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Research Paper

Bioutilization of whey for ethanol production using yeast isolate

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Abstract

Whey, the liquid remaining after milk fat and casein separated from whole milk, is one of the major disposal problem of the dairy industry, and demands simple and economical solutions. The utilization of whey for ethanol production can not only solve the whey disposal problems but also address environmental concern effectively. In the present study, lactose in whey served as a good substrate for the ethanol production by using yeast cultures. During screening of different yeast cultures, isolated yeast strain (*Kluyveromyces marxianus* WIG2) gave maximum ethanol production and lactose utilization. Among the various nitrogen and salt sources tested, corn steep liquor and sodium chloride resulted in maximum ethanol production and lactose utilization. During optimization of process conditions, maximum ethanol production (1.95%) has been observed at temperature of 35 °C and pH 5 after 40 hr of incubation with lactose utilization of 94.5%.

Keywords: whey, lactose, fermentation, *K. marxianus* WIG2, ethanol

Whey is a liquid byproduct of dairy processing industry, which is obtained by coagulating and separating the casein proteins from whole or skim milk. The composition of whey varies according to its origin and to the cheese-making technique employed. Most abundant nutrients present in whey are lactose (4.5-5%, w/v), soluble proteins, lipids, mineral salts besides small amounts of organic acids, non-protein nitrogen compounds, vitamins etc. (Siso, 1996). It has been reported that out of total cheese-whey produced by dairy industry, 50% of its production is treated and transformed into various food products of which 45% is used directly in liquid form, 30% in form of powdered cheese whey, 15% as lactose and delactosed by products, and rest as cheese-whey protein concentrates (Marwaha and Kennedy, 1998). However, the search for a satisfactory solution for disposal of the unutilized whey produced is an area of intense concern for the dairy industry.

Whey represents an important environmental problem because of the high volumes produced and its high organic matter content. It exhibits both high biochemical and a chemical oxygen demands (Guimaraes *et al.,* 2010). Moreover, excessive lactose and mineral proportions limit whey to use in feeding as agricultural fertilizer (Sienkiewicz and Riedel, 1990). To overcome the problems associated with whey disposal, a better alternative is to develop value added products from it through biotechnological means (Nagar and Nagal, 2013). The presence of

lactose and other nutrients essential for the growth of microorganisms makes it one of the potent raw materials for bio-products production (Panesar *et al.*, 2007).

The fermentation of whey to produce ethanol has received wide attention globally; as the lactose content makes it suitable material for ethanol production (Marwaha and Kennedy, 1984). Different strategies have been employed for fermentation of whey to ethanol production and various micro-organisms employed in ethanol fermentation are *K. fragilis, K. marxianus, Candida pseudotropicalis, Saccharomyces cerevisiae* (Marwaha and Kennedy, 1988; Panesar, 2008; Panesar and Kennedy, 2012). However, to achieve a good utilization of lactose from whey, it is important to choose a strain of yeast with suitable physiological characteristics for maximum product formation.

Designing a fermentation medium is a critical process, as the medium composition can significantly affect the product yield. Keeping in view these facts, the present study has been carried out for ethanol production from whey by using yeast cells as well as optimization of media composition and process parameters to enhance ethanol production.

Materials and Methods

Procurement of microbial cultures

Different yeast strains namely Kluyveromyces marxianus MTCC 1388, Kluyveromyces marxianus var. marxianus MTCC 1389, Kluyveromyces marxianus var. lactis NCIM 3551, Kluyveromyces marxianus NCIM 346 have been procured from Institute of Microbial Technology (IMTECH), Chandigarh (India) and National Chemical Laboratory (NCL), Pune (India), respectively. Kluyveromyces marxianus WIG 2 was isolated in Food Biotechnology Laboratory, Sant Longowal Institute of Engineering and Technology (SLIET), Longowal, Punjab, (India). The cultures were grown on agar slants containing (w/v) malt extract (0.3%), yeast extract (0.3%), peptone (0.5%), glucose (1.0%) and agar-agar (2.0%), adjusted to pH 5.0. The agar slants were incubated at 30 °C for 24 hr. The culture was maintained by sub-culturing, aseptically at fortnight intervals and stored at 4°C, until further use.

Screening of yeast cultures

Different yeast strains were screened for their potential in production of ethanol using whey as fermentation medium and pH was adjusted to 5.0. After sterilization, the flasks were inoculated with starter culture and incubated at 30°C for 48 hr. The samples were taken after 48 hrs and were analysed for ethanol production and lactose consumption.

Screening of nitrogen sources

To investigate the influence of different nitrogen sources on ethanol production, different synthetic and natural sources such as yeast extract, corn steep liquor, peptone, urea and ammonium sulphate were added individually in the fermentation medium.

Screening of salts

Different salts such as monopotassium phosphate (KH_2PO_4), manganese sulphate ($MnSO_4$), sodium chloride (NaCl), calcium chloride ($CaCl_2$), ammonium chloride (NH_4Cl) and magnesium sulphate ($MgSO_4$) were supplemented in whey in addition to nitrogen source to study the effect of ethanol production and lactose utilization.

Optimization of process parameters

The optimization of different process parameters like pH, temperature, and incubation time was carried out using the above optimized fermentation medium at flask level, to enhance the ethanol production. The pH of fermentation medium was adjusted to 4.0-6.0, whereas temperature range was adjusted between 25-40°C. The flasks were incubated at 30°C for 48 hr and the samples were drawn at 4 hr time intervals starting from 8 hr incubation.

Analytical techniques

The lactose estimation was carried by the procedure of Nickerson *et al.* (1976) and ethanol was analysed by

using the Gas Chromatograph (NUCON, Series 5700, New Delhi, India) equipped with flame ionization detector and glass column.

Statistical Analysis

All the experiments were carried out in triplicates for statistical analysis. Mean values and standard deviations ($P<0.05$) were calculated by using Microsoft Office Excel 2007.

Results and Discussion

Screening of microbial cultures

All the yeast cultures namely *Kluyveromyces marxianus* MTCC 1388, *Kluyveromyces marxianus* var. *marxianus* MTCC 1389, *Kluyveromyces marxianus* var. *lactis* NCIM 3551, *Kluyveromyces marxianus* NCIM 3465 and *Kluyveromyces marxianus* WIG 2 were screened for the production of ethanol using whey as a fermentation medium. The samples were taken after 48 hr and were analyzed for ethanol production and lactose consumption. The results (Table 1) revealed that the maximum ethanol production (1.53%) and lactose utilization (77.5%) was shown by isolated yeast strain *K. marxianus* WIG 2. Variations in ethanol production and lactose utilization by different yeast strains may due to strain specificity or metabolism.

Table 1. Screening of different yeast cultures for ethanol production utilizing whey

Yeast culture	Ethanol production (%)	Lactose Utilization (%)
K. *marxianus* MTCC 1388	1.21±0.02	71.5±1.12
K. *marxianus* MTCC 1389	1.05±0.02	66.25±0.52
K. *marxianus* MTCC 3465	1.29±0.03	73.25±1.32
K. *marxianus* MTCC 3551	0.99±0.01	65.5±1.02
K. *marxianus* WIG 2	1.53±0.03	77.5±2.02

Screening and optimization of nitrogen sources

Among all the nitrogen sources tested, corn steep liquor displayed maximum ethanol production and lactose utilization, which was 1.79% (v/v) and 90.25% (w/v), respectively (Figure 1). Nitrogen is a major component of proteins and nucleic acids, which plays an important role in the growth of microbes. Corn steep liquor, a major by-product of corn starch processing, has been reported to be low-cost source of proteins, amino acids, minerals, vitamins and trace elements, which can be used as a effective nutritional supplement for replacement of yeast extract and peptone in alcoholic fermentations (Seo *et al*., 2009; Periera *et al*., 2010).

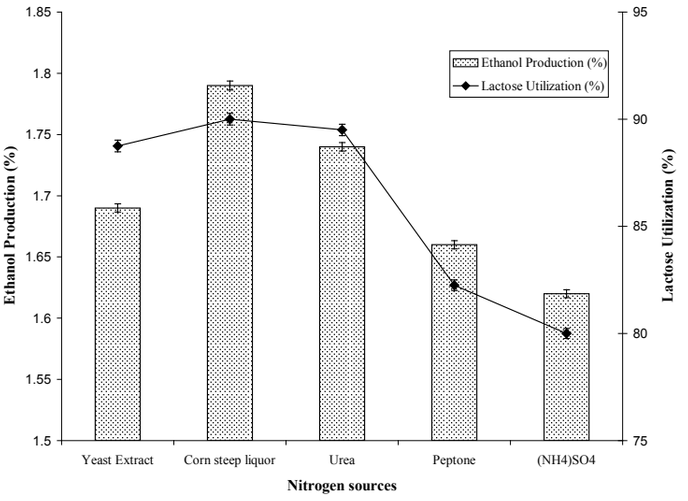

Figure 1: Screening of nitrogen sources for ethanol production and lactose utilization from whey using *K. marxianus* WIG 2

Furthermore, corn steep liquor was supplemented in different concentrations (0.5% - 4%) to whey and the results revealed that maximum ethanol production and lactose utilization was at 1% corn steep liquor supplementation. After this, there was no further increase on the production of ethanol and lactose consumption was observed (Figure 2).

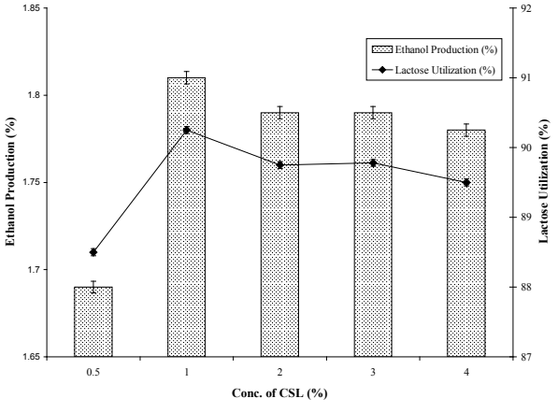

Figure 2: Effect of corn steep liquor concentration on ethanol production and lactose utilization from whey using *K. marxianus* WIG 2

Screening and optimization of salt source

Different salts such as KH_2PO_4, $MnSO_4$, NaCl, $CaCl_2$, NH_4Cl, and $MgSO_4$ were supplemented in whey in addition to corn steep liquor to study the effect of salts on ethanol production and lactose utilization (Figure 3).

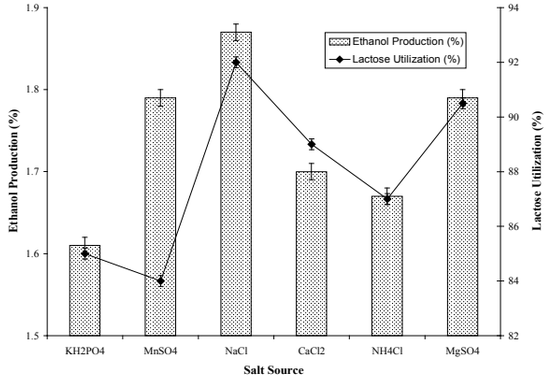

Fig 3: Effect of different salts on ethanol production and lactose utilization from whey using *K. marxianus* WIG 2

It has been observed that maximum ethanol production (1.87%) and lactose utilization has been observed with NaCl. Further, during optimization of NaCl concentration, maximum production and lactose utilization had been shown by 1% NaCl as shown in Figure 4. The higher concentration of the salt used did not show any further improvement in ethanol production and lactose utilization, which

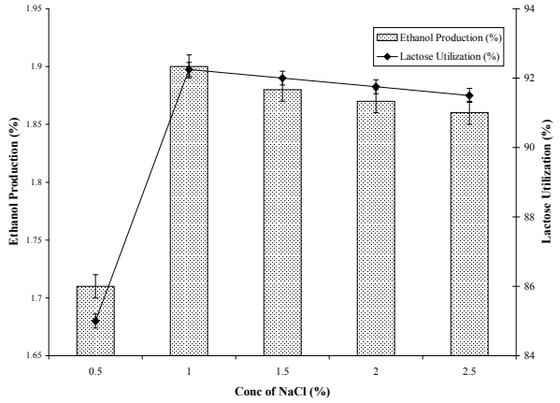

Figure 4. Effect of NaCl concentration on ethanol production and lactose utilization from whey using *K. marxianus* WIG 2

may be due to the inhibitory effect of high salt concentrations on the growth of yeast cells due to osmolytic effect (Gawel and Kosikowski, 1978).

Effect of pH

The pH of fermentation media was varied from 4-6 to find out the optimum pH for the ethanol production.

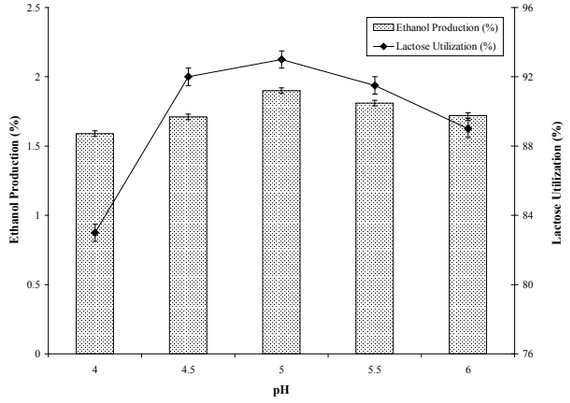

Figure 5. Effect of pH on ethanol production and lactose utilization from whey using K. marxianus WIG 2

The maximum ethanol (1.9%) has been produced at pH 5 with maximum lactose consumption of 93% (Figure 5). It has been reported earlier that the optimum pH range was 4.0-6.0 with variation depending on the strain of yeast used as well composition of fermentation media components (Marwaha and Kennedy, 1984; Vienne and von Stocker, 1985; Hajar *et al*., 2012).

Effect of temperature

The effect of temperature on ethanol production was studied by cultivating *K. marxianus* WIG 2 in whey fermentation media supplemented with corn steep liquor and sodium chloride at temperature range of 25-40 °C for 48 hrs. The results (Figure 6) revealed that maximum ethanol production (1.94%) and lactose utilization (94.25%) was observed at 35 °C. Temperature has been reported to significantly affect the enzymatic activity as well membrane turgidity of yeast cells, which in turn affects the activity of one or more enzymes involved in growth of yeast and hence, ethanol production (Tesfaw and Assesfa, 2014).

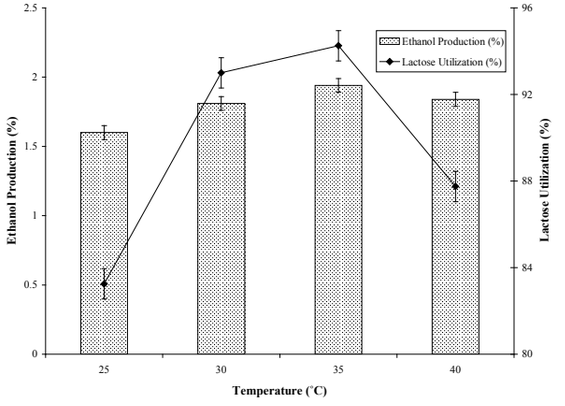

Figure 6. Effect of temperature on ethanol production and lactose utilization from whey using K. marxianus WIG 2

The temperature range of 30°C - 40°C with most of the reports identifying 35°C as the optimal for ethanol production has been reported by various researchers (Marwaha and Kennedy, 1984; Tu *et. al*., 1985; Hashem *et al*., 2013).

Effect of incubation time

To find out the optimal incubation time for the maximal ethanol production, whey fermentation medium inoculated with yeast culture was incubated for 48 hr under the above optimized conditions. The samples were drawn at 8 hr time intervals and the results obtained are presented in Figure 7. As evident from the results, an increase in ethanol content was found up to 40 hr and thereafter no further

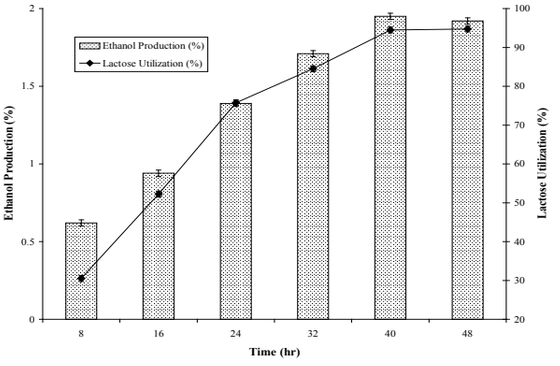

Figure 7. Effect of time on ethanol production and lactose utilization from whey using K. marxianus WIG 2

improvement in this function was observed. The maximum ethanol production and lactose utilization after 40 hr was 1.95% and 94.5%, respectively. This may be attributed to the growth of the culture reach to the stationary phase and as a consequence of metabolism, microorganisms continuously change the characteristics of the medium and the environment.

Similarly, an incubation period of 48 hr has been reported for the maximal ethanol production from cheese whey powder using *Kluyveromyces marxianus* NRRL-1195 (Kargi and Ozmihci, 2012). However, the optimum incubation time for *Kluyveromyces marxianus* ATCC8554 and *Candida kefyr* ATCC 14245 from unconcentrated and concentrated cheese whey permeate was reported to be 60 hr (Koushki *et al*., 2012).

Conclusion

Cheese whey, a main dairy by-product is increasingly recognized as a source of added value products, instead of just a waste stream. However, lactose rich stream remains after separation of proteins and other bioactive components is still a major environmental concern and demands for its effective utilization. Therefore, whey can be utilized for ethanol production utilizing lactose as carbon source using yeast cells. During screening of yeast cultures, *K. marxianus* WIG 2 was observed to be efficient strain for ethanol production by utilizing whey. In media components, corn steep liquor and sodium chloride were observed to be the best nitrogen and salt source for ethanol production, respectively. The optimization of process parameters revealed that temperature of 35°C with pH 5, gave maximum ethanol production and lactose utilization after 40 hr of incubation.

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