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FACTORS AFFECTING THE PRODUCTION OF PROTEINS BY AZOTOBACTER CHROOCOCCUM

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Abstract. The aeration intensity as well as different organic supplements were tested as factors affecting the utilization of glucose and the synthesis of proteins by *A. chroococcum* when cultivated in a laboratory fermentor. The best aeration intensity was 1.0 litre air/1.0 litre of medium/min. Addition of vitamins to the fermentation medium was not effective in this respect. Acetate, α-ketoglutarate, succinate, malate and fumarate accelerated the utilization of glucose and reduced the fermentation period.

In a previous communication,[1] a series of experiments were carried out in order to evaluate the possibility of the commercial production of proteins using a locally isolated strain of *Azotobacter chroococcum*. Aeration was found to be effective for the production of proteins. It reduced the time required for the utilization of 2% glucose by the organism from seven days in surface cultures to two days in aerated cultures. It was also observed that 8% glucose was utilized after seven days' incubation under aerated conditions.

In the present work, trials were carried out, using a laboratory fermentor, in order to reduce the time required for the utilization of 8% glucose by the local *A. chroococcum* strain.

Experimental

The Experimental Organism. The cultures used in this work were inoculated with descendants from a single slant of a locally isolated pure culture of *Azotobacter chroococcum*.

Cultivation, fermentation medium, determination of bacterial dry weight and lipid content were carried out according to the technique previously adopted by El-Sayed *et al.*[1] The fermentor used was described in the same communication.

The fermentation medium which contained 8% glucose was supplemented with a mixture of vitamins. This mixture comprised cyanocobalamine, nicotinic acid, thiamine, riboflavin and pantothenic acid in a concentration of 0.005 mg% for each vitamin. The other organic supplements, namely acetate, α-ketoglutarate, succinate, malate and fumarate were added to the fermentation medium in carbon concentration equivalent to the carbon content of 1.0% glucose. Therefore, the amounts used were 1.4, 1.1, 1.2, 1.3 and 1.2% for acetate, α-ketoglutarate, succinate, malate and fumarate, respectively.

Determination of Total Nitrogen. The total nitrogen content of the cultures was estimated by the microkjeldahl method.[2,3]

Determination of Glucose. The utilization of glucose by the microorganism was determined daily by using the modified Luff-Schoorl method.[4]

Results and Discussion

The present work is an endeavour towards the achievement of high yields of the bacterial cell-mass, lipids and nitrogen. The latter was taken as an indication for the total protein content of the fermented cultures. The role of aeration intensity as well as the effect of different organic supplements were studied aiming at the reduction of the time required for the utilization of the supplemented glucose and the subsequent production of proteins. The results obtained with the different experimental treatments were compared with the fermentation medium containing no additive substances and aerated with 1.0 litre air/1.0 litre medium/min as control (Table 1).

The results presented in Table 1 show that the consumption of glucose in cultures subjected to 0.5, 1.0 and 2.0 litre of air/1.0 litre medium/min lasts 8, 7 and 7 days, respectively. The yields of bacterial dry weight, nitrogen and lipids, at the lowest aeration intensity were almost one-half those obtained with the highest intensities of aeration. The produced cell-mass, nitrogen and lipids at the second day of fermentation increased with the increase of the aeration intensity. The final yields of dry weight and lipids increased also with the increase of the aeration intensity. However, the nitrogen content did not appreciably affected by the changes of aeration. The economic coefficient (E.C.) increased from 40% in the lowest intensity of aeration to 53 and 55% in higher intensities.

The aforementioned results revealed that the lower aeration, intensity (0.5 litre air/1.0 litre medium/min) was not sufficient for supplying the optimal oxygen tension in the culture medium. The organism adapted itself more rapidly in cultures subjected to the higher aeration intensities. The rapid adaptations were reflected on the shorter lag period of growth in case of the aeration intensities of 1.0 and 2.0 litre air/1.0 litre medium/min and on the sudden increase of the bacterial dry weight, nitrogen and lipids in cultures subjected to the highest aeration intensity. These results coincided with those of Lorenz and Rippel-Balds,[5] and Khmel *et al.*[6] The same situation also met El-Sayed[7] in his work on acetic acid bacteria. He observed that the higher the aeration intensity, the more efficient was the

acetification process. Although the aeration intensity affected appreciably the cell-mass yield and the lipid content of the organism, it did not affect the nitrogen fixing capacity. Nilsson and Jonsson[8] also recorded an increase of the bacterial dry weight without a parallel increase of the nitrogen fixation.

Under all the tested aeration intensities, the increase of the lipid content throughout the fermentation period, was rather high compared to the bacterial dry weight and nitrogen content. While

TABLE 1. THE DRY WEIGHT, TOTAL NITROGEN AND TOTAL LIPIDS (mg/100 ml cultured medium) PRODUCED BY THE EXPERIMENTAL ORGANISM CULTIVATED IN LABORATORY FERMENTOR USING NITROGEN-FREE MEDIUM CONTAINING 8% GLUCOSE, AS AFFECTED BY DIFFERENT AERATION INTENSITIES.

Days	Dry wt (mg)	Total nitrogen (mg)	Total lipids (mg)
0.5 *litre air*/1.0 *litre medium*/*min**			
1	338	29.7	12.4
2	520	45.6	15.4
3	690	50.0	24.5
4	1614	71.9	139.1
5	2140	74.3	340.0
6	2954	97.3	492.0
7	3531	118.3	510.0
8	3787	127.4	614.2
9	3787	127.9	615.5
1.0 *litre air*/1.0 *litre medium*/*min*†			
1	625	51.0	32.1
2	914	64.2	82.3
3	2314	93.2	211.2
4	3414	111.2	582.0
5	4122	130.0	723.4
6	4132	131.1	725.1
7	4242	132.5	734.2
8	3990	129.1	730.2
2.0 *litre air*/1.0 *litre medium*/*min***			
1	640	52.8	40.6
2	2311	101.2	218.7
3	2812	115.2	371.5
4	3616	122.6	418.6
5	4400	137.2	692.8
6	4395	132.8	747.8
7	4396	132.1	810.7
8	4112	129.1	811.1

$$\text{Economic coefficient (E. C.)} = \frac{\text{Dry weigth}}{\text{Consumed sugar}} \times 100$$

Glucose was practically not detected after : *8 days, E. C. about 40%; †7 days, E.C. about 53%; and **7 days, E. C. about 55%.

TABLE 2. THE DRY WEIGHT, TOTAL NITROGEN AND TOTAL LIPIDS (mg/100 ml cultured medium) PRODUCED BY THE EXPERIMENTAL ORGANISM CULTIVATED IN THE LABORATORY FERMENTOR USING NITROGEN-FREE MEDIUM CONTAINING 8% GLUCOSE and Supplemented with Different Organic SUPPLEMENTS. (Aeration intensity was 1.0 litre air/1.0 litre of medium/min).

Days	Dry wt (mg)	Total nitrogen (mg)	Total lipids (mg)
Mixture of Vitamins[a]			
1	340	28.9	15.1
2	481	42.4	16.7
3	704	52.6	94.7
4	1618	73.2	228.1
5	2210	95.6	292.1
6	3016	117.6	412.1
7	3292	130.7	618.1
8	3288	129.3	674.0
Acetate[b]			
1	640	38.4	54.5
2	1465	72.3	219.2
3	3112	111.4	414.5
4	3248	117.4	618.3
5	3216	127.4	711.9
α-Ketoglutarate[c]			
1	574	32.3	52.4
2	2660	91.6	189.4
3	3779	109.6	380.0
4	3914	128.4	584.6
5	3920	124.6	681.8
Succinate[d]			
1	414	28.1	42.4
2	830	48.8	140.4
3	1710	52.6	190.0
4	3220	93.2	311.2
5	4412	112.2	568.0
6	4215	121.2	671.0
Malate[e]			
1	398	28.4	45.1
2	830	51.8	99.2
3	1821	82.4	181.1
4	3012	113.6	324.8
5	4210	119.1	544.1
6	4112	122.2	614.1
Fumarate[f]			
1	722	39.1	39.9
2	1428	62.3	120.2
3	3334	88.1	324.6
4	3329	115.8	568.4
5	3318	119.7	663.2

Glucose was practically not detected after : (a) 7 days, E. C. 41%; (b) 5 days, E.C. 40%; (c) 5 days, E.C. 49%; (d) 6 days, E.C. 53%; (e) 6 days, E.C. 51%; and (f) 5 days, E.C. 42%.

the increase of the dry weight was about 8–10 times and that of nitrogen was almost 4 times, the increase of lipids was 40–50 times. Moreover, the highest rate of lipid accumulation was at the late stages of growth. During the early stages of growth (log phase) the organism was comparatively more active. In this period, the yields of the bacterial cell-mass, nitrogen and lipids increased steadily. At the beginning of the fourth or fifth day of the fermentation period, the rate of increase in cell-mass yield as well as the content of nitrogen lowered down indicating that the organism is joining the stationary phase of growth. Consequently, the energy released as a result of glucose consumption was utilized in other metabolic activities, perhaps in the synthesis of more lipids.

Upon leaving the fermentation process to proceed after the complete utilization of glucose (Table 1), a noticeable decrease in the yields of the bacterial cell-mass, nitrogen and lipids was observed with the higher rates of aeration. In case of the lowest intensity, the yields of the three parameters remained almost unaffected. This may be explained on the bases that the high rates of aeration accelerated the autolysis of some bacterial cells and the utilization of the liberated proteins and lipids as sources of energy, instead of glucose, by the remained cells. The lowest rate of aeration seemed to be not sufficient in this respect.

The addition of a mixture of vitamins to the aerated culture medium (Table 2) did not reduce the fermentation period. However, noticeable decreases of the bacterial dry weight and lipid content were recorded at the end of the fermentation period. The final yield of nitrogen was almost equal to that of the control. Moreover, the amounts of cell-mass yield, nitrogen and lipids at the first, second and third days' fermentation were noticeably lower, compared to the yields obtained at the same period with the control. This means that the organism could not adapt itself rapidly on vitamin-enriched medium. The organism was unable to obtain a complete benefit of the supplemented vitamins, especially at the early stages of growth, perhaps because it is considered a vitamin producer.[9–11] On the other hand, the presence of vitamins might impart conditions of nutritional imbalances which exerted an adverse effect on the growth and lipid formation. The same situation was met by some investigators in their work on several bacteria like acetic acid bacteria[7] and nodule bacteria.[12]

The other organic supplements (Table 2) greatly enhanced the utilization of glucose and consequently reduced the fermentation period. The fermentation process lasted 5 or 6 days in media enriched with the organic acids against 7 days in the control. The yields of dry weight, nitrogen and lipids at the first day of fermentation were the same or little less compared to those of the control at the same period. However, the yields of the three parameters suddenly increased at the second day. At the end of the fermentation period, the E.C. was about 40, 49, 53, 51 and 42% in presence of acetate, α-ketoglutarate, succinate, malate and fumarate, respectively. The presence of these supplements seemed to cause a reduction in the lag period of growth, in spite of the little decrease of the final yield of nitrogen. The only plausible explanation is that these supplements acted as 'sparkers' for the utilization of glucose.[7,9,13,14] The sparking effect was reflected on the shorter time at which glucose was utilized. In presence of acetate, the initial accumulation of lipids was higher compared to the control. The other additives increased the initial accumulation of lipids but at different rates, perhaps due to the different sparking effects of these supplements. This is true in so far the E.C. was fluctuating between 40–53% according to the supplemented organic acid.

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