PRODUCTION OF SINGLE CELL PROTEIN FROM BREWERY SPENT GRAINS

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ABSTRACT

The production of single cell protein (SCP) by the propagation of the yeast, *Saccharomyces cerevisae* obtained from the Federal Institute of Industrial Research Oshodi was studied by using the extract of spent grains obtained from the International Beer and Beverage Industries, Kaduna, Nigeria as a substrate in a medium which was also supplemented with other essential nutrients at a temperature of 30°C, pH of 4.6, air flow rate of 1.2 scf/h and agitation rate of 200rmpm for 15h in a bench scale laboratory fermentor. Seed cultures for each of the three experimental fermentation runs A, B, C undertaken belong to the first, second and third generations respectively. The specific growth rate of SCP obtained for each of the runs were determined to be $0.088h^{-1}$ $0.090h^{-1}$, $0.093h^{-1}$ whilst the corresponding doubling times were determined to be 7.88h, 7.70hr, 7.45h. The specific growth rate values fall within the range of $0.07\text{-}0.11h^{-1}$ reported for most strains of *Saccharomyces cerevisiae*. The yield of yeast from each of the runs were determined to be 0.85, 0.875 and 0.90 whilst the corresponding protein contents were also determined be 49%, 52.5% and 54.25%. The observed increase in the specific growth constants, yield and protein content and the decrease of the doubling times for the successive generation of yeast suggest an increase in the adaptation of the cells to the media from generation to generation.

INTRODUCTION

Developing countries continue to experience shortage of cheap proteins for both human and animal consumption due to poverty. This problem may become severe during the next decade as a result of an alarming rise in population. It is for this reason that attention is now shifting from the customary food and feed sources of protein (agriculture and fishery) to other sources like single cell protein (SCP); whose production from hydrocarbons is one of the great development in modern applied microbiology (1). Single cell protein is a generic term for crude or refined sources of protein whose origin is unicellular or simple multicellular organism such as bacteria, yeasts, fungi, algae, protozoa, and even bacteriophages generally cultivated on substrates such as hydrocarbons, lower alcohols, industrial and agricultural wastes etc (2).

Pilot plant production of single cell proteins now take place in several centres in Europe, Japan, Northern America, Finland, and Germany. Production is of special interest to areas having ample sources of cheap carbohydrate and scarcity of proteins and vitamins. Most of the yeast used in the United States for feed and pharmaceutical have been recovered by brewers and distillers after the alcoholic fermentation. But now, increasing quantities of yeast are being grown directly for such purposes (3).

The rise in the set-up of agro-allied industries has brought about a corresponding rise in the general of both liquid and solid wastes. These waste are potential causes of environmental pollution particularly if alternative usage are not found for them. Nigeria has a number of breweries that utilize maize and sorghum as one of their raw materials for beer production; replacing or as an adjunct to malted barley as part of a strategy to save foreign exchange and promote the use of locally available materials,. Spent grains obtained from International Beer and Beverages Company, Kaduna is one of the solid wastes arising from brewing activities of the company and it is essentially the mash after filtration and consists of mainly sorghum grits, maize grits, chaff and little water. It has found use as

animal feed but little or no information has been documented as per its explication for the production of single cell proteins. This report considered the results of a preliminary investigation on its potential for use as source of substrate for the production of single cell protein.

EXPERIMENTAL

Materials: The yeast - *Saccharomyces cerevisiae* used for this work was obtained from the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria; Spent grains was obtained from IBBI (i.e International Beer and Beverage Industries), Kaduna; other chemicals or reagents used were either of analar or biochemical grade.

Methods:

Medium preparation: Five hundred grams of the spent grains was measured and soaked in 6 liters of water in a conical flask which was maintained at a temperature of 60°C in a hot water bath for a period of 2 h, after which it was cooled, filtered and the residue was discarded while the filtrate or extract was stored in a refrigerator (4±2°C) for subsequent experiments.

The extracts obtained served as a carbon and energy source for the growth of the microorganisms. Other growth requirements added to the medium per litre were 2.0g $(NH_4O)_2SO_4$, 0.5g KH_2 PO_2 0.05g Na_2 $HP0_4$ 0.2g $MgSO_4$ $7H_2$ O, 0.1g FesSQ. $7H_2$ 0 and 1.0g sucrose.

Inoculum preparation: One hundred milliliters of the medium was measured in a conical flask and sterilized at a pressure of 20 psi, temperature of 121°C for 15 min after which it was cooled and inoculated with the yeast. The flask was then attached to a Gallenkamp shaker operated at 200rpm to ensure proper mixing of the contents. Here the yeast was grown for 18h at 32°C (room temperature). Ten milliliters of this inoculum were added to 10ml of the sterilized 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.

Fermentation: One thousand milliliters of the media was prepared and poured into a bench scale 2 litres fermentor (Bioflo Model F-2000, New Brunswick Scientific Co Inc, U.S.A.). The contents were then sterilized at 121°C under a pressure of 20 psi for 15 min and then cooled to room temperature. The inoculum prepared above then cooled to room temperature. The inoculum prepared above was then aseptically transferred into the contents of the fermentor, the agitation and air flow rates were set at 200 rpm and 1.2 scf/h respectively whilst the temperature was maintained at 30°C and pH at 4.6 (4).

At the start of the experiment, sampling was done every 1.5h for a period of 16h. For each sample withdrawn, it was centrifuged and the supernatant separated whilst the biomass was washed with distilled water, filtered and dried to a constant weight at 80°C in a hot air oven. The same procudure was repeated for each of the second and third generations. The pH of the broth was kept at 4.6. by the injection of either hydrochloric acid or sodium hydroxide solution.

After the sampling, the remaining content of the fermentor 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 Bradstreet (5). The method involves determination of total nitrogen and subsequently obtaining the crude protein as 6.25 multiplied by the total nitrogen; the figure 6.25 being an empirical factor.

RESULTS AND DISCUSSION

Figure 1 shows a plot of cell concentration versus time for runs A,B and C at 30°C and a pH of 4.6. From the plot, a lag period of about 3h was obtained for each of the runs. This lag period is the time required for the cells to adapt to the media constituents. This period was followed by an exponential growth period during which the cells had to undergo rapid division. The first order growth kinetics, r=uC was tested by plotting InC versus time for the exponential growth phase of each of the

runs (Fig.2). The linearity of the plot confirms that the growth kinetics is describable by a first order kinetics.

The specific growth rate constants(u) were determined from the slopes of the lines in Fig. 2 and the values obtained are shown in Table 1. These values fall within the range of 0.07-0.11h^{-1} reported for most strains of *Saccharomyces cerevisiae* (6).

Thevalues of the doubling time(t) were obtained from the equation, ln(2)/u (8) and are similarly tabulated in Table 1. The doubling times obtained here, 7.88-7.45h, appear to be higher than reported literature values of 2-4h for bacteria and yeasts by Keith Steinkraus (7) but in agreement with that of Bijkerk(6) for yeasts.

The values of the protein content of yeasts from the fermentation runs was determined by the Kjeldahl method are also show in Table 1. The percent protein values are in good agreement with literature value of 50% reported for yeasts suitable for use as food supplement or as animal feed. This finding attests to the suitability of the yeast produced here for use as food supplement or as animal feed.

TABLE 1: Specific growth rate constants(u), doubling times (t) and % protein contents.

Sampling	u(h^{-1})	t_d(h)	% protein
RUN A	0.088	7.88	49
RUN B	0.09	7.7	52.5
RUN C	0.093	7.45	54.2

The yield of yeast as SCP for each of the runs A, B and C were determined to be 0.85, 0.875 and 0.90 respectively. These yield values are higher than 0.45g dry wt/g-glucose reported by Bijerk *et al.*, (9) and 0.42g dry wt/g-glucose reported by Nwosah (10). The higher yield values obtained here suggest a better utilization of the extracts for biomass production compared to the media used by the other workers (9,10).

The increase in the specific growth rate constant, the yield, and the percentage protein content and the decrease in the doubling times for each of the runs show an increasing adaptation and adjustment of the yeast cells to the medium from generation to generation.

The SCP produced here can be assumed safe for both human and animal consumption on grounds that no carcinogenic, mutagenic or embryotoxic effects have been observed from the previous use of yeasts produced from various carbohydrate sources, molasses, sulphite liquors and vegetable wastes (1).

The major limitation of the use of SCP as food and feed is the concentration of nucleic acids which is known to cause gout and the accumulation of uric acid stones in kidney if its serum level is high. Since the concentration of this acid depends on the composition of the media in which the yeast cells are propagated, further work will be done to ascertain the effect of various constituents on its accumulation. However, there are various ways of reducing the nucleic acid contents of cells but all involve additional costs(1).

Nigeria has about 32 breweries whose combined solid wastes as spent grains should be able to sustain a plant for the production of SCP to satisfy some of the needs of their consumers. In the design of such a plant, the metabolic parameters obtained in the present study i.e. the specific growth rate constant and the yield coefficients, are not likely to change during scale-up; but the determination of the oxygen transfer coefficient, the power requirement and the physical dimensions of the bench scale fermentor are needed in order to apply the relevant scale up criteria which could be geometric similarity or constant volummetric oxygen transfer coefficient or constant power per unit volume of liquid (11).

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