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ETHANOL AS A SUBSTRATE FOR SINGLE CELL PROTEIN PRODUCTION

Growth Studies of Ethanol-Utilizing Yeast Strain *Candida utilis* EUY-G2*

Salah-ud-Din, M. Yaqoob Choudry and M.I.D. Chughtai+

PCSIR Laboratories, Lahore-16

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Candida utilis EUY-G2 was cultivated in batch culture on ethanol as a sole source of carbon. The optimum growth of this yeast strain was observed at 30 to 33° and at pH 5.0. Trace amounts of Fe^{++}, Mg^{++} and Ca^{++} ions were required. Diammonium hydrogen phosphate was the most effective nitrogen source. The growth rate was dependent on the concentration of ethanol, reaching a maximum at 1.0 % (w/v). The maximum specific growth rate and cell yields were 0.46 hr^{-1} and 75 % (w/v) respectively. The biomass contained 53.4 % crude protein, 0.7 % crude fat and 8.1 % nucleic acid. The amino acid profile, except for methionine, compared well with FAO reference levels.

Key words: Ethanol, Protein, Candida utilis.

INTRODUCTION

The advantages of employing ethanol-utilizing micro-organisms as a source of single-cell protein (SCP) has been discussed in the literature [1-3]. In addition, much of the recent (SCP) work has centred on yeasts [4-9], because yeast cells can be easily harvested because of their larger particle size, low nucleic acid contents, their ability to grow at a low pH (4.0 to 5.0) which minimizes chances of contamination, and of better acceptance as an edible material.

The present paper deals with experiments to establish optimal growth conditions, and the most economical medium composition for the strain, *C. utilis* EUY-G2, capable of utilizing ethanol as a sole carbon source. Protein, amino acids, nucleic acid and lipid contents of the biomass were determined.

MATERIALS AND METHODS

Microorganism. The microorganism used in this study was Candida utilis EUY-G2, which was locally isolated and identified in the Lahore Laboratories. The taxonomical description of the strain has been reported elsewhere [10].

Cultivation medium. The composition of the medium M-1 used for growth studies is shown in Table 1. pH of the medium was adjusted with 0.1 N NaOH or 0.1N HCl solutions. The solution was autoclaved and cooled to room temperature before the addition of ethanol.

Table 1. Composition of medium M-1.

Component	Amount
$(NH_4)_2SO_4$	5.0 g/l
KH_2PO_4	1.0 g/l
$MgSO_4.7H_2O$	0.5 g/l
$CaCl_2.2H_2O$	0.1 g/l
NaCl	0.1 g/l
Ethanol	as desired
Distilled water	to 1-litre
pH	4.0

Procedure for cultivation. The yeast strain was cultured on yeast extract – malt extract agar slant for 48 hr at 30 ± 1°. Inoculated flasks were incubated on a rotary shaker at 125 r.p.m. Reproducibility of data was examined over at least two serially inoculated flasks under identical conditions.

Analytical methods. Optical density (OD) of the suspension was measured with a photometer (EEL-Model 197) at a wavelength of 610nm, using distilled water as reference. Dry weight of the cells was then read from a corresponding caliberation curve (OD readings at 610nm vs dry cell weight). Cell yield was calculated on the basis of cell mass formed after ethanol consumption. Specific growth rate (μ) in batch culture was estimated as follows (Miller and Hougton) [11].

$$\mu\ (hr)^{-1} = \frac{0.693}{\text{doubling time}}$$

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+Corresponding address: Institute of Chemistry, Punjab University, Lahore 1.

The doubling time was determined graphically.

Ethanol in the fermentation medium was determined by oxidation with acid potassium dichromate according to Barnard and Karayannis [12].

The total protein content of dried cells was determined by multiplying values of the cellular nitrogen [13] by a factor of 6.25.

The amino-acid composition was determined with an auto-amino acid analyzer (EEL, High Speed Amino Acid Analyzer, Model 193). The procedure employed for the hydrolysis of proteins was that given by Block and Weiss [14].

The total nucleic acid content of cells was estimated according to Levine and Cooney [15] by extracting a cell sample with perchloric acid.

The crude fat of the yeast sample was determined by Soxhlet's method.

RESULTS AND DISCUSSION

Effect of pH. The organism grew best in the pH range of 4.0 to 5.5 (Fig. 1). The optimum pH for growth was found to be 5.0. The growth was very poor below pH 2.0 or above pH 7.0.

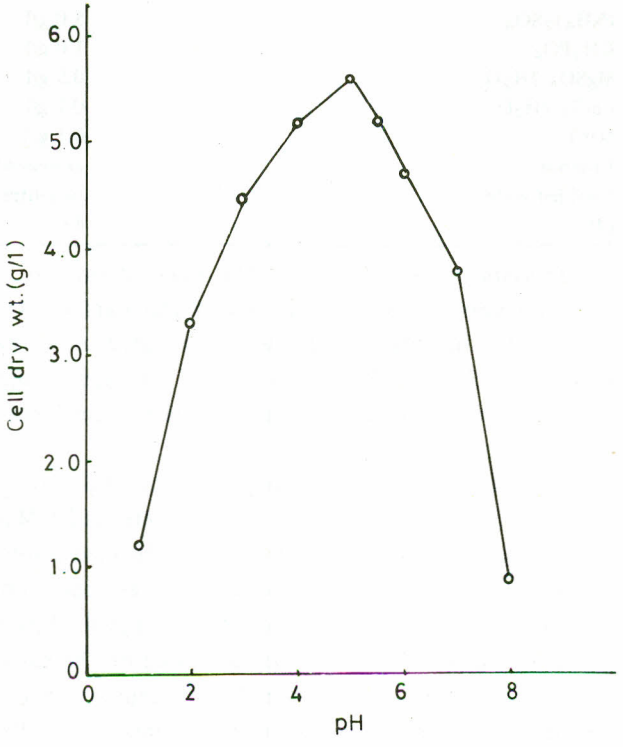

Fig. 1. The effect of initial pH on the growth of *C.utilis* EUY-G2. Shake culture at 30° for 30 hr in medium M-1, containing 1.0 % (w/v) ethanol.

Effect of temperature. At 20° or below, very little growth was observed. It was found that the yeast grew over the range of 20–45° (Fig. 2). The optimum temperature range for growth was 30-33° and there was no difference in results at this range.

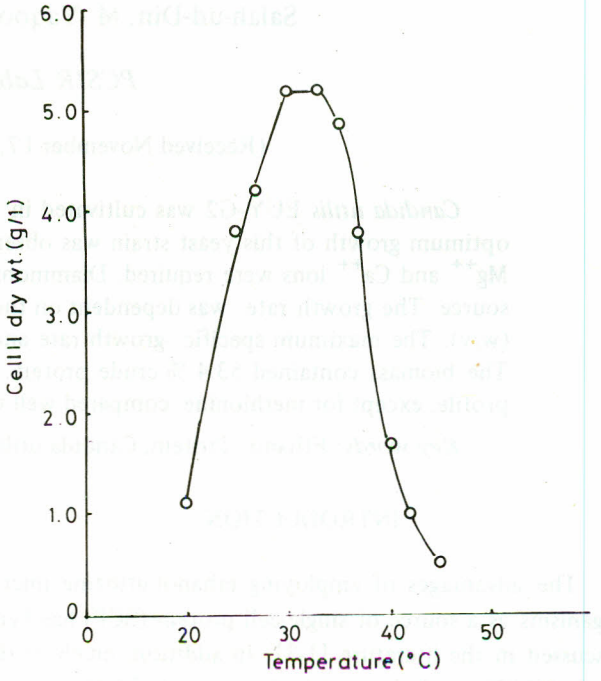

Fig. 2. Effect of temperature on the growth of *C.utilis* EUY G2. Shake culture was carried out in medium M-1, containing 1.0 % (w/v) ethanol.

Effect of mineral ions. The effect of different concentrations of metal ions on the specific growth rate and cell yield of *C. utilis* EUY-G2 was also examined (Tabe 2). It is evident that I^-, Zn^{++} and Mn^{++} ions had no effect on growth, but Fe^{++} ion at a concentration of 0.2 mg/litre stimulated growth slightly. Potassium, Mg^{++}, Ca^{++} and Na^+ ions stimulated growth.

Effect of Organic Nutrients. The effect of complex organic nutrients at a concentration of 10, 50 and 100 mg/litres is given in Table 3. Only yeast extract and peptone at a concentration of 100 mg/litre were effective in enhancing the growth of the organism. Other organic nutrients (malt extract, cas-amino acids and vitamin mixture) exercised no significant effect on the growth.

The cell concentration of the yeast strain showed increase upto the concentration of 100 mg/litre of both the yeast extract and peptone, while it remained constant at above 100 mg/litre concentration (Fig. 3).

Effect of carbon sources. A variety of carbon sources other than ethanol were examined for their ability to support the growth of *C. utilis* EUY-G2 (Table 4). It was

Table 2. Effect of mineral ions on the growth of *C. utilis* EUY-G2.

Metallic compound used	Concentration of metallic compound (g/1)	Growth rate (μ) hr.$^{-1}$	Cell yield (%)
KH_2PO_4	0.000	0.184	46.2
	0.500	0.289	46.2
	1.000	0.380	62.0
	2.000	0.365	60.0
$MgSO_4.7H_2O$	0.000	0.204	33.3
	0.200	0.285	46.7
	0.500	0.385	62.2
	1.000	0.315	51.4
$FeSO_4.7H_2O$	0.0000	0.385	62.5
	0.0001	0.385	63.4
	0.0002	0.385	64.5
	0.0003	0.365	60.2
KI	0.000	0.385	62.5
	0.001	0.365	60.3
	0.002	0.346	56.1
	0.003	0.315	51.4
$ZnSO_4.7H_2O$	0.000	0.385	62.5
	0.001	0.365	60.2
	0.002	0.247	40.5
	0.003	0.204	33.7
$MnSO_4.7H_2O$	0.000	0.385	62.5
	0.001	0.385	62.5
	0.002	0.346	56.4
	0.003	0.315	50.6

Shake culture at 30 ± 1° for 30 hr. using medium M-1, with 1.0 % (w/v) ethanol.

found that the isolate could also grow in media containing sodium acetate and glucose. The other substrates did not support growth.

Effect of different nitrogen sources. Five inorganic compounds $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$, NH_4Cl, NH_4NO_3 and $NaNO_2$ at a fixed nitrogen concentrations 0.1 % were employed as the sole nitrogen source in the growth medium. Results (Table 5) show that $(NH_4)_2HPO_4$ was the most suitable nitrogen source. No growth was observed with sodium nitrite.

Effect of ethanol concentration. The effect of ethanol concentrations (0.5-8.0 % (w/v)) on the growth of the yeast isolate was investigated. It is apparent from Fig. 4 that the specific growth rate increased rapidly with an increase in ethanol concentration upto 1.0 % (w/v) and decreased almost linearly thereafter. No growth was observed at ethanol concentration above 7.0 % (w/v) Table 6).

Table 3. Effect of organic nutrients on the growth of *C. utilis* EUY-G2.

Organic nutrient used	Concentration of orgnic nutrient (mg/1)	Dry cell weight (g/1)
Peptone	0	6.20
	10	6.20
	50	6.28
	100	6.50
Yeast extract	0	6.20
	10	6.20
	50	6.35
	100	6.68
Malt extract	0	6.20
	10	6.20
	50	6.25
	100	6.39
Cas-amino acid	0	6.20
	10	6.20
	50	6.28
	100	6.42
Vitamine mixture*	0	6.20
	10	6.28
	50	6.37
	100	6.70

*Vitamin mixture contained thiamine HCl 4 mg, riboflavin 1 mg, pyridoxine-HCL 4 Ca pantothenate 4 mg, inositol 20 mg, p-amino-benzoic acid 1 mg, niacin 4 mg and biotin 0.1 mg in 1 litre.

Shake culture at 30 ± 1° for 30 hr. using medium M-1 with 1.0 % (w/v) ethanol.

Table 4. Effect of different carbon sources on the growth of *C. utilis* EUY-G2.

Substrate	Concentration g or ml/100 ml	Growth	Final pH
Ethanol	0.5 ml	++++	2.6
Sodium	1.0 g	+++	5.4
Acetaldehyde	1.0 ml	+	4.6
Methanol	1.0 "	–	5.0
Isopropanol	1.0 "	–	5.0
Isobutanol	1.0 "	–	5.0
Glycerol	1.0 "	++	4.6
Glucose	1.0 g	++++	2.6

Shake culture at 30 ± 1° for 30 hr. using medium M-1.

Composition of the improved medium M-2. Based upon a series of the above experiments, an improved medium was developed shown in Table 7. The effect of medium components on the growth of *C. utilis* EUY-G2 is depicted in Fig. 5. A maximum cell biomass of 7.50 g/litre was obtained with improved medium M-2 as compared with a cell concentration of 6.20 g/litre obtained with the original growth medium M–1.

Typical growth curve. A typical growth curve of the organism is shown in Fig. 6. The yeast strain was inoculated into Medium M-2 and incubated at 30 ± 1° with shak-

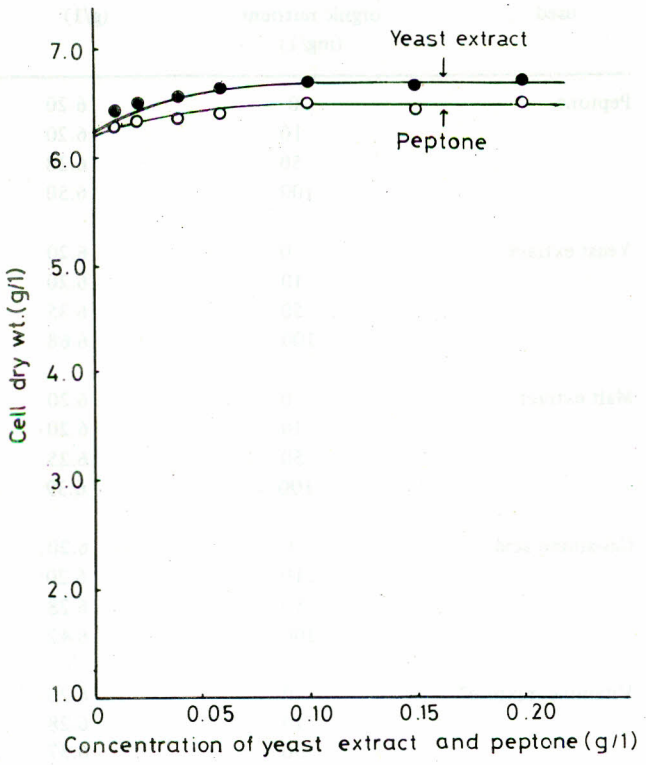

Fig. 3. Effect of yeast extract and peptone on the growth of *C.utilis* EUY-G2.Shake culture at 30° for 30 hr using medium M-1 with 1.0 % (w/v) ethanol.

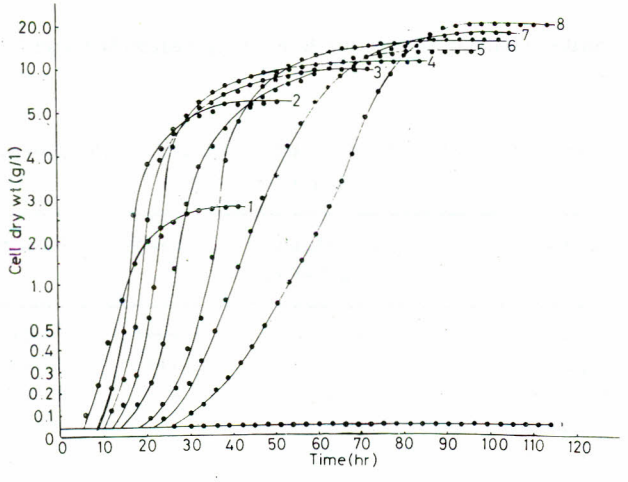

Fig. 4. Effect of ethanol concentration on the growth of *C.utilis* EUY-G2. Shake culture at 30° in medium M-1, using various concentration of ethanol. Curve 1, 0.5 %; 2, 1.0 %; 3, 2.0 %; 4, 3.0 %; 5, 4.0 %; 6, 5.0 %; 7, 6.0 %; 8, 7.0 %; 9, 8.0 % (w/v) ethanol.

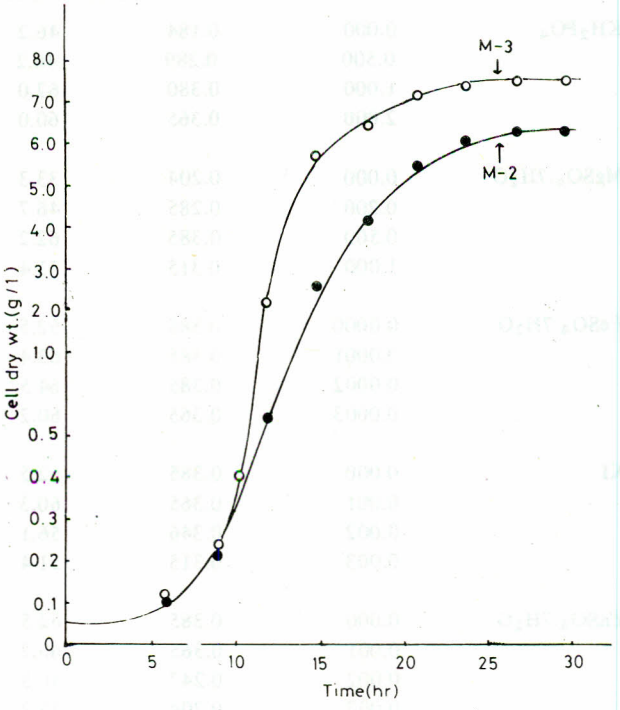

Fig. 5. Effect of medium M-1 and M-2 on the growth of *C.utilis* EUY-G2: Shake culture at 30° for 30 hr using medium M-1 and M-2, with 1.0 % (w/v) ethanol.

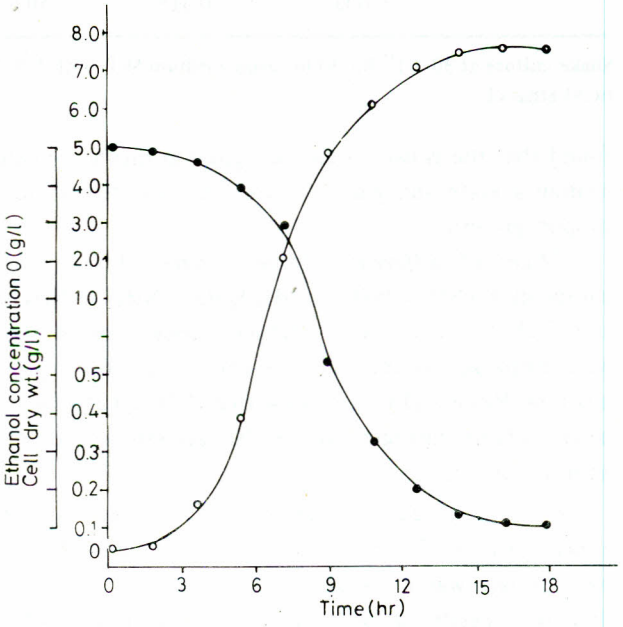

Fig. 6. Typical growth curve of *C. utilis* EUY-G2 in batch culture, containing medium M-2 with 1.0% (w/v) ethanol.

Table 5. Effect of nitrogen sources on the growth of *C. utilis* EUY-G2.

Nitrogen source	Concentration of N_2 (g/1)	Cell dry wt. (g/1)	Ethanol consumed (g/1)	Yield %
$(NH_4)_2HPO_4$	1.0	7.10	9.4	75.5
$(NH_4)_2SO_4$	1.0	6.40	9.0	71.1
$(NH_4)Cl$	1.0	6.17	8.7	70.9
NH_4NO_3	1.0	5.03	8.0	62.8
$NaNO_2$	1.0	–	–	–

Shake culture at 30 ± 1° for 30 hr. using medium M-1, with 1.0 % (w/v) ethanol.

Table 6. Growth characteristics of *C. utilise* EUY-G2 in batch culture on ethanol at various initial concentrations.

Intial conc. of ethanol g/l	Maximum dry cell weight g/l	Cell yield (%)	Time lag (hr.)	Doubling time (hr)	μ (hr^{-1})
5.0	2.80	56.0	5.0	2.3	0.300
10.0	6.20	62.0	8.0	1.8	0.385
20.0	9.80	49.0	10.0	2.0	0.346
30.0	11.20	37.3	12.0	2.2	0.315
40.0	13.60	34.0	14.0	2.6	0.266
50.0	15.60	31.2	18.0	3.5	0.198
60.0	17.20	28.6	22.0	4.8	0.144
70.0	18.80	26.8	25.0	7.5	0.092
80.0	–	–	–	–	–

Shake culture was carried out at 30 ± 1° in medium M-1, using various concentration of ethanol.

Table 7. Composition of medium M-2.

Component	Amount
Ethanol	1.0 % (w/v)
$(NH_4)_2HPO_4$	5.0 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
$CaCl_2.2H_2O$	0.1 g
NaCl	0.1 g
$FeSO_4.7H_2O$	0.0002 g
Yeast extract	0.100 g
Distilled water	to 1-litre

pH: adjusted to 5.0.

ing. The maximum cell concentration obtained was 7.50 g/litre after 15 hr. The cell yield in this experiment was calculated to be 75 %. The maximum growth rate obtained during the logrithmic growth period was 0.46 hr^{-1}.

Cell composition. The crude protein content of the cells was found to be 47.2 to 53.4 %. This value was closely related to the typical value of protein content (45–52 %) for yeast [16] The total nucleic acid content was estimated to be about 8.4 % of the dry cell weight. This value is lower than the nucleic acid contents in ethanol-utilizing yeasts which ranged from 6 to 10 % of the total biomass as reviewed by Masuda [4]. The amount of crude fat (0.7 %) was below the lower limits of the value observed in yeasts (1 to 6 %) [5].

Table 8. Amino acid composition of ethanol fermenting yeast and other protein sources (g/100 g of protein).

Amino acid	FAO reference*	*C. utilise* EUY-G2 (ethanol fermenting yeast)
Lysine	4.2	7.8
Threonine	2.8	4.2
Valine	4.2	5.2
Methionine	2.2	1.1
Isoleucine	4.2	4.3
Leucine	4.8	7.5
Phenylalanine	2.8	4.0
Tryptophane	1.4	1.6

*Masuda [5] (1974).

The total essential amino acid content was 36.0 % of the crude protein. A comparison of amino acid profile obtained from *C. utilis* EUY-G2 and FAO standard is given in Table 8. As is typical for yeasts, sulphur-amino acid methionine was below the standard requirements, while all other essential amino acids were in excess.

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