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Ethanol Production from Waste Potato Mash By Using *Saccharomyces cerevisiae*

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Abstract. *Ethanol is one of the bio-energy sources with high efficiency and low environmental impact. Various raw materials have been using as carbon sources for ethanol production. In this study, waste potato mash was chosen as a carbon source; however, a pretreatment process is needed to convert starch of potato to fermentable carbon sources through liquefaction and*

saccharification process. Then, the effect of pH, inoculum size and various nitrogen sources to obtain maximum ethanol from waste potato mash was studied. The maximum ethanol concentration and production rates were 27.7 g/L and 5.47 g/L/h, respectively, at controlled pH 5.5, whereas 22.75 g/L and 2.22 g/L/h were obtained at uncontrolled pH. Optimum inoculum size was determined as 3% for maximum ethanol concentration and production rate. Furthermore, five different nitrogen sources (yeast extract, poultry meal, hull and fines mix, feather meal, and meat and bone meal) were evaluated to determine an economical alternative nitrogen source to yeast extract. In conclusion, this study demonstrated the potential for utilization of potato waste for ethanol production.

Keywords. Enzyme hydrolysis, response surface method, bio-ethanol, Saccharomyces cerevisiae, fermentation, waste potato mash

Introduction

In the 21st century, global warming is one of the greatest problems that threaten the world. While the demand for energy for transportation, heating, and industrial processing is increasing day by day, environmental issues are another point of concern (Hahn-Hagerdal et al., 2006). Renewable energy sources receive attention not only to protect the environment but also to supply the energy needs by reducing dependence on foreign oil, and bio-energy sources have become more important as a viable and economical alternative source. Bio-ethanol, which is one of the renewable energy sources, is known to be a potential alternative to petroleum-derived fuels and has potential to meet the increasing demand for energy for industrial processes, heating and transportation (Balat et al., 2008).

Ethanol is one of end products of fermentation, which can be performed by either bacteria or yeasts. Bio-ethanol can be produced from various sources, such as corn, sugar cane, cellulose, potato, etc. Sugar cane, as a raw material, is used for 60% of global ethanol production, and 40% of global production of ethanol comes from other crops. However, corn grain is the main raw material of ethanol production in the United States (90%) whereas in Brazil, sugar cane is the major source (Balat et al., 2008). These carbon sources are high value products as a food source (Nalley and Hudson, 2003; USDA, 2008).

Potato is a high value crop as a food source and currently, utilized 34% frozen, 28% fresh, 12% chip, 10% dehydrated, 15% potato seed and on farm consumption, and 1% canned in the US (NPC, 2008). Potato can be an alternative for ethanol production, although it also is a high value crop because a significant percent of potato (18% in the potato chips industry) is lost as a waste during the processing (Fadel, 2000). By-products of potato industry are currently; utilized as animal feed. However, waste of potato industry could be a cheap carbon source for ethanol fermentation, because high glucose content of potato wastes in the industry. Waste potato can be fermented to the ethanol by yeast, *Saccharomyces cerevisiae.*

Ethanol production from other sources, such as corn, has already been studied. However, ethanol production from waste potato is a relatively new topic and limited research has been conducted about the utilization of potato waste for ethanol production. Therefore, this study was undertaken to evaluate hydrolysis of potato starch and fermentation parameters, such as pH, inoculum size, and nitrogen sources.

Materials and methods

Microorganism and Medium

Saccharomyces cerevisiae (ATCC 36858) was obtained from the American Type Culture Collection (Manassas, VA). To prepare inoculum, *S. cerevisiae* grown in medium composed of 20 g/L of glucose, 6 g/L of yeast extract, 0.3 g/L of $CaCl_2.2H_2.O$, 4 g/L of $(NH_4)_2SO_2$, 1 g/L of $MgSO_4.7H_2O$, and 1.5 g/L of KH_2PO_4 at 30°C for 24 hour. In order to maintain viability, the culture was stored at 4°C and sub-cultured biweekly, whereas stock cultures kept in 20% glycerol at -80°C for long-term storage.

Hydrolysis of Starch and Response Surface Analysis

Waste potato mash was obtained from Keystone Potato Products, LLC (Hegins, PA). In order to hydrolyze starch, enzyme hydrolysis was chosen because of the high conversion yield. α-amylase (18.8 mg protein/ml) for liquefaction and amyloglucosidase (300 unit/ml) for saccharification, were used. These enzymes were manufactured by Novozyme Corporation (Saint Louis, MO). Box- Behnken Surface Response Method was performed to determine the optimum conditions for liquefaction and saccharification (Box and Behnken, 1960). Box-Behnken response surface design was created to determine optimum combinations of

temperature, enzyme concentration and dry weight concentrations for liquefaction, whereas, temperature, amount of enzyme concentration and time were the parameters for saccharification. Minitab (version 13.3; Minitab Inc., State College, PA) was used to design combination of variables and to evaluate the data. For liquefaction, first pH of the slurry was adjusted to 6.5 by 1 N NaOH, and α-amylase solution was added 0.2 -1 ml per kg solid according to design. The mixture was incubated at 50, 72.5, and 95ºC for at 120 rpm agitation for 3 hours. Initial and final samples were taken and analyzed for residual sugar contents and non-dissolved solid in the solutions to determine optimum temperature- time-enzyme concentration combination which yields the maximum loss in dry weight. The optimum combination of temperature, dose of enzyme (α-amylase), and amount of potato mash was determined as 95°C, 1 ml of enzyme (18.8 mg protein/ml), and 4.04 g dry-weight potato mash /100 ml DI water, respectively with a 68.86% loss in dry weight during liquefaction process. At sachharification process, amyloglucosidase solution, 0.2 - 1 ml, was added to the liquefied solution and incubated at 30, 45, and 60ºC in a shaker incubator at 120 rpm for 72 h. Samples were taken at every 24 h according to Box-Behnken design and analyzed for glucose concentration to determine optimum temperature- time- enzyme concentration combination which yields the maximum glucose yield. For saccharification, dose of enzyme, temperature, and saccharification time were determined by Box-Behnken Surface Response Optimizer as 60°C-72 h-0.8 ml of amyloglucosidase enzyme, which produced 30.7g/L of glucose which was the maximum concentration (Izmirlioglu and Demirci, 2010).

Fermentation Media

The base-line fermentation (glucose/yeast extract) medium used in these experiments contained 50 g/l of glucose, 6 g/l of yeast extract, 0.3 g/L of $CaCl_2 \cdot 2H_2O$, 4 g/L of $(NH_4)_2SO_2$, 1 g/L of $MgSO_4 \cdot 7H_2O$, and 1.5 g/L of KH_2PO_4 per liter of deionized water. For waste potato mash studies, hydrolyzed waste potato mash was used as the base medium supplemented with all other ingredients except glucose. Furthermore, four different alternative nitrogen sources were evaluated to investigate an economical substitute of yeast extract.

Ethanol Fermentation

Sartorious Biostat B Plus Bioreactors (Allentown, PA) with 2.5 L vessel (working volume of 1.5 L) equipped with pH, temperature, and agitation controls were used. Temperature was maintained at 30°C and agitation was maintained at 3,800 x *g*. pH was controlled at 5.5 by adding 4 N NaOH or H2SO4. Inoculum was grown for 24 h at 30ºC. After inoculation, 48 h fermentation was carried out and samples were taken every one or two hour first 12 hour of fermentation whereas every 6 h during the remaining of the fermentation time.

Analysis

Spiral plating was performed to analyze the samples for biomass determination by using a spiral auto-plater (Model 4000, Spiral Biotech, Norwood, MA). After plating, 24 h incubation was carried out at 30°C.Q-count software (Version 2.1, Spiral Biotech, Norwood, MA) was used for microbial enumeration. Samples were analyzed for glucose and ethanol by using YSI 2700 Analyzer (Yellow Springs, OH).

Statistical Analysis

Statistical analysis was conducted to test the significant differences between each treatment. In particular, two-sample t test, ANOVA, and Dunnett tests were used to test for significant differences between the mean of production rate and growth rate values of each treatment. Level of significance was set at 0.05. Statistical analysis was performed using statistical software Minitab (State College, PA).

Results and Discussions

This study was designed to evaluate the potential of waste potato mash for ethanol production. The obtained data revealed waste potato mash can be a raw material for ethanol procudtion.

Effect of pH on Ethanol Production

By using control medium, ethanol fermentation was evaluated at two different pH profiles to determine the effect of pH; uncontrolled pH and controlled pH at 5.5. Figure 1 shows the cell population (log CFU/ml) and glucose and ethanol concentrations (g/L) in the fermentation broth with pH 5.5 control and uncontrolled pH.

The results clearly indicated that a higher growth rate for biomass was obtained with the controlled pH at 5.5 (0.496 CFU/ml/h) than uncontrolled pH (0.289 CFU/ml/h). Furthermore, the maximum ethanol concentration and production rates were 27.7 g/L and 5.47 g/L/h, respectively at controlled pH 5.5, whereas 22.75 g/L and 2.22 g/L/h were obtained at uncontrolled pH. Therefore, determination of optimum pH was relied on growth rate, production rate, and ethanol concentrations, which indicated that controlled pH at 5.5 is better for ethanol fermentation; however, there was no statistically significant difference in the means growth rate and production rates ($p>0.05$).

It was reported that high ethanol production was obtained by using initial pH 5.0 to 6.0 [6]. It was also shown that no ethanol production exits lower than pH 4.0 (Graves et al., 2006). Turhan et al. (2008) reported that maximum ethanol yield, maximum growth rate, and biomass concentration were obtained at pH 5.5 on carob as a medium for ethanol production. Thus, pH 5.5 was found to be the best pH level, and used for rest of study.

Effect of Inoculum Size on Ethanol Production

Three different inoculum sizes was investigated; 1%, 3%, and 5% (v/v) to determine the effect of inoculum size on kinetic parameters on ethanol fermentation from waste potato mash. Figure 2 illustrates the ethanol production (g/L), glucose consumption (g/L), and the cell population (log CFU/ml) over 48 h fermentation periods. The maximum ethanol productivity (6.48 g/L/h) and maximum growth rate (0.3 CFU/ml/h) were obtained when 3% inoculum inoculated with a 30.99 g/L ethanol concentration. Among 1, 3, and 5 % inoculum sizes, 3% was determined the optimum inoculum by comparing production rate, maximum growth rate and produced ethanol. The highest production rate, growth rate and produced ethanol were 6.48 g/l/h, 0.3 log CFU/ml/h, and 30.99 g/L, respectively, which were produced by 3% inoculum size. There was no statistically significant difference in mean production rate among the inoculum sizes ($p>0.05$), however, growth rates were statistically different for inoculum sizes ($p<0.05$).

The results reveal that there is an increase on ethanol yield up to 3%, however 5% inoculum causes a decrease on kinetic parameters of ethanol fermentation by *S. cerevisiae*. Fadel reported that ethanol production increases by inoculum up to 4% (Fadel 2000). Furthermore, it was reported that 3% inoculum size was the optimum for ethanol production from carob (Turhan et al., 2008). The 3% inoculum size was found the optimum for ethanol production and used for following fermentations.

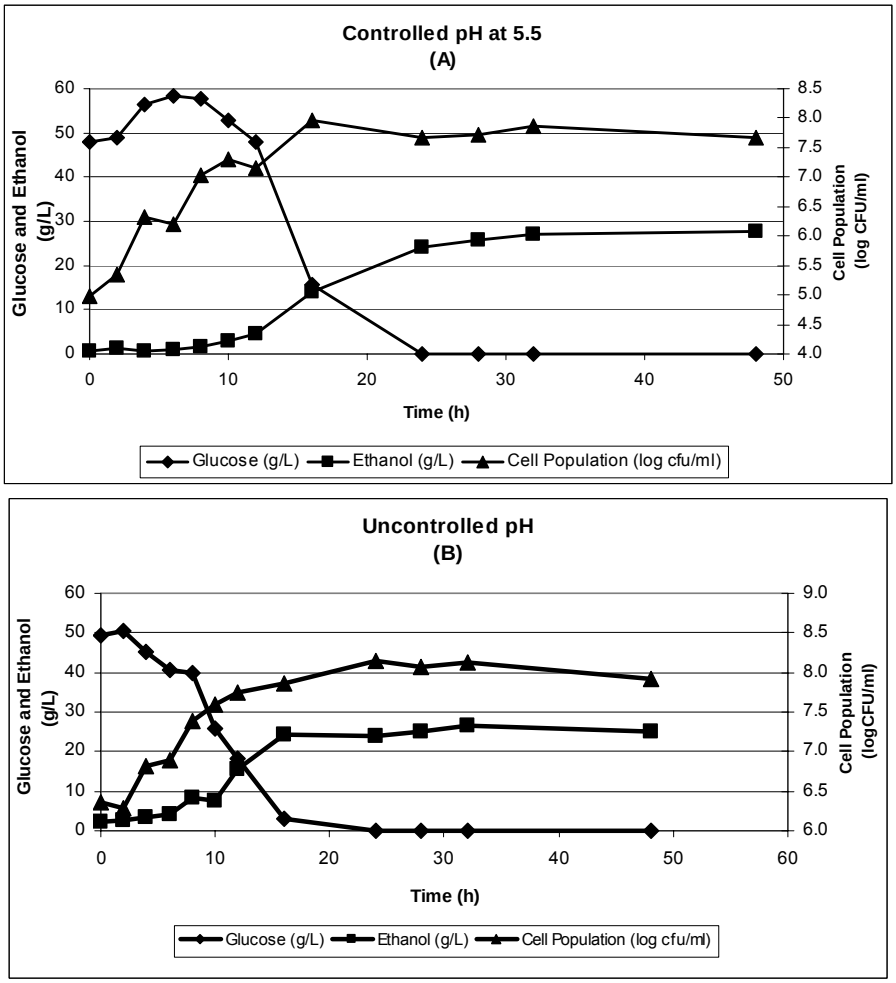


Figure 1. Glucose and ethanol, and biomass concentrations at pH 5.5 (A) and uncontrolled pH (B).

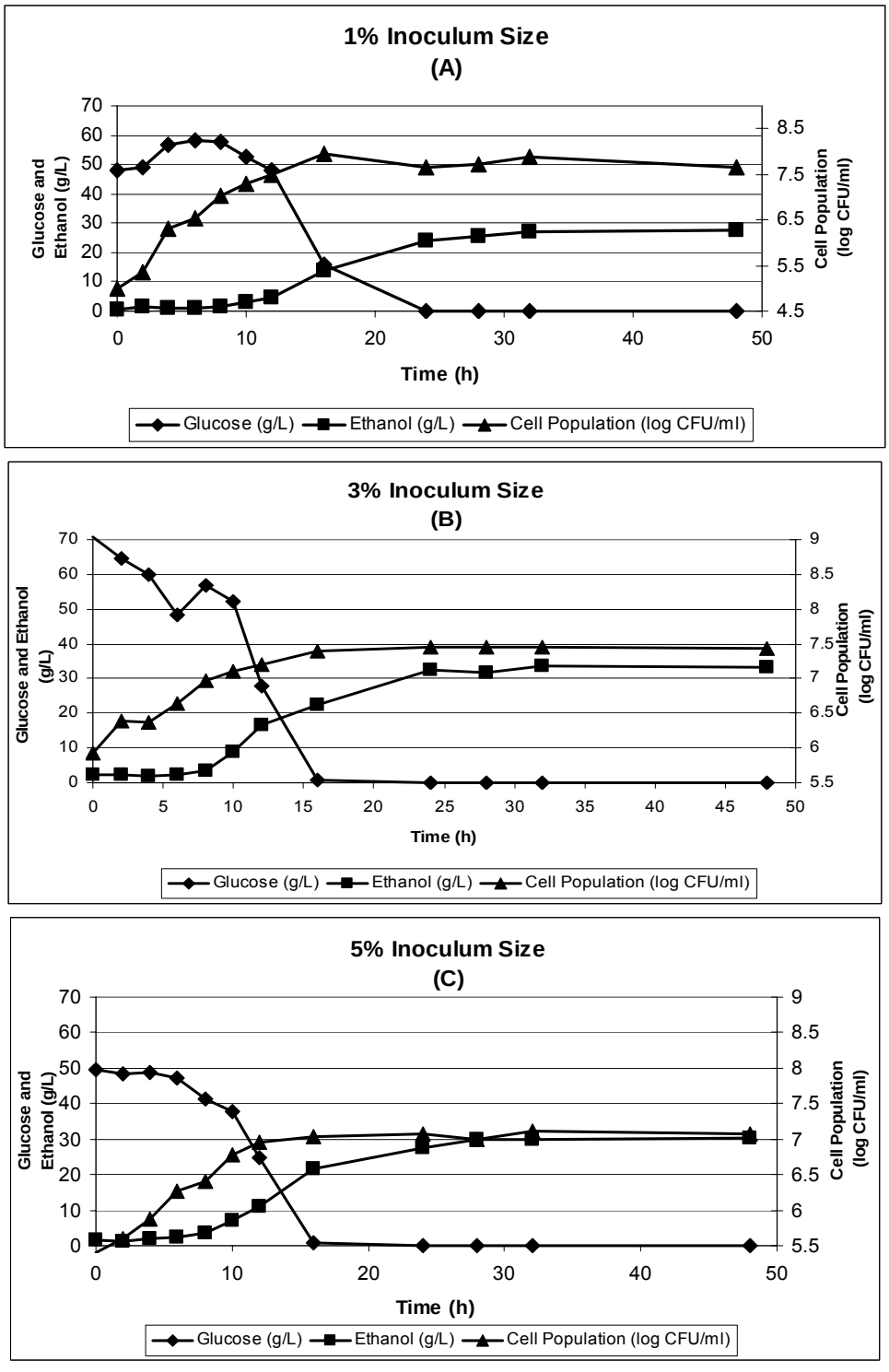
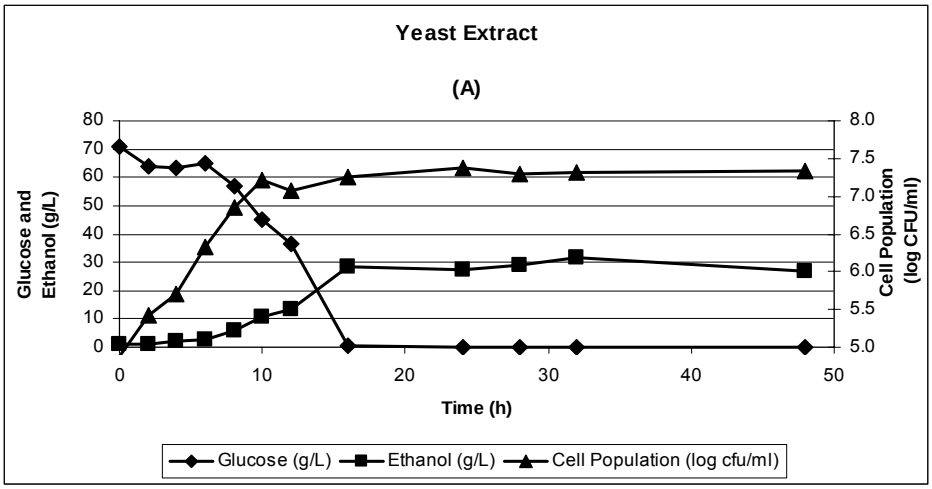


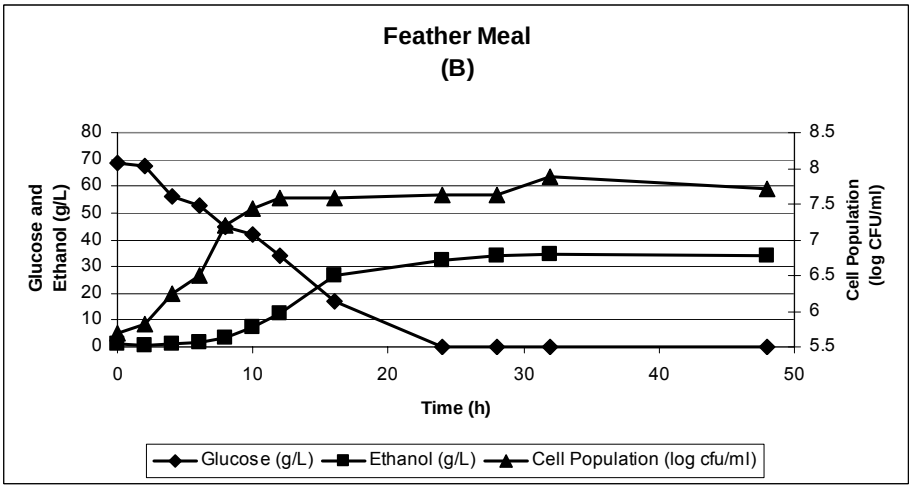
Figure 2. Ethanol, glucose, and cell population in the fermentation broth with different inocula sizes.

Effect of Nitrogen Sources

Fermentation was performed on media included poultry meal, hull and fines mix, feather meal, and meat and bone to evaluate ethanol yield on media which has different nitrogen sources rather than yeast extract. Because the yeast extract is expensive, an alternative nitrogen source was investigated. Figure 3 is a representation of ethanol, glucose and cell population on four different nitrogen sources contained fermentation medium.

A limitation of using alternative nitrogen sources was that these animal-by-products were not 100% soluble. Although, sterilization was done properly, a contamination occurred with meat bone meal at all replications.





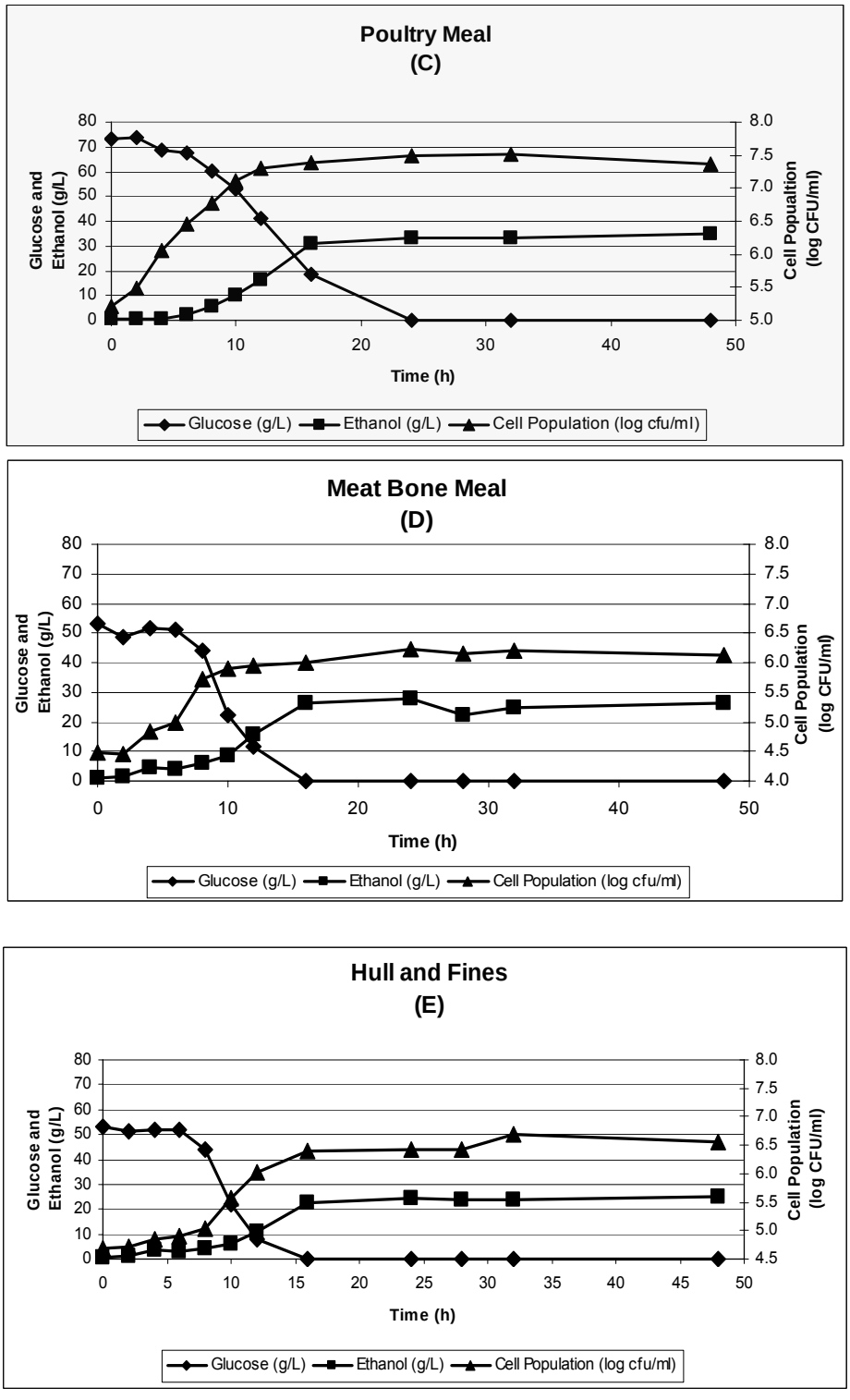


Figure 3. Ethanol, glucose, and cell populations in the fermentation broth with different nitrogen sources: Yeast extract (A), Feather meal (B), Poultry meal (C), Meat bone meal (D), Hull and fines mix (E).

Based on the results, it can be concluded that all of the animal by-products investigated in this study supply nitrogen for growth of yeast in ethanol fermentation. Although the maximum ethanol yield was attained from poultry meal with 35 g/L ethanol; growth rate, and production rate of poultry meal fermentation (0.211 log CFU/ml/h, and 3.2 g/l/h, respectively) were lower compared to yeast extract. Second highest ethanol yield with 32 g/L ethanol concentration in final broth was observed with feather meal which also had very good results for growth rate (0.28 log CFU/ml), and production rate (3.59 g/L/h). Hull and fines mix and meat bone meal were less than yeast extract with a 24.59 and 25.54 g/L ethanol, respectively. Moreover, these two nitrogen sources fell behind in growth and production rates (0.194 log CFU/ml/h and 1.97 g/l/h and 0.13 log CFU/ml/h and 2.36 g/l/h, respectively). Overall, the maximum growth rate was observed with yeast extract and feather meal (both 0.28 log CFU/ml/h). However, maximum production rate was obtained with yeast extract (3.68 g/L/h) with a 30.8 g/L ethanol production.

Statistical analysis showed that there is a significant difference among production rates of different nitrogen sources ($p<0.05$), however, for growth rates, no significant difference occurs. Each nitrogen source was compared to yeast extract by Dunnett Test, and it was concluded that no nitrogen source produces ethanol less or more than yeast extract.

Conclusion

Ethanol fermentation from waste potato mash with pH control at 5.5, inoculum amount of 3%, and yeast extract in the reactor was the best choice. The maximum production rate was obtained at pH 5.5, 30°C, 400 rpm agitation, and 3% inoculum size. The addition of alternative nitrogen sources instead of yeast extract into the fermentation medium resulted promising ethanol production.

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