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Effect of pH in the bioethanol production from cheese whey using the yeast *Kluyveromyces marxianus*

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Abstract

Ethanol fermentation of cheese why by using *Kluyveromyces marxianus* MTCC 242 in batch experiments showed that pH 5 to be most suitable for maximizing ethanol concentration. The maximum yield coefficient of ethanol i.e., 0.425 g EtOH g sugar-^{1} was observed at pH 5.

Keywords : bioethanol, fermentation, *Kluyveromyces*, pH, yeast

INTRODUCTION

The world production of cheese whey is estimated to be over 10^{8} tons per year (Grba, *et al.,* 2002) The predicted value for whey production in India is estimated at 4.84 million tonnes per annum (Raju *et al.,* 2005). Typical cheese whey contains 5-6 percent lactose, 0.8 1 percent protein, and 0.06 percent fat, constituting an inexpensive and nutritionally rich raw material for ethanol fermentation (Zafar and Owais 2006). Cheese whey represents an important source of environmental pollution due to its enormous global waste generation rate (to make 1 kg of cheese, 9 kg of whey is generated) and high organic matter content, exhibiting BOD and COD values of 50 and 80 g l^{-1}, respectively (Ozmihci, and Kargi, 2007). The bioconversion of lactose to ethanol is a promising alternative that would not only reduce the environmental impact of cheese whey but also present an alternative way of production of ethanol as a valuable fuel resource (Rapin *et al.,* 1994). Extensive attention has been paid during the last 15 years to the evaluation of whey permeate as a potential alternative fermentable substrate for alcohol production. Among the various operating parameters influencing ethanol production, initial pH of the substrate is reported to be important. Kargi, and Ozmihci (2006) studied the effect of varying initial pH on ethanol production using cheese whey powder solution and reported that pH 5 was found as an ideal pH to maximize the final ethanol yield. However, the effect of pH on cheese whey solution as a substrate for ethanol production is not known. Hence, this paper ascertains the fermentation of cheese whey solution at different pH levels in order to find the most suitable pH level for ethanol production using the yeast strain *Kluyveromyces marxianus* MTCC 242.

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MATERIAL AND METHODS

Experimental system

Batch experiments were performed by using sterile conical flasks. One hundred ml of sterilized cheese whey was taken in 500 ml conical flasks and 4 ml of 0.1 N NaOH was added in order to adjust the pH levels to optimum conditions and freshly prepared 10 ml pure *K. marxianus* culture was added. The conical flasks were prepared in duplicates. Inoculated conical flasks were prepared in triplicates and were placed in an incubator shaker at 30± 2°C. Samples were withdrawn aseptically from the conical flasks periodically (24 h) for analysis of total sugar, and ethanol production. Experiments were conducted at four different pH levels *viz.,* 4, 5, 6 and 7.

Procurement and maintenance of Microorganism

K. marxianus strain MTCC 242 was procured from the culture collection centre of the Institute of Microbial Technology (MTCC), Chandigarh India. The strain was maintained on agar medium having the following composition: lactose, 20g l^{-1}; bactopeptone, 10 g l^{-1}; malt extract 3g l^{-1}; yeast extract, 5 g l^{-1}; agar, 20 g l^{-1}. Slants were kept for 48 h at 30°C for the growth of the yeast cells and then they were preserved at 4°C for further use.

Analytical methods

Biomass was measured in terms of dry weight. Yeast cells were harvested by centrifugation for 10 min at 5,000 rpm and then washed twice with distilled water and weighed after 24 h at 100°C. (Dubois *et al.,* 1956) Amount of reducing sugars was measured to using the phenolacid method. Ethanol was estimated by the dichromate colorimetric method (William and Reese, 1950), which is based on the complete oxidation of ethanol by dichromate in the presence of sulphuric acid to form acetic acid. The fermentation efficiency was calculated as:

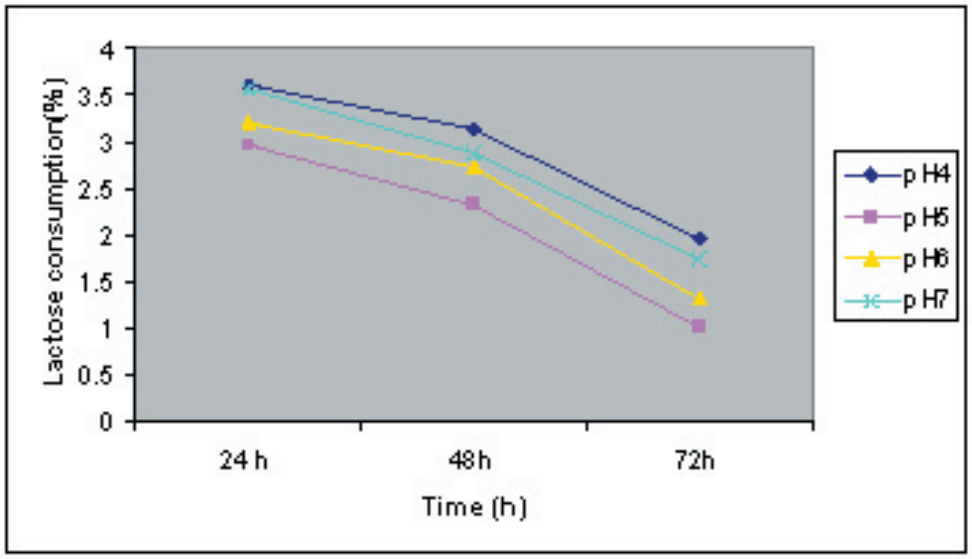

Figure 1. Lactose consumption

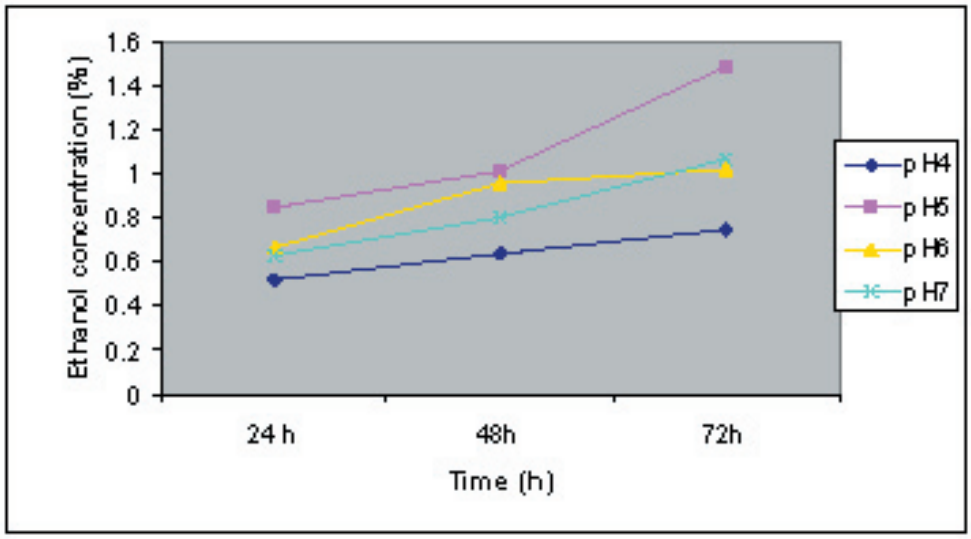

Figure 2. Ethanol concentration

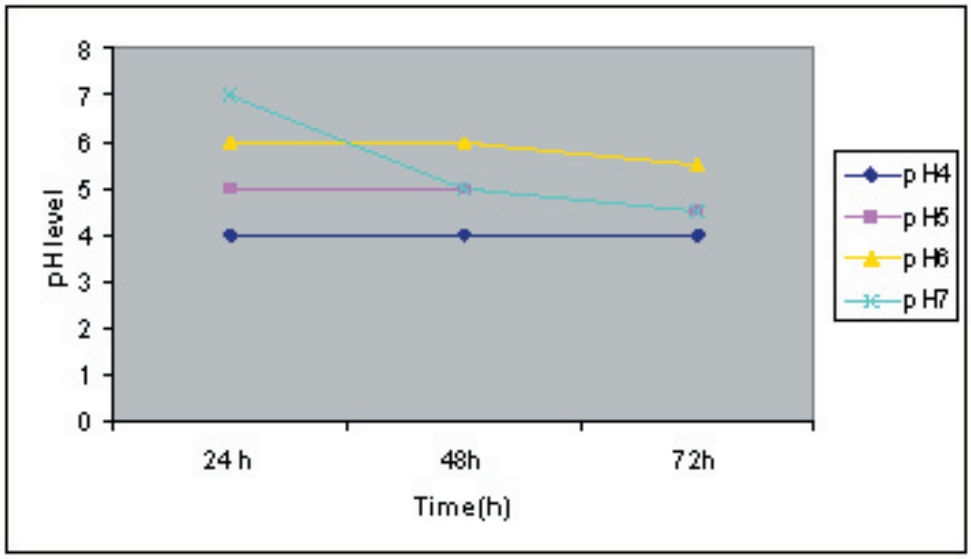

Figure 3. Changes of pH levels at different timings of fermentations

Ethanol produced/Theoretical maximum ethanol yield from sugar x 100 (Theoretical maximum ethanol yield = 0.54 g ethanol per gram sugar).

RESULTS AND DISCUSSION

In all the experiments total lactose concentration (4.2 percent) level decreased with increase of time with the lactose consumption level being faster in the pH 5 when compared to the other pH levels of 4, 6 and 7,

Table 1. Lactose concentrations at different times of fermentations of cheese whey solution by the yeast *Kluyveromyces marxianus.*

pH	24 h (%)	48h (%)	72h (%)
4	3.62	3.13	1.96
5	2.97	2.32	1.02
6	3.2	2.73	1.32
7	3.57	2.89	1.75

(Table 1).

The ethanol concentration also reached the maximum

Table 2. Ethanol concentration at different times of fermentation of cheese whey solution by the yeast *Kluyveromyces marxianus*

pH	24 h (%)	48h (%)	72h (%)
4	0.52	0.64	0.75
5	0.842	1.013	1.48
6	0.667	0.954	1.02
7	0.625	0.801	1.06

level of 1.48 percent at 72 h in pH 5 (Fig. 2).

There is no change in the level of pH in the experiment having the initial pH level 4, (Fig 3). But in the other remaining pH levels eventhough there is no change in the first 24 h, after 72 h pH 5 and 6 had a slight decrease

Table 3. Changes of pH levels at different times of fermentations of cheese whey solution by the yeast *Kluyveromyces marxianus*

pH	24 h	48h	72h
4	4	4	4
5	5	5	4.5
6	6	6	5.5
7	7	5	4.5

of 0.5. In the same way pH 7 was stable up to 24 h. But after 48h the level got reduced to pH 5 and then to pH 4.5 after 72 h.

Ethanol yield coefficient was calculated and the maximum ethanol yield co efficient was obtained at initial pH of 5 (0.42 percent, v/v ethanol) followed by pH 7 (0.343 percent, v/v). Ethanol yield coefficients at other pH levels of 4 and 6 were considerably lower than that obtained at pH of 5 and 7. Fermentation efficiency indicated, that pH 5 has given good efficiency of 60.9 per cent followed by pH 6 (43.6%) 4 and 7 (39.% and 38%, respectively). On the basis of the overall results it is concluded that pH 5 is suitable for good ethanol production from cheese whey solution.

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