DETERMINATION OF GROWTH RATE OF SINGLE CELL PROTEIN IN YAM PEEL

Mohammed, A., Akpan, U. G. and Abubakar, M. I
Chemical Engineering Department, F.U.T., Minna.

ABSTRACT

The growth of a single cell protein (SCP) on carbohydrate based food waste (yam peel) has been determined by spectrophotometric method at 540nm. The results obtained show that the specific growth rate(μ) of SCP produced in this way was 0.41 hr^{-1} Substrate consumption rate was 0.021mg/(ml)(hr) while maximum specific growth rate was 1.113hr^{-1} which was achieved within the first one hour of fermentation. The concentration of single cell protein reached its peak at the 24th and 25th hour of the fermentation period, which stabilizes for 30 minutes. The yield for a sterilized feed of carbohydrates based food (yam peek) was determined to be 3.37mg/ml, and a linear relationship was developed to predict the concentration of SCP of given absorbance at 540nm.

Key words: Single cell protein, specific growth rate, substrate consumption, yield, fermentation time, and saccharamyces cerevisae.

INTRODUCTION

Determination of growth rate of single cell protein using micro organism required a medium which will provide all the essential nutrients needed for the cell growth. In this relatively new route, microorganisms are fed on simple and relatively in expensive substance like hydrocarbon, and carbohydrate. Cell growth involves consumption of substance which provides energy and raw material required for the synthesis of additional cell mass. Once they have multiplied many folds, the cells are removed by filtering from the liquid in which they are grown (Silvio, 1977).

Agricultural crops with high concentration of carbohydrate, which are not properly utilized, wasted away after processing. In this work, the growth rate of single cell protein in carbohydrate based food waste (yam peel) using *saccharamyces cerevisae* was determined. This will be a major step toward better utilization of the important resources in the production of single cell protein (SCP). The production of single cell protein via organism action on carbohydrate waste becomes important as it will be a source of protein to carter for the exploding world's population and will also serve as a means of checking the population control. Many scientists believe that the use of microbial fermentation and the development of an industry to produce and supply SCP are possible solutions to primary or expensive animal protein (Mohammed,1999 and Shaba, 1998).

The use of fungi as a single cell protein is of important to the nutritionist because of the possibilities of using non-human food (i.e. waste) as the substrate for SCP production. It is nutritionally balance because it contains essential nutrients required for animal and human growth, again, it has rapid increase in cell growth due to extremely short generation time and the production of SCP does not depend on climatic conditions, but requires a minimum area for cultivation, and it is equally acceptable to the public (Peter, 1984).

It is known that growth of an organism is an orderly increase in all its chemical constituents and result in increase in the number of cell as well as the mass of all cellular material and that the growth rate depends on the nutrient supply, and the culture media which may be synthetic (one with a known composition) or complex (one with undefined composition). Other common complex media include beef broth, blood infusions broth, corn steep liquor, and sewage.

The function of a medium is to support good growth/or high rate of product synthesis. Excessive concentration of a nutrient can inhibit or even poison cell growth. Moreover, if the cell grows too extensively, their accumulated metabolites and toxic products will often disrupt the normal biochemical process of the cell. Consequently, it is a common practice to limit total growth by limiting the amount of one nutrient in the medium.

GROWTH CYCLE PHASES FOR BATCH CULTIVATION

In a typical batch process, the number of living cells varies with time, as shown below.

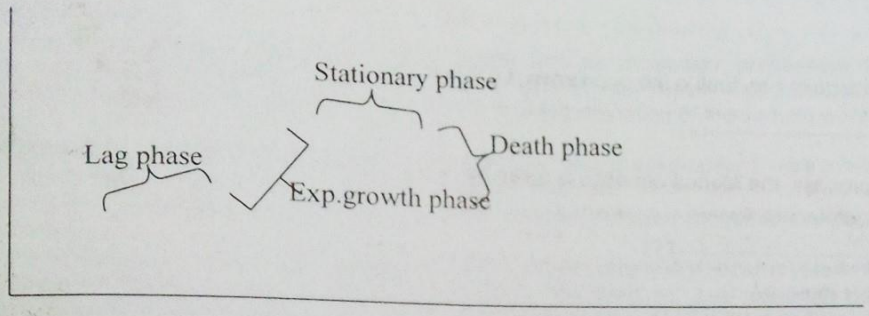

Figure1: Typical growth curve for batch cell cultivation

After a lag phase, where no increase in cell number is evident, a period of rapid growth ensures, during which the cell number increase exponentially with time. Naturally in a closed vessel the cell cannot multiply indefinitely, and a stationary phase follows the period of exponential growth where population achieves its maximum size. Eventually a decline in cell number of living individuals is often observed.

Each phase is of potential importance in microbial processes. For example, the general objective of a good design may be to minimize the length of the lag phase and to maximize the rate and length of the exponential phase. The last objective is achieved by slowing the onset of the transition to stationary growth phase. The length of lag phase observed when fresh medium is inoculated depends on both the changes in nutrient composition (if any) experienced by the cells, the age and the size of inoculums, and the shock of rapid switch to a new environment.

Multiple lag phases may sometimes be observed when the medium contains multiple carbon sources. This phenomenon is known as diauxic growth. After one carbon substrate is exhausted, the cell must divert its energies from growth to "retool" for the new carbon supply.
Design to minimize culture and process times normally includes minimization of the lag times associated with each new batch culture. The following generalization can be made in attempting to minimize lag phase:

- The inoculation culture should be as active as possible and the inoculation carried out in exponential growth phase.
- The culture medium used to grow the inoculums should be as close as possible to the final full – scale fermentation.
- Use of reasonably large inoculums (order of 5-10% of new medium volume) is recommended to avoid undue loss by diffusion of required intermediate or activators.

At the end of lag phase the population of microorganism is well adjusted to a new environment. The cell can then multiply rapidly and cell mass double with time as depicted by the equation.

$$\frac{dx}{dt} = \mu x \text{ or } \frac{1}{x}\frac{dx}{dt} = \mu \text{ ---------- (1)}$$

$X = x_1$ at $t = t_{lag}$ ---------- (2)

Upon integration equation (1) become

$\ln X = \mu (t - t_{lag})$ or

$$X = X_1 e^{\mu(t - t_{lag})} \text{ ---------- (3)}$$

$t > t_{lag}$

The time interval required to double the population is given by

$t_d = \ln\frac{2}{\mu}$ ----------(4)

Also, in a batch process, the Monod equation is given by

$\frac{dx}{dt}\alpha = \frac{dx}{dt}$ ----------(5)

$\frac{dx}{dt} = -Y_{\frac{x}{s}}\frac{ds}{dt}$ ---------- (6) Where $\frac{dx}{dt}$ = cell growth rate, $\frac{ds}{dt} = r_s$ = rate of substrate consumption,

$Y_{\frac{x}{s}}$ = yield constant

Integrating equation (6) yield.

$(x-x_o) = Y_{\frac{x}{s}}(S-S_o)$. ---------- (7)

If the rate of substrate disappearance is $-r_S = \frac{\mu_{max} Sx}{Y^1_{x/s}(S+k_s)}$ ---------- (8)

Where $Y^1_{\frac{x}{s}}$ is the " true" coefficient, $Y_{\frac{x}{s}}$ is the overall stoichiometric coefficient equal to the total mass of cell produced divided by the total mass of substrate consumed. For the common case of sterile feed ($x_i = 0$), these equation can readily be solved for x and s.

X sterile feed = $Y^1_{\frac{x}{s}}[S_0 - \frac{Dk_s}{\mu_{max} - D}]$ ---------- (9)

And Xsterile feed = $\frac{Dks}{\mu_{max} - D}$ ----------(10)

The rate of cell production per unit reactor volume is Δx. This quantity can be compared with cell output rate by solving $\frac{d(\Delta x)}{d\Delta t} = 0$ ----------(11)

Where equation (9) gives (D_{max} out) = $\mu\left(1 - \sqrt{\frac{k_s}{k_s + S_o}}\right)$ ---------- (12)

If $S_o >> K_s$, as often the case, the value of D max output approaches μ_{max} as consequently is near washout (Dundee, 1993; Encyclopedia, 1992 and James, 1986).

Recent studies have established microbial growth in aerated fementers using substrates such as gas oil, paraffin, natural gas, molasses and celluloses. Human consumption occurs at much smaller level where production of baker's yeast continues to be the prime example. Yeast generally grows at temperature between 20°C and 35°C, and pH between 3.0 and 8.0, the most suitable culture condition are found at about 25°C and pH = 4.5 – 6.5(Anthony, 1992) Specific growth of between 0.11 – 0.7 μ^{-1} are obtainable (Encyclopedia,1992).

METHODOLOGY

The investigation involves collection of yam waste and its necessary preparation for fermentation process. Laboratory instrument such as milling machine, pH meter, spectrophotometer, weighing balance, foil paper, and yeast utilized for the growth rate determination of the sample were used.

In order to obtain relevant parameters for the investigation, some assumptions were made due to the fact that the investigation was carried out in a batch reactor. They are:

- Environmental conditions such as temperature, dissolved oxygen concentration were assumed to be constant.
- Complexity of the medium was ignored and that a single component became rate limiting nutrient.
- Change in inhibitory product, which accumulate in the medium, was assumed not to significantly affect microbial kinetic within the range of variation encountered in the investigation.

Preparation of Standard Calibration for Protein

The standard protein solution was prepared by weighing 10mg of protein solution and making it up to a final volume of 1ml with distilled water in a measuring cylinder. Various milliliters of the standard protein solution were measured and diluted with distilled water. 4ml of buiret reagent was added to the different dilutions of the standard protein. The solution was thoroughly mixed and allowed to develop a color change at room temperature for 30 minutes.

The absorbance of the developed solution was measured at the wavelength of 540 nm against a blank solution (i.e. solution containing the entire reagent except the sample). The absorbance of the different dilution of standard protein solution (mg/ml) was recorded, and a graph of absorbance against concentration was plotted for the standard as calibration graph.

Preparation of Yam Peel

The yam peel was sun dry for 5 days. After drying, it was ground into powder form; 20g of the powder was suspended in 200ml of hot water in a 500ml beaker. The suspension was filtered through a Whitman number one filter paper. The extract obtained was concentrated to 100ml and kept in the refrigerator for further use.

Sacccharamyces cerevisae (dry yeast) was used for making fermentation inoculums for the yam peel.

Preparation of Inoculum

500ml distilled water was added to 20g of ground yam peel. It was boiled until it became semisolid, then the semisolid substance was sterilized in an autoclaved at 126^0C for 11 minutes and then kept in the refrigerator for further use. 5ml of this autoclaved sample residue was added to 20ml of basal medium (composition in g/1 $MgSO_4$-1.2, Na_2HPO_4 – 0.05, $FeCl_3$ – 0.5, Zn SO_4 – 1, Difco yeast extract 0.1, $CuSO_4$). The medium pH was adjusted to 5.1 before sterilization in autoclave for 11 minutes at 126^0C. 2g of *S._cerevisae* (dry yeast) was introduced into 25ml of the medium and allowed to incubate for 72 – 96 hrs at 30^0 C. The inoculum was kept in the refrigerator for fermentation purpose.

Fermentation

Yam peel residue was prepared following the same procedures described in the preparation of the inoculums. 20ml of basal medium (composition is the same as that of inoculum medium) was taken in 100ml conical flask and sterilized at 45^0C. Then 10ml of the yam peel residue was also prepared the same way. The solution of the prepared yam peel was added to the fermentation medium, which was incubated for 4 hours. At the intervals of 30 minutes for two hours thirty minutes, 1ml of the fermented medium was withdrawn, and 4.0ml of buiret reagent was added to it. The sample solution was kept for 30 minutes and allowed to develop a light green colour. The absorbance of the sample withdrawn was measured by the use of spectrophotometer at room temperature, and hence the concentration of the protein produced evaluated from the calibration curve. This procedure was repeated for 5 days at an interval of 24hrs and the results obtained are presented below.

RESULTS

The result of various analyses conducted on single cell production is presented in Figures 2-8.

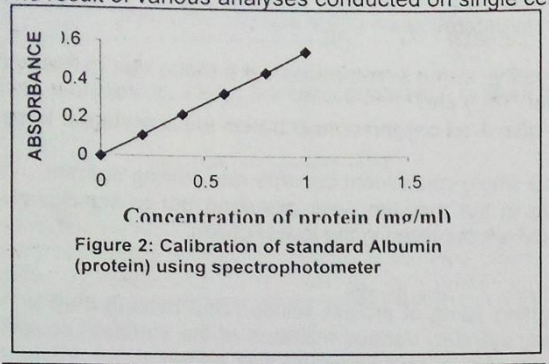

Figure 2: Calibration of standard Albumin (protein) using spectrophotometer

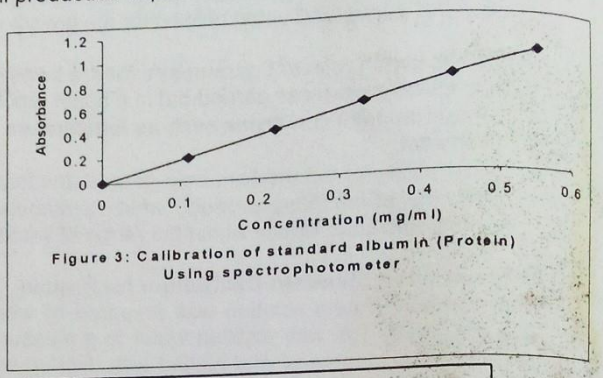

Figure 3: Calibration of standard albumin (Protein) Using spectrophotometer

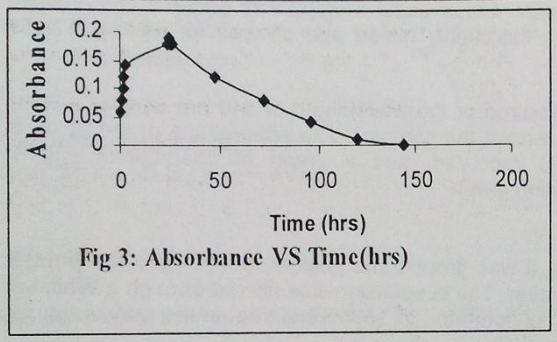

Fig 3: Absorbance VS Time(hrs)

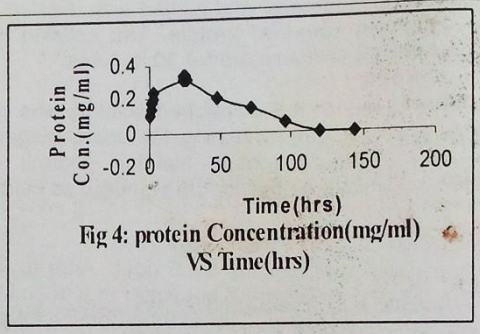

Fig 4: protein Concentration(mg/ml) VS Time(hrs)

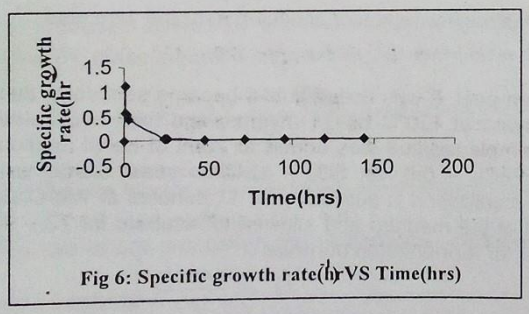

Fig 6: Specific growth rate(hr) VS Time(hrs)

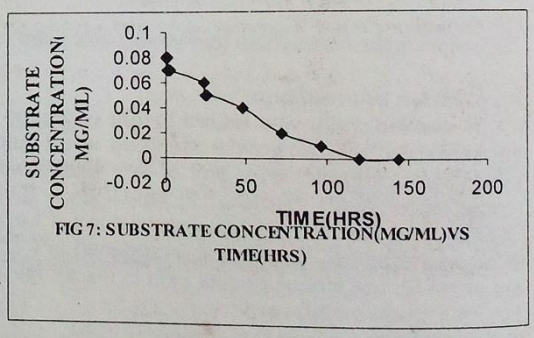

FIG 7: SUBSTRATE CONCENTRATION(MG/ML)VS TIME(HRS)

DISCUSSION OF RESULTS

Growth rate of single cell protein from yam peel depend on time of fermentation, substrate concentration, and temperature. Figure 2 shows that absorbance of protein increased continuously with increase in Concentration of protein, given a linear graph which can be represented by the equation.

Concentration of single cell protein = ½ × *Absorbance* on the electron photometer.

The relationship can be used to predict single cell protein concentration at any given absorbance of 540nm wavelength. From Figure 3 it can be observed that the population density of single cell protein increased with increasing fermentation time between 0 – 24th hrs of fermentation and stabilizes for 30minute thereafter continued to decrease until it reaches zero. This signifies that high SCP was obtained between 24.5hr and 25th hrs of fermentation time.

Figure 4 shows a similar observation on the SCP growth rate as that depicted in figure 3. Concentration of protein increased to its peak during the 24hr 30min and was stable for another 30min and continued to decease again.

As shown in figure 7, the specific growth rate (μ) was at maximum ($1.113hr^{-1}$) during the first 1hr of the fermentation time and declined drastically to almost zero. From the same graph, the point at which specific growth rate half its maximum value was determine to be 0.033 mg/ml. The yield on substrate and the rate of substrate consumption was mathematically determines to be 3.375 and 0.021mg/ml hr^{-1} respectively.

Substrate concentration decreases with increase in time as depicted in Figure (6); however this is as expected. The decrease was a bit stable within the first 1hr and further stability in the decrease was observed between 1hr 30minutes of fermentation time and continue to increase again until the substrate concentration is completely exhausted at 120hr of fermentation time (Dundee, 1993; Encyclopedia, 1992 and James, 1986).

CONCLUSION

Single cell protein (SCP) was produced from carbohydrate base food waste at temperatures of 33°C and pH between 5.1 and 6.7. The growth rate of SCP was observed to be dependent on medium which will provide the entire necessary nutrient required for the growth.
The rate of substrate consumption($-r_s$) was determined to be 0.021mg/(ml)(hr) and the specific growth rate (μ) was found to be 0.41 hr^{-1} while the maximum specific growth rate (μ_{max}) was determined to be equal to 1.113 hr^{-1}. For a sterilized feed of carbohydrate based food waste (yam peel), the yield was determine to be 3.37 and a linear relationship was developed to predict the concentration of SCP of any given absorbance at 540nm.

REFERENCES

1. *Anthony R.R et al.: Protein Engineering. pp 334 – 336, IRL Press, Oxford, 1992.*
2. *Dundee G. H, Protein Phosphorylation. pp 282 – 283, IRL Press, Oxford, 1993.*
3. *Encyclopedia of Physical Science and Technology, 2nd Edition, pp. 79– 402, San Diego Academic press, Inc, 1992.*
4. *James, E.B and David, F.O. Biological Engineering Fundamentals, 2nd Edition, pp. 373 – 402, 839, 844, New York, McGraw Hill Book Company, 1986.*
5. *Mohammed A., "Production of single cell protein from petroleum and petroleum components", A seminar paper presented at the biological science Dept. (Micro – biology unit). FUT. Minna, pp 7 – 13, 1999.*
6. *Peter F S and Allan W, Principle of fermentation Technology, PP.1-17, 108, Pergamon Press, Oxford, 1984.*
7. *Silvio G. et al Single Cell Protein Safety for Animal and Human Feeding, pp. 47, 142, 158, Pergamon Press, Oxford, 1977.*
8. *Shaba A.M., "Protein Enrichment of Carbohydrate Based Food Wastes Using Saccharanmyces Cerevisiae", a project reported to the biological science Dept. FUT. Minna, pp 1- 14, 1998.*

LIST OF ABRIVIATIONS AND SYMBOLS

Ks --Limiting nutrient concentration
r_s------Rate of substrate consumption
S----------Substrate concentration
S_o------Initial substrate concentration
SCP---------- Single Cell Protein
ΔS-Change in Substrate concentration
t---------- Time
t_{lag}---------- Time lag
t_d----------doubling Time
μ----------Growth Rate
μ_{max}----------Max growth Rate
X---------- Protein Concentration
X_1-------Initial Protein Concentration
ΔX--Change in Protein Concentration
ΔX_{max}. ----- Output Max dilution rate
$Y_{x/s}$----------Yield Factor