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Screening and optimization of trace elements supplement in sweet sorghum juice for ethanol production

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ABSTRACT

Eight trace elements were screened for increasing efficiency of ethanol yield from sweet sorghum juice using the Plackett-Burman design method. $MnCl_2 \cdot 4H_2O$, $CoCl_2 \cdot 6H_2O$ and biotin was screened as the significant variables which have positive effects on ethanol production from sweet sorghum juice. The values of $MnCl_2 \cdot 4H_2O$, $CoCl_2 \cdot 6H_2O$ and biotin, optimized by Box-Behnken design method, were 7.70 mg L^{-1}, 15.74 mg L^{-1} and 11.97 mg L^{-1}, respectively. The experimental efficiency of ethanol yield under optimal conditions was 89.30 $\pm$ 0.10%, which enhances the efficiency of ethanol yield by 5.63% by the addition of $MnCl_2 \cdot 4H_2O$, $CoCl_2 \cdot 6H_2O$ and biotin. The results from this study have identified optimal conditions as a foundation for pilot scale ethanol production.

1. Introduction

1.1. Background

Bioethanol is an alternate energy source to fossil-derived oil and is, as well, a hot research topic in many countries because of its perceived renewable and eco-friendly features. Sweet sorghum with 15–20% (wt.) fermentable sugar in the juice of its stalk can be directly converted into bioethanol by yeast. As a non-food grain crop, it is deemed as an excellent raw material for production of fuel ethanol against a background of fossil liquid fuels and food crop concerns. Although sweet sorghum juice is plentiful in nitrogen and is an excellent medium for growth of *Saccharomyces cerevisiae*, lack of inorganic constituents, vitamins, carbohydrates and some biogenic elements may limit fermentation [1]. Microelements can play an important role in cellular metabolism, primarily as cofactors for a large number of enzymes [2], even though the amount of trace elements may be very low. Fermentation of a carbohydrate to ethanol with yeast is an intricate process involving numerous metabolic reactions. Also, ethanol production is related to factors, such as temperature, pH, dissolved oxygen and presence of enzyme modulators. Metal ions can affect the rate of glycolysis and conversion of pyruvate to ethanol [3]. Copper (Cu) may act as a cofactor for some enzymes such as cytochrome coxydase, lactase, and Cu, Zn-superoxide dismutase [4]. Zinc, copper and manganese ions are very interesting because they have a positive effect on the respiratory activity and the growth rate of *S. cerevisiae* [5]. Zinc is essential as a catalytic cofactor of many enzymes, including alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase and several carboxypeptidases [6]. Iron (Fe) is one of the compositions of cytochrome, cytochrome oxidase and ironporphyrin which are the active group of peroxidase. Iron (Fe) is required as a cofactor for several metabolic pathways and is an important biogenic element for microbial

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metabolism [7]. Cobalt is a vital element because it can improve yeast cell proliferation and addition of 0.02 g L^{-1} cobalt has been shown to increase ethanol production [8]. Biotin is necessary for yeast growth and it plays a leading role in the citric acid cycle, a process by which biochemical energy is generated during aerobic respiration [9]. In addition, sodium molybdate (Na_2MoO_4) and boric acid (H_3BO_4) have been added to the ethanol fermentation medium [10].

In order to increase the efficiency of ethanol yield, some researchers have reported adding dosages of nutrient salts such as amino acid, Ammonium sulfate, Magnesium sulfate, Monopotassium phosphate, Calcium chloride to improve ethanol production [11]. Also, the literature has shown that trace elements such as Cu, Zn, Mn, Co and Fe in the synthetic media have positive effects on yeast cell growth and efficiency of ethanol yield [6,12,13]. However, it has been shown that sensitivity and tolerance of yeast to trace elements are related to numerous factors such as the species of yeast, syrup and the fermentation conditions. The ideal dosage of certain trace elements will vary among various raw materials. Besides, the endurance capacity of yeast to some trace elements is different.

1.2. *Objectives*

The main objectives of this study were: 1) to determine trace elements from respective salt compounds which have significant effects on efficiency of ethanol yield in fermented liquors; 2) to determine optimum levels of trace elements for fermentation of sweet sorghum juice with *Saccharomyces cerevisae* to maximize efficiency of ethanol yield.

2. Materials and methods

2.1. *Sweet sorghum juice*

Sweet sorghum (Chongming No.1) was planted in April and harvested in September, 2009 from the farm of Shanghai JiaoTong University, P.R.China. Stalks were squeezed to obtain the juice by a laboratory scale squeezer. The juice was kept at −20 °C before it was used. The main compositions of the sweet sorghum juice are shown in Table 1. The test methods are described in analytical methods.

Table 1 – The main compositions of the sweet sorghum juice.

Composition	Concentration
Total soluble sugar content (g L^{-1})	168.20 ± 0.45
Water content (%)	82.29 ± 0.13
Ash content (%)	0.88 ± 0.08
K (g L^{-1})	3.23
Ca (g L^{-1})	0.35
Na (mg L^{-1})	10.17
Mg (mg L^{-1})	98.78
Cu (mg L^{-1})	0.14
Fe (mg L^{-1})	19.52
Zn (mg L^{-1})	2.35
Mn (mg L^{-1})	0.80
B (mg L^{-1})	2.78
Mo (mg L^{-1})	0.04
Co (mg L^{-1})	0.13
P (mg L^{-1})	61.65
Cr (mg L^{-1})	0.52
Pb (mg L^{-1})	0.09
Se (mg L^{-1})	0.66
As (mg L^{-1})	undetected

2.2. *Microorganism and growth conditions*

S. cerevisae was bought from Hubei Angel Yeast Co., Ltd, China for ethanol fermentation because of its characteristic high rate of fermentation and high sugar resistance. The yeast was rehydrated and activated for 20 min at 38 °C and propagated at 31 °C for 2 h. The enrichment medium composition consisted of (g L^{-1}): glucose, 50; yeast extract, 5; peptone, 5; $MgSO_4 \cdot 7H_2O$, 1; K_2HPO_4, 1. The medium was sterilized by autoclaving at 121 °C for 20 min.

2.3. *Ethanol fermentation conditions*

100 mL of sweet sorghum juice with addition of different concentrations of trace elements, according to the experimental design, was put into a 250 mL conical flask. The pH value of the juice was adjusted to 5.0 by addition of 6 mol L^{-1} HCl or 6 mol L^{-1} NaOH, and then sterilized by autoclaving at 121 °C for 20 min before it was inoculated aseptically with the enrichment medium at the volume ratio of 1:10 of the fermentation broth. The fermentation tests were conducted at 30 ± 0.5 °C with a shaker at 140 rpm. All the fermentation flasks were weighted by balance (0.001 g) every 2 h, and weight loss for each flask was calculated. The fermentation was considered as termination when the conical flask weight loss was less than or equal to 0.1 g h^{-1} [14].The samples for analysis were centrifuged for 10 min at 6000 rpm before the supernatant was filtered by 0.45 μm micromembrane for ethanol determination. Fermentation samples were kept at −20 °C before analysis.

2.4. *Analytical methods*

The pH value was determined directly by a pH meter (Mettler-Toledo International Inc.). The reducing sugar content was determined by the 3, 5-dinitrosalicylic acid (DNS) method [15]. The total soluble sugar content was measured by the DNS method after hydrolysis to convert the sucrose to glucose and fructose [16]. The main composition of sweet sorghum juice was determined according to the method presented in the literature [17]. The metallic elements in sweet sorghum stalk juice were determined by inductively coupled plasma (ICP) [18]. Ethanol concentration was analyzed by gas chromatography (Shimadzu GC-2010) with a flame ionization detector and n-propanol was used as an internal standard [14]. All runs were carried out in duplicate and the results were averaged. The experimental data were statistically analyzed using Design-Expert (Stat-Ease Inc., V7.1.6, Minneapolis, USA).

The efficiency of ethanol yield (E_y) is calculated by Equation (1)

$$E_y = \frac{Y_i}{S_0 \times 0.511} \times 100 \quad (1)$$

where E_y is the efficiency of ethanol yield (%), Y_i is the actual ethanol concentration (g L^{-1}), S_0 is the initial total soluble