Influence of the Level of the Carbon Source on the Autolysis of *Aspergillus niger*

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SUMMARY

The degree of autolysis of *Aspergillus niger* cultures grown and autolysed on 2.5, 5, 10, 20 and 40 g./l. initial glucose was 64, 60, 43, 43 and 43 %, respectively. The greatest loss (73 %) of mycelial nitrogen took place with the lowest initial concentration of glucose. The greatest disappearance of lipids occurred in the period up to the 8th day of autolysis irrespective of the initial sugar concentration. The percentage loss of lipids was practically constant for the first four concentrations of initial glucose assayed. The amount of total free reducing substances contained in the mycelium at the beginning of the autolytic phase increased rapidly to a high level if the initial concentration of glucose was 40 g./l., but not if it was 20 g./l. or less. The total mycelial carbohydrates determined by the anthrone reaction declined continuously irrespective of the initial sugar concentration. The percentage of mycelial constituents released during autolysis increased with initial concentration of glucose.

INTRODUCTION

The influence of certain imposed conditions on the autolytic phase of growth in cultures of filamentous fungi has been studied by Behr (1930), who was the first to describe the influence of the two types of media, physiologically acid and alkaline, upon different aspects of the autolytic phase of growth in cultures of *Aspergillus niger*. The effect of the addition of certain growth factors upon the autolytic processes of several filamentous fungi was investigated by Ritter (1955). Tandon & Chandra (1962) studied the nature of the changes induced in autolysing cultures of *Colletotrichum gloeosporioides* by the addition of antibiotics. Changes in the general pattern of autolysis brought about by a variety of different cultural conditions in *A. flavus* and in *A. terreus* were described by Lahoz (1967), Lahoz, Reyes & Beltrá (1966), Lahoz, Reyes, Beltrá & García-Tapia (1967) and Lahoz & González Ibeas (1968). In our search through the literature on the filamentous fungi we were unable to find any report of the effect of alteration of concentration of carbon source upon autolysis. The present work analyses the effect on autolysis of *A. niger* cultures of changes in the initial amounts of glucose.

METHODS

Organism. We used *Aspergillus niger* van Tieghem no. 120.49 from the Centraal-bureau voor Schimmelcultures, Baarn, Holland.

Chemicals. Mineral salts used in the preparation of culture media were of analytical

grade from Probus S.A., Barcelona, Spain. Organic reagents were of analytical purity either from the British Drug Houses Ltd, Poole, Dorset, England, or from Sigma Chemical Co., St Louis, Mo., U.S.A.

Conditions of culture. Jena conical flasks (300 ml.), each containing 80 ml. of Czapek-Dox medium (g./l. distilled water: NaNO_3 , 2; KCl , 0.5; KH_2PO_4 , 1; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5) with glucose (anhydrous) at 2.5, 5, 10, 20 and 40 g./l., were prepared and sterilized as previously described (Lahoz *et al.* 1966, 1967). At convenient intervals sets of 80, 100 or 200 flasks were in turn inoculated with 1 or 2 ml. of a spore suspension of *Aspergillus niger* prepared as previously described (Lahoz *et al.* 1966, 1967). Each batch was incubated on a reciprocating shaker at 90 oscillations/min. with an amplitude of 10 cm. at 24 to 26° in the dark. At the time of the maximum yield of mycelium at least five flasks were withdrawn from the shaker; the number of flasks taken at any particular time depended on the amount of mycelium present when harvesting. In the set containing the lowest glucose concentration samples of 10, 15 or 20 flasks had to be taken and their contents combined since growth was very scanty. During the later periods 45 flasks were needed to give sufficient mycelium for detailed analysis. In all cases the mycelium was filtered free of culture fluid, washed with water and dried at 70° to constant weight. The culture fluid was discarded after the residual glucose and the pH had been estimated.

Analytical methods. Total nitrogen, carbohydrates and lipids in mycelium were determined as in previous work (Lahoz *et al.* 1966, 1967, 1968). Methods of extraction of total free reducing substances from defatted mycelium and their estimation were as previously described (Lahoz *et al.* 1966, 1967; Lahoz & Gonzalez Ibeas, 1968; Lahoz, Beltrá & Ballesteros, 1970). The alkali soluble protein from the residue was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) using hot (80°) N-NaOH (100 mg. mycelium/25 ml. NaOH) extracts.

RESULTS

The highest yield of mycelium was attained at the 3rd, 3rd, 4th, 7th and 11th day of incubation for media containing 2.5, 5, 10, 20 and 40 g./l. initial glucose, respectively. As in previous work, the loss in mycelial dry weight was adopted as a criterion of autolysis. Therefore the day that the maximum amount of mycelium formed in the different concentrations tested was taken as the 'zero day of autolysis' and samples to be analysed were withdrawn from the shaker 8, 23, 38 and 53 days later.

The loss of weight in autolysing mycelium of *Aspergillus niger* grown at different initial glucose concentrations can be seen in Table 1. The percentage autolysis for the 2.5, 5, 10, 20 and 40 g./l. concentrations were 64, 60, 43, 43 and 43 %, respectively. Above 10 g./l. the degree of autolysis remained uniformly low as the concentration of the carbon source increased.

The total nitrogen in the autolysing mycelium of *Aspergillus niger* is shown in Table 2. With each initial concentration of glucose the nitrogen content of the mycelium (expressed as nitrogen/unit dry weight and as nitrogen/culture) at first diminished as the culture aged, but became practically constant later. The greatest loss (73 %) of mycelial nitrogen took place at the lowest initial glucose concentration (Table 3).

The greatest disappearance of lipids from autolysing mycelium of *Aspergillus niger*

took place before the 8th day of autolysis at all the initial glucose concentrations except 40 g. glucose/l. at which the amount of fat in the mycelium remained constant throughout the whole period of autolysis (Table 4). The total fat lost during 38 days

Table 1. *Influence of initial glucose concentration on mycelial weight during autolysis of Aspergillus niger*

Initial glucose concentration (g./l.)	Time of autolysis (days)				
	0	8	23	38	53
	Weight of mycelium/flask (mg.)				
2.5	76	43	33	30	27
5	150	86	72	61	59
10	205	140	123	116	116
20	409	276	252	238	232
40	730	579	496	460	410

Table 2. *The effect of initial glucose concentration on the total nitrogen in autolysing mycelium of Aspergillus niger*

Initial glucose concentration (g./l.)	Time of autolysis (days)									
	0		8		23		38		53	
	A*	B†	A	B	A	B	A	B	A	B
2.5	4.5	3.4	3.6	1.5	3.3	1.1	3.1	0.9	—	—
5	4.9	7.3	4.0	3.4	3.7	2.6	3.7	2.3	—	—
10	4.0	8.2	3.5	4.9	3.4	4.2	3.2	3.7	3.3	3.8
20	3.8	15.4	3.3	9.1	3.1	7.7	3.0	7.3	3.0	7.0
40	2.8	20.1	2.9	16.5	2.2	11.0	2.4	11.2	2.5	10.3

* Mg. N/100 mg. dry mycelium.

† Mg. mycelial N/flask.

Table 3. *Percentage loss of mycelial nitrogen during the first 38 days of autolysis of Aspergillus niger at different initial glucose concentrations*

Initial glucose (g./l.)	Zero day (mg./flask)	38th day (mg./flask)	Difference	% loss
2.5	3.4	0.9	2.5	73
5	7.3	2.3	5.0	68
10	8.2	3.7	4.5	54
20	15.4	7.3	8.1	52
40	20.1	11.2	8.9	44

Table 4. *Influence of initial glucose concentration on the amount of fat in autolysing mycelium of Aspergillus niger*

Initial glucose concentration (g./l.)	Time of autolysis (days)				
	0	8	23	38	53
	Mg. fat per flask				
2.5	3.4	0.8	1.2	1.3	—
5	6.1	3.2	2.6	2.3	—
10	8.4	4.2	5.0	3.3	2.6
20	27.9	10.0	10.1	11.0	10.1
40	18.9	17.4	19.3	18.3	17.6

Fig. 1. Influence of initial glucose concentration on free reducing substances in the mycelium during autolysis of *Aspergillus niger* cultures. Mg./100 mg. dry weight mycelium, -----; mg./flask, ———; 2.5 g./l. glucose, ○; 5 g./l. glucose, △; 10 g./l. glucose, □; 20 g./l. glucose, ●; 40 g./l. glucose, ▲.

Table 5. *Relation of total carbohydrates in autolysing mycelium of Aspergillus niger to initial glucose concentration*

Initial glucose concentration (g./l.)	Time of autolysis (days)				
	0	8	23	38	53
	Mg. anthrone positive carbohydrate/flask				
2.5	23	20	17	15	—
5	47	37	35	29	—
10	73	58	56	57	55
20	165	129	121	116	113
40	310	263	207	187	143

Table 6. *Final balance sheet of the loss of partial cellular constituents from autolysing mycelium of Aspergillus niger in cultures with different initial glucose concentrations*

mg./100 mg.	Initial concentration of glucose (g./l.)				
	2.5	5	10	20	40
Total nitrogen	5.2	5.5	5.0	4.7	3.1
Crude fat	4.2	4.2	6.5	1.0	—
Total carbohydrates	17.0	19.7	20.1	29.2	52.1
Total loss (%)	26.4	29.4	31.6	34.9	55.2

of autolysis of *A. niger*, grown and autolysed at 2.5, 5, 10 and 20 g. glucose/l. was practically uniform at 61, 62, 60 and 60% respectively. The change in total carbohydrates during autolysis can be seen in Table 5: they declined continuously at all of the initial glucose concentrations. The alkali soluble protein in the mycelium remained constant during autolysis for 53 days.

The percentage of the constituents released by autolysing cultures grown at different initial glucose levels increases with initial glucose concentration (Table 6).

DISCUSSION

Behr (1930), in his work on autolysis of *Aspergillus niger*, showed that the percentage of mycelial nitrogen continuously increases or remains constant with the ageing of the culture in physiologically alkaline media. It is shown here that the nitrogen in the mycelium decreased when expressed as N/100 mg. dry weight and N/flask (Table 2).

The increase in the content of the free reducing substances in mycelium during autolysis, which in previous work (Lahoz, 1967; Lahoz *et al.* 1970) has been attributed to an enzymic hydrolysis due to autolytic enzymes, is confirmed by the present work for the 40 g./l. level of initial glucose. The experiments showed that the initial concentration of the carbon source exerted a marked influence upon the behaviour of free reducing substances in the mycelium. A fall in free reducing substances with time was characteristic of all initial glucose levels except for the 40 g/l. (Fig. 1).

Similarly, the quantitative changes in lipids (Table 4) in autolysing mycelium seemed to be related to the initial level of the carbon source. Lipids in autolysing mycelium of *Aspergillus niger* grown in the highest initial glucose concentration (40 g./l.) remained at a constant level. A similar constancy was observed while studying autolysis in still cultures of *A. flavus*, in which a concentration of 50 glucose/l. was used. On the other hand the change in the amount of the carbon source did not exert a noticeable influence on the behaviour of total carbohydrates contained in mycelium of *A. niger* during autolysis. The pattern of continuous diminution, as autolysis proceeds, has been found previously (Lahoz & Gonzalez Ibeas, 1968; Lahoz *et al.* 1970).

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REFERENCES

- BEHR, G. (1930). Über Autolyse bei *Aspergillus niger*. *Archiv für Mikrobiologie*, 418.
LAHOZ, R. (1967). Quantitative changes in the content of non-nitrogenous compounds during autolysis of *Aspergillus terreus*. *Journal of General Microbiology* 46, 451.
LAHOZ, R., BELTRÁ, R. & BALLESTEROS, A. M. (1970). Biochemical changes in cultures of *Nectria gal- ligena* during the autolytic phase of growth. *Annals of Botany* 34, 205.
LAHOZ, R. & GONZALEZ IBEAS, J. (1968). The autolysis of *Aspergillus flavus* in an alkaline medium. *Journal of General Microbiology* 53, 101.
LAHOZ, R., REYES, F. & BELTRÁ, R. (1966). Some chemical changes in the mycelium of *Aspergillus flavus* during autolysis. *Journal of General Microbiology* 45, 41.
LAHOZ, R., REYES, F., BELTRÁ, R. & GARCIA-TAPIA, C. (1967). The autolysis of *Aspergillus terreus* in a physiologically acid medium. *Journal of General Microbiology* 49, 259.

- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265.
- RITTER, R. (1955). Physiologische Untersuchungen an Zygomyceten. *Archiv für Mikrobiologie* **22**, 248.
- TANDON, R. N. & CHANDRA, S. (1962). Changes in amino acids, sugars and organic acids in the mycelium of *Colletotrichum gloeosporioides* Penz. during the autolytic phase of growth. *Phyton. International Journal of Experimental Botany, Buenos Aires* **19**, 127.