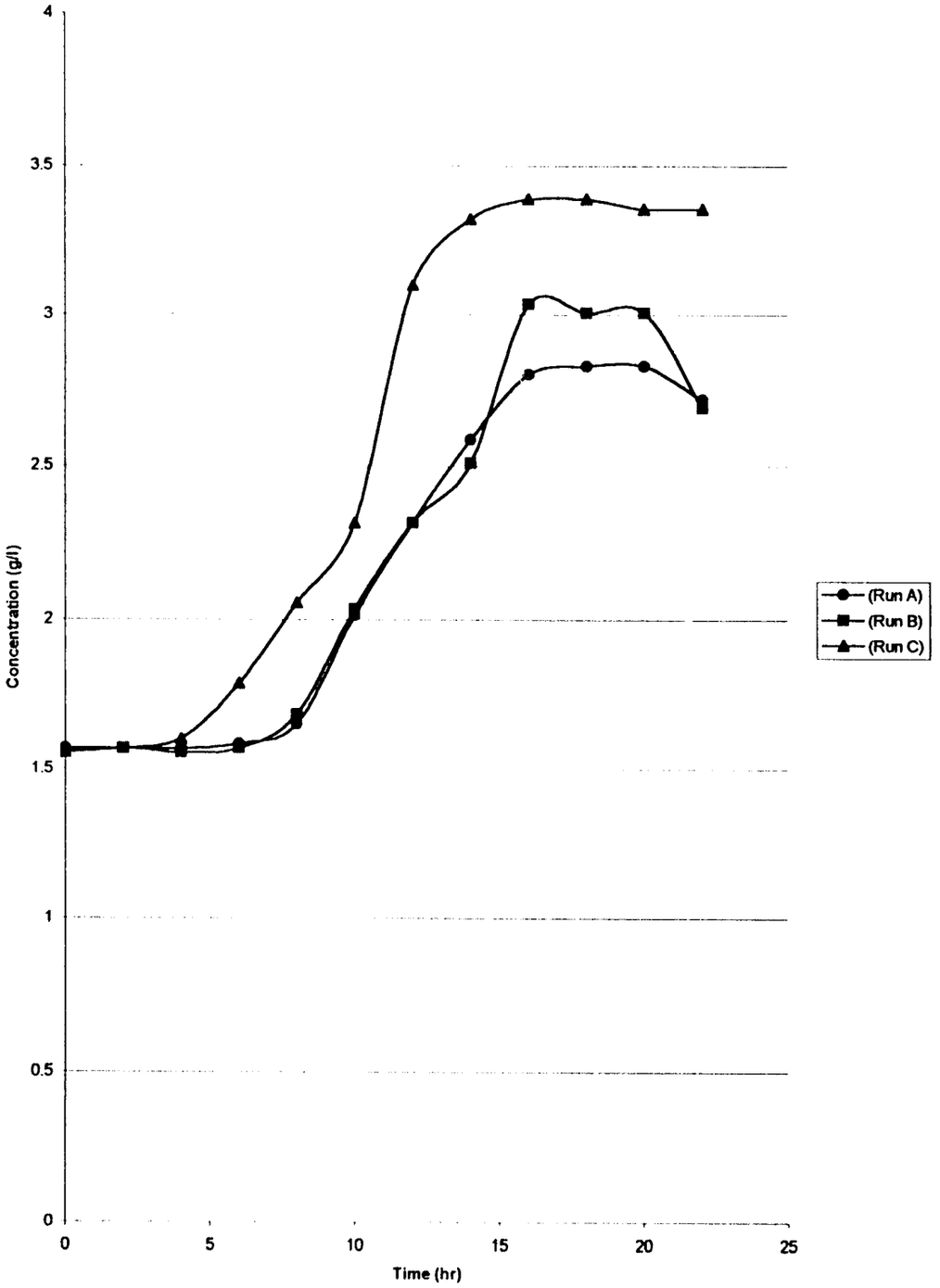
The results of fermentation Runs A, B, and C with crude effluent are shown in Figure 4.1 – 4.3, where

Run A Represent generation obtained from pipetting 20ml of the inoculum into 200ml of sterilized medium (first generation)

Run B Represent generations obtained by pipetting 20ml of the inoculum from first generation into another 200ml of the sterilized medium (Second generation).

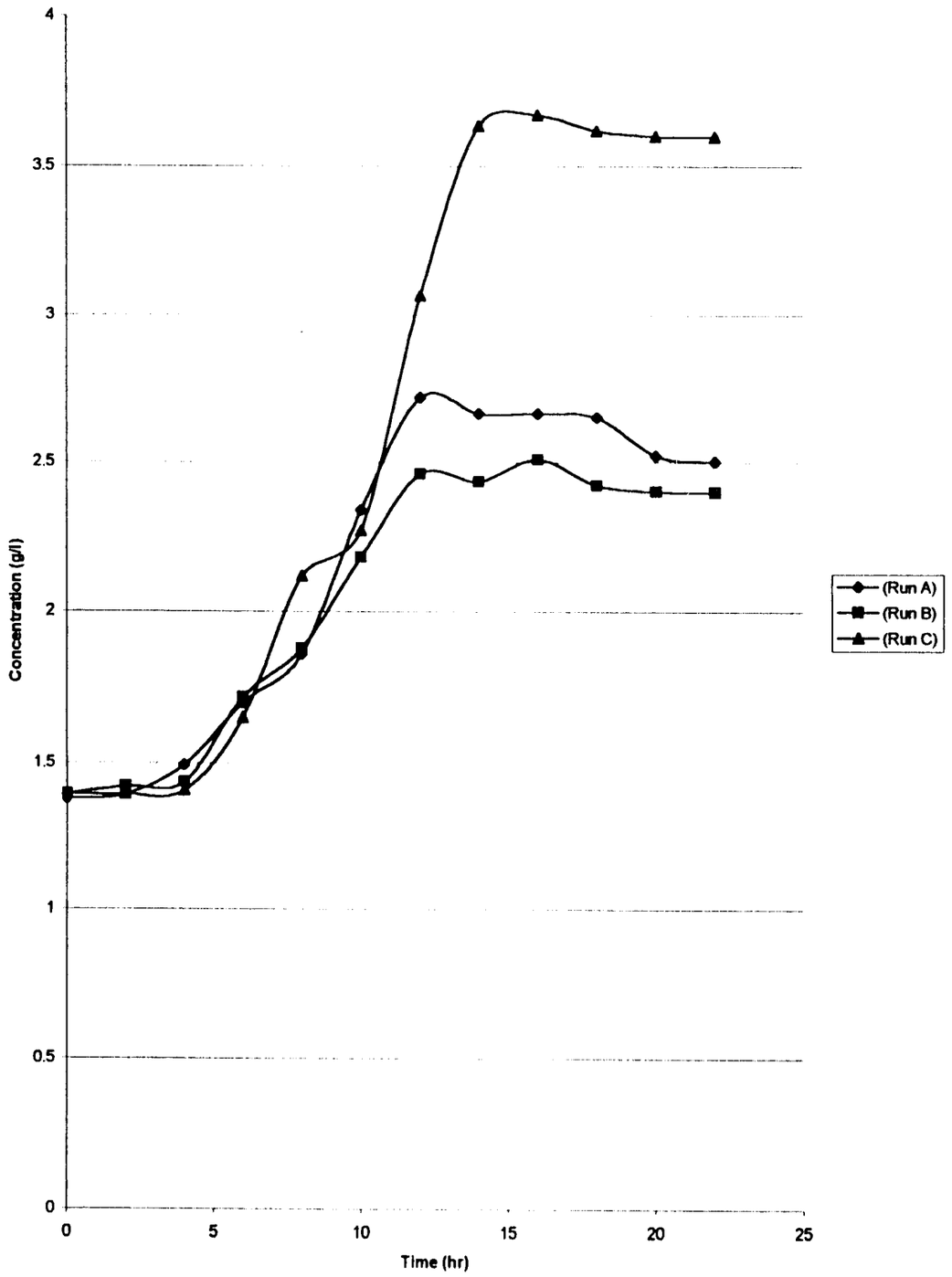
Run C Represent the third generation obtained by further pipetting 20ml second generation into another 200ml of the sterilized medium.

Fig. 4.1: CONCENTRATION VERSUS TIME AT 0% DILUTION LEVEL



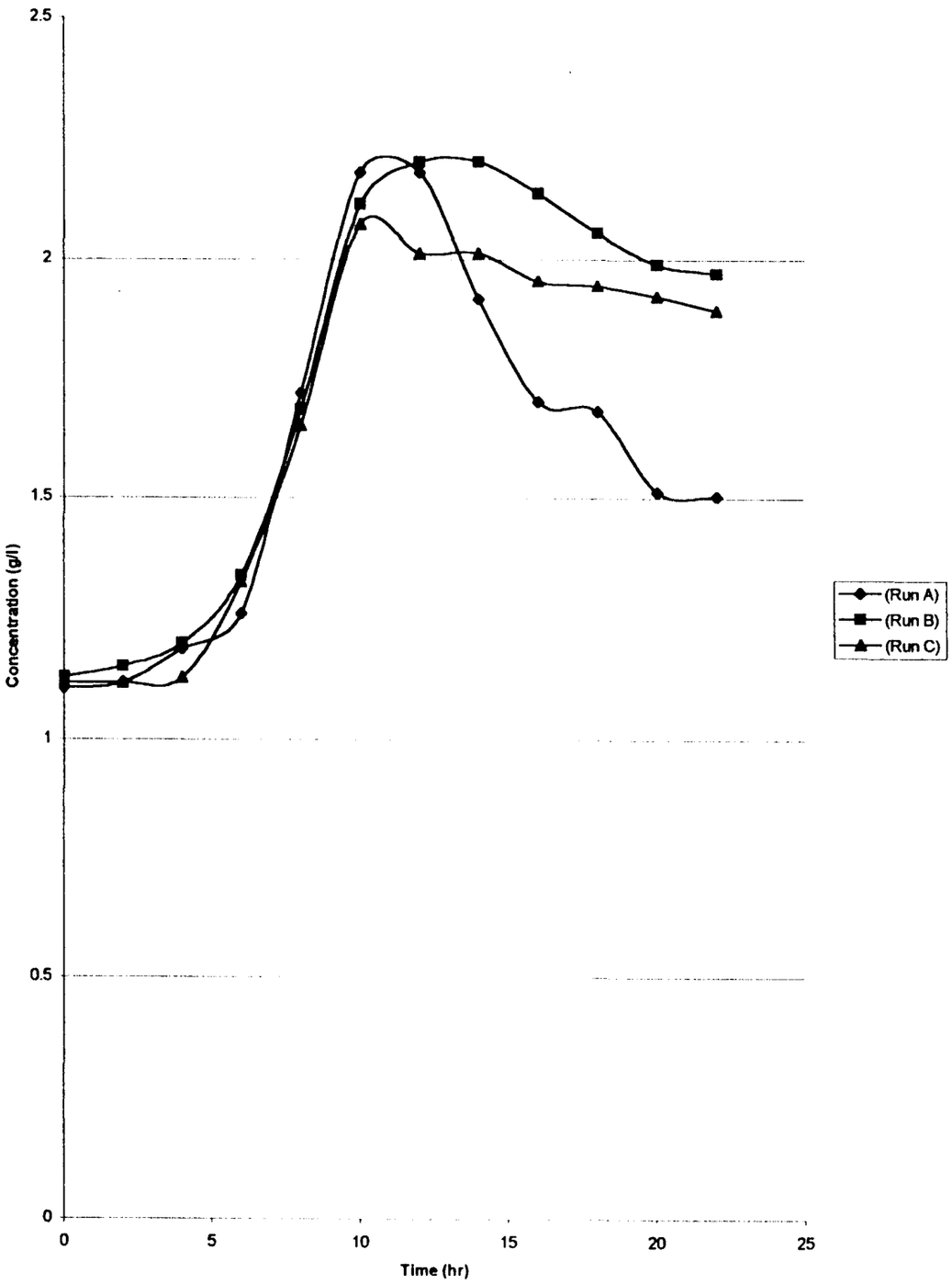
It can be deducted from figure 4.1 (The plot of concentration (g/l) against time (hr) for Run A, B and C at 0% dilution level) That the result gave a lag period of 8 hrs for Run A, 6 hrs for Run B and 4 hrs for Run C, after which there were exponential growth in the course of fermentation. The result showed an increase in concentration for fermentation Run A from 1.5683 (g/l) at 0hr to 2.8292 (g/l) at 18 hrs, for Run B from 1.5527 (g/l) at 0hr to 3.0343 (g/l) at 14 hr, for Run C from 1.5527 (g/l) at 0hr to 3.3872 (g/l) at 16hr. Fermentation Run A, B and C experienced a drop in concentration after a situational period of 4hrs.

Fig. 4.2: CONCENTRATION (g/l) VERSUS TIME (hr) AT 50% DILUTION LEVEL



It can also be deduced from figure 4.2 (the plot of concentration (g/l) against time (hr) at 50% dilution level) that the lag period for each Runs was 4hrs (the time that the yeast were trying to adopt to the medium before initiation of growth), but immediately after this period, micro-organism started to grow exponentially as from 1.4918 (g/l) at 4hrs to 2.7183 (g/l) at 12hrs for Run A, from 1.4333 (g/l) at 4hrs to 2.5093 (g/l) at 16 hrs for Run B, and from 1.4049 (g/l) at 4hrs to 3.6693 (g/l) at 16 hrs for Run C. Different period of stationary was deduced from the figure after which is a decrease

Fig. 4.3: CONCENTRATION (g/l) VERSUS TIME (hr) AT 75% DILUTION LEVEL



. It can be shown from figure 4.3 (which is the plot concentration (g/l) against time (hr) at 75% dilution level) that there was a lag period of 4hrs for all Runs. The concentration increased from 1.1252(g/l) at 0hr to 2.1815 (g/l) at 10hr for Rum A, from 1.1275(g/l) at 0hr to 2.1383 (g/l) at 10hr for Rum B and from 1.1160(g/l) at 2hr to 2.0750 (g/l) at 10hr for Rum C. Immediate decrease in concentration is shown in figure 4.3 as the concentration reached highest point.

Kinetic parameters are tabulated in table 4.1 whilst the % protein content are tabulated in tableTable 4.1 kinetic parameters

RUN	Percentage Dilution		Of Crude Liquid		Effluent	
	0%		5%		75%	
	$\mu(hr^{-1})$	tg(hr)	$\mu (hr^{-1})$	tg(hr)	$\mu (hr^{-1})$	tg(hr)
A	0.0625	11.09	0.0783	8.85	0.1375	5.04
B	0.0737	9.39	0.090	7.702	0.1150	6.03
C	0.09167	7.56	0.1025	6.762	0.1125	6.16

Table 4.2 protein content

% Dilution	Run	protein percentages %	Volume of substrate used (ml)
0%	A	48.50	800
	B	58.50	1000
	C	60.00	1200
50%	A	45.80	800
	B	48.00	1000
	C	55.50	1200
75%	A	19.00	800
	B	29.50	100
	C	30.50	1200

CHAPTER FIVE

5.0 DISCUSSION OF RESULT

The plot of cell concentration versus time for Runs A, B and C, at a temperature of 32^0C and pH of 4.6-4.8 and at different level of crude liquid effluent dilution (25%, 50% and 75% using distilled water as diluent) are shown in figure 4.1 – 4.3. From the plot, different lag period, t_g was obtained;

At 0% dilution, Run A has a lag period of 7 hrs, Run B = 6 hrs and Run C = 4hrs, while at 50% dilution, Run A = 4hrs, Run B = 3.5hrs and Run C = 2hrs and the corresponding value at 75% dilution, Run A = 6hrs, Run B = 4hrs and Run C = 3.8hrs. It was hence observed that concentration of the medium affects the microbial growth and determines the time that it will take for the cells to adopt to the media constituents.

The first order growth kinetic, $r = \mu C$, was tested by plotting lnC versus time for each of the Runs at different level of dilution of the crude liquid effluent of cassava processing. The plots are shown in Figure 4.4 – 4.6. The specific growth rate was determined from the slope of the exponential growth line to obtain the following values:-

At 0% dilution, $\mu_A = 0.0625hr^{-1}$, $\mu_B = 0.0737hr^{-1}$, $\mu_C = 0.0916hr^{-1}$ and at 50% dilution, $\mu_A = 0.0783hr^{-1}$, $\mu_B = 0.09hr^{-1}$, $\mu_C = 0.1025hr^{-1}$ while at 75% dilution, $\mu_A = 0.1375hr^{-1}$, $\mu_B = 0.115hr^{-1}$, $\mu_C = 0.1125hr^{-1}$. Clearly the specific growth rate for Run A, B, and C at different dilution are in the following order;

$\mu_{Ao} < \mu_{Bo} < \mu_{Co}$, $\mu_{A50} < \mu_{B50} < \mu_{C50}$, $\mu_{A75} > \mu_{B75} \geq \mu_{C75}$,

This is inconformity with the literature (Biochemical Engineering Fundamentals by James E. Bailey and David F. Ollis second Edition, 381-390.) At dilution level above average, specific growth rate for the Run A, B and C (generations) will follow an increasing order.

The doubling times t_g for each Runs at different dilution level of the substrate are; At 0% dilution, t_{dA} = 11.09hr, t_{dB} = 9.39hr, t_{dC} = 7.56hr while at 50% dilution, t_{dA} = 8.85hr, t_{dB} = 7.70hr, t_{dC} = 6.76hr and at 75% dilution, t_{dA} = 5.04hr, t_{dB} = 6.02hr, t_{dC} = 6.16hr. The generation time are in different order viz:

$t_{dAo} > t_{dBo} > t_{dCo}$, $t_{dA50} > t_{dB50} > t_{dC50}$, and $t_{dA75} < t_{dB75} > t_{dC75}$. These implies that the generation time increase with Run but at high level of dilution reverse is the case which may be due to large variation in the concentration of the medium or insufficient substrate required for efficient growth of yeasts.

The protein contents for Runs A, B and C were determined to be 48.50%, 58.5% and 60% respectively for zero dilution. The corresponding value at 50% dilution are 45.80, 48.6% and 55.5% which that at 75% dilution are 19.0%, 29.5% and 30.5%.

At Runs B and C, the protein content of the yeast appear to be higher than literature value of 50% (Food micro-biology, Willham C. F and D. C. Westhoff, (1978); 398-404) This thus suggest that cassava processing liquid effluent is a reliable source of protein SCP.

Linear regression was used to compute for the line of best fit for the exponential growth phase, which is a linear model to the observed value and the results are;

For 0% dilution

$C_A = 1.917e^{0.04003t}$, $C_B = 1.36e^{0.0429t}$, $C_C = 1.37e^{0.057t}$

For 50% dilution,

$C_A = 1.25e^{0.05893t}$ $C_B = 1.28e^{0.0507t}$ $C_C = 1.19e^{0.072t}$

For 75% dilution

$C_A = 0.98e^{0.0678t}$ $C_B = 1.02e^{0.0637t}$ $C_C = 0.99e^{0.0633t}$

It was observed that the modelled equations provided very minimal error for the exponential growth phase only.

Error computation were made and the results obtained are as stated below:

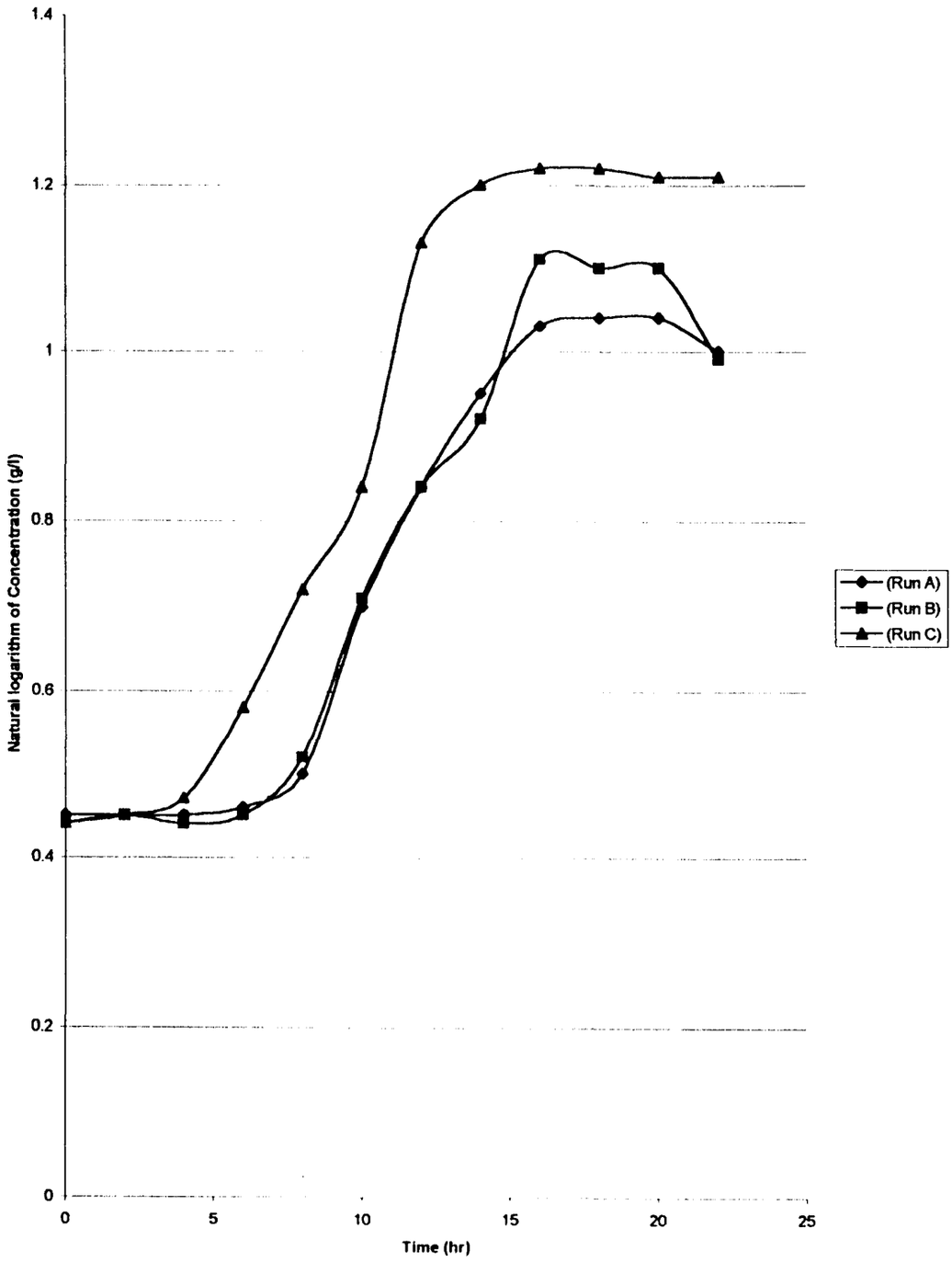
At 0% dilution the slope, Error in slope, variance and Regression for Run A were $0.0625hr^{-1}$, 0.0042, 0.111, 0.9467, For Run B are $0.07375hr^{-1}$. 0.0035, 0.00746, 0.9317-, For Run C are $0.0917hr^{-1}$, 0.0087, 0.00652, 0.9674 respectively.

At 50% dilution, the slope, error in slope, variance and regression are; for Run A, $0.0783hr^{-1}$, 0.00015, 0.00385, 0.9566; For Run B, $0.09hr^{-1}$, 0.0037, 0.034, 0.977; For Run C, $0.1025hr^{-1}$, 0.0085, 0.00839, 0.971, all respectively

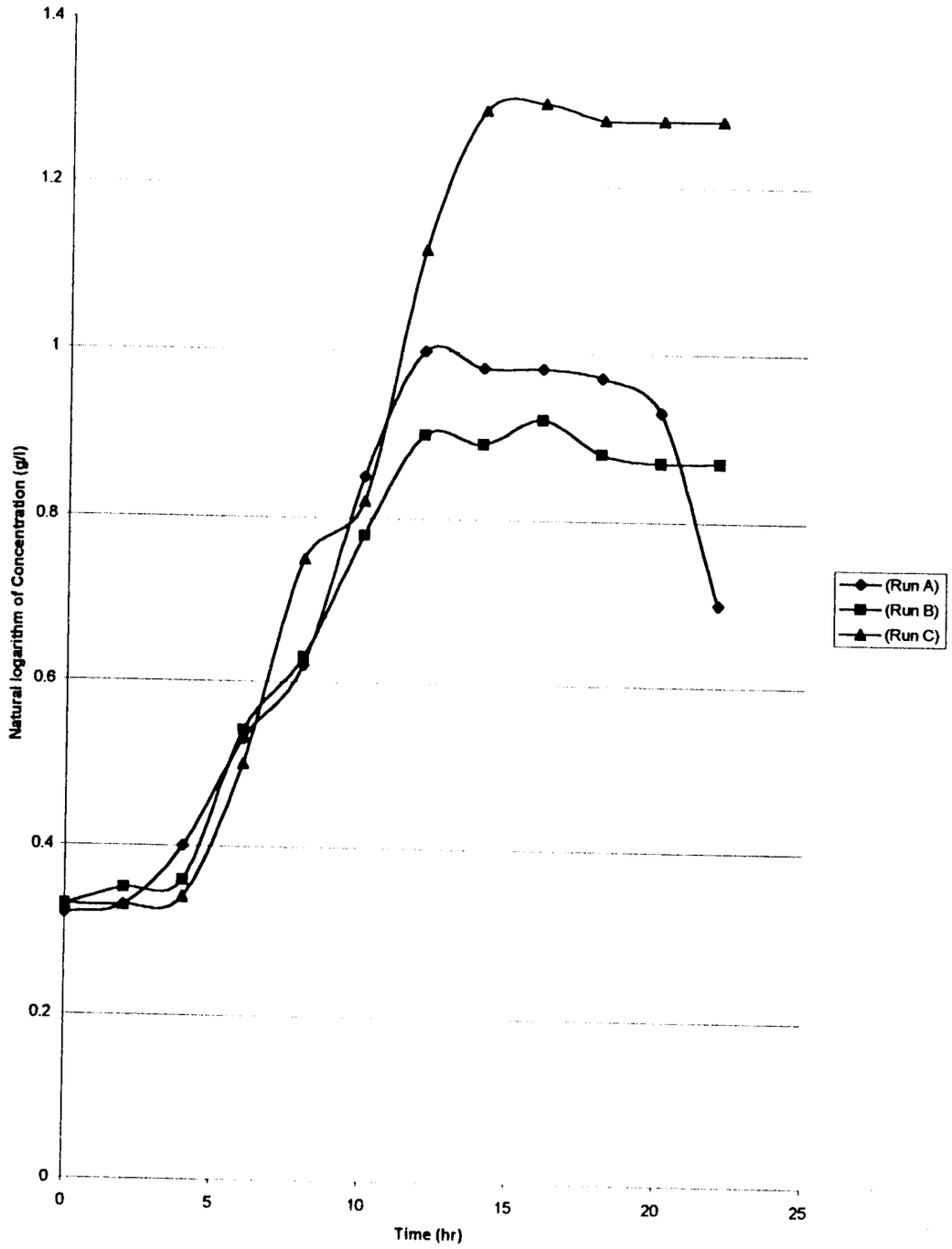
At 75% dilution the slope, error in slope, variance and regression are; For Run A $0.1375hr^{-1}$, 0.0015, 0.01013, 0.977, For Run B, $0.115hr^{-1}$, 0.0009, 0.00576, 0.967; For Run C, $0.1125hr^{-1}$, 0.0056, 0.00818, 0.982 respectively.

Increase with Runs but at high level of dilution the reverse is the case which may be due to large variation ion the concentration of the medium or insufficient minimum substrate required for efficient growth of yeast.

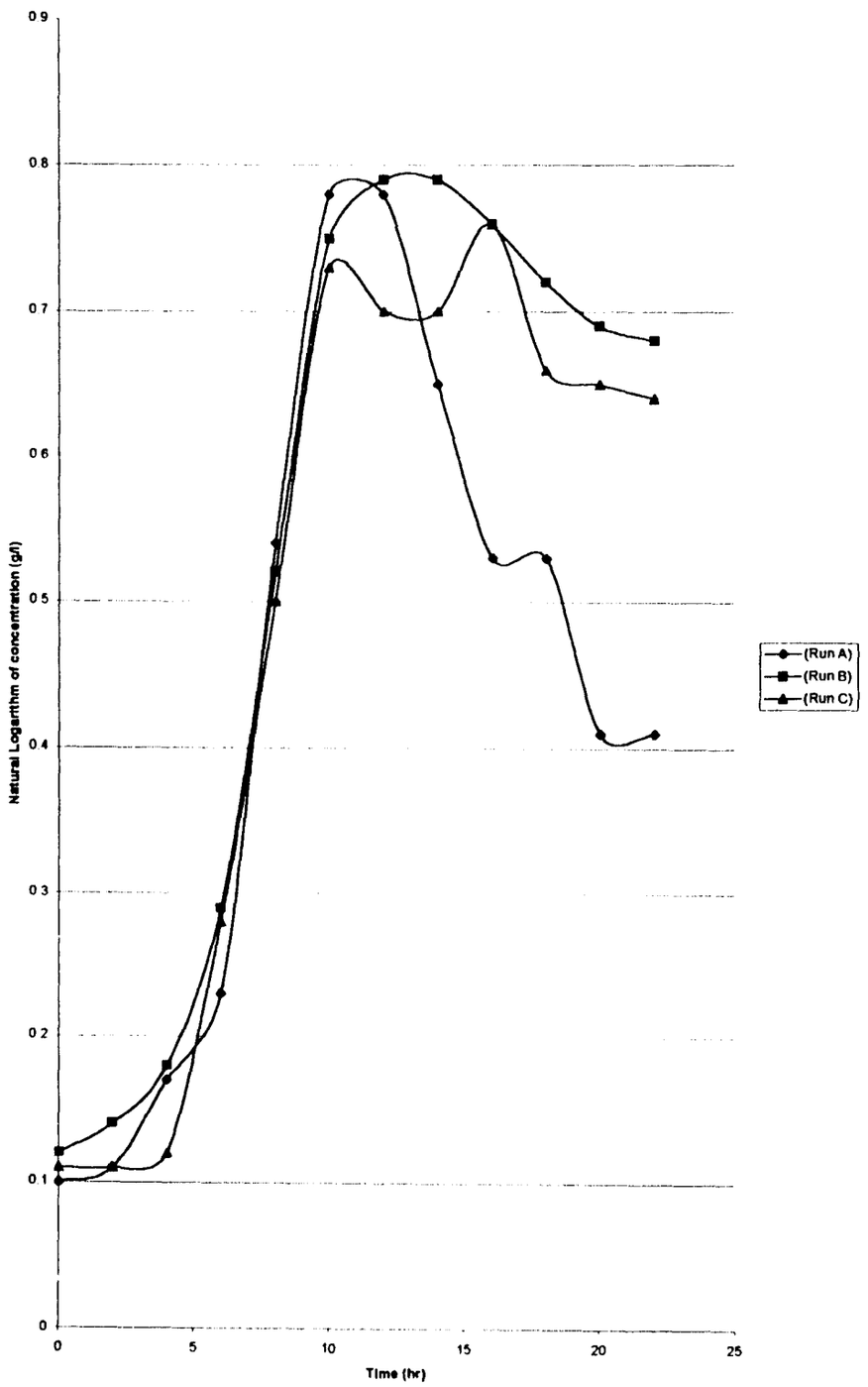
Fi.g. 4.4: NATURAL LOGARITHM OF CONCENTRATION (g/l) VERSUS TIME (hr) AT 0% DILUTION LEVEL



Fi.g. 4.5: NATURAL LOGARITHM OF CONCENTRATION (g/l) VERSUS TIME (hr) AT 50% DILUTION LEVEL



Fl.g. 4.6: NATURAL LOGARITHM OF CONCENTRATION (g/l) VERSUS TIME (hr) AT 75% DILUTION LEVEL



CHAPTER SIX

6.0 CONCLUSION

The results of this study have shown that SCP of high protein yield which is comparable to commercial SCP can be provided using the effluents from a local gari processing mill.

6.1 RECOMMENDATION

Based on the experiment performed on this project work, cassava liquid effluent can be of higher advantage for production of single-cell protein with high protein content than other substrate can be produced provided the optimum limit of substrate dilution level is not exceeded and if the micro-nutrients needed for the medium are well supplied.

General there were some limitations faced during the course of this work which resulted into deviation of some of the estimated parameters from literature values. Such limitations include equipment inadequate, unavailability of some of the chemicals required for the medium, the method of sampling as well as method of taking readings.

Despite all researches so far on the utilization of SCP in human subject, such as the area of toxicity and palatability, there is still some gaps, the gas intestinal syndrome is yet to be explained, as are the potential difficulties associated with high Purina feedings

Extensive researches are required in some areas like continuous system for SCP production as opposed to a batch system of production, effect of heat on the

removal of water from product SCP, metabolism of SCP and its possible chronic and acute toxic effect are needed, with infant animals, malnourished animals or those challenged with appropriate infectious disease. Effect of alcohol and carbondioxide formed during fermentation and investigation of aeration and agitation to obtain optimum yield in SCP production.

APPENDIX I

FERMENTATION RESULT TABLES

TABLE 6.1 : Fermentation Run A at 0% dilution of the crude liquid effluent.

S/N	Time, t (hr)	X(g)	Y(g)	Z (g)	C_A (g/L)	ln C_A
1	0	0.65	0.6735	0.0235	1.5683	0.45
2.	2	0.65	0.6735	0.0235	1.5683	0.45
3.	4	0.65	0.6735	0.0235	1.5683	0.45
4.	6	0.65	0.6738	0.0238	1.5841	0.46
5.	8	0.65	0.6747	0.0247	1.6487	0.50
6.	10	0.65	0.6802	0.0302	2.0137	0.70
7.	12	0.65	0.6847	0.0347	2.3164	0.84
8	14	0.65	0.6888	0.0388	2.5857	0.95
9.	16	0.65	0.6920	0.0420	2.8011	1.03
10	18	0.65	0.6924	0.0424	2.8292	1.04
11	20	0.65	0.6924	0.0424	2.8292	1.04
12	22	0.65	0.6908	0.0408	2.7183	1.00

TABLE 6.2 Fermentation Run B at 0% dilution of the crude liquid effluent.

S/N	Time, t (hr)	X(g)	Y(g)	Z (g)	C_B (g/L)	ln C_B
1	0	0.65	0.6733	0.0233	1.5527	0.44
2.	2	0.65	0.6735	0.0235	1.5683	0.45
3.	4	0.65	0.6733	0.0233	1.5527	0.44
4.	6	0.65	0.6735	0.0235	1.5683	0.45
5.	8	0.65	0.6752	0.0252	1.6820	0.52
6.	10	0.65	0.6805	0.0305	2.0340	0.71
7.	12	0.65	0.6847	0.0347	2.3164	0.84
8	14	0.65	0.6876	0.0376	2.5093	0.92
9.	16	0.65	0.6955	0.0455	3.0343	1.11
10	18	0.65	0.6951	0.0451	3.0042	1.10
11	20	0.65	0.6951	0.0451	3.0042	1.10
12	22	0.65	0.6904	0.0404	2.6912	0.99

TABLE 6.3 Fermentation Run C at 0% dilution of the crude liquid effluent.

S/N	Time, t (hr)	X(g)	Y(g)	Z (g)	C_C (g/l)	ln C_C
1	0	0.65	0.6733	0.0233	1.5527	0.44
2.	2	0.65	0.6733	0.0235	1.5683	0.45
3.	4	0.65	0.6740	0.0240	1.6000	0.47
4.	6	0.65	0.6768	0.0268	1.7860	0.58
5.	8	0.65	0.6808	0.0308	2.0544	0.72
6.	10	0.65	0.6847	0.03475	2.3164	0.84
7.	12	0.65	0.6964	0.0464	3.0956	1.13
8	14	0.65	0.6998	0.0498	3.3200	1.20
9.	16	0.65	0.7008	0.0508	3.3872	1.22
10	18	0.65	0.7008	0.0508	3.3872	1.22
11	20	0.65	0.7003	0.0503	3.3535	1.21
12	22	0.65	0.7003	0.0503	3.3535	1.21

TABLE 6.4 Fermentation Run A at 50% dilution of the crude liquid effluent.

S/N	Time, t (hr)	X(g)	Y(g)	Z (g)	C_A (g/l)	ln C_A
1	0	0.65	0.6706	0.0206	1.3771	0.32
2.	2	0.65	0.6709	0.0209	1.3910	0.33
3.	4	0.65	0.6724	0.0224	1.4918	0.40
4.	6	0.65	0.6755	0.0255	1.6989	0.53
5.	8	0.65	0.6779	0.0279	1.8589	0.62
6.	10	0.65	0.6851	0.0351	2.3396	0.85
7.	12	0.65	0.6908	0.0408	2.7183	1.00
8	14	0.65	0.6900	0.0400	2.6644	0.98
9.	16	0.65	0.6900	0.0400	2.6644	0.98
10	18	0.65	0.6898	0.03977	2.6510	0.97
11	20	0.65	0.6878	0.03783	2.5223	0.93
12	22	0.65	0.6875	0.3752	2.5012	0.70

TABLE 6.5 Fermentation Run B at 50% dilution of the crude liquid effluent.

S/N	Time, t (hr)	X(g)	Y(g)	Z (g)	C_B (g/l)	ln C_B
1	0	0.65	0.6709	0.0209	1.3910	0.33
2.	2	0.65	0.6713	0.0213	1.4191	0.35
3.	4	0.65	0.6715	0.0215	1.4333	0.36
4.	6	0.65	0.6757	0.0257	1.7160	0.54
5.	8	0.65	0.6782	0.0282	1.8776	0.63
6.	10	0.65	0.6827	0.0327	2.1815	0.78
7.	12	0.65	0.6869	0.0369	2.4595	0.90
8	14	0.65	0.6865	0.0365	2.4351	0.89
9.	16	0.65	0.6841	0.03406	2.5093	0.92
10	18	0.65	0.6863	0.03632	2.4217	0.88
11	20	0.65	0.6860	0.03603	2.4025	0.87
12	22	0.65	0.6800	0.03600	2.4001	0.87

TABLE 6.6 Fermentation Run C at 50% dilution of the crude liquid effluent.

S/N	Time, t (hr)	X(g)	Y(g)	Z (g)	C_C (g/l)	ln C_C
1	0	0.65	0.6709	0.0209	1.3910	0.33
2.	2	0.65	0.6709	0.0209	1.3910	0.33
3.	4	0.65	0.6711	0.0211	1.4049	0.34
4.	6	0.65	0.6747	0.0247	1.6487	0.50
5.	8	0.65	0.6817	0.0317	2.1170	0.75
6.	10	0.65	0.6840	0.0340	2.2705	0.82
7.	12	0.65	0.6960	0.0460	3.0648	1.12
8	14	0.65	0.7045	0.0545	3.6328	1.29
9.	16	0.65	0.7050	0.0550	3.6693	1.30
10	18	0.65	0.70426	0.05426	3.6172	1.28
11	20	0.65	0.70399	0.05399	3.5993	1.28
12	22	0.65	0.70396	0.05396	3.5972	1.28

TABLE 6.7 Fermentation Run A at 75% dilution of the crude liquid effluent.

S/N	Time, t (hr)	X(g)	Y(g)	Z (g)	C_A (g/l)	ln C_A
1	0	0.65	0.6666	0.0166	1.1052	0.10
2.	2	0.65	0.6667	0.01674	1.1163	0.11
3.	4	0.65	0.6678	0.0178	1.1853	0.17
4.	6	0.65	0.6689	0.0189	1.2586	0.23
5.	8	0.65	0.6757	0.0257	1.7160	0.54
6.	10	0.65	0.6827	0.0327	2.1815	0.78
7.	12	0.65	0.6827	0.0327	2.1815	0.78
8	14	0.65	0.6787	0.0287	1.9155	0.65
9.	16	0.65	0.6755	0.0255	1.6989	0.53
10	18	0.65	0.6752	0.0252	1.6778	0.52
11	20	0.65	0.6727	0.02268	1.5124	0.41
12	22	0.65	0.6725	0.0225	1.5025	0.41

TABLE 6.8 Fermentation Run B at 75% dilution of the crude liquid effluent.

S/N	Time, t (hr)	X(g)	Y(g)	Z (g)	C_B (g/l)	ln C_B
1	0	0.65	0.6669	0.0169	1.1275	0.12
2.	2	0.65	0.6672	0.0172	1.1503	0.14
3.	4	0.65	0.6680	0.0180	1.1972	0.18
4.	6	0.65	0.6700	0.0200	1.3364	0.29
5.	8	0.65	0.6752	0.0252	1.6820	0.52
6.	10	0.65	0.6817	0.0319	2.1170	0.75
7.	12	0.65	0.6830	0.0330	2.2034	0.79
8	14	0.65	0.6830	0.0330	2.2034	0.79
9.	16	0.65	0.6831	0.0321	2.1383	0.76
10	18	0.65	0.6808	0.0308	2.0568	0.72
11	20	0.65	0.6798	0.0298	1.9897	0.690
12	22	0.65	0.6795	0.0295	1.9699	0.68

TABLE 6.1 Fermentation Run C at 75% dilution of the crude liquid effluent.

S/N	Time, t (hr)	X(g)	Y(g)	Z (g)	C_C (g/l)	ln C_C
1	0	0.65	0.6667	0.0167	1.1160	0.11
2.	2	0.65	0.6667	0.0167	1.1160	0.11
3.	4	0.65	0.6669	0.0169	1.1270	0.12
4.	6	0.65	0.6698	0.0198	1.3230	0.28
5.	8	0.65	0.6747	0.0247	1.6490	0.50
6.	10	0.65	0.6811	0.0311	2.0750	0.73
7.	12	0.65	0.6802	0.0302	2.0140	0.70
8	14	0.65	0.6802	0.0302	2.0140	0.70
9.	16	0.65	0.6793	0.0293	1.9540	0.67
10	18	0.65	0.6791	0.0291	1.9435	0.66
11	20	0.65	0.6788	0.0288	1.9206	0.65
12	22	0.65	0.6783	0.0283	1.8906	0.64

APPENDIX II

To obtain slope (μ) of the fermentation run at 0% dilution level

Run A

Volume of the substrate = 800ml

Therefore: $\mu_{A0} = \frac{\text{change in Y – axis}}{\text{change in X – axis}} = \frac{0.95 - 0.7}{14 - 10}$

$= 0.0625\ hr^{-1}$

Therefore the generation time for Rum A (from Biochemical Engineering Fundamental, page 577 – 565

$$tg_{A0} = \frac{\ln 2}{\mu_{A0}} = \frac{0.693}{0.0625}$$

$= 11.09\ hr$

Rum B

Volume of the substrate = 1000ml

Therefore $N_{B0} = \frac{DY}{DX} = \frac{1.11 - 0.52}{16 - 18} = 0.07375\ hr^{-1}$

Then $t_{gB0} = \frac{\ln 2}{0.07375} = 9.39\ hr$

Rum C

Volume of the substrate = 1200ml

Therefore, $\mu_{C0} = \frac{1.13 - 0.58}{12 - 6} = 0.09167\ hr^{-1}$

$$t_{gC0} = \frac{\ln 2}{0.09167} = 7.56 \text{ hr}$$

To obtain the slope (μ) of the fermentation rum at 50% dilution level

Rum A

Volume of the substrate are 800ml, 1000ml and 1200ml for Rum A, B and therefore, $\mu_{A50} = \frac{1.00 - 0.53}{12 - 6} = 0.0783 \text{ hr}^{-1}$

$$\mu_{B50} = \frac{0.78 - 0.36}{10 - 4} = 0.09 \text{ hr}^{-1}$$

$$\mu_{C50} = \frac{0.75 - 0.34}{8 - 4} = 0.1025 \text{hr}^{-1}$$

Then,

$$t_{gA50} = \frac{\ln 2}{0.0783} = 8.849 \text{hr}$$

$$t_{gB50} = \frac{\ln 2}{0.09} = 7.702 \text{hr}$$

$$t_{gC50} = \frac{\ln 2}{0.1025} = 6.76 \text{hr}$$

To obtain the slope (μ) of the fermentation rum at 75% dilution level

Volume of the substrate are 800ml, 1000ml and 1200ml for rum A, B and C respectively.

$$\mu_{A75} = \frac{0.78 - 0.23}{10 - 16} = 0.1375 \text{hr}^{-1}$$

$$\mu_{B75} = \frac{0.75 - 0.29}{10 - 16} = 0.115 \text{hr}^{-1}$$

$$\mu_{C75} = 0.1125 \text{hr}^{-1}$$

Then ,

$$t_{gA75} = \frac{\ln 2}{} - 5.04 \text{hr}$$

$$\frac{}{0.1375}$$

$$t_{gB75} = \frac{\ln 2}{0.115} - 6.027hr$$

$$tg_{B75} = \frac{\ln 2}{0.1125} - 6.16hr$$

PROTEIN CONTENT ANALYSIS

Equation for the reactions are:-

$2\ NH_3 + H_2SO_4 \longrightarrow (NH_4)_2So_4$ ..... (1)

$(NH_4)_2SO_4 + NaoH \longrightarrow 2NH_3 + Na_2\ SO_4 + 2H_2$..... (2)

$2\ NH_3 + H_2SO_4 \longrightarrow (NH_4)_2S0_4$ ..... (3)

<u>For Rum A at 0% dilution</u>

Molarity of the acid for digestion = 1M

Volume of acid used = $1.39cm^3$

Number of moles of acid in $1.39cm^3 = \frac{1.39 \times 1}{1000}$

= 0.00139 mole

Number of moles of NH_3 = 0.00139 x 2

= 0.00277 moles

But, Mass of NH_3 = 17 x 0.00277

= 0.0477lg

Mass of nitrogen in NH_3 = 14/17 x 0.0477lg

= 0.0338g

0. 5g of the sample contain 0.0338g of nitrogen

1. 1g of the sample will contain 0.0338g x 1g nitrogen

Mass of nitrogen in 1g of sample = 0.0776g

The % protein content = 0.0776g x 6.25 x 100

= 48.50%

Run B at 0% dilution level

M_A = 1M V_A = 1.67cm^3

$n_A = \frac{M_A V_A}{1000} = \frac{1.67 \times 1}{1000}$ = 0.00167 moles

Stoichiometry acid to NH_3 ratio is 1: 2

Number of moles of NH_3 = 2 x 0.00167 mole

= 0.0334 mole

Mass of ammonia = 17 x 0.00334

= 0.05683g

Mass of nitrogen in ammonia = 14/17 x 0.05683

= 0.0468g

0.5g of the sample contain 0.0468g of nitrogen

1g of the sample will contain $\frac{0.0468 \text{ of nitrogen}}{0.5}$

= 0.0936g of nitrogen

The % protein = 6.25 x 100 x 0.0936

= 58.5%

Run C at 0% dilution level

N_A = 1M V_A = 1.71cm^3

$$n_A = \frac{M_A V_A}{1000} = \frac{1 \times 1.71}{1000} = 0.00171 \text{ mole}$$

Acid to ammonia ratio is 1.2

Therefore,

Number mole of ammonia = 2 x 0.00171

= 0.0343 mole

Mass of ammonia = 17 x 0.00343

= 0.05829g

Mass of nitrogen in ammonia = 14/17 x 0.05829

= 0.048g

0.5g of the sample contain 0.048g of nitrogen

1g of the sample contain $\frac{0.048g \times 1g}{0.5g}$ of nitrogen

= 0.096g of Nitrogen

The % protein = 6.25 x 100 x 0.096

= 60.0%

The same calculation was done to determine the percentage protein content which was repeated for Rum A, B, and C at 50% dilution and 75% dilution level. The results are as stated below:

At 50% dilution.

Run A, % protein content = 45.8%

Rum A, % protein content = 48.0%

Rum C, % protein content = 55.5%

At 75% dilution,

Rum A, % protein content = 19.0%

Rum B, % protein content = 29.5%

Rum C, % protein content = 30.5%

LINE OF BEST FIT EVALUATION

For Rum A at 0% dilution

t	ln C_{A0}	t ln C_{A0}	t^2	$(\ln C_{A0})^2$
0	0.45	0.00	0	0.2025
2	0.45	0.90	4	0.2025
4	0.45	1.80	16	0.2025
6	0.46	2.76	36	0.2116
8	0.50	4.00	64	0.250
10	0.70	7.00	100	0.490
12	0.84	10.08	144	0.7056
14	0.95	13.30	196	0.9025
16	1.03	16.48	256	1.0609
18	1.04	18.72	324	1.0816
90	6.87	75.04	1140	5.3097

Constants,

$$B = \frac{n\Sigma (t \ln C_{A0}) - \Sigma t \Sigma (\ln C_{A0})}{n\Sigma t^2 - (\Sigma t)^2}$$

$$= \frac{10\,(75.04) - 90\,(6.87)}{10(1140) - (90)^2}$$

$$B = 0.04003$$

$$A = \frac{\Sigma (\ln C_{A0}) - B\Sigma t}{n}$$

$$= \frac{6.87 - 0.04003\ (90)}{10}$$

$A = 0.06507$

Therefore. from $\ln C = A + Bt$ where $A = \ln C_0$

$\ln C_{A0} = 0.65097 + 0.04003t$ $B = \mu$

This is equivalent to

$C_{A0} = 1.912e^{0.04003t}$

For Run B at 0% dilution,

t	$\ln C_{B0}$	$t \ln C_{B0}$	t^2	$(\ln C_{B0})^2$
0	0.44	0	0	0.1936
2	0.45	0.90	4	0.2025
4	0.44	1.76	16	0.1936
6	0.45	2.70	36	0.2025
8	0.52	4.16	64	0.2704
10	0.71	7.10	100	0.5041
12	0.84	10.08	144	0.7056
14	0.92	12.88	196	0.8464
16	1.11	17.76	256	1.2321
72	5.88	57.34	816	4.3508

To obtain the constants

$$B = \frac{n\Sigma (t \ln C_{B0}) - \Sigma t\ (\ln C_{B0})}{n\Sigma (t)^2 - (\Sigma t)^2} \qquad A = 5.88 - \frac{0.0429\ (72)}{9}$$

$B = 0.0429$ $A = 0.3099973$

The equation becomes

$\ln C_{B0} = 0.3099973 + 0.042917t$

Therefore.

$C_{B0} = 1.35e^{0.042917}$

For Run C at 0% dilution

t	ln C_{C0}	t ln C_{C0}	t^2	$(\ln C_{C0})^2$
0	0.44	0	0	0.1936
2	0.45	0.90	4	0.2025
4	0.47	1.88	16	0.2209
6	0.58	3.48	36	0.3364
8	0.72	5.76	64	0.5184
10	0.84	8.40	100	0.7056
12	1.13	13.56	144	0.2760
14	1.20	16.80	196	1.440
16	1.22	19.52	256	1.4884
72	7.05	70.30	816	6.3827

$$B = \frac{9(70.30) - 72(7.05)}{9(816) - (72)^2} \qquad A = \frac{7.05 - 0.05792(72)}{9}$$

$B = 0.05792$ $\qquad$ $A = 0.31997$

$$\ln C_{C0} = 0.31997 + 0.05792\,t$$

Which is simply

$$C_{C0} = 1.38e^{0.05792t}$$

Run A at 50% dilution

t	ln C_{A50}	t ln C_{A50}	t^2	$(\ln C_{A50})^2$
0	0.32	0	0	0.1024
2	0.33	0.66	4	0.1089
4	0.40	1.60	16	0.160
6	0.53	3.18	36	0.2809
8	0.62	4.96	64	0.3844
10	0.85	8.50	100	0.7225
12	1.00	12.00	144	1.000
90	4.05	30.90	364	2.75910

$$B = \frac{7(30.90) - 42(4.05)}{7(364) - (42)^2} = 0.05893; \quad A = \frac{4.05 - 0.05893(42)}{7} = 022499$$

Therefore, $\ln C_{A50} = 0.22499 + 0.05893t$

For Run B at 50% dilution,

t	$\ln C_{B50}$	$t \ln C_{B50}$	t^2	$(\ln C_{B50})^2$
0	0.33	0	0	0.1089
2	0.35	0.70	4	0.1225
4	0.36	1.44	16	0.1296
6	0.54	3.24	36	0.2916
8	0.63	5.04	64	0.3969
10	0.78	7.80	100	0.6084
12	0.90	10.80	144	0.8100
42	4.05	29.02	364	2.4679

$$B = \frac{7(29.02) - 42(3.89)}{7(364) - (42)^2} \qquad A = \frac{3.89 - 0.05071(42)}{7}$$

$B = 0.05071$ $\qquad$ $A = 0.025145$

Therefore,

$\ln C_{B50} = 25145 + 0.05071t$

For Run C at 50% dilution

t	$\ln C_{C50}$	$t \ln C_{C50}$	t^2	$(\ln C_{C50})^2$
0	0.33	0	0	0.1089
2	0.33	0.66	4	0.1089
4	0.34	1.36	16	0.1156
6	0.50	3.00	36	0.2500
8	0.75	6.00	64	0.5625
10	0.82	8.20	100	0.6724
12	1.12	13.44	144	1.2544
14	1.29	18. 60	196	1.6641
16	1.30	20.80	256	1.6900
72	6.78	17.52	816	6.4262

$$B = \frac{9(71.52) - 72(6.78)}{9(816) - (72)} = 0.072; \qquad A = \frac{6.78 - 0.072(72)}{9} = 0.17733$$

$$\ln C_{C50} = 0.17733 + 0.072t$$

Run A at 75% dilution

t	$\ln C_{A75}$	$t \ln C_{A75}$	t^2	$(\ln C_{A75})^2$
0	0.10	0	0	0.01
2	0.11	0.22	4	0.012
4	0.17	0.68	16	0.0289
6	0.23	1.38	36	0.0529
8	0.54	4.32	64	0.2916
10	0.78	7.80	100	0.6084
30	1.93	14.40	220	1.0038

$$B = \frac{6(14.40) - 30(1.93)}{6(220) - (30)^2} = 0.06786; \qquad A = \frac{1.93 - 0.06786(30)}{6} = -0.01763$$

$$\ln C_{A75} = -0.01763 + 0.06786t$$

Run B at 75% dilution

t	$\ln C_{B75}$	$t \ln C_{B75}$	t^2	$(\ln C_{B75})^2$
0	0.12	0	0	0.0144
2	0.14	0.28	4	0.0196
4	0.18	0.72	16	0.0324
6	0.29	1.74	36	0.0841
8	0.52	4.16	64	0.2704
10	0.72	7.50	100	0.5625
12	1.79	9.48	144	0.6241
42	2.79	23.88	364	1.6075

$$B = \frac{7(23.880) - 42(2.79)}{7(364) - (42)^2} = 0.06375; \qquad A = \frac{2.79 - 0.06375(42)}{7} = 0.01607$$

$$\ln C_{B75} = 0.01607 + 0.06375t$$

For Rum C at 75% dilution

t	ln C_{C75}	t ln C_{C75}	t^2	$(\ln C_{C75})^2$
0	0.11	0	0	0.0121
2	0.11	0.22	4	0.0121
4	0.12	0.48	16	0.0144
6	0.28	1.68	36	0.0784
8	0.50	4.00	64	0.2500
10	0.73	7.30	100	0.5329
30	7.05	13.68	220	0.8999

$$B = \frac{6\,(13.68) - 30\,(1.58);}{6\,(220) - (30)^2} = 0.06329; \quad A = \frac{1.85 - 0.06329}{6} = -0.00812$$

$$\ln C_{C75} = -0.00812 + 0.06329t$$

NEW EVALUATED VALUES FOR RUN A, B AND C AT DIFFERENT DILUTION LEVEL.

For Run A, B, and C at 0% dilution level.

t	C_{A0}
0	0.65097
2	0.73103
4	0.81109
6	0.89115
8	0.97121
10	0.05127
12	1.13133
14	1.21139
16	1.29145
18	1.37151

t	C_{B0}
0	0.30997
2	0.39681
4	0.48164
6	0.56747
8	0.65331
10	0.73914
12	0.82498
14	0.91081
16	0.99664

t	C_{C0}
0	0.31997
2	0.43581
4	.55165
6	0.66749
8	0.78333
10	0.89917
12	1.01501
14	1.13085
16	1.24669

For A, B, and C at 50% dilution level

t	C_{A0}
0	0.22499
2	0.34285
4	0.46071
6	0.57857
8	0.69643
10	0.81429
12	0.93215

t	C_{B50}
0	0.25145
2	0.35287
4	0.45429
6	0.55571
8	0.65713
10	0.75855
12	0.85997

t	ln C_{C50}
0	0.17733
2	0.32133
4	0.46533
6	0.60933
8	0.75333
10	0.89733
12	1.04133
14	1.18533
16	1.32933

For Run A, B, and C at 75% dilution

t	C_{A75}
0	- 0.01763
2	0.11809
4	0.25381
6	0.38953
8	0.52525
10	0.66097

t	C_{B75}
0	0.01607
2	0.14357
4	0.27107
6	0.39857
8	0.52607
10	0.65357
12	0.78107

t	C_{C75}
0	-0.00812
2	0.11846
4	0.24504
6	0.37162
8	0.4982
10	0.62478

STANDARD DEVIATIONS FOR RUN A AND A, B AND C

At 0% dilution level, (For exponential growth only)

w = deviation

= Evaluation value – observed value

Subscript 'A', 'B', 'C', represent Rums A, B and C

w_A	w_B	w_C	w_A^2	w_B^2	w_C^2
0.20000	0.1300	0.1200	0.0404	0.0169	0.0144
0.2809	0.0541	0.0141	0.0789	0.00293	0.0002
0.3611	0.0471	0.0817	0.13039	0.00174	0.00667
0.4311	0.1175	0.0875	0.18589	0.01381	0.00765
0.4713	0.1333	0.0633	0.2222	0.01778	0.00401
0.3511	0.0291	0.0592	0.1233	0.00085	0.00350
0.2913	0.0089	0.1149	0.08487	0.00022	0.01322
0.2614	0.0148	0.0691	0.06832	0.00008	0.00478
0.8268	0.1133	0.1133	0.6836	0.01284	0.00071
0.3315			0.1099		

Standard deviation, $\sigma = \Sigma w^2$ where N = number of observations

$\sigma_A = 0.111$; $\sigma_B = 0.00746$; $\sigma_C = 0.00652$

At 50% dilution level

Subscript 'A', 'B', 'C', represent Rums A, B and C

w_A	w_B	w_C	w_A^2	w_B^2	w_C^2
0.09500	0.3239	0.1527	0.00903	0.09885	0.02331
0.00894	0.00017	0.04216	0.00008	0.01304	0.2064
0.0607	0.0889	0.1253	0.00369	0.00791	0.01571
0.0484	0.1414	0.1093	0.0036	0.020	0.01195
0.0764	0.1039	0.0032	0.00584	0.0108	0.00001
0.0357	0.1262	0.0773	0.00128	0.01593	0.00598
0.0678	0.2464	0.0786	0.00460	0.06073	0.00619
0.1047	0.01096	0.0293	0.00086		

$\sigma_A = 0.00385$; $\sigma_B = 0.034$; $\sigma_C = 0.00839$

At 75% dilution level

W_A	W_B	W_C	W_A^2	W_B^2	W_C^2
0.1039	0.1181	0.01384	0.0108	0.01395	
0.1176	0.00316	0.00836	0.00007	0.00001	0.00007
0.0084	0.0910	0.1250	0.00702	0.00829	0.01564
0.0838	0.1086	0.0916	0.02545	0.01179	0.00839
0.1595	0.00632	0.0000	0.00022	0.00004	0.0000
0.0148	0.1092	0.1052	0.01417	0.01193	0.01107
0.1190	0.00894	0.00008			

$\sigma_A = 0.01013$; $\sigma_B = 0.00576$; $\sigma_C = 0.00818$

REGRESSION

For Run A at 0% dilution

$$R_{A0} = \frac{\Sigma xy - \Sigma x \, \Sigma y}{\sqrt{(n\Sigma x^2 - (\Sigma x)^2)\,(n\Sigma y^2 - (\Sigma y)^2)}}$$

$$= \frac{10\,(75.07) - 90\,(6.87)}{\sqrt{(10\,(1140) - (90)^2\,(10\,(5.3097) - (6.87)^2)}}$$

$$R_{A0} = 0.9467$$

For Run B at 0% dilution

$$R_{B0} = \frac{9\,(57.34) - 72\,(5.88)}{\sqrt{(9\,(816) - (72)^2\,(9\,(4.3508) - (5.88)^2)}}$$

$$R_{B0} = 0.9317$$

For Rum C at 0% dilution

$$R_{C0} = \frac{9\,(70.30) - 72\,(7.05)}{\sqrt{(9\,(816) - (72)^2\,(9\,(6.3827) - (7.05)^2)}}$$

$$R_C = 0.96741$$

For Run A at 50% dilution

$$R_{A50} = \frac{7\,(30.90) - 42\,(4.05)}{\sqrt{(7\,(364) - (42)^2)\,(7\,(2.7591) - (4.05)^2)}}$$

$$R_{A50} = 0.9566$$

For Rum B at 50% dilution

$$R_{B50} = \frac{7\,(29.02) - 42\,(3.89)}{\sqrt{(7\,(2.4679) - (3.89)^2)\,(7\,(364) - (42)^2)}}$$

$$R_{B50} = 0.977$$

For Run C at 50% dilution

$$R_{C50} = \frac{9\,(71.52) - 72\,(6.78)}{\sqrt{(9(816) - (72)^2)\,(9\,(6.4268) - (6.78)^2)}}$$

$$R_{C50} = 0.971$$

For Run A at 75% dilution

$$R_{A75} = \frac{6\,(14.40) - 30\,(1.93)}{\sqrt{(6(1.0038) - (1.93)^2)\,(6\,(022) - (30)^2)}}$$

$$R_{A75} = 0.977$$

For Rum B at 75% dilution

$$R_{B75} = \frac{7\,(23.88) - 42\,(2.78)}{\sqrt{(7(1.6075) - 42\,(2.79)^2)\,(7\,(364) - (42)^2)}}$$

For Run C at 75% dilution

$$R_{C75} = \frac{6\,(13.68) - 30\,(1.85)}{\sqrt{(6(0.8999) - (1.85)^2)\,(6\,(220) - (30)^2)}}$$

$$R_{C75} = 0.982$$

NOMENCLATURE

μ	Specific growth rate of the micro-organism
tg/td	Generation time or Doubling time of the micro-organism
Run A	Fermentation Run A
Run B	Fermentation Run B
Run C	Fermentation Run C
I	Intensity of light striking the suspension
Io	Intensity of light transmitted by the suspension
A	Absorbency
Y	Growth Yield
X	Dry weight per ml of cells
Xo	Dry weight per ml of cells immediately after inoculating
X	Weight of fitter paper
Y	Weight of fitter paper + dry sample
Z	Weight of dry sample
W	Width between the highest point and the lowest point on the graph
R	The horizontal separation between these two points called the range of the abscissas
N	Number of point of experiments or number of generations

No	Number of organism inoculated at time zero
N	Total number of organism at the end of a given period
S	Standard error
S,D	Standard Deviation
δ	Variance
R.	Regression
w	Difference between the evaluated and the observed values
CO_2	Carbondioxide
V	Volume of old medium transferred
a	Concentration of critical substance per unit volume
n_o	Number of cells per unit new volume
a"	Increase in critical substance due to internal cell
a'	Average increase in cell critical substance due to production by other cells, per time per cell
t_l	lag time
C'	Critical substances in the cell
μ	Specific growth rate
t_d	Doubling time
t_g	Generation time
Subscripts	'A', 'B' and 'C' represent run A, B. and C respectively
Subscripts	'0', '50' and '75' represent dilution level of effluent at 0%, 50% and 75%

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