



Ethanol Production from Cheese Whey with Sweet Sorghum

KEYWORDS

Ethanol; cheese whey; sweet sorghum; *Saccharomyces cerevisiae*.

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ABSTRACT *Saccharomyces cerevisiae* was used for ethanol production from cheese whey supplemented with sweet sorghum in different concentrations in batch experiments. Effects of sweet sorghum substrate concentrations on the rate and extent of ethanol production were investigated. The maximum fermentation time was fixed as 72 hours. The temperature and pH values were taken as 30°C and 5 respectively. Under the above said experimental conditions results were obtained. The results indicated that when cheese whey supplemented with sweet sorghum at the level of 50g/l the ethanol production was found significant.

1. Introduction

Study on ethanol production from different raw materials containing carbohydrates has created much interest among the researchers because of their wide range of applications (Hari KS et al., 2001). Utilization of waste materials for ethanol formation offer special advantages by providing cheap raw materials and simultaneous waste treatment with ethanol production. The common raw materials for ethanol fermentations are cellulosic materials (straw, baggase, and waste paper), starch containing materials (corn, wheat, and rice), sugar cane, sugar beet and molasses. Among the raw materials, sweet sorghum and cheese whey which are the waste by-products of dairy industries and agricultural fields are inexpensive and highly available. In addition, cheese whey is an important source of environmental pollution because of the high organic matter content with biological oxygen demand ranging from 40-50 g/l and a chemical oxygen demand of 60-80 g/l (Ghaly AE, El-Taweel AA (1997). In India, the estimated production of whey from cheese is about 4.84 million tonnes per annum (Rajesh K and Aniruddha B (2007). Hence, the production of ethanol from whey has gained importance because it is not only used to satisfy fuel demand but reduce the environmental pollution. Sweet sorghum (*Sorghum bicolor*) is similar to grain sorghum with a sugar-rich stalk, almost similar to sugarcane. Besides having wide adaptability, rapid growth, high sugar accumulation and biomass production potential, sweet sorghum is also tolerant to drought, water logging, soil salinity and acidity toxicity. It has great potential for jaggery, syrup and alcohol (most importantly Gasohol, which is ethanol blended with petrol) production (Davis L et al., 2005). *Kluyveromyces* species have been the most widely used yeast strain for ethanol production from cheese whey. Recently, Zafar and Owasis (2006) have studied production of ethanol from crude cheese whey. Kargi and Ozmichi (2006) reported 1.28% ethanol productions from cheese whey powder. To the best of the author's knowledge there is no literature study on ethanol production from cheese whey with sweet sorghum. In the present study, an attempt has been made for ethanol production from cheese whey supplemented with sweet sorghum in the presence of *Saccharomyces cerevisiae*.

2. Materials and methods

2.1 Preparation of Samples

Cheese whey produced in our laboratory contained 4.5% of lactose. Sweet sorghum stalks were collected from the Tamilnadu Agricultural University, Coimbatore, Tamil Nadu. The outer skin present in the sweet sorghum stalk was removed. Then the stalk was chopped in to very small pieces and crushed well using mixer and this preparation was used for further experiment.

2.2 Organism

Saccharomyces cerevisiae MTCC 178 was procured from the Culture Collection Centre of the Institute of Microbial Technology (MTCC) at Chandigarh, India. *Saccharomyces cerevisiae* MTCC 178 was maintained in Yeast Peptone Dextrose (YPD).

2.3 Substrate composition

Cheese whey (containing 4.5% lactose, 0.05 % fat, 0.52 % protein and trace amount of ash) and sweet sorghum (containing 15% sucrose, 0.35% glucose, 11.5% cellulose, 5.0% hemicellulose and 66% moisture) were used in the present study.

2.4 Experimental methods

One hundred ml of cheese whey supplemented with sweet sorghum at the rate of 50, 100, 150 and 200 g were adjusted to pH 5 by using sodium thio-glycolate (98%). This medium was sterilized by autoclaving at 15 psi for 20 min. The autoclaved media were inoculated with 10 ml of *Saccharomyces cerevisiae* in different experimental flasks incubated at 30°C for 72 hours. 10 ml of the above sample was centrifuged for 15 min at 5,000 rpm and the supernatant was used for estimating ethanol and reducing sugar. The maximum fermentation time is fixed as 72 hours. The temperature and pH values were taken as 30°C and 5 respectively the experiment was repeated ten times.

2.5. Analytical methods

Yeast cells harvested by centrifugation were weighed after drying at 105°C for 24 hrs. Lactose in cheese whey was estimated by the method of Lane-Eynon method (1977), the total reducing sugar by phenol-acid method (1956), and ethanol by the dichromate colorimetric method (1950). Ethanol yield coefficient was calculated in terms of g of ethanol produced per g of substrate consumed. The fermentation efficiency was calculated as shown below

$F/E = \text{Ethanol produced} \times 100$

Theoretical maximum ethanol yield from sugar

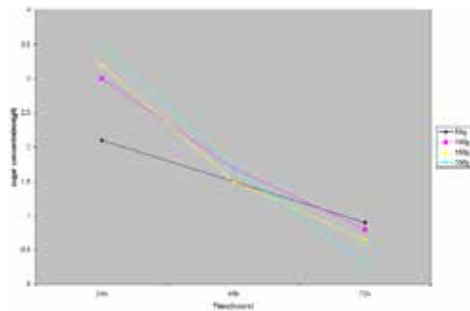
(Theoretical maximum ethanol yield = 0.54 g ethanol per gram of lactose).

3. Results and discussion

Batch experiments were carried out with the cheese whey supplemented with sweet sorghum in different concentrations between 50 to 200g/l with total sugar contents between 5.25 to 7.50g/l by keeping the experimental conditions of the pH 5, temperature 30°C and incubation maximum time 72 hours as a constant. Variations of total soluble sugar and ethanol production with time are shown in Figure 1 and Fig-

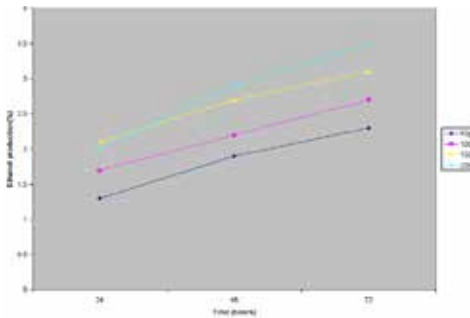
ure 2 respectively for cheese whey supplemented with sweet sorghum in different concentrations.

Fig.1 sugar consumption for different concentration of sweet sorghum with different time intervals



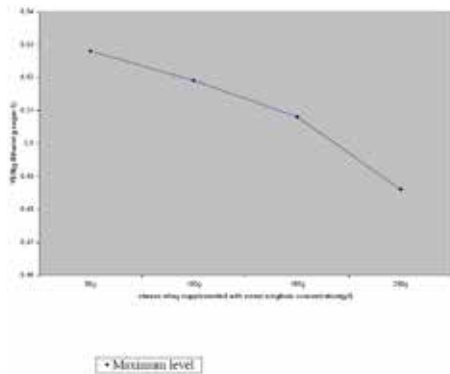
Total sugar concentration decreased with increasing time and fermentation was completed in 72 h in all experiments. Total sugar consumption was faster in cheese whey supplemented with 200g/l (total sugar 7.5g/l) as compared to others. An incubation time of 72 h was considered in all further calculations like the ethanol yield coefficient, fermentation efficiency and conversion efficiency. Time course of variations of ethanol production is depicted in Fig.2.

Fig.2 Ethanol production for different concentration of sweet sorghum with different time intervals



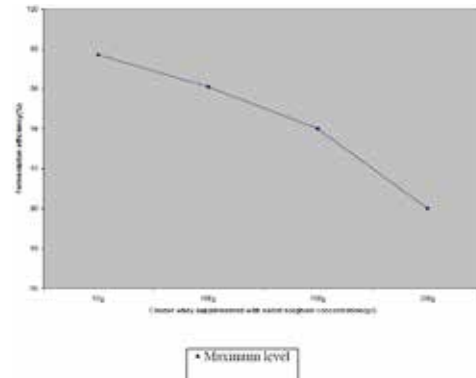
The ethanol production increased with time and reached the maximum level at 72h. The ethanol production was obtained at 3.5% as cheese whey supplemented with 200g/l (total sugar 7.5g/l) followed by cheese whey supplemented with 150g/l (total sugar 6.75g/l) were considerably lower than that obtained with cheese whey supplemented with 100g/l (total sugar 6.0 g/l) and cheese whey supplemented with 50g/l (total sugar 6.25 g/l) Variations of the ethanol yield coefficient with the cheese whey supplemented with sweet sorghum in different concentrations are shown in Figure 3.

Fig.3 Maximum ethanol yield coefficient for different concentration of sweet sorghum



The maximum ethanol yield coefficient was obtained at cheese whey supplemented with sweet sorghum concentrations in 50g/l(TS=5.25g/l)YE/S (0.528gEtOHgLactose-1) almost constant at the theoretical value (0.54gEtOHgLactose-1) followed by that cheese whey supplemented with sweet sorghum concentrations in 100 g / l (Total Sugar =6.0g/l) YE/S (0.519gEtOHgLactose-1) were considerably lower than those obtained at cheese whey supplemented with 150g/l (total sugar 6.75 g/l) and cheese whey supplemented with 200g/l (total sugar 7.50 g/l) because of the inhibitory effects of high sugar concentrations Variations of fermentation efficiency to the cheese whey supplemented with sweet sorghum in different concentrations are shown in Fig4.

Fig.4 Maximum fermentation efficiency for different concentration of sweet sorghum.



Fermentation efficiency was calculated at 72 h incubation time. The fermentation efficiency was 97.7% obtained at cheese whey supplemented with sweet sorghum concentrations in 50g/l (Total Sugar =5.25 g/l) followed by cheese whey supplemented with sweet sorghum concentrations in 100g/l (Total Sugar =6.0g/l) that was considerably lower than those obtained at cheese whey supplemented with 150g/l (total sugar 6.75 g/l) and cheese whey supplemented with 200g/l (total sugar 7.50 g/l) due to the substrate inhibition. There are no literature studies on ethanol production of cheese whey supplemented with sweet sorghum concentrations for comparison.

4. Conclusion

Ethanol production from cheese whey supplemented with sweet sorghum in different concentrations was investigated by using batch experiments. The rate and extent of ethanol production and sugar utilization increased with increasing the concentration of cheese whey supplemented with sweet sorghum concentrations. The results indicated that there is a gradual increase in ethanol production with increasing the level of sweet sorghum from 50g/l to 200g/l. However the fermentation efficiency and ethanol yield coefficient was found higher at the cheese whey supplemented with sweet sorghum 50g/l. The ethanol yield co-efficient was also equal to the theoretical yield (0.54gEtOHgLactose -1) for cheese whey supplemented with sweet sorghum concentration 50g/l. From this study it could be concluded that cheese whey supplemented with sweet sorghum concentration should be kept below 50g/l in batch experiments to avoid substrate inhibition.

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