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KINETICS OF FERMENTATION OF OIL BEAN SEED

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Abstract
The design of large plants for the processing of oil bean seed, which is abundant in the rain forest zones of Nigeria, has been hampered by the dearth of kinetic and thermodynamic parameters for the scale-up of pilot plants This work addresses part of this problem by determining some kinetic constants. During the fermentation of oil bean seed, the action of endogenous protease on protein constituents of the seed coagulated by boiling result in the formation of amino acid and liberation of NH3 gas. This work utilizes the rate of NH3 gas liberated to monitor the kinetics of the process. To monitor the rate of release of NH3 gas, four different samples were prepared, two with the same boiling time but different sizes and the other two with the same size but different boiling time. One sample was set aside as a control experiment. The samples were incubated at 30 ^{0}C. From the results obtained both the boiling time and particle size of the sliced seeds affected the rate of fermentation. There was also a noticeable change in the pH from acidic to basic condition during the period of fermentation. At the end of the experiment the sample B gave the best results with $y=0.0015x+5.299$ while $R^2=0.989$ for the plot of pH against time and $y=0.381x-5.9h$ with $R^2=0.938$ for the plot of percentage concentration of NH_3 against time.

Keywords: oil bean seeds, fermentation, kinetics, reaction rate, pH

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1.INTRODUCTION

Many tropical underutilized crop plants have seeds that are recently attracting worldwide attention because of their high nutrient potential especially with regard to solving the prevalent protein-energy malnutrition (PEM) that has ravaged populations in the less developed parts of the world, an example of such crop is the oil bean seed. Oil bean seed (*Pentaclethra macrophylla*) belongs to the leguminous family mimosa cease found mostly in tropical Africa, it is cultivated in forest areas, with about eight flat glossy brown edible seed per pod. The pods explode at maturity and disperse the seeds. The raw seed is a potential source of protein, energy and fatty acids (Enujiugha and Agbede 2000, Enujiugha 2003).

Chemical composition of the oil bean seed

The oil bean seed contains 4-117% carbohydrate; 44-47% oils which has been found to be rich in oleic acids (Nwokedi, 1975, Odoemena, 2005) and lignoceric acid (Onwuliri et al, 2004). Onwuliri also found out that the saturated fatty acid, lignoceric acid occurred in very high amount constituting about 10% of the total fatty concentration, they also reported that the oil contains about 75% saturated fatty acid and 25% unsaturated fatty acid. Fermentations are reactions wherein a raw organic feed is converted into product by the action of microbes or by the action of enzymes. In this study microbial fermentation is involved.

Organisms involved in fermentation of oil bean seed.

Several workers have investigated the micro-organisms involved in fermentation of oil been seed, only bacteria are involved in the fermentation (Obeta et al,Odunfa and Oyinyola,Ogueke and Aririatu 2004) The main fermenting micro-organisms have been identified to be *proteolytic Bacillus sp.*(Obeta, 1983) which include *B. subtilis* (most predominant), *B. licheniformis*, *B. megaterium, B. macerans* and *B. circulans*.
Microbial fermentation can be represented by:
Microbe C: Organic feed A → Product, R + more cells, C

In microbial fermentation the catalytic agent, the cell, or microbe reproduces itself; the cells `manufacture its own enzymes (Fred Deindoerfer, 1960). Kinetic equations, which describe the activity of a micro-organism on a particular substrate, are crucial in understanding many phenomena in biotechnological processes. Quantitative experimental data is required for the design and optimization of biological transformation processes. Studies of batch fermentation process in nearly all development programs involve periodic observation of growth, carbohydrate utilization and product formation throughout the course of fermentation. The fermentation literature abounds such data for large number of processes, and also for a wide variety of operating conditions for a particular process. Kinetic analysis is the interpretation of these data and the factors which influence them to shed light on the proposed reaction schemes or fermentation patterns (Achinewhu, S.C., 1986). Kinetic information coupled with biochemical evidence, provides a sound basis for studying reaction mechanisms in fermentation processes. Such studies can eventually lead to improvement in batch fermentations through a program of control that optimizes the rate determining steps occurring during fermentation once they are established and can also lead to the successful design and operation of multistage continuous system. The crux of any kinetic analysis lies in determining how the rate of product formation and its stoichiometric coefficient vary with respect to the chemical and physical factors that influence them. Really meaningful quantitative knowledge is lacking for practically all fermentation process, with the exception of processes where the primary product is cellular tissues.

FACTORS INFLUENCING REACTION RATE

There are a number of controllable features of chemical reactions that can be used to influence the course of events during fermentation process. They include; concentration, temperature, surface area and catalytic agents.

2. MATERIALS AND METHOD

This experimental work was carried out at an atmospheric pressure of 1 atmosphere and a temperature 31^0C**.** Fresh oil bean seeds was purchased from local market (Oil mill Rumukurusi, Port Harcourt) and used for the experiment. The seeds were parboiled for four hours with the help of a pressure cooker and then dehauled (to remove the hard brownish convert). It was then sliced into different sizes of about 5.0cm long × 1.0cm broad and samples boiled for 2 hours and another for 4hours at a temperature of 35^0C.The samples were soaked in distilled water for 2hours and the water drained out. The two samples boiled (boiled at different times) were blended into paste using (Phillips electric blender) and named as sample A and B, while the size of 5.0cm long by 1.0cm broad boiled at different boiling time saved as sample C and D as represented in table 1.

Table 1 Specification of experimental samples

Sample	Temp,^{0}C	Time,h	Size
A	35	2	Paste, microns
B	35	4	Paste, microns
C	35	4	5.0 by 1.0cm
D	35	2	5.0 by 1.0cm

100gs of each sample was weighed using mettler weighing balance and placed in a water bottle. Water bottle was used because it is airtight and posses a thin enough outlet convenient for expelling the ammonia gas liberated. The bottle was held in place with the help of a clamp and the outlet directed into a test tube containing 2% boric acid (H_3BO_4) and was placed into a test tube rack. The test tube was covered to avoid the absorption of external ammonia gas. The samples were allowed to ferment at 29^oC for 72 hours. The first reading was taken at 24hours interval while the subsequent ones were taken at 8 hour's interval. The percentage weight of NH_4^+ that reacted with the H_3BO_4 was evaluated using titration method and the ph noted for the various samples. Also the pH was measured by collecting the same specimen from different samples and testing for pH with a pH meter at an interval of 24hour.

3. RESULTS AND DISCUSSION

The results will be analyzed using Microsoft Excel to do a regression analysis of the data displaced above figures 1 and 2.

Sample A
% Concentration of NH_3 = 0,301t - 6,91
R^2 = 0,962
This implies that the graph is linear with a slope of 0,301 and a negative intercept.

Sample B
% Concentration of NH_3 = 0,318t - 5,9
R^2 = 0,994
This is also linear graph.

Sample C
% Concentration of N Sample B
pH = 0,015t + 5,299
R^2 = 0,989
This graph is linear
H_3 = 0,264t - 6,99
R^2 = 0,938

Sample D
% Concentration of NH_3 = 0,286t - 7,2 ,
R^2 = 0,946

Also analyzing figure 2
Sample A
pH = 0,000t^2 + 0,11t + 5,382 ,
R^2 = 0,969
This graph is polynomial.

Sample C
pH = 0,029t - 5,504 ,
R^2 = 0,817
This graph is linear.

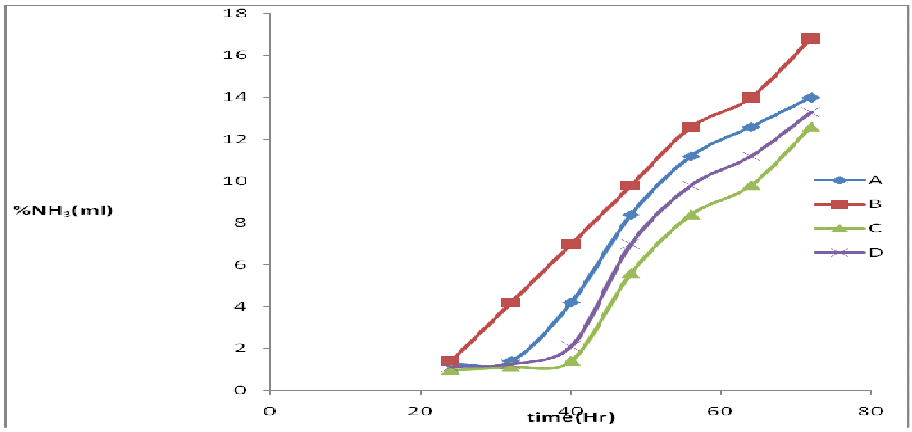

Fig 1 Percentage concentration of ammonium ion in the solution

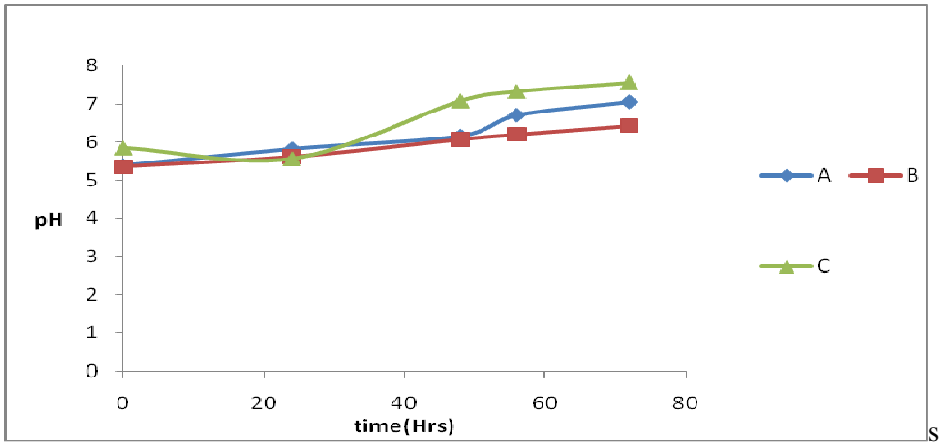

Fig 2 Changes in pH for the different samples

By reducing the size of the oil bean seeds to paste, the surface area is increased and fermentation rate also is enhanced, this is observed in the result shown in the fig 1, the regression was done using Microsoft excel, the sample that was reduced to paste and with a longer reaction time gave the best fit (sample B) when compared with the results got from the other samples. The rate of such reaction could be defined in terms of moles of reactants or products per surface area. The rates of most reactions are altered by the presence of a catalyst. The fermentation of oil bean seed occurred through existing microbes such as *Bacillus subtilis*. These microbes contain enzymes such as protease which converts the protein in the seed to amino acid and ammonia gas by the reaction;

Protein component $\xrightarrow[Protease]{Bacillus}$ amino acid + NH_3

Sample B also gave a better result when compared to the other samples from fig 2, this implies that reducing a given sample to paste, allows for more of the reactants to take place in the reaction thereby affecting the pH of the reaction. From there results it can be deduced that both size reduction, reaction time play an important role in determining how well chemical reaction takes place. Generally the pHs of the samples becomes alkaline as the process proceeds, from 5.37 to 7.20.

4. CONCLUSIONS

This work is centered on the effects of certain variables such as particle size, concentration, and the boiling time on the rate of fermentation of oil bean seed. From the analysis of the data obtained, it can be said that the rate of fermentation of oil bean seed is directly proportional to time and concentration, inversely proportional to particle size.

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