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Optimization of Sucrose Ethanol Fermentation for K, Na, Ca, and Cu Metal Contents

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ABSTRACT

The effect of the trace metals Cu, K, Na, and Ca, separately or in mixture, on fermentation time, ethanol production rate, and cell growth in the fermentation of synthetic media containing sucrose is discussed. The results are related to the range of contents found in raw materials, molasses and raisins, in order to determine their optimum concentrations for alcohol production.

Index Entries: Copper; K; Na; Ca; ethanol; fermentation.

INTRODUCTION

Raw materials used in alcohol production contain trace metals such as K, Na, Ca, and Cu, often in large amounts. Many investigators have focused their interest on the effect of Ca on alcohol fermentation. Specifically phosphates in the fermentation media decrease the uptake of Ca (*1*), which protects yeast cells from the toxic effect of Cd (*2*). The uptake of mono and divalent cations by *Saccharomyces cerevisiae* cells has been correlated to the rejection by the cells of calcium ions (*3*). It was found that calcium chloride activates the enzyme pyruvic decarboxylase (*4*). Relatively high concentrations of Ca^{2+} result in an uptake by yeast cells that

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has a harmful effect (*2,5*), reducing cell growth (*6*). Recently it was reported that the fermentability of raisins is correlated with traces of Cu (*7*); relatively high Cu concentrations reduces the fermentation rate from batch to batch in the cell recycle process (*8*). Although a number of publications have appeared discussing the effect of Ca and some other elements on ethanol fermentation, a systematic study concerning the ethanol production rates obtained in the presence of the metals Cu, Ca, K, and Na contained in various high concentrations in raw materials, is not found in the literature. Due to relative high concentrations in raw materials of the metals K, Na, and Ca and the inhibitory effect caused by the trace element Cu (*8*), an optimization study of ethanol fermentation for Cu and K, Na, and Ca contents is also necessary. The latter is the subject of the present work.

METHODS

Growth of Culture and Fermentations

Using baker's yeast, a mono cell culture was isolated and it was grown on complete medium containing yeast extract agar (*9*). This was inoculated in a liquid minimal medium. All batch fermentations were carried out at 30°C, using sucrose solutions with a density of 7°Be and yeast wet wt cell concentration of 20 g/L obtained as described earlier. Before the addition of cells and sucrose, solutions of copper sulfate, calcium chloride, potassium chloride, and sodium chloride were added separately or in mixture at the required concentrations. In order to obtain for each of the metals the concentration at which the fermentation time becomes lower, batches containing various concentrations of each were performed. Likewise, batch fermentations were made in the presence of Cu, K, Na, and Ca in mixture at their optimum concentration, found by the curves of fermentation time vs concentration of each of the metals. The kinetics of fermentation was followed by measuring the °Be density at various time intervals.

Biomass Estimation

The biomass was determined by measuring the optical density at 700 nm. Standard curves of optical density vs wet wt cells (g/L) were prepared for the estimation of the biomass concentration in the sample.

Determination of K, Na, Ca, and Cu in Raisin and Molasses

The determination of K and Na were made by flame photometry using a flame photometer (Turner, model 510). Ca and Cu were determined by atomic absorption spectrometry with a Perkin Elmer instrument model

560, air acetylene flame, and hollow cathode lamp. The treatment of samples was carried out according to a previous study (*7*). In the case of Ca to avoid interference of phosphates, sulfates, aluminum, and silicon, a solution of lanthanum oxide treated with hydrochloric acid was used (*10*). This solution was added to the solution of samples in a percentage proportion 0.1–1%. For the preparation of the lanthanum oxide solution, 58.64 g lanthanum oxide were added to 50 mL distilled water and treated with 250 mL hydrochloric acid. This solution was diluted to 1:1.

RESULTS

The raw materials, beet molasses and raisins, used in ethanol production are rich in metals such as K, Na, and Ca and contain the trace element Cu at various concentrations (Table 1). The metal concentrations used in synthetic sucrose media in this work are within the range of the concentrations found in molasses and raisins.

Figure 1 shows that the fermentation time decreases as the concentration of metal increases from 0 to 5 ppm Cu, 1000 ppm Ca, 500 mg K_2O/100 g, and 500 mg Na_2O/100 g. At higher concentrations the fermentation time increases. These concentrations of Cu, Ca, K, and Na are optimum for the alcoholic fermentations of sucrose solutions. The improvement of the ethanol production rate in the presence of each of the metals separately at their optimum concentrations, as compared in the absence of metals, is clearly shown in Fig. 2B. Potassium, used alone, has the major effect and copper the lowest. When all of these metals were mixed at their optimum concentration, a drastic drop of the fermentation time was obtained, as compared with the absence of metals (Fig. 2A). In this case the fermentation time achieved is about equal to that obtained by Ca and Na separately and clearly lower than that of Cu.

Figure 3A indicates the increase of cell growth in the solution of the metals containing their optimum concentrations as compared to those obtained by the absence of it. This increase of cell growth is estimated to be 135%. The highest augmentation of cell growth is achieved by potassium (Fig. 3B), yielding to a greater final cell concentration than that obtained by the mixture of the metals at their optimum concentrations (Fig. 3A). The other metals used alone gave a lower increase in cell growth as shown in Fig. 3B.

DISCUSSION

From the results, it is shown that potassium increases the fermentation of sucrose-containing media, than the other metals used in this study. Sodium and calcium have a moderate effect and copper the lowest. The

Table 1
Concentrations of K, Na, Ca, and Cu in Greek Molasses and Raisins

Samples from	Molasses K ($mg\ K_2O$/100 g)	Molasses Na ($mg\ Na_2O$/100 g)	Molasses Ca ppm	Molasses Cu ppm	Samples	Raisins K ($mg\ K_2O$/100 g)	Raisins Na ($mg\ Na_2O$/100 g)	Raisins Ca ppm	Raisins Cu ppm
Plati	3385±30	1560±19	5160±40	9.3±0.3	Trechumena	546-964*	19-37	1117-1634	5.4-18.6
Xanthi	3342±28	1467±17	2285±36	4.2±0.2	Chondrada	763-823	17-35	1510-1793	11.9-16.2
Larissa	2795±22	1340±17	3608±35	6.5±0.3	Piotiki dialogi	792-842	24-43	1610-1949	17.3-20.2
Serres	1486±19	1914±17	2947±31	10.6±0.4	Psila	757-1027	20-51	1827-3870	14.8-38.7
Orestiada	525±16	1739±16	2536±42	10.9±0.4	Sultana	1105-2156	46-131	783-4653	8.3-48.5

*Concentration ranges were obtained, in all cases, from analytical results of samples collected from 5 different areas of Greece.

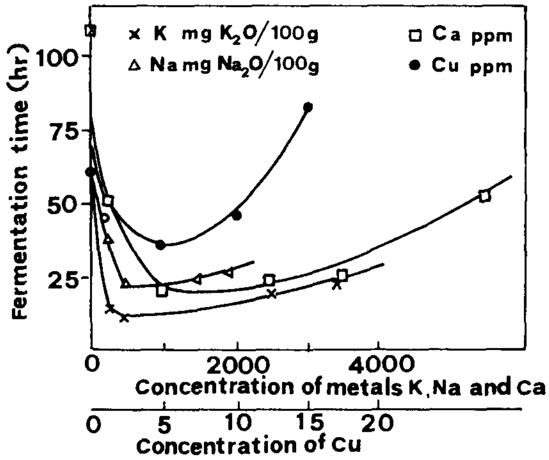

Fig. 1. Fermentation time vs concentration of K, Na, Ca, and Cu obtained in the alcoholic fermentation of synthetic media containing sucrose and each of the metals separately.

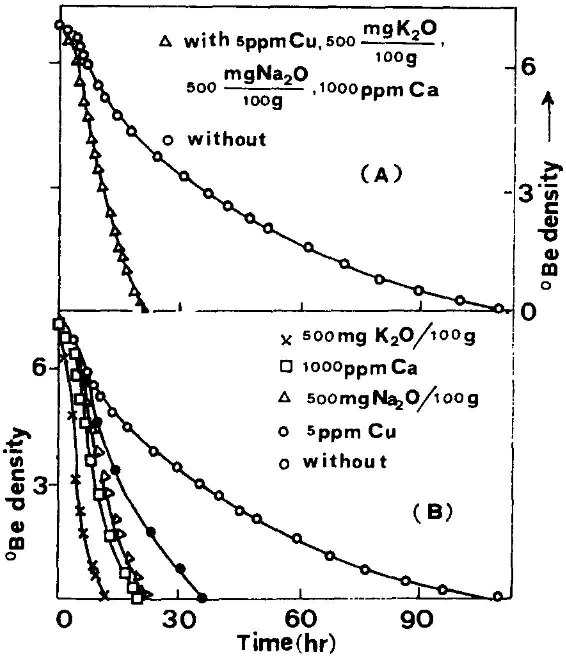

Fig. 2. Fermentation kinetics observed in the presence of the metal mixture K, Na, Ca, and Cu (A) as well as of each of the metals separately and (B) at their optimum concentrations, as compared with the kinetics of fermentation in the absence of metals.

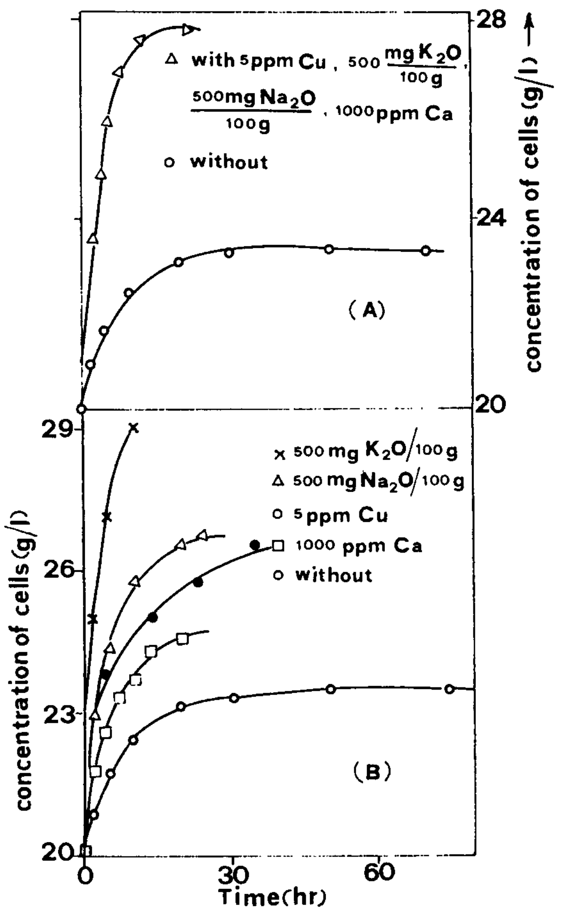

Fig. 3. Cell growth vs time during fermentation in the presence of the metal mixture K, Na, Ca, and Cu (A) as well as of each of the metals separately and (B) at their optimum concentrations, as compared with cell growth of fermentation in the absence of metals.

smallest reduction of the fermentation time was observed with the trace element copper as compared to those achieved by the other metals. This may be attributed to a decrease in the rate of glucose uptake, caused by the uptake of copper by cells (*8*). In all cases the decrease of the fermentation time obtained in the presence of K, Na, Ca, and Cu separately or in mixture, parallels the respective increase of cell growth. However, to our knowledge, this increase of cell growth can only in part explain the drastic drop of the fermentation time. The results obtained in the present study may support the notion that the differences in ethanol production rates observed in industrial scale fermentations, in the case of molasses and other raw materials, are mainly caused by different contents of the metals described. Furthermore, the presence of Cu, K, Na, and Ca in suitable concentrations in raisins (e.g., Trechumena) is one of the reasons for the better vitality of *S. cerevisiae* (*7*) and *Z. mobilis* cells grown in raisin extract and for their higher fermentation rates observed as compared to those of synthetic media (*11*). Finally, the results of the present study may be employed for the preparation of solid supported biocatalysts, prepared by the immobilization of *S. cerevisiae* on a solid, having to their surface K, Na, Ca, and Cu at the appropriate concentrations.

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