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MEDIUM OPTIMIZATION FOR BIOMASS PRODUCTION AND PROTIEN CONTENT OF CANDIDA UTILIS K50

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ABSTRACT

Molasse medium containing different concentrations of $(NH_4)_2 SO_4$, $(NH_4)_3 PO_4$, urea, KCI, and P_2O_5 were compared with the medium used for commercial production of *C. utilis* in a factory south of Iraq. An efficient medium, which produced 19. 16% dry wt. and 5. 78% protein, was developed. The effect of adding various concentrations of micronutrients ($FeSO_4$, 7T20, $MnSO_4$. 7H20, $ZnSO_4$. 7H20) was also studied. Results showed that $FeSo_4$. 7H20 caused a noticeable increase in both dry wt. and protein content of the yeast.

INTRODUCTION

Yeasts, as other microorganisms, require carbon, nitrogen, phosphur. and other sources for growth and reproduction (Reed, 1982). Adding macronutrients (e.g. NPK) and micronutrients (e.g. Fe, Mn, Zn stlts) to the propagation medium of torula yeast improve biomass yield and protein content.

Lorenze (1969) found that productivity of *C. utilis* decreases upon decreasing amounts of phosphate salt in the medium, while protein content of cell was not effected. Good multiplication of yeast cells was achieved when medium was enriched with NPK salt (Lugauskiene, 1968; Valavicious, 1967 a). A direct relationship was found between macro- and micronutrient salts in improving cell yield and protein content (Valavicious, 1967b).

This study was performed to improve yield efficiency and protein content of *C. utilis* K50 which is used in a factory for commercial production of fodder yeast.

MATERIALS AND METHODS

Yeast and substrate :

Candida utilis mutant K50 which is used the commercial production of fodder yeast factory (FYF) south of Iraq was used in this study. Black strap cane molasse, after being diluted to the desirable concentration, was used as main substrate. Molasse was obtained, as a by-product, from a sugar factory.

Macro- and micronutrients :

Portion of 0.1 and 0.2g of each of $(NH_4)_2SO_4$, $(NH_4)_3PO_4$, urea, KCl, and P_2O_5 were added as macronutrient salt to each 50 ml of FYF molasse medium as shown in table (1). Micronutrient salt included $FeSO_4 \cdot 7H_2O$, and $ZnSO_4 \cdot 7H_2O$ and were separately added to each liter of medium in the levels of 2.5, 5, 7.5, and 10 mg (table 2).

FYF first inoculum medium :

Composed of 2g yeast ext., 0.5g amm. diphosphate, 100g sucrose in 1L distilled water. After adjusting pH to 4.5, the medium was sterilized at 100 c°/30 min. Sterilization was repeated twice after 24 and 48 hrs.

FYF secod inoculum medium :

Molasse of 12° Brix was prepared by diluting 1200 g-molasse of 40° Brix with 2800 ml dist. water.

It is composed of 1200g molasse (12 Brix) and 0.5g amm. diphos. with pH4.5. The medium was sterilized three times at 100° C/30 min.

Seed Yeast preparation :

First inoculum medium was inoculated in a flask, with *C. utilis* K50 and incubated at 32°C/48 hrs., content of the flask was added to the second inoculum medium and incubated at 32°C/48 hrs.

Propagation procedure :

After molasse medium (4% sugar) was distributed in 50 ml portions into 250 ml flask, nitrogen salt, P_2O_5, and KCl (to give 3.5 g (N), 1.5g (P), and 1g (K), respectively/100g sugar) were added to the FYF medium as shown in table (1). Each 50 ml of the medium was inoculated with 0.1 ml seed yeast suspension (previously diluted to 0.9 absorbance in Bousch and lomb spectroph. 20 at 625 nm). **FYF** medium (no. 1) was enriched with the same micronutrients which usually are used in FYF. All media were incubated in a shaking incubator (Gallenkamp) of 100 times /min. at 30 ± 1°C/18 hrs. Cells were centrifuged (2000g) for 5 min. and washed with water twice.

Micronutrients were added to the FYF medium (no. 1) and the modified medium no. 5.

Determination of dry wt., efficiency and protein :

Cell dry wt. of yeasts was determined after drying in an air oven at 105 C and until constant weight. Efficiency of yield was calculated according to Harrison (1968) equation :

$$\% \text{ Yield effi.} = \frac{\text{Net dry wt. (g)}}{\text{Theoritical yield (g)}} \times 100$$

Semimicrokjeldahl method (AOAC, 1970) was used to determine total nitrogen of yeast. According to Majonnier *et al* (1955), crude protein was calculated after multiplying (N) by 6.25.

RESULTS AND DISCUSSION

Effect of macronutrients :

Table (1) shows the effect of macronutrient salts on dry wt. and yield efficiency of C. *utilis* K50 grown in molasse medium. Dry wt. and efficieney of FYF medium was 0.2186g/50 ml molasse and 60.72%, respectively, $(NH_4)_2SO_4$ and $(NH_4)_3PO_4$ are the only salts to be added to the FYF propagation medium. Medium no. 5, on the other hand, was selected among other eight modified media due to its dry wt (0.2605 g/50ml) and yield efficiency (72.36%). This was contained by subsituting 1/2 the quantity of $(NH_4)_2SO_4$ with urea in addition to KCI. An increase of 19.195% in dry wt. and 11.64% in efficiency was achieved by medium no. 5 compared to FYF medium.

Crude protein of media was ranged from 42.52% to 50.08% (table 1). In fact. more than one medium were better than FYF medium in protein content, but highest percentage (50.08) was achieved by medium no. 5; 2.83% increase from FYF medium.

Effect of micronutrients on dry wt. :

Biomass Production and Protein Content of Candida Utilis K 50

Table (1)

Effect of Macronutrients (NPK) on some Technological

Parameters of Production.

Medium* No.	gm/50 ml molasse (NH4)2SO4	(NH4)3PO4	Urea	K C1	P2O5	DryWt. of yeast (X 10-2)	Protein (Kjeldalh) (%)	Efficiency (%)
1 (FYF)	0.2	0.2	—	—	—	21.86	47.25	60.72
2	0.2	0.2	—	0.2	—	21.07	46.46	58.53
3	—	0.2	0.2	—	—	19.43	47.77	53.97
4	—	0.2	0.2	0.2	—	47.25	47.25	60.77
5	0.1	0.1	0.2	0.2	—	26.05	50.08	72.36
6	0.1	0.1	0.1	0.2	0.2	15.11	45.67	41.97
7	0.1	—	0.1	0.2	0.2	13.05	44.57	36.25
8	0.2	0.2	0.1	0.2	—	15.36	47.25	42.67
9	0.1	—	0.2	0.2	—	12.35	46.77	34.30
14.80	42.52	41.11	10	0.2	0.2	0.2	0.2	0.2

* No. 1 medium is FYF propagation medium, other nine media are modified propagated media

FYF medium (no. 1) and modified medium no. 5 were compared for dry wt. and protein after the addition of three micronutrients. Table (2) shows the increases in cell dry wt. Generally, dry wt. of both media was improved after adding the micronutrients. However, medium no. 5 produced highest increases in dry wt. at all concentrations used compared to medium no. 1.

Highest increases in dry wt.; 32.511, 32.165, and 30.462% were obtained when 10 mg $FeSO4.\ 7H2O$. 10 mg $ZnSO4.\ 7H2O$. and 2.5 mg $MnSO4.\ 7H2O$. respectively, per IL were seperately added to medium no. 5. Increases in dry wt. improved as Fe and Zn levels increased in the medium, but this was not so with Mn salt.

Effect of micronutrients on protein :

Highest increase in protein content (11.109%) of medium no. 5 was obtained after adding 10mg of $FeSO4.\ 7H2O$ IL molasse, while an increase of only 5.452% was achieved by FYF medium, under same conditions. Despite the addition of various concentrations of Mn and Zn salt caused a noticeable increase in protein content of both media, remarkable increase was obtained with Fe salt. A direct relationship was found between Fe level and protein content of yeasts.

Considerable increase in dry wt., yield efficiency, and protein content of modified medium no. 5 could be referred to the effect of some nutritional salts added to the medium.

Table (2)

Increases (%) In the cell dry wt. after addition of micronutrients to the propagation medium

Micronutrients	Concentrients mg/L	(%) Increase in Dry Wt FYF medium (No. 1)	(%) Increase in Dry Wt Modified medium (No. 5)
		0.000*	19.165*
$FeSO_4.\ 7H_2O$	2.5	0.228	24.928
	5.0	0.548	29.685
	7.5	7.500	32.292
	10.0	7.730	32.521
$Mn\ SO_4.\ 7H_2O$	2.5	5.671	30.462
	5.0	5.900	30.051
	7.5	6.174	25.843
	10.0	6.083	24.013
$Zn\ SO_4.\ 7H_2O$	2.5	0.686	24.013
	5.0	7.272	31.789
	7.5	7.455	31.926
	10.0	7.547	32.155

* Medium (1) was fixed as a control treatment for evariuation of the increases in dry wt. after addition of both macro-and micronutrien,s. This means that each number in last column represents the increase in the cell wt. after the addition of macronutrient (19.165) plus the increase after addition off micronutrients.

Table (3)

Increases in the total crude protein after addition of micronutrients to the propagation medium

Micronutrients	Concentrients mg/L	% Increase in total crude Protein FYF medium (No. 1)	Modified medium (No. 5)
		0.000*	2.83*
FeSO4. 7H2O	2.5	1.438	5.988
	5.0	1.523	6.178
	7.5	4.147	9.839
	10.0	5.459	11.109
Mn SO4. 7H2O	2.5	2.221	7.088
	5.0	1.523	6.051
	7.5	3.639	5.967
	10.0	1.798	6.051
Zn SO4. 7H2O	2.5	2.031	7.659
	5.0	3.914	9.881
	7.5	4.358	6.051
	10.0	6.051	9.818

* Medium (1) was fixed as a control treatment to evaluate the increases in protein content after addition of both macro- and micronutrients.

using various sources of (N) improved growth of yeasts and reproduction. Van Uden (1971) stated that urea is better than $(NH_4)_2SO_4$ in enhancing yeast growth.

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وسط زرعي مثالي لانتاج الكتلة الحيوية وتحسين المحتوى البروتيني لخميرة *Candida utilis* K50

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الخلاصة

تمت مقارنة وسط المولاس الحاوى على تراكيز مختلفة من كبريتات الامونيوم ، فوسفات الامونيوم ، يوريا ، كلوريد البوتاسيوم ، وخامس اوكسيد الفسفور مع الوسط المستخدم لانتاج خميرة التوريلا في مصنع يقع جنوب العراق. امكن تطوير وسط زرعي ذو كفاءة انتاج عالية وذلك عندما ازداد الوزن ١٦ر١٩٪ والمحتوى البروتيني ٧٨ر٥٪ عن الوسط المستخدم في المصنع . كما ودرس تأثير املاح التغذية الثانوية (املاح الحديد والمنغنيز والخارصين) على كل من الوسط المطور في الدراسة هذه والوسط المستخدم في المصنع . اظهرت النتائج ان املاح الحديد ادت الى حصول أعلى الزيادات في الوزن الجاف والمحتوى البروتيني للخميرة مقارنة ببقية الاملاح الثانوية