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Full Length Research Paper

A study on some efficient parameters in batch fermentation of ethanol using *Saccharomyces cerevesiae* SC1 extracted from fermented siahe sardasht pomace

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Siahe sardasht grape is famous variety of grape in Iran that is used for red grape juice concentrate. The pomace of this grape (as byproduct of fruit juice concentrate industry) is a favorable medium for growth of all type of yeasts. The ethanol production using *Saccharomyces cerevesiae* SC1 extracted from fermented siahe sardasht grape pomace was studied in batch fermentation. The best ethanol production rates are observed at pH 4.5, temperature 32°C and sugar concentration equal to 100 g/L. According to the results, KH_2PO_4 is a better phosphorous source in comparison with K_2HPO_4, and $(NH4)_2SO_4$ is the best nitrogen source.

Key word: Ethanol, *Saccharomyces cerevesiae*, sardasht grape pomace.

INTRODUCTION

Ethanol has been described as one of the most exotic synthetic oxygen-containing organic chemicals because of its unique combination of properties as a solvent, a germicide, an antifreeze, a fuel, a depressant and especially of its versatility as a chemical intermediate for other organic chemicals. Since the energy crisis of the 1970, the development of low-cost, sustainable and renewable energy sources such as ethanol has been a major focus in scientific research (Favela et al., 1986; Ingledew, 1999; Pramanik, 2003; Pramanik, 2005). Traditionally, ethanol is produced from a liquid or a fluid mash via submerged microbial fermentation (Hang et al., 1981). The used microorganisms to carry out the fermentation process are just as important as the substrate and they have also been the target of many researches. *Saccharomyces cerevesia,* also known as brewers yeast, is the most widely used fermentation microbe because of the baking and beer brewing industries (Gunasekaran and Chandra, 1999; Michilka, 2007; Roehr, 2001). Many of the sugar crops that would be suitable for industrial fermentation include sugarcane, sugar beets, fruits, sweet potato, sweet sorghum, Jerusalem artichokes and agricultural wastes (Atiyeh and Duvnjak, 2002; Hang et al., 1981; Ingledew and Kunkee, 1985; Joshi et al., 2001; Mancilha et al., 1984; Michilka, 2007; Muttara and Nirmala, 1982; Pramanik, 2005; Rousseau et al., 1992; Singh and Jain, 1995; Torres et al., 1986). There have been numerous studies concerning the effects on the fermentation kinetics of temperature, ethanol concentration, assimilable nitrogen, nutrients, oxygen and inhibitors. Importance in ethanol fermentation has been focused on taking up of renewed interest in research works in several areas such as use of improved mutant strains, yeast strain development from cheaper source, use of cheaper source of raw materials, optimum reactor design, better nutrients for optimum cell growth, optimization of fermentation factors, e.t.c. (Bisson, 1991; Converti et al., 1985; Gregory et al., 1984; Gregory et al., 1984; Insa et al., 1995; Jones et al., 1981; Mancilha et al., 1984; Mendes et al., 2004; Michilka, 2007; Pramanik, 2003; Pramanik, 2005; Rousseau et al., 1992). Four different fermentation operations are currently used in industry: batch, continuous, fed batch, and semi-continuous. The batch process is the classical method that has stood for hundreds of years, and is currently the most commonly used method of ethanol production (Green, 2002; Mendes et al., 2004). In batch processing, cell slurry is grown separately from the fermentation substrate, and

then slurry and substrate are combined in a reactor along with any required enzymes or nutrients. In this study, conver-sion of sugar to ethanol and affect of various conditions in alcoholic fermentation process by *S. cerevesiae* SC1 isolated from fermented siahe sardasht grape pomace was investigated.

MATERIALS AND METHODS

Microorganism and its extraction

S. cerevesiae SC1 was extracted from fermented siahe sardasht grape pomace by YGC agar (Redžepović et al., 2002). The extracted yeast was maintained on agar slant containing 1% glucose, 0.5% peptone, 3% beef extract, 3% malt extract and 2% Agar-Agar (Pramanik, 2005). The cultured yeast on Agar Slant was kept at 30°C for 72 h, and then it was stored at refrigerator temperature (4°C) (Pramanik, 2003).

Preparation of inoculums for fermentation process

The medium was prepared with 10 g glucose, 0.2 g beef extract, 0.2 g $(NH_4)_2SO_4$, 0.04 g $MgSO_4$, and 0.5 g KH_2PO_4. After pH adjustment of medium with sulfuric acid to 4.5, it was sterilized for in 121.1°C for 15 min. After cooling of medium to room temperature, the colonies of S. *cerevesiae* SC1 were introduced into it. Then the culture was kept for growth in a shaker incubator in 30°C and speed of the agitator was maintained at 110 rpm (Pramanik, 2003).

Installation of fermentation system and fermentation procedure

Construction of one-liter batch fermenter used in this study is shown in Figure 1 (Michilka, 2007; Roehr, 2001). This fermenter was equipped with magnet agitator. The fermentation process was done in thermo stable condition. All sections of fermenter and medium were autoclaved in 121.1°C for 15 min. Sulfuric acid was used to adjust the initial pH prior to inoculation. The agitator speed was maintained constant through out the experiment at 200 rpm (Pramanik, 2003; Roehr, 2001).

Investigation of pH, temprature, sugar concentration effect on ethanol fermentation

According to significant influence of pH, temperature and initial sugar concentration on fermentation processes, this study was carried out at various amount of above parameters.

Efficiency evaluation of initial pH values 3.5,4, 4.5,5 and 5.5 on ethanol concentration, sugar conversion and ethanol yield was carried out. The pH adjusting in all fermentations was done by sulfuric acid 0.1 N and sodium hydroxide 0.1 N. In this experiment, temperature and sugar concentration adjusted to 32°C and 100 g/L, respectively (Pramanik, 2005).

The temperature of fermentation can affect the development of different Saccharomyces strains. The yield of ethanol and other fermentation byproducts are also related to temperature. Usually the rate of fermentation increases with temperature to an optimum value between 30 and 40°C using conventional baker's yeast (Ingledew and Kunkee, 1985; Jones et al., 1981; Pramanik, 2003; Rousseau et al., 1992; Torres et al., 1986). However, both optimum and temperature tolerance for cell growth and fermentation are strongly strain dependent. Therefore efficiency evaluation of temperature values (28, 30, 32, 34, 36, 38 and 40°C) on ethanol concentration, sugar conversion and ethanol yield was investigated in this study. In this experiment pH, sugar and temperature adjusted to 4.5, 100 g/L and 32°C, respectively.

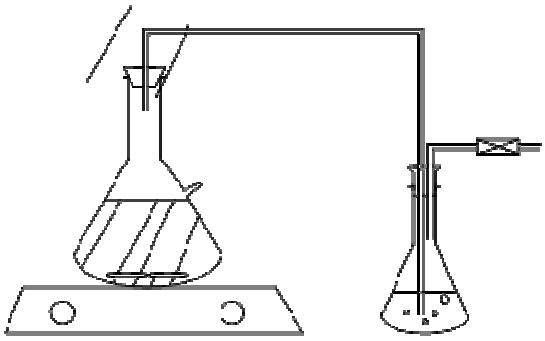

Figure 1. Anaerobic fermentation system for ethanol production.

An interesting research field in alcoholic fermentation is the study of yeast strains that are able to utilize sugar solutions more concentrated than those generally fermented in usual practice (Converti et al., 1985; Pramanik, 2003). To investigate about sugar concentration efficiency on fermentation process, different batches of fermentation medium with various initial concentration of sugar (50, 100, 150, 200, 250 g/L) were prepared. In this experiment pH and temperature adjusted to 4.5 and 32°C, respectively.

To investigate about efficiency of phosphorus source type on the fermentation process, KH_2PO_4 and K_2HPO_4 were used. And to investigate about efficiency of nitrogen source type on the fermentation process, $NaNO_3$, $(NH_4)H_2PO_4$, NH_4NO_3 and $(NH_4)_2SO_4$ were used.

Analytical methods

Samples were taken periodically and aseptically during fermentation for analysis of the ethanol and sugar concentrations. The measurement of sugar concentration was done by DNS method (Miller, 1959; Srinorakutara et al., 2008). The measurement of ethanol concentration was done by spectrophotometrically at 600 nm (Pramanik, 2003; Pramanik, 2005).

RESULTS AND DISCUSSION

Effect of initial pH

Figures 2 and 3 show the initial pH effect on ethanol concentration and sugar conversion in fermentation process. According to results shown in Figure 2, ethanol concentration was increased steadily with time with all pH values though the rate of production varied considerably. The maximum ethanol concentration was achieved with pH 4.5 followed by pH 4 (Table 1). The lowest achieved ethanol concentration was at pH 3.5 indicating that it has lower enzyme activity at this pH. According to results shown in Figure 3, sugar conversion decreased steadily with time at all pH values. The time taken for maximum sugar conversion was 72 h at pH 4.25, whereas the fermentation time for other pH values was found to be more.

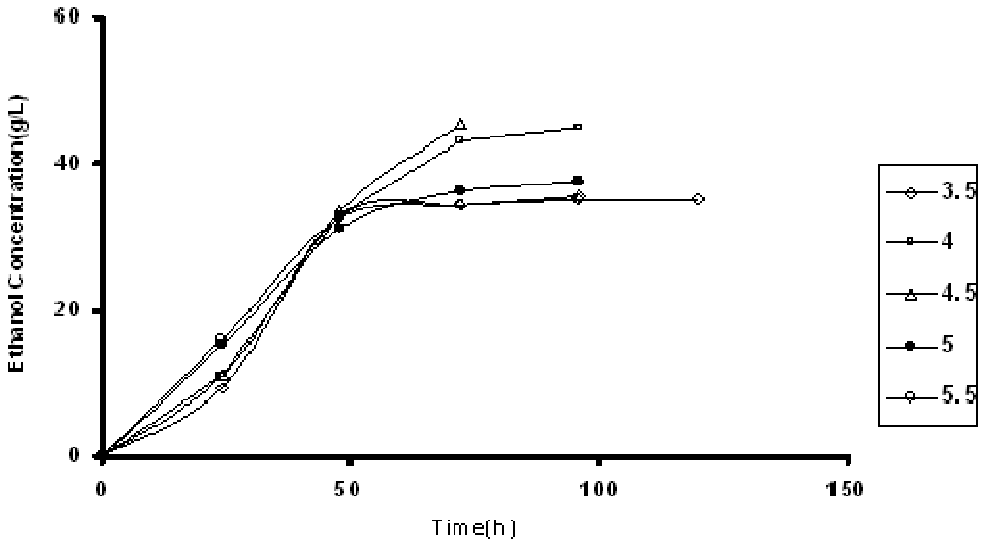

Figure 2. Effect of pH on ethanol production.

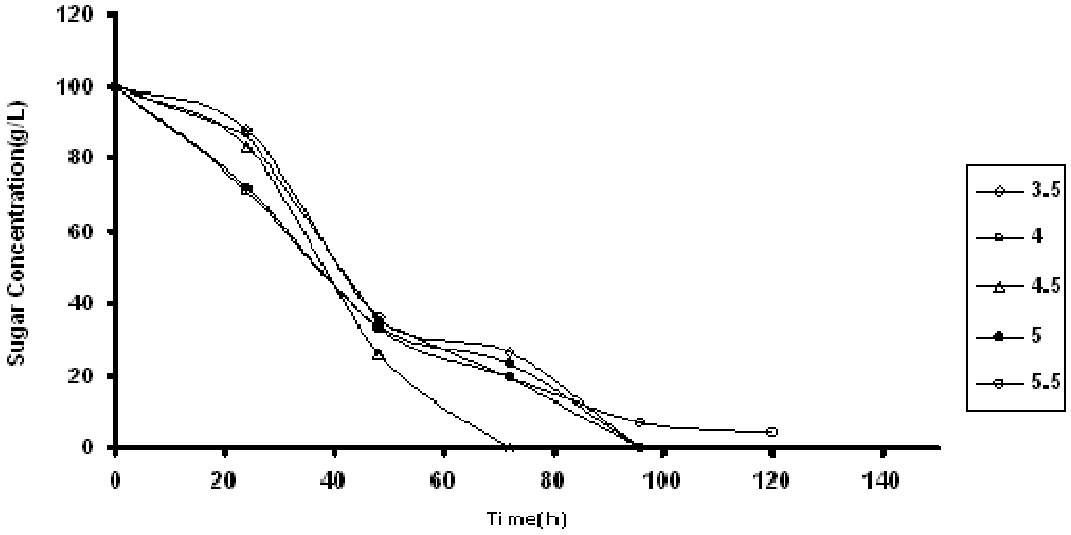

Figure 3. Effect of pH on sugar conversion in fermentation process.

Table 1. Effect of pH on ethanol yield.

pH	Ethanol yield
3.5	0.357
4.0	0.447
4.5	0.453
5.0	0.375
5.5	0.364

$$\text{Ethanol yield} = \frac{\text{Produced ethanol (g)}}{\text{Consumed sugar (g)}}$$

Effect of temperature

Figures 4 and 5 show the temperature effect on ethanol concentration and sugar conversion in the fermentation process. According to the results ethanol concentration increased steadily with time and remained, while sugar concentration decreased steadily with time in all temperatures. The maximum ethanol concentration was achieved in 32°C followed by 30°C. The lowest achieved ethanol concentration was in 40°C.

According to the results, the percentage conversion of sugar was found to be 100% in 30, 32 and 34°C. Minimum percentage conversion was found to be 90.7% in 40°C.

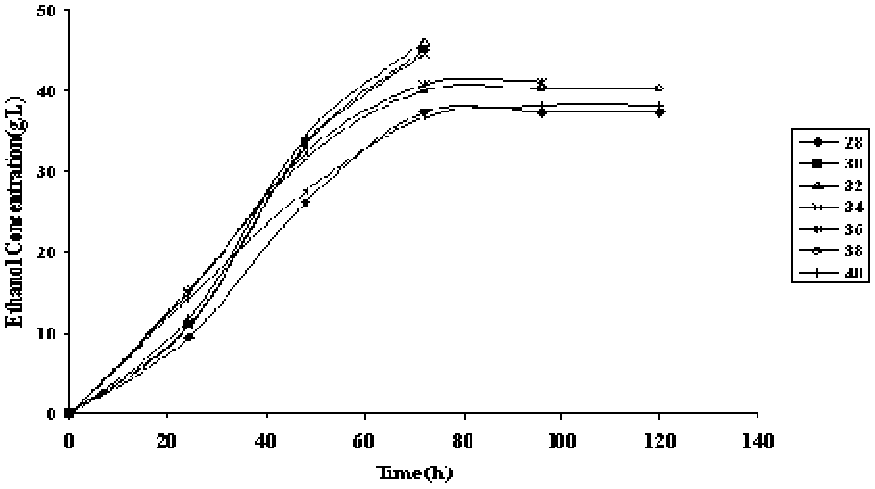

Figure 4. Effect of temperature on ethanol production.

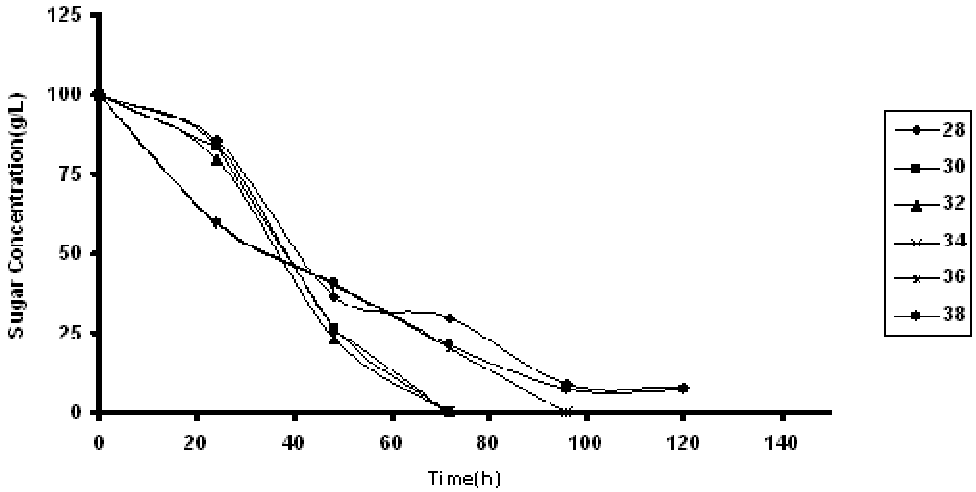

Figure 5. Effect of temperature on sugar conversion.

Table 2. Effect of temperature on ethanol yield.

Temperature (°C)	Ethanol yield
28	0.405
30	0.451
32	0.461
34	0.446
36	0.41
38	0.434
40	0.421

These findings are in agreement with last studies about temperature effect on ethanol fermentation and a little difference refers to this fact that yeast strains differ in response to temperature (Muttara and Nirmala, 1982; Pramanik, 2003; Redžepović et al., 2002; Roehr, 2001). The optimum temperature for vinification can vary widely (Jackson, 2000).

According to the results, maximum and minimum ethanol yield was observed at 32 and 28°C, respectively (Table 2).

Effect of initial sugar concentration

Figures 6 and 7 show the initial sugar concentration effect on ethanol concentration and sugar conversion in the fermentation process. According to the results, ethanol concentration increased with increase in substrate concentration but there was wide variation in time taken

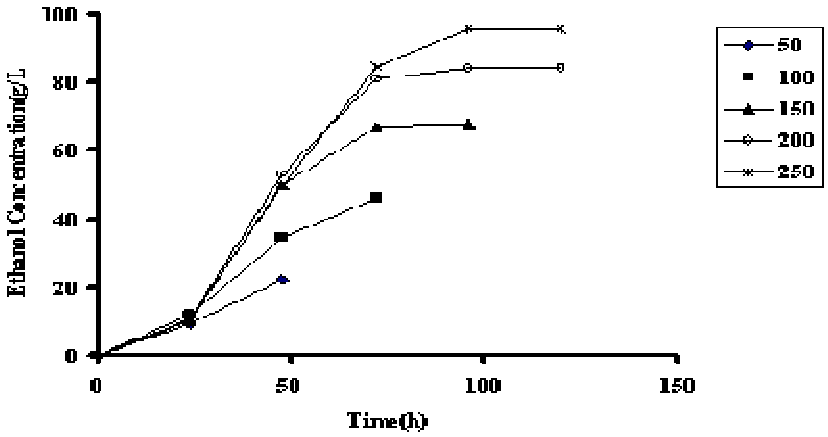

Figure 6. Effect of initial sugar concentration on ethanol production.

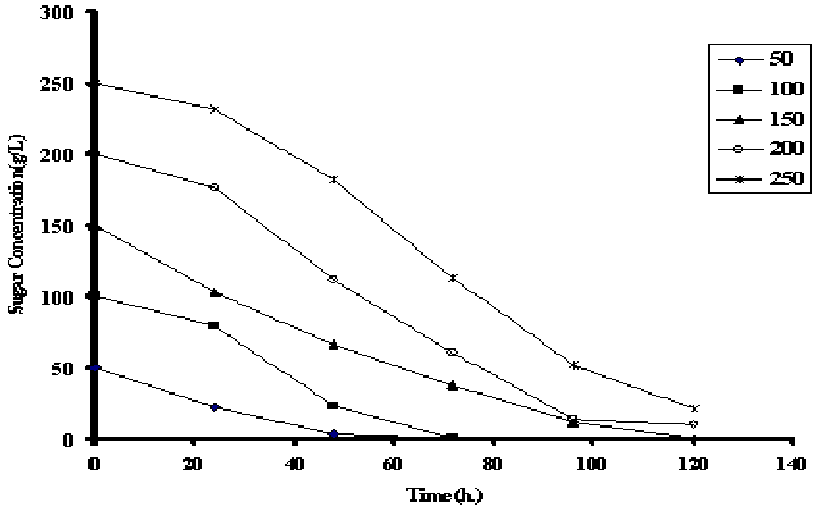

Figure 7. Effect of initial sugar concentration on sugar conversion.

Table 3. Effect of initial sugar concentration on ethanol yield.

Initial sugar conc. (g/L)	Ethanol yield
50	0.446
100	0.461
150	0.453
200	0.442
250	0.416

for complete fermentation. Maximum ethanol concentrations of 23, 46.1, and 95.4 g/L were obtained in 48, 72, and 120 h with 50, 100, and 250 g/L sugar solutions. As was observed for the lower concentration of sugar, the production of ethanol was associated with yeast growth only for a short period of time and hence required less fermentation time. Pramanik (2003) research observed that ethanol became inhibitory when its concentration reached about 95 g/L, but in this research this phenomenon was not observed.

According to the results, the percentage conversion of sugar was found to be 100% in 50 and 100 g/L. Minimum percentage conversion was found to be 89.5% in 250 g/L.

According to results, maximum and minimum ethanol yield were observed at 100 and 150 g/L, respectively (Table 3).

Effect of phosphorus source type

According to results (Figure 8), KH_2PO_4 with 46.1 g/L ethanol concentration is a better phosphorus source in comparing with K_2HPO_4 with 45.3 g/L ethanol concentration.

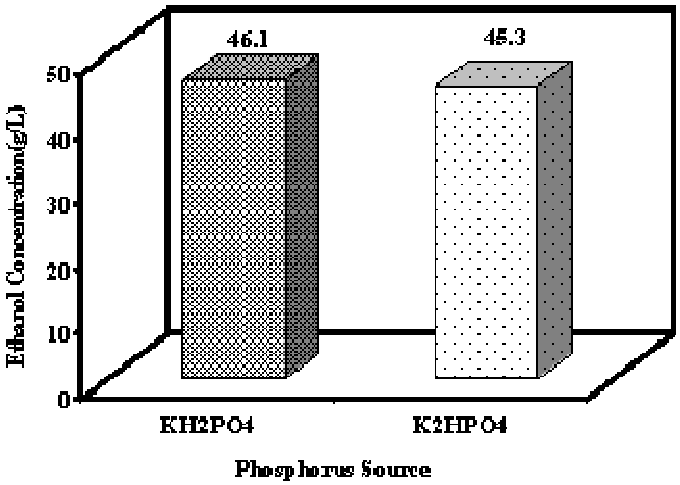

Figure 8. Effect of phosphorus source type on ethanol production.

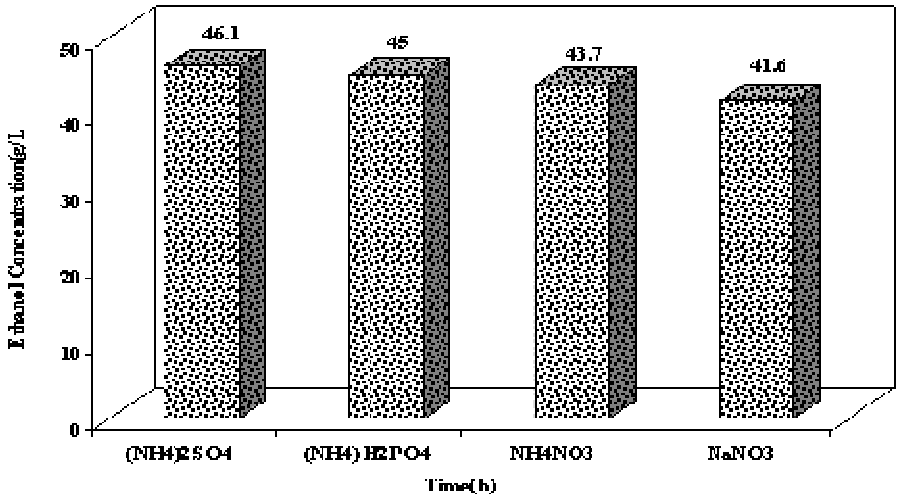

Figure 9. Effect of nitrogen source type on ethanol production.

Effect of nitrogen source type

According to results (Figure 9), $(NH4)_2SO_4$ with 46.1 g/L ethanol concentration is the best nitrogen source in ethanol fermentation by *S. cerevesiae* SC1.

Conclusion

S. cerevesiae SC1 is able to convert sugar to ethanol and other chemicals such as CO_2 (these chemicals were not measured in this study) in fermentation medium. Optimum parameters for ethanol fermentation by this strain are pH 4.5, temperature at 32°C, initial sugar concentration of 100 g/L, KH_2PO_4 as phosphorus source, and $(NH4)_2SO_4$ as nitrogen source.

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