PRJ, Vol. 4. (January 1992); P. 56–58, 2 FIGS, 2 TABLES.

CHARACTERIZATION OF METHANOL-UTILIZING BACTERIA AND YEAST AS A SINGLE CELL PROTEIN PRODUCT

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تقييم الخميرة والبكتريا (النامية على الميثانول) كمصدر لبروتين وحيد الخلية

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إستعملت تقنية النمو المستمر (المخمر) للكائنات الدقيقة الحية لدراسة معدل النمو ومحتوى الخلية لكل من البكتريا مثايلوموناس كلارا Methylomonas Clara والخميرة كانديدا بويوديني Candida boidinii. وجد أن أحسن معدل للنمو كان عند التركيز 2 % من الميثانول مع البكتريا وإنخفض المعدل عندالتركيز الأعلى من الميثانول.

معدل النمو للخميرة تحسن مع إزدياد تركيز إضافة الميثانول فازداد المعدل مع إزدياد التركيز (1 % ، 2 % و3 %).

كما وجد أن أعلى نسبة من البروتين والدهن كانت عند التركيز 2 % من الميثانول بالنسبة للبكتريا (مثايلوموناس كلارا) فكانت نسبة البروتين (71.6 %) ونسبة الدهن (4.1 %). ولم تظهر إختلافات في نسبة المحتوى للخلية بالنسبة للخميرة (كانديدا بويوديني).

ABSTRACT

A continuous culture technique was used to study the growth rate and cell contents of methanol-utilizing bacteria Methylomonas clara and yeast Candida boidinii. The changes in propagation of the culture have been investigated. The optimal growth and biomass yield of Methylomonas clara occured at 2% methanol concentration, beyond that an inhibition in growth was observed. However, the specific growth rate and cell yield for Candida boidinii increased with increasing methanol concentration 1%, 2% and 3%. The maximum contents of protein and fat were observed at 2% methanol concentration for Methylomonas clara (71.6%) and (4.1%) respectively. While, no differences in cell contents were observed with Candida boidinii.

INTRODUCTION

Microorganisms are protein sources of growing importance. The microorganisms cultivated on carbohydrates and hydrocarbons will be produced on a large scale in the near future. The importance of yeast and bacteria as test organisms for the study of biological phenomena are well known. Many investigation have been devoted to reveal the effect of nutritional, environmental, and genetical factor on the growth of yeast and bacteria cells, behaviour of the cells and their cell composition (Levin and Cooney, 1973; Haber *et al.*, 1983; Jara *et al.*, 1983; Hamdani *et al.*, 1987; Tani *et al.*, 1988; Lim and Tani, 1988).

Methanol, ethanol and *n*-paraffine are attractive raw materials for the production of fermentation products such as single cell protein, amino acids, organic acids and antibiotics because of their low cost, high purity, high water solubility (methanol) and because they can be easily and cheaply produced.

The existence of methanol assimilating yeast and bacteria is relatively a recent subject of investigation. The microbial technology is still lagging, especially the growth physiology of the involved organism.

Some efforts have been made to illustrate some of the parameters affecting the methanol utilization by yeast (Fonda and El-Masry, 1981) and bacteria (Abu-

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Ruwaida *et al.*, 1990). This work is undertaken with the objective to determine the growth propagation rate and cellular contents of Methylomonas clara and Candida boidinii for a biomass production as a single cell protein products.

MATERIAL AND METHODS

Microorganisms

Bacteria, Methylomonas clara strain ATCC No. 31226 and Yeast, Candida boidinii strain ATCC No. 56507 were used throughout this study.

Cultivation

The cultivation was carried out at 30°C in a 10 liter MBR fermentor (MCS 10, MBR Bio reactor Ltd. Switzerland). The dissolved oxygen concentration in the medium expressed in percentage was (20%). The rotation speed of the impeller was between 200 to 400 rpm.

The minimal salts medium used for the growth of bacteria contained, (per liter of distilled water),: $Na_2HPO_4 \cdot 2H_2O$, 2.75 g; KH_2PO_4, 1.36 g; $MgSO_4 \cdot 7H_2O$, 0.04 g; $(NH_4)_2SO_4$, 2.0 g; Fe_3C_2, 10 mg; NaCl, 2 g. The trace elements were added to the medium at 1 ml per liter which contains (in mg) $ZnSO_4 \cdot 7H_2O$, 0.08; $MnSO_4 \cdot 4H_2O$, 0.03; $CuCl_2 \cdot 2H_2O$, 0.03; $NiCl \cdot 6H_2O$, 0.02; $CaCl_2 \cdot 6H_2O$, 0.1; $CaCl_2 \cdot 2H_2O$, 0·2 and H_3BO_3, 0·2.

The pH of the medium was adjusted between 7 to 7.1.

The yeast was grown on the following medium (g/liter):

Bacto-yeast extract	3
Malt extract	3
Bacto pepton	5
Bacto dextrose	10

The pH of the medium was set up at 4.44. The methanol concentrations in the feeds were 1%, 2% and 3%.

Analytical Methods

Growth was measured turbidimetrically at 610 nm while referring to a standard free media curve. Dry weight was estimated after drying overnight at 80°C. The protein was estimated by the macro-Kjedahl method (AOAC, 1980). The fat content was determined by diethyl ether extraction method (AOAC, 1980). The soluble carbohydrates was estimated as nitrogen free extract (NFE) method (AOAC, 1980).

RESULTS AND DISCUSSION

The results showed that a methanol feed concentration up to 2% produced the highest growth and yield coefficient, but also high cellular protein content when Methylomonas clara was used.

Changes of cell concentration of Methylomonas clara during the batches cultivation at 1% and 2% methanol concentration in the biomass fermentor are illustrated in Fig. 1. The lag phase has extended with increase in the methanol concentration.

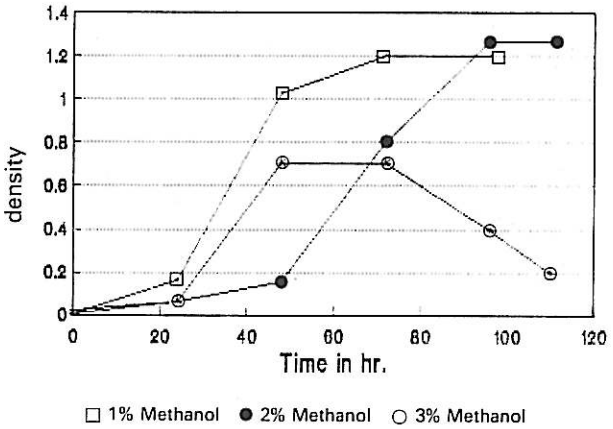

FIG. 1. Effect of methanol on growth rate of Methylomonas clara.

The specific growth rate and cell yield decreased with increasing methanol concentration, while the maximum content of crude protein was observed at the concentration of 2% methanol. However, 3% methanol concentration inhibited the growth rate of this organism. Changing the methanol concentration from 1% to 2% increased the crude protein and fat 62 to 71.63% and 3.05 to 4.1% respectively, while the carbohydrate and ash were decreased. The cellular contents of the protein, fat, carbohydrates and ash were determined (Table 1).

Growth behaviour of Candida boidinii is illustrated in Fig. 2.

The lag time became longer with increasing methanol concentration. The growth rate increased with increasing methanol concentration, while no differences were observed on cellular contents such as crude protein and fat (Table 2). Inhibition of

Table 1. The Effect of Methanol Concentrations on Crude Protein, Fat, Carbohydrates, Ash and Moisture Contents in Methylomonas Clara (in Percentage)

Methanol	1%	2%
Moisture	4.74	6.49
Protein	62.00	71.60
Fat	3.05	4.10
Carbohydrates	26.26	17.30
Ash	8.69	7.00

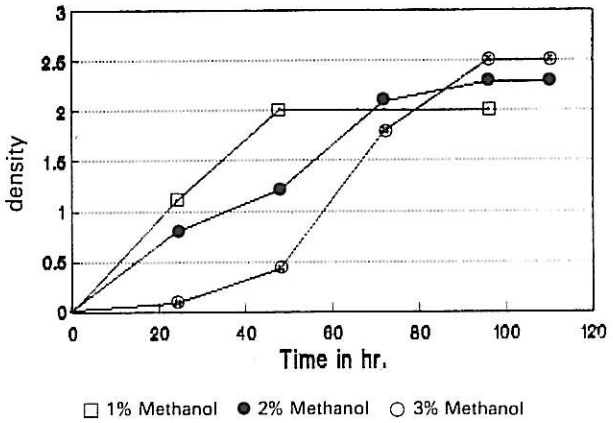

FIG. 2. Effect of methanol on growth rate of Candida Boidinii.

Table 2. The Effect of Methanol Concentrations on Crude Protein, Fat, Carbohydrates, Ash and Moisture Contents in Candida Boidinii (in Percentage)

Methanol	1%	2%	3%
Moisture	5.3	4.1	3.4
Protein	40.25	40.20	39.2
Fat	8.07	8.10	7.5
Ash	7.01	6.3	5.0
Carbohydrates	44.67	45.4	46.9

growth rate was observed at 3% methanol concentration. A similar observation has been reported for other methanol utilizing bacteria (Kim and Ryu, 1976). However, unlike Methylomonas clara, the 3% methanol concentration in the feed did not inhibit the growth rate of Candida boidinii.

CONCLUSION

The Methylomonas clara culture gave a maximum yield at 2% methanol concentration beyond which the yield decreased. The decrease in yield at high methanol concentration is associated with a slight increase in the cellular contents of protein and fat.

The optimal concentration for Candida boidinii was not defined and at 3% methanol concentration did not inhibit their growth.

The present investigation resulted in defining the optimal concentration of methanol as the sole carbon source in the medium used for growing Methylomonas clara. The methanol inclusion in the medium supported satisfactory levels of growth, yield and product. The cellular contents of crude protein (71.6%) for Methylomonas clara and (40%) for Candida boidinii was within acceptable levels for single cell protein producing cultures (Krug *et al.* 1979).

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