XA7601925

REPORT NO. IAEA-R-935-F

TITLE

Use of ionizing irradiation to increase rates of production and yield of yeast from paraffins, (part of a coordinated programme on radiation microbiology)

FINAL REPORT FOR THE PERIOD

1 October 1970 - 31 March 1975

AUTHOR(S)

J.Meyrath

INSTITUTE

Institute of Applied Microbiology
Vienna, Austria

INTERNATIONAL ATOMIC ENERGY AGENCY

DATE September 1975

HOCHSCHULE FÜR BODENKULTUR
Institut für angewandte Mikrobiologie
Vorstand: Prof. Dr. J. Meyrath

A-1190 Wien, Peter-Jordan-Str. 82
Telefon 34 25 00

CERTIFIED BY: C.K. O'Neal

Final PROJECT REPORT

Research Contract 935/~~RB~~/RB

Use of ionizing irradiations to increase rates of production and yield of yeast from paraffins (part of a coordinated programme of research on radiation microbiology)

1) The role of carbon dioxide in the assimilation of n-alkanes by Candida tropicalis

In previous reports it has been mentioned that upon addition of carbon dioxide to young cultures assimilating n-alkane the respiratory quotient (ratio O_2/CO_2) is decreased considerably so much so that a net assimilation of CO_2 takes place. These observations have been confirmed by determining CO_2-uptake using $^{14}CO_2$ as tracer. For example, at a partial pressure of CO_2 of 0.05 atm in an enclosed system the following relative activities of ^{14}C were measured using a liquid sctintillation counter:

Yeast strain	counts per minute per ml of standardized suspension		
	Glucose	Acetate	n-Hexadecane
Candida sp. 10 002	12 700	20 000	22 700
Candida sp. 10 111	12 600	20 500	32 800

Thus the carboxylating reactions are considerably higher on n-hexadecane and acetate than on glucose. Acetate reacts similarly to alkane which is quite understandable considering that alkanes are broken down to acetate by ß-oxidative degradation. Synthesis of compounds with more than two C-atoms is likely to take place at

least in part by carboxylation.

Investigations also showed that raising the partial pressure of CO_2 above 0,1 atm does not lead to an increased fixation of carbon dioxide. As much as 3 % cell carbon can be derived from carbon dioxide.

2) Distribution of CO_2-carbon in intermediary metabolism

Fixation of carbon dioxide proceeds very rapidly, since a few minutes contact time suffice to show the uptake of CO_2. Tricarboxylic acid cycle intermediates and amino acids have been measured in the cell pool. Using thin layer chromatography combined with autoradiography separation and identification were carried out.

There was no difference in the ^{14}C label of tricarboxylic acid cycle intermediates when Candida was cultured on either glucose, acetate or n-hexadecane. Succinate, malate and citrate were the only labelled compounds although all other intermediates of the cycle could be shown to be present.

The labelling pattern of pool-aminoacids was also largely similar when using the three different carbon sources as above. However, labelled threonine with acetate and n-hexadecane could be detected only after about 60 min. cultivation, whereas with glucose this amino acid was labelled after about 30 min. On the other hand labelled alanine, which with acetate and n-hexadecane could be detected after 30 min., was present as labelled compound on glucose only after about 120 min. This difference in labelling can very well provide us a clue as to the carboxylating reactions on acetate or hydrocarbon.

Not only the lower molecular compounds are labelled within

very short times, also such compounds as proteines were found to be radioactive. Upon hydrolysis of the proteines it was shown that again the activity of alanine is considerably smaller on glucose than on n-hexadecane or acetate.

3) Determination of ATP in microbial cells

In the assimilation of n-alkanes a large amount of energy is wasted if compared to the growth on carbohydrates. Thus ATP measurement will be useful to start investigations on the mechanisms why and where this energy is waisted and to examine whether by selecting specific mutants this energy can be used to assimilate carbon dioxide to a greater extent. After examining a number of methods two groups, i.e. luciferin-luciferase methods and phosphoglycerate methods proved to be suitable for measuring ATP in bacteria and yeast. This cannot be said from the hexokinase methods. As an example the following results can be mentioned:

1. ATP-pool in fermenting yeasts (Saccharomyces sp):

Conditions	ATP µMol/g
anaerobic fermentation with CO_2 added	5, 2
anaerobic fermentation with 95 % N_2, 5 % CO_2	5, 9
anaerobic fermentation with 92 % N_2, 8 % CO_2	8, 5
aerobic fermentation	5, 2

2. ATP-pool in Acetobacter utilizing acetate:

Conditions	ATP µMol/g	
	intracell.	extracell.
yeast extract-broth	7, 2	0, 88
yeast extract broth with pyruvate added	6, 7	0, 76

Further investigations along this line will follow. The above results should demonstrate above all the suitability of the methods used.

4) The use of flocculating yeasts to produce biomass

In the production of single-cell-protein one of the major tasks which is economically very important is the recovery of the cells. In a published paper (Process Biochemistry, April 1975) it has been shown that flocculating yeasts are of considerable advantage in the production of scp. The procedure can be used with carbohydrates or other soluble compounds as well as with hydrocarbons. It is also particularly suitable for the production of proteins from industrial wastes.

11 August 1975
J. Myrdal.