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PRODUCTION OF PROTEINS BY AZOTOBACTER CHROOCOCCUM

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Abstract. Cultivation of a locally isolated nitrogen fixer strain of *Azotobacter chroococcum* in laboratory fermentor was successfully achieved. Composition of the medium and methods of fermentation were recorded. The growth, as well as the nitrogen and lipid of the cultures were greatly affected by aeration as well as the carbohydrate level of the fermentation medium. The chemical composition of the cell proteins revealed the presence of sixteen different amino acids.

Large scale production of *Azotobacter* for many commercial purposes, created much controversy among the investigators. *Azotobacter* can compete now with yeasts as source of proteins and is preferred for utilization of atmospheric nitrogen and production of the essential biofactors in considerable amounts.

In our study on the physiological and biochemical aspects of the nitrogen fixation, a locally isolated strain *Azotobacter chroococcum* proved to be the best nitrogen fixer among twenty different cultures of *Azotobacter*.[1] The role of certain factors affecting the fixation of atmospheric nitrogen by this organism was also investigated.[2] The present work describes cultivation of the organism in a laboratory scale fermentor, in a trial to explore the possibility of the commercial production of proteins through the agency of this organism.

Experimental

The Experimental Organism. The culture was inoculated with descendants from a single slant of a locally isolated pure culture of *Azotobacter chroococcum.*

Cultivation. The fermentor described by Hormatka[3] was used. It consisted of a glass tube 12 cm dia and 80 cm long. To one end of this tube a sintered glass disc (porosity G4) was fused. This end was drawn to form a funnel-shaped part. A delivery tube was adjusted at a distance of 2 cm over the sintered glass disc and was used for the withdrawal of samples of liquid cultures. Sterile air was passed through the opening of the funnel-shaped end. The flowing air, passing through the sintered glass disc, was divided into small bubbles which were finely dispersed into the liquid culture medium. The air passing through the culture medium resulted in proper mixing and uniform distribution of oxygen in the culture medium.

The composition of the fermentation medium was: (g/l; w/v) glucose (different levels); KH_2PO_4, 1.0; NaCl, 0.5; $CaCO_3$, 2.0; $MgSO_4.7H_2O$, 0.5; $FeSO_4$, 0.05; sodium molybdate, 0.005. The pH was adjusted to 7.0. The fermentor was sterilized separately from the culture medium and then charged with the sterile medium. It was found better to sterilize glucose and salt solution separately. The medium was then inoculated with standard inoculum suspension (5 ml/100 ml medium). This inoculum contains about 30 mg bacterial cell dry weight and 2 mg total nitrogen content. Aeration intensity was 1.0 l of air/1.0 l of medium/min. A small quantity of an antifoam (commercial mixture of cetyl and stearyl alcohols) was preferably added to the culture medium. Such conditions were found to be more convenient.[4]

Fermentation was carried out in a thermostatically controlled incubator room, adjusted at 30°C±2. Samples for analysis were taken daily.

Determination of Dry Weight. Samples of the cultures were treated with dil HCl to remove the precipitated $CaCO_3$. The diluted samples were then transferred to a colorimeter and the values obtained were compared with those of the previously prepared standard curve.[5]

Analysis of Nitrogenous Compounds. The total nitrogen contents of the cultures were estimated by microkjeldahl method.[6,7] Paper chromatographic technique[8,9] was also employed to identify the amino acids of the cells.

Determination of the Lipid Content. The lipid fraction of the bacterial cells was estimated by exhaustive extraction of the dried ground cells in Soxhlet units.[10]

Results and Discussion

The production of cell mass, nitrogen and lipid substances by the experimental organism were determined by adding different levels of glucose to the fermentation medium. From the results it is clear that glucose level of the medium exerted a great effect on the metabolic activities of the organism (Table 1). When 2 g% glucose was used (Table 1), good growth and nitrogen yields were obtained after 2 days of incubation. The growth as well as the nitrogen yields exhibited constant values after 3 and 4 days of fermentation. However, the biosynthesis of lipid fraction was concurrently increased as the culture aged.

Upon using 4 and 6 g% glucose the growth values were gradually increased during the different fermentation periods. However, the rate of increase was particularly observed between 2–3 days of fermentation. Higher yields of nitrogen were obtained after 3 days of growth. Maximal lipid yield was obtained after 4 days of fermentation.

When higher levels of glucose, namely 8.0 and 10.0 g% were used the period of growth phase proved to be longer compared with the other experimental

treatments and maximal growth values were obtained after 6 and 7 days. Under these experimental conditions, best nitrogen contents were achieved after 6 and 7 days. Again, the lipid contents gradually increased during the investigated fermentation periods.

The economic coefficient (E.C.)††† increased from about 27.7 to 52.6 when glucose level increased from 2.0 to 4.0 g%. The E.C. attained similar values at the glucose levels 6.0 and 8.0 g%. However, it exhibited lower values (32.4) with 10.0 g% glucose.

TABLE 1. THE DRY WEIGHT, TOTAL NITROGEN AND TOTAL LIPIDS (mg/100 ml CULTURED MEDIUM) PRODUCED BY THE EXPERIMENTAL ORGANISM CULTIVATED IN THE LABORATORY FERMENTOR USING THE NITROGEN-FREE MEDIUM CONTAINING DIFFERENT AMOUNTS OF GLUCOSE.

Days	Dry wt (mg)	Total nitrogen (mg)	Total lipids (mg)
Medium containing 2% (*w*/*v*) *glucose**			
1	412	32.3	22.2
2	555	43.8	51.1
3	552	43.0	71.8
4	550	42.8	82.5
Medium containing 4% (*w*/*v*) *glucose*†			
1	755	52.5	49.1
2	915	65.3	65.7
3	2105	75.4	282.2
4	2101	75.4	313.6
Medium containing 6 g% (*w*/*v*) *glucose***			
1	785	56.0	48.6
2	1120	75.6	118.4
3	2950	86.8	396.4
4	3050	86.8	418.5
Medium containing 8 g% (*w*/*v*) *glucose*††			
1	735	59.0	41.8
2	1023	71.6	94.2
3	2556	93.2	271.2
4	3384	103.6	578.2
5	3922	126.0	676.5
6	4055	126.0	728.3
7	3898	118.0	732.0
Medium containing 10 g% (*w*/*v*) *glucose****			
1	330	29.2	12.2
2	480	41.4	15.4
3	680	46.4	36.1
4	1465	65.2	131.8
5	2955	87.3	389.2
6	3145	87.5	497.3
7	3245	87.5	520.2
8	3115	86.5	523.3

Glucose was not practically detected after:* 2 days of fermentation; † 3 days of fermentation; ** 4 days of fermentation; †† 5 days of fermentation; *** 7 days of fermentation.

††† $\text{E.C.} = \frac{\text{Dry wt}}{\text{Consumed sugar}} \times 100$

The time required for complete consumption of the added glucose was increased according to the concentration of the glucose used. Thus, the organism required 2, 3, 4, 5, and 7 days to utilize 2.4, 6, 8 and 10 g% glucose, respectively. It is worth mentioning that when the experimental organism was grown on the culture medium containing 2.0 g% glucose, under stagnant culture conditions, glucose was practically consumed after 7 days of growth.[2] As has been pointed out before, such glucose concentration was consumed after only 2 days under aerated conditions. It could be concluded, therefore, that aeration reduced the time of fermentation nearly to one-third. Such results showed the importance of the glucose level of the medium as well as the aeration on the metabolic activities of the organism. The favourable effect of aeration was studied by Lorenz and Rippel[11] and Khmel *et al.*,[12] who stated that the increase of oxygen supply through aeration stimulated the growth and the protein formation by *A. chroococcum*. Overbeck[13] also noticed a rapid consumption of carbohydrates by *A. chroococcum* in shake cultures.

The chromatographic analysis of the acid hydrolysate of the cell proteins revealed the presence of the following amino acids: cystine, lysine, aspartic acid, histidine, serine, arginine, glutamic acid, glycine, threonine, alanine, tyrosine, methionine, valine, proline, phenyl alanine and leucine. Amino acid tryptophan was also detected in the alkaline hydrolysate of the cell proteins.[14]

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