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EFFECT OF AERATION ON THE LIPIDS OF *CANDIDA UTILIS*

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Lipid production by *Candida utilis* was studied by growing cells substituting glucose with "maizgur" sugar. Cultural conditions related to the oxygen supply, glucose concentration, pH of the medium and temperature were controlled. It was observed that changes in concentration of "maizgur" sugar from 2% to 3% enhanced the lipid content. of *C. utilis.* Further increase in "maize gur" sugar had no effect on lipid content. The fatty acid composition of the lipid by gas liquid chromatography showed an increase in polyunsaturated acids with aeration.

INTRODUCTION

Lipid producing (oleaginous) organisms have been known for many years and their potential as alternative sources of animal or plant oil has been periodically assessed [1-2]. Yeasts seem to be the most likely condidates for bio-oil production, having been associated with man's diet for many years. Studies concerning the nature of yeast have been extensively carried out. In earlier studies carbohydrates were shown to be converted into fat by yeast [3]. The changes in lipid composition during the yeast cell cycle have also been reported [4]. *C. utilis* has already been studied with further changes in lipid composition during the cell cycle. An oleaginous yeast *Candida 107* has been successfully grown in a single-stage continuous culture producing as high as 40% of lipid contents [5].

Ethanol has been shown to be suitable carbon source for the growth of *Rhodotorula gracilis* to give higher oil levels. It has already been observed that *Candida utilis* accumulates upto 8% of the dry weight of its biomass as lipid under most propitious growth conditions [6-7].

In Pakistan considerable quantities of "maize gur" are available as a by-product of maize processing industries. In view of this and our interest in microbial lipids it was thought proper to use "maize gur" as a source of carbon instead of glucose for the growth of *C. utilis.*

The present studies, therefore, report results of both qualitative and quantitative changes in the lipid content of *C. utilis* obtainable by its growth on "maiz gur" (hydrolysed starch) under different environmental conditions.

MATERIALS AND METHODS

Organisms. C. utilis was used throughout the study. Stock culture was maintained on MY-PG agar slopes (malt extract, 0.3%; yeast extract, 0.3%; peptone, 0.5%; glucose, 2.0%; and agar, 2.0%.

Substrate. Hydrol ("maize gur"), a by-product of glucose industry, was used as a carbon source. It is the mother liquor left over after the crystallization of dextrose monohydrate with the composition given in (Table 1).

Maiz gur was purified by double treatment, i.e. first with lime to pH 9 and after decantation from the sediment adjusted filtrate to pH 5 with sulphuric acid.

Composition of the medium. A simple, chemically defined medium of the following composition was used for the growth of the organism (under conditions of nitrogen limited growth).

Glucose	30.0 g, $MgSO_4$, $7H_2O$ 0.5 g, $CaCl_2$ 0.05 g
KH_2PO_4,	2.5 g, $(NH_4)_2SO_4$ 1.0 g and KI per litre.
Winzler salt solution	10 ml per litre of H_2O.

Winzler salt solution contains 5 μg $FeCl_3$; 1 μg $CuSO_4$, $5H_2O$, and 1 μ gk per litre

Glucose was substituted by "Maiz gur".
Growth was carried out at 30°C at pH 4.5 – 5.5.

The product sold under the name "Maiz gur" is the mother liquor left over after the crystallization of dextrose monohydrate. It is also referred in the trade as *Hydrol.*

Table 1 Composition of maize gur:

Description:	A yellow to brownish yellow semi solid material with characteristic odour.
Gravity at 60°F	42° to 43° Baume
Sugar	60%
Phosphorus	0.30%
Iron	140-150 ppm.
Ash	2%
Colour: 50% solution in 1/4 cell.	Not more than 5.0 yellow 1.5 red.
Dry wt.	83%

Inoculum preparation. 24 hr. old culture of yeast was transferred from the slants to inoculate 100 ml of sterilized medium in 500 ml flask and placed on a rotary shaker with 120 rpm at 30° for two days. The contents were transferred to a 1000 ml Erlnmeyer flask containing 250 ml cultivation medium using different concentrations of maiz gur both under aeration and stationary conditions to see the difference in lipid contents. Samples were removed at 24 hr. intervals for biomass assay and the sugar consumed.

Analytical methods. Harvesting of biomass was done by centrifuging for 20 min at 3000 rpm for dry weight determination. The cell mass was washed with water and dried at 105° for 24 hr and weighed.

Lipid extraction. 0.500 g of dried cells were ground in a mortar, to which were added 50 ml of 2N HCl, refluxed on a water bath for 2 hr. and the mixture was filtered. The debris was washed with 2 x 25 ml water, transferred to a Soxhlet apparatus and extracted with redistilled chloroform and methanol (2:1 v/v) for 18 hr. The solvent was removed in the rotary evaporator and the residue was mixed with fresh chloroform-methanol mixture and shaken occasionally for about 30 min. The solution was filtered and the filtrate was dried in a rotary evaporator. The resulting material was lipids.

RESULTS

The effect of different concentrations of Maiz Gur" on Lipid Production. The effect of different concentrations of "maiz gur" as the sole source of carbon on the growth an lipid production of *C. utilis* in shake flask culture has been reported in Table 2. *Candida utilis* increased its lipid content when glucose concentration was increased upto 4%. The specific rate of glucose utilization increased with increasing growth rate. Dry cell weight increased almost linearly till nitrogen in the broth was exhausted. After that, lipid production was stimulated, while the biomass weight remained constant.

The effect of aeration on the fatty acid composition of C. utilis. Studies were made on the yeast grown in stationary as well as under shaking conditions using the same composition of the medium. The yield of the lipids were more or less the same on dry weight basis under both conditions. Lipids were extracted from both cultures.

The lipids so obtained were further processed for saponification. Esterification was also processed with a methanol, benzyl chloride and acetyl chloride mixture [8]. The esters so prepared were run for GLC with the column PEGS at 210°. The injector and detector temperatures were 220 and 250° respectively. Nitrogen was used as carrier gas with a flow rate of 40 ml per min. Analysis showed some changes in the fatty acid composition of *C. utilis* with aeration. It showed an increase in the level of polyunsaturated acids with aeration, e.g. the percentages of linolenic acid and oleic acid increased but the percentage of linoleic acid remained unchanged. Stearic acid showed a slight decrease but palmitic acid also remained constant.

DISCUSSION

The choice of carbon substrate is crucial for the economic viability of oil production by fermentation. "Maize gur" is a good substrate for lipid production. It was used because it contains about 60% sugar and is available at a low price. "Maize gur" as the sole source of carbon gave almost the same percentage of lipids as given by using

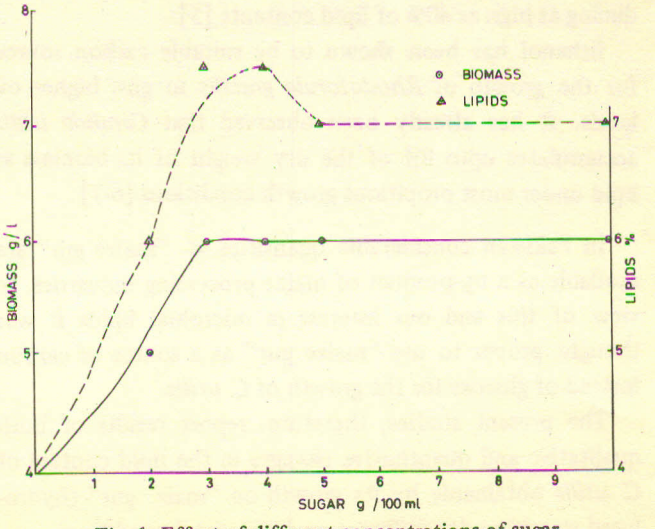

Fig. 1. Effect of different concentrations of sugar.

glucose. The efficiency with which lipid was synthesised is expressed as lipid yield in grams or fat co-efficient.

Lipid production can be viewed as a two-stage process. The first stage is the multiplication of the cells followed by the accumulation of fat globules under the conditions of growth limitation. It has been noted that lipid accumulation is caused by nitrogen depletion, inducing a high rate of lipid synthesis due to the energy sparing effect caused by the slow rate of protein synthesis. However, it has also been observed that an adequate supply of oxygen and the removal of CO_2 is essential to maintain growth and metabolic activity of fat synthesising microorganisms [9]. Aeration appears to be an important factor influencing the composition of the lipids extracted. It was observed that at low glucose concentration excessive aeration was not necessary. However, at high glucose concentration the aeration was found to be critical. In the present studies it has been observed that the fatty acid composition of *C. utilis* was dependant to a large extent on aeration. Aeration increased the level of polyunsaturated acids such as linolenic and oleic acids. Linoleic acid remained constant. Among the saturated acids stearic acid showed a slight decrease with aeration Palmitic acid was constant under both the conditions (Table 3). This observation is supported by the view that changes in glucose or oxygen concentration had little effect on the amount or degree of desaturation of palmitic acid [10]. It has been concluded that under conditions of extreme aeration the enzyme system responsible for multiple desaturation tends to catalyse an attack on the methyl end of oleic acid to induce two methylene double bonds carbon atoms 12 and 15.

It is also important to note that the oxygen demand of an organism accumulating lipids is much less than for the same organism being grown for protein because lipid needs no input of oxygen for its synthesis.

Table 2. Effect of different concentrations of "maiz gur" (Hydrol)

Sugar concentration in "maiz gur" (g/100 ml.)	Biomass (g/l)	Lipid (%) "maiz gur" (dry wt. basis)	Lipid (%) glucose (dry wt. basis)
1.0	3.5	5.0	5.5
1.5	4.5	5.0	5.6
2.0	5.0	6.0	6.0
3.0	6.0	7.5	7.8
4.0	6.0	7.0	7.6
5.0	6.0	7.0	7.0
10.0	6.0	7.0	7.0

Table 3. Relative fatty acid composition

	$C_{16:1\%}$	$C_{18:0\%}$	$C_{18:1\%}$	$C_{18:2\%}$	$C_{18:3\%}$	$C_{22:0\%}$
Without aeration	25.0	6.5	23.0	18.1	6.4	2.0
With aeration	25.0	5.3	27.0	18.50	8.5	1.8

Table 4. Sugar (%) during cultivation

Time	Surgar (%)
Initial	4.0
After 8 hr.	3.5
" 24 hr.	2.9
" 32 hr.	2.0
" 48 hr.	1.0
" 56 hr.	0.5
" 72 hr.	0.2

REFERENCES

1. C. Ratledge, *Microbiological production of lipids and fatty acids,* p. 98, in G.G. Birch, K.J. Parker and J.T. Worgan (eds.) "Food from waste" Applied Science Publishers, Barking, England (1976).
2. M. Woodbine, *Microbial fat: Microorganisms as potential producers,* p. 181, in D.J.D. Hockenhull (ed.), *Progress in Industrial Microbiology,* Heywood, London, (1959), Vol. 1.
3. C. Naegeli, and Loew, Ann., 193: 322 (1978).
4. P.S.S. Dawson, and B.M. Craig, *Lipids of Candida utilis: Changes with growth* Can. J. Microbiol, 12 pp. 775 (1966).
5. C. Ratledge, *Production of fatty acids and lipids by a Candida sp. growing on a fraction of n. alkanes predominating in tridecane,* Biotechnol. Bioeng, **10**, 511 (1961).
6. T. Babij, F.J. Moss and B.J. Ralph, *Effects of oxygen and glucose levels on lipid composition of the yeast Candida utilis growing in continuous culture.* Biotechnol. Bioengin., XI, 593-603. (1969).
7. R.F. Thorpe, and C. Ratledge, *Fatty acid distribution in triglycerides of yeasts grown on glucose or n-alkanes* J. Gen. Microbiol. 72, 151. (1972).
8. C.A. Brignoli, E. Kinsella, and J.L. Weihrau, J. Am. Diabet. Assoc. 68, (1976).
9. D.A. Whitworth, and C. Ratledge, *Microorganisms as a*

potential source of oils and fats, Process Biochem. November, p. 21. (1974).

10. T. Babij, F.J. Moss, and B.J. Ralph. *Effect of oxygen and glucose levels on lipid composition of yeast Canadida utilisgrownin continuous culture,* Biotechnol. Bioengin. Vol. XI, p. 602.