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Kinetic Study of the Fermentation of Cassava Whey

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ABSTRACT: *This project work is about the qualitative analysis and fermentation of cassava whey (a milky liquor that results from the pressing of freshly ground cassava) to produce ethanol using saccharomyces cerevisiae yeast. Qualitative analysis was carried out on the cassava whey to confirm the presence of starch, reducing sugar, protein and lipid. Also, the pH of the cassava whey was obtained to be 5.83 showing that it was slightly acidic. The density was obtained to be 1.028g/l and the moisture content was obtained as 82.3%. Acid hydrolysis was carried out using distilled water and 2.5M HCl on the cassava whey to break down the bonds of the starch. The pH was afterwards neutralized using 2.5M NaOH to correct the pH. The hydrolyzed cassava whey was sterilized using an autoclave, and yeast was introduced into the medium to begin the fermentation process. During the fermentation, samples were collected every 24 hours to monitor the concentration of the glucose, ethanol and yeast. From the results, it was noticed to follow the supposed pattern. The glucose depleted because it is the substrate that the yeast feeds on, the yeast increased and the product formed was the ethanol. The log phase of the yeast growth was used to obtain the Michelis Menten constant as 29.85 and the maximum reaction rate as 0.025g/l.hour.*

KEY WORDS*: Fermentation, Kinetics*

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I. INTRODUCTION

In a country like Nigeria and in a time like this, processors need to think of a way to turn things around and also enrich their pocket at the same time. Nigeria is expected to double its population in the coming years as the sizes of its cities explode. To meet up with the demands and needs of this rising population, raw materials are depleted without proper replacement, and also different wastes are being generated during the processing of these raw materials. Raw materials depletion without an alternative source or support has led to much anxiety about the fate of processes and products that depend solely on them when they eventually hit levels below their replacement. This triggered researchers into the area of alternative raw material discovery and it beamed a searchlight on cassava. Cassava was then discovered as an alternative source of starch to wheat which has been over-burdened over the years. Cassava production is vital to the economy of the country as Nigeria is the world's largest producer of the commodity. It is widely consumed in the country in the form of starch, traditionally-prepared fufu, garri, etc. The processing of cassava to obtain these products has led to wastes. These wastes include the peels, sievate, stumps and whey. Cassava whey is the liquid pressed out of the tuber after it has been crushed mechanically, it is regarded as a total waste and its disposal is an added burden.Turning these wastes into something useful gives an edge as an engineer. The peels, sievate and stumps can be used as animal feed, while the whey can be converted to ethanol. Ethanol can be used as fuel, as a major component of petroleum liquid fuel-ethanol blend and as a source of energy used solely on its own as fuel. It is obtained from starch via enzymatic hydrolysis and then fermentation or through acid hydrolysis and fermentation. Many models describing microbial activities during alcohol fermentation has been proposed, the most common being Monod kinetics. The verification of this model will be carried out using cassava whey as our substrate of concern.

II. KINETIC STUDY FOR THE PRODUCTION OF ETHANOL FROM CASSAVA WHEY

Ethanol, also called ethyl alcohol; can be used as fuel alcohol, drinking alcohol, and grain alcohol. The common type of ethanol is the one found in alcoholic beverages. It is also used as fuel for cars and often called alcohol or spirit. Ethanol is generally produced by the fermentation of sugar, cellulose, or converted starch and

has a long history. In Nigeria, local production of ethanol from maize, guinea corn, millet, other starchy substrates, and cellulose is as old as the country itself. The opportunity for ethanol production in Nigeria is amazing. Right now, our demand locally is being met by importation. Nigeria is the largest producer of cassava in the world, producing about 40 million tons per of the product annually. Cassava is one of the richest fermentable substances for the production of crude alcohol/ethanol.

Fermentation is a metabolic process that converts sugar to acids, gases, or alcohol. It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product. French microbiologist Louis Pasteur is often remembered for his insights into fermentation and its microbial causes. The science of fermentation is known as zymology.

Fermentation takes place when the electron transport chain is unusable (often due to lack of a final electron receptor, such as oxygen). In this case it becomes the cell's primary means of ATP (energy) production (Klein, 2006). Fermentation turns NADH and pyruvate produced in glycolysis into NAD+ and an organic molecule which varies depending on the type of fermentation. In the presence of O2, NADH and pyruvate are used to generate ATP in respiration. This is called oxidative phosphorylation, and it generates much more ATP than glycolysis alone. For that reason, cells generally benefit from avoiding fermentation when oxygen is available, the exception being obligate anaerobes which cannot tolerate oxygen.

In oxidative phosphorylation the energy for ATP formation is derived from an electrochemical proton gradient generated across the inner mitochondrial membrane (or, in the case of bacteria, the plasma membrane) via the electron transport chain. Glycolysis has substrate-level phosphorylation (ATP generated directly at the point of reaction). Humans have used fermentation to produce drinks and beverages since the Neolithic age. For example, fermentation is used for preservation in a process that produces lactic acid as found in such sour foods as pickled cucumbers, kimchi and yogurt , as well as for producing alcoholic beverages such as wineand beer. Fermentation can even occur within the stomachs of animals, such as humans.

Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to aerobic respiration, as long as sugars are readily available for consumption (a phenomenon known as the Crabtree effect) (Dickinson, 1999). The antibiotic activity of hops also inhibits aerobic metabolism in yeast. The chemical equation below shows the alcoholic fermentation of glucose, whose chemical formula is C6H12O6. One glucose molecule is converted into two ethanol molecules and two carbon dioxide molecules:

$C6H2O6 \rightarrow 2\ C2H5OH + 2\ CO2$

C2H5OH is the chemical formula for ethanol.

Before fermentation takes place, one glucose molecule is broken down into two pyruvate molecules. This is known as glycolysis (Stryer, 1975).

CASSAVA

Manihot esculenta (commonly called cassava (/kəˈsɑːvə/), yuca, manioc, "mandioca" andBrazilian arrowroot) is a woody shrub native to South America of the spurge family, Euphorbiaceae. It is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root, a major source of carbohydrates. Though it is often called yuca in Spanish and in the United States, it differs from the yucca, an unrelated fruit-bearing shrub in the family Asparagaceae. Cassava, when dried to a powdery (or pearly) extract, is called tapioca; its fermented, flaky version is named garri.

Cassava is the third-largest source of food carbohydrates in the tropics, after rice and maize (Fauquet, 1990).Cassava is a major staple food in the developing world, providing a basic diet for over half a billion people (Food and Agriculture Organization of the United Nations 1995). It is one of the most drought-tolerant crops, capable of growing on marginal soils. Nigeria is the world's largest producer of cassava, while Thailand is the largest exporter of dried cassava.

Cassava is classified as either sweet or bitter. Like other roots and tubers, both bitter and sweet varieties of cassava contain anti-nutritional factors and toxins, with the bitter varieties containing much larger amounts. It must be properly prepared before consumption, as improper preparation of cassava can leave enough residual cyanide to cause acute cyanide intoxication, goiters, and even ataxia, partial paralysis, or death. The more toxic varieties of cassava are a fallback resource (a "food security crop") in times of famine or food insecurity in some places. Farmers often prefer the bitter varieties because they deter pests, animals, and thieves (Linley, et al., 2002).

CHEMICAL KINETICS

Chemical kinetics, also known as reaction kinetics, is the study of rates of chemical processes. Chemical kinetics includes investigations of how different experimental conditions can influence the speed of a

chemical reaction and yield information about the reaction's mechanism and transition states, as well as the construction of mathematical models that can describe the characteristics of a chemical reaction.

2.8.2 Factors affecting reaction rate

Nature of the reactants

Depending upon what substances are reacting, the reaction rate varies. Acid/base reactions, the formation of salts, and ion exchange are fast reactions. When covalent bond formation takes place between the molecules and when large molecules are formed, the reactions tend to be very slow. Nature and strength of bonds in reactant molecules greatly influence the rate of its transformation into products.

Physical state

The physical state (solid, liquid, or gas) of a reactant is also an important factor of the rate of change. When reactants are in the same phase, as in aqueous solution, thermal motion brings them into contact. However, when they are in different phases, the reaction is limited to the interface between the reactants. Reaction can occur only at their area of contact; in the case of a liquid and a gas, at the surface of the liquid. Vigorous shaking and stirring may be needed to bring the reaction to completion. This means that the more finely divided a solid or liquid reactant the greater its surface area per unit volume and the more contact it has with the other reactant, thus the faster the reaction. To make an analogy, for example, when one starts a fire, one uses wood chips and small branches — one does not start with large logs right away.

Concentration

The frequency with which molecules or ions collide depends upon their concentrations. The more crowded the molecules are, the more likely they are to collide and react with one another. Thus, an increase in the concentrations of the reactants will usually result in the corresponding increase in the reaction rate, while a decrease in the concentrations will usually have a reverse effect. For example, combustion will occur more rapidly in pure oxygen than in air (21% oxygen).

Temperature

Temperature usually has a major effect on the rate of a chemical reaction. Molecules at a higher temperature have more thermal energy. Although collision frequency is greater at higher temperatures, this alone contributes only a very small proportion to the increase in rate of reaction. Much more important is the fact that the proportion of reactant molecules with sufficient energy to react (energy greater than activation energy: $E > Ea$) is significantly higher and is explained in detail by the Maxwell–Boltzmann distribution of molecular energies.A reaction's kinetics can also be studied with a temperature jump approach. This involves using a sharp rise in temperature and observing the relaxation time of the return to equilibrium. A particularly useful form of temperature jump apparatus is a shock tube, which can rapidly jump a gas's temperature by more than 1000 degrees.

Catalyst

The presence of the catalyst opens a different reaction pathway with a lower activation energy. The final result and the overall thermodynamics are the same. A catalyst is a substance that alters the rate of a chemical reaction but remains chemically unchanged afterwards. The catalyst increases the rate of the reaction by providing a different reaction mechanism to occur with a lower activation energy. In autocatalysis a reaction product is itself a catalyst for that reaction leading to positive feedback. Proteins that act as catalysts in biochemical reactions are called enzymes. Michaelis–Menten kinetics describes the rate of enzyme mediated reactions. A catalyst does not affect the position of the equilibrium, as the catalyst speeds up the backward and forward reactions equally. In certain organic molecules, specific substituent can have an influence on reaction rate in neighboring group participation.

Pressure

Increasing the pressure in a gaseous reaction will increase the number of collisions between reactants, increasing the rate of reaction. This is because the activity of a gas is directly proportional to the partial pressure of the gas. This is similar to the effect of increasing the concentration of a solution. In addition to this straightforward mass-action effect, the rate coefficients themselves can change due to pressure. The rate coefficients and products of many high-temperature gas-phase reactions change if an inert gas is added to the mixture; variations on this effect are called fall-off and chemical activation. These phenomena are due to exothermic or endothermic reactions occurring faster than heat transfer, causing the reacting molecules to have non-thermal energy distributions (non-Boltzmann distribution).Increasing the pressure increases the heat transfer rate between the reacting molecules and the rest of the system, reducing this effect. A reaction's kinetics

can also be studied with a pressure jump approach. This involves making fast changes in pressure and observing the relaxation time of the return to equilibrium.

The Beer-Lambert Law

The Beer-Lambert law relates the attenuation of light to the properties of the material through which the light is traveling.

The Absorbance of a Solution

For each wavelength of light passing through the spectrometer, the intensity of the light passing through the reference cell is measured. This is usually referred to as IoIo - that's I for Intensity.

III. MATERIALS AND METHODOLOGY

3.1 EQUIPMENTS USED are
Autoclave.
pH meter.
Glass wares (beaker, conical flask, measuring cylinder, etc)
Spatula.
Weighing balance, Ultra Violet Spectrophotometer, Constant temperature water bath, Stir rod, Inoculation vessel, Electric heater.
MATERIALS include Cassava whey, Yeast, 2.5M HCl, 2.5M NaOH, Distilled water, Benedict's reagent, Biuret's solution, Sudan III solution, Lugol's solution. And the experiments were carried out in the chemical engineering laboratory at Federal University of Technology, Owerri (FUTO).

3.3 QUALITATIVE TEST PROCEDURE.

3.3.1 Lugol's test for starch.

Lugol's solution, also known as aqueous iodine and strong iodine solution, is a solution of potassium iodine with iodine in water. 2-3 drops of Lugol's iodine solution was added to 5ml of the cassava whey and shaken thoroughly to obtain a dark blue colouration.

3.3.2 Benedict's test for reducing sugar.

Benedict's reagent, also called Benedict's qualitative solution is a chemical reagent used to detect the presence of reducing sugars. 3ml of Benedict's solution was added to same quantity of cassava whey, and then allowed to boil for few minutes and cooled. A green colour precipitate formed indicates that a reducing sugar is present.

3.3.3 Biuret's test for protein.

It is a chemical test used to detect the presence of peptide bonds, i.e. protein. In the presence of peptides, a copper(II) ion forms violet-coloured coordination complexes in an alkaline solution. 5ml of the cassava whey is added to an equal volume of 1% 2.5M NaOH followed by few drops of $CuSO_4$. The colour change to purple indicates that protein is present.

3.3.4 Sudan III test

Sudan III is a dye used for Sudan staining. 2-3 drops of Sudan III was added to 2ml of cassava whey and shaken vigorously. A brick-red coloration obtained indicates that lipid is present.

3.4 HYDROLYSIS OF CASSAVA WHEY

100ml of 2.5M HCl was added to 600ml of cassava whey. To this mixture, 50ml of distilled water was added and placed in a bath of boiling water for 30 minutes. The solution was afterwards cooled and the pH corrected using 2.5M NaOH to neutralize the excess acid.

3.5 FERMENTATION OF THE HYDROLYSED CASSAVA WHEY

The cassava whey was sterilized using an autoclave at $121^{O}C$, for 15 minutes. It was brought out and allowed to cool to room temperature. Under sterile condition, inoculation of the medium was carried out with 0.1g of yeast. This was done in a fermenter, and samples were collected every 24 hours and analyzed for glucose, ethanol, and microbial concentrations using the absorbance obtained by the ultra violet spectrophotometer at their respective wavelengths.

3.6 QUANTITATIVE METHODS

3.6.1 Obtaining the glucose concentration.

At a wavelength of 488nm, the absorbance of different concentrations of glucose was obtained using the ultra violet spectrophotometer. A plot of absorbance against concentration was obtained and it is represented by

$$y = -0.063x + 0.912$$

Where, y is the absorbance and
x is the concentration.
At different time intervals, the sample collected is placed in the ultra violet spectrophometer and the absorbance is obtained at a wavelength 488nm. The absorbance obtained is inserted in the equation and the resulting concentration is obtained.

3.6.2 Obtaining the ethanol concentration.

At a wavelength of 410nm, the absorbance of different concentrations of ethanol was obtained using the ultra violet spectrophotometer. A plot of absorbance against concentration was obtained and it is represented by

$$y = 0.978x$$

Where, y is the absorbance and
x is the concentration.
At different time intervals, the sample collected is placed in the ultra violet spectrophometer and the absorbance is obtained at a wavelength 410nm. The absorbance obtained is inserted in the equation and the resulting concentration is obtained. It has no intercept because there was no ethanol initially.

3.6.3 Obtaining the yeast growth.

At a wavelength of 610nm, the absorbance of different concentrations of glucose was obtained using the ultra violet spectrophotometer. A plot of absorbance against concentration was obtained and it is represented by

$$y = 0.10x$$

Where, y is the absorbance and
x is the concentration.
At different time intervals, the sample collected is placed in the ultra violet spectrophometer and the absorbance is obtained at a wavelength 610nm. The absorbance obtained is inserted in the equation and the resulting concentration is obtained.

3.7 MOISTURE CONTENT

The moisture content of the cassava whey was obtained by measuring the following weights and inserting it into the required formular.
Weight of empty beaker = w_1
Weight of beaker + cassava whey = W_1
Weight of cassava whey = $W_1 - w_1$
Weight of beaker + dried cassava whey = W_2
Weight of cassava whey after drying = $W_2 - w_1$
Percentage moisture content = $\frac{(W_1 - w_1) - (W_2 - w_1)}{W_1 - w_1}$

3.8 DENSITY OF CASSAVA WHEY

Density $= \frac{\text{mass}}{\text{volume}} = \frac{W_1 - w_1}{\text{volume used}}$

IV. RESULTS AND ANALYSIS

4.1 CONSTITUENTS OF CASSAVA WHEY

Lugol's test: Starch present.
Benedict's test: Reducing sugar present.
Biuret's test: Protein present.
Sudan III test: Lipids present.

4.2 RESULTS OF QUANTITATIVE ANALYSIS.

Moisture content of cassava whey is 82.3%
pH of cassava whey is 5.82
Density of cassava whey is 1.028 g/ml

4.3 KINETIC RESULT AND DISCUSSION.

The table below shows the relationship between glucose, ethanol and microbial concentration with time.

Time (hours)	Glucose abs.	Ethanol abs.	Yeast abs.
0	0.016	0.001	0.010
24	0.203	1.897	0.073
48	0.512	3.188	0.105
72	0.715	5.037	0.121
96	0.855	6.377	0.115

The corresponding values of the concentration of glucose, ethanol and biomass are obtained by inserting the absorbance values into their respective equations. It leads to the following values:

Time (hours)	Glucose conc.(g/l)	Ethanol conc.(g/l)	Yeast conc.(g/l)
0	14.22	0.00	0.10
24	11.26	1.94	0.73
48	6.21	3.26	1.05
72	3.13	5.15	1.21
96	0.91	6.52	1.15

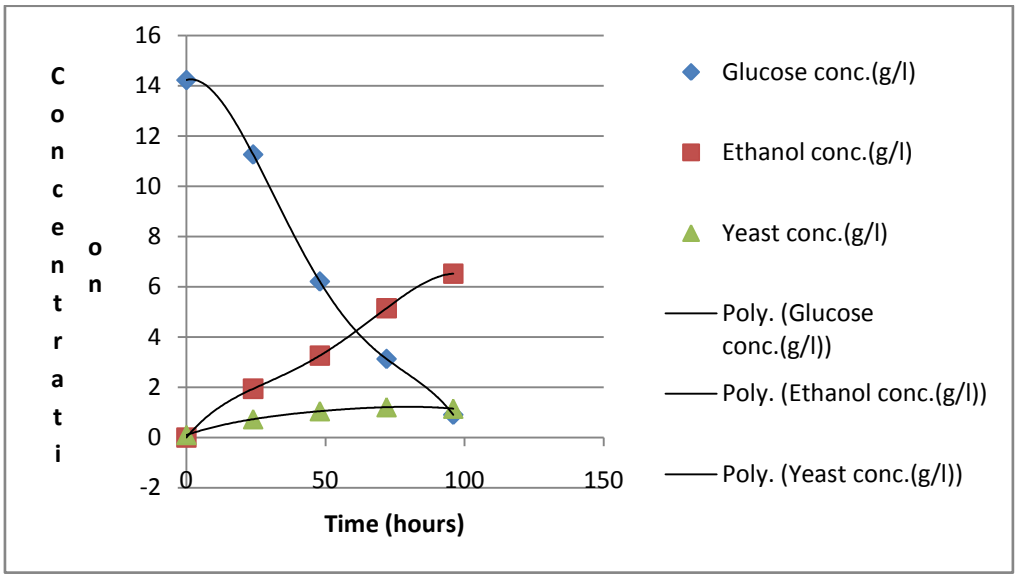


Fig 4.1 Concentration profile for glucose, ethanol and microbial biomass.

4.4 OBTAINING THE KINETIC PARAMETERS OF THE LOG PHASE

According to Michelis Menten, the substrate consumption at the log or exponential phase is expressed as follows:

$$V = \frac{V_{max}\,[S]}{K_m + [S]}$$

Where, V is the reaction velocity

V_{max} is the maximum reaction velocity
[S] is the substrate concentration
K_m is the Michelis Menten constant

Reaction Velocity (V)	Substrate Concentration [S]
0.026	14.22
0.013	11.26
0.007	6.21

Linearizing the equation using the Lineweaver-Burk equation, we have

$$\frac{1}{V} = \frac{K_m}{V_{max}\,[S]} + \frac{1}{V_{max}}$$

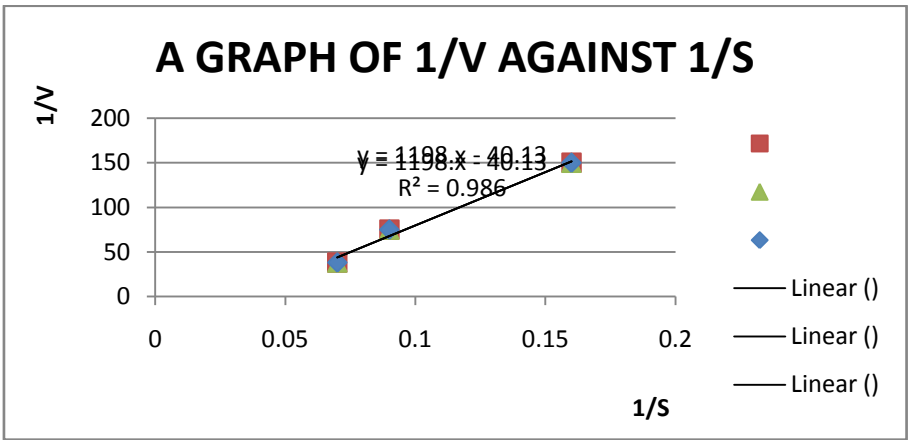
Where, $\frac{1}{V}$ represents the y axis

$\frac{1}{[S]}$ represents the x axis

$\frac{1}{V_{max}}$ is the intercept

$\frac{K_m}{V_{max}}$ is the slope

$\frac{1}{V}$	$\frac{1}{[S]}$
38.10	0.07
75.00	0.09
150.00	0.16



The kinetic parameters obtained are:
Maximum reaction velocity, $V_{max} = 0.025$
Michelis Menten constant, $K_m = 29.85$

4.5 DISCUSSION

From the qualitative analysis of the cassava whey it has starch, reducing sugar, protein and lipid present which correspond to literature. The moisture content of the cassava whey was obtained to be 82.3%, which is in the range for most cassava tubers harvested in the raining season. This was done by weighing a 20ml of cassava whey which was afterwards dried and weighed again. The difference in weight divided by the initial weight gives the fractional moisture content. The pH obtained was 5.82 which indicate that the cassava whey is slightly acidic. The density of the cassava whey was obtained to be 1.028g/l which indicates that it denser than water, the density was obtained by dividing the mass of 20ml of cassava whey by its volume.

Form the graph above, it can be seen that the glucose content of the cassava whey is the substrate, the yeast is the biomass that is inoculated, and the ethanol is being formed by the process. After introducing 0.1g of yeast into the sterile hydrolyzed cassava whey, the inoculation process began. The yeast fed on the substrate and increased while the glucose reduced; this process led to the formation of ethanol. The yeast did not experience much lag phase because the medium is rich and also because the cassava whey is sterile. It experiences the log phase, where it divides by binary fission to increase its number consuming the glucose as a source of energy. Towards the end of the process, the yeast decreased which can be as a result of product accumulation or nutrient depletion.

Also, from the graph the log phase was fitted with the Michelis Menten equation and also linearized to obtain the kinetic parameters. The maximum reaction rate was obtained to be 0.025, while the Michelis Menten constant was obtained to be 29.85. This was obtained from our plot and it represents the concentration of the substrate when the reaction velocity is equal to one half of the maximal velocity for the reaction.

V. CONCLUSION/RECOMMENDATION

After obtaining the cassava whey by crushing the cassava and compressing to extract the liquid content, the moisture content was obtained as 82.3% from characterization. This moisture content is relatively high because the cassava whey was obtained during the raining season. The density was obtained to be 1.028g/l and the pH obtained was 5.83 meaning that it was slightly acidic.

The cassava whey was hydrolyzed using 2.5M acid for acid hydrolysis, which was later neutralized with 2.5M NaOH to correct the pH. The hydrolyzed cassava whey was sterilized and subjected to fermentation

using yeast as the enzyme. And samples were collected every 24 hours to monitor growth of yeast, depletion of glucose and ethanol production. The yeast increased in number until it reached the death phase, where the substrate present was not sufficient and also the ethanol concentration was high. It was observed to follow the normal trend.

The log phase of the yeast growth was monitored using the Michelis Menten equation, and the maximum reaction velocity was obtained to be 0.025g/l.hour, and the Michelis Menten constant obtained was 29.85.

From the knowledge and experience from the project, I highly recommend the following;

Cassava whey should be used to produce ethanol, instead of discarding them improperly to cause environmental pollution. Also, engineers should look into other wastes that can be turned into something useful.

The production of ethanol from cassava whey should be commercialized to serve as a source of employment and revenue to the society at large.

Based on the fact that the cassava used goes a long way in determining the concentration of the cassava whey. Further works should be carried out using improved varieties of of cultivar.

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