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# UTILIZATION OF POTATO WASTE FOR PRODUCTION OF BIOETHANOL: A NOVEL TECHNOLOGY

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**ABSTRACT : This study was conducted on the exploration of the potential use of potato as feedstock for bioethanol production, through saccharification and fermentation. The saccharification was performed via thermal and enzymatic hydrolysis through the use of commercial enzymes such as cellulase, α-amylase, β-amylase and glucoamylosidase. The potato mash was mixed with 100 mL distilled water. The mixture was treated with different enzyme concentrations 10 U, 15 U and 20 U. The mixture was incubated at the temperature of 75 °C and pH 5.0. The saccharification process was completed with 10 U of amyloglucosidase at 60 °C, pH 4.5 for 72 h. Samples were withdrawn at different time intervals and the ethanol and consumed sugar assays were carried out by YSI Analyser. The use of commercial α-amylase led to higher reducing sugars release (69.21 ± 0.34 ± 0.32 g/100 g ) and in the combination of enzymes found the maximum efficiency of saccharification i.e. 81.40 ± 0.56 g/100 g. The results showed that the enzymatic treatment led to higher saccharification efficiency (81.40%) equivalent to 96% of the maximum theoretical. The fermentation process by using various strains of Yeast at different time intervals. The maximum ethanol yield was obtained by *Saccharomyces cerevisiae* MTCC 178 after 72 h was 84.19 g/L.**

**KEYWORDS:** Potato, bioethanol, fermentation, saccharification, microorganisms

## INTRODUCTION

Biofuel is a liquid green energy source that is both renewable and sustainable, and it is a viable alternative to petroleum-derived fossil fuels. Its market is promising in near future because of the energy security of fossil fuels and environmental problems (Joshi *et al;* 2017; Niphadkar *et al;* 2018; Usmani 2020). In 2009, the Union Ministry of New and Renewable Energy drafted the National Policy on Biofuels to promote biofuels in India. The Ethanol Blended Petrol (EBP) initiative was started by the Indian government in January 2013, making it necessary for oil firms to sell petrol that contains at least 5% ethanol. The government of India reintroduced the Biofuel policy in 2018 and this policy has set the target bioethanol blending 10% and 30% in petrol up to 2022 and 2030 respectively (Saravanan *et al;* 2018). Currently, in India, the total production of bioethanol is 1.9 billion litres which are 57.6% of the total demand of 3.3 billion litres. Presently the major portion comes through the sugarcane industry. The sugarcane for the production of biofuel is not the sustainable solution because it is an exhaustive and long duration crop (18 months) also sugar mills has a balance sheet problem. Therefore in Biofuel policy, 2018 recommended much feedstock for bioethanol production one of them is potato (Singh *et al;* 2017; Saravanan *et al;* 2020; Usmani 2020). Potato (*Solanum tuberosum* L.) is the world's third most significant food crop in terms of human consumption, after wheat and rice. After China, India is the world's second-largest producer of overall potato production. The potato has

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the highest energy per time per space for any other existing crops (photosynthetic assimilation potential) and less duration crop (3 Months). Approximately 68 % of potato production is consumed fresh, 8.5 % as seed, 7.6% for processing, and approximately 16 % go to waste (At harvesting level 3-4%, at post-harvest handling 3-8% and storage level 6-8%) (Kumar *et al*; 2020; Singh *et al;* 2020). Presently potato processing industries are blooming and they create waste of potatoes in the range of 20-40% in various potato processing based products such as chips, french fries, potato flakes, crisps and potato hash etc. (Srichuwong *et al;* 2009; Khawla *et al;* 2014; Thatoi *et al;* 2016). Potato shows an attractive feedstock for bioethanol production in some regions in Europe, because ethanol yields from potato tubers are approximately 1400–1800 L/ha or more than 74 L/t (Arapoglou *et al;* 2010). Potatoes are carbohydrate-rich sources. In a nutshell, potatoes have a huge potential for industrial bioethanol production because they are ecologically sound, green fuel, highly productive, cost-effective, and manageable. There is a dire need for technology to the alternative use of waste potatoes (by-product in processing and surplus production of potatoes). Using these waste /surplus potatoes for bioethanol production can be an as useful approach by creating wealth from the waste of potatoes.

In this work, we examine how the process conditions such as the type of potato tuber, the type and concentration of enzymes, type of microorganisms, optimum conditions for saccharification and fermentation such as temperature, pH, inoculum size etc. All affect the rate of fresh potato tuber mash hydrolysis, saccharification and fermentation. The main goal was to define the kinetics of the fresh potato tuber starch enzymatic and thermal hydrolysis and the optimal process conditions concerning the total reducing sugars and ethanol formation. In addition, an enzymatic potato tuber hydrolyzate was tested as a substrate for bioethanol production using commercial various bakery yeast strains.

## MATERIALS AND METHODS

### Microorganism, starchy feedstock and chemicals

The commercially various bakery yeast strains (*Saccharomyces cerevisiae*) were purchased from CSIR-Institute of Microbial Technology, Chandigarh, India. These yeast strains were screened for ethanol production prom potato i.e. *Saccharomyces cerevisiae* MTCC 170, *S. cerevisiae* MTCC 171, *S. cerevisiae* MTCC 173, *S. cerevisiae* MTCC 174, *S. cerevisiae* MTCC 177 and *S. cerevisiae* MTCC 178. The processing varieties of potatoes which have more than 20% dry matter such as Kufri Chipsona 1, Kufri Chipsona 2, and Kufri Frysona are mainly grown in India, were used for bioethanol production. Potato tubers were washed, peeled, sliced and milled into a mash before use. Enzymes were obtained from Merck (Darmstadt, Germany). All products were analytical. The *Saccharomyces cerevisiae* strains were was maintained on a malt agar slant. The agar slant consisted of beef extract (3 g $L^{-1}$), yeast extract (3 g $L^{-1}$), peptone (5 g $L^{-1}$), agar (18 g $L^{-1}$) and distilled water (up to 1 L). For the inoculum, the culture was grown aerobically in 250 mL flasks in a shaking water bath at 32 °C for 48 h. The liquid media consisted of yeast extract (3 g $L^{-1}$), peptone (3.5 g $L^{-1}$), $KH_2PO_4$ (2 g $L^{-1}$), $MgSO_4 7H_2O$ (1 g $L^{-1}$), $(NH_2)_2SO_4$ (1 g $L^{-1}$), glucose (10 g $L^{-1}$) and distilled water. Three to six per cent of inoculum was used for the fermentation of potato (Tasić *et al*; 2009; Delgado *et al*; 2009; Khawla *et al*; 2014; Thatoi *et al*; 2016; Wang *et al*; 2016).

### Thermal and enzymatic hydrolysis of fresh potato tubers

Saccharification denotes the breakdown of complex carbohydrates into a simpler form of carbohydrate. The saccharification needs liquefaction and hydrolysis of the substrate. Hydrolysis was performed in a 0.5 L glass three-necked round-bottom flask, equipped with a stirrer and a reflux condenser, immersed in a boiling water bath. Then, the potato tuber mash was added, so that the total volume of the reaction mixture was 200 mL. The different units of enzymes were poured into the flask and heated until boiling. During the hydrolysis process, samples of 3–5 mL were periodically taken from the reaction mixture, centrifuged at 3000 $min^{-1}$ for 15 min. After appropriate dilution of the supernatant, the concentrations of reducing were estimated by YSI analyser (Delgado *et al*; 2009; Thatoi *et al*; 2016; Wang *et al*; 2016; Sheikh *et al*; 2016).

### Fermentation for bioethanol production

Fermentation is the conversion of glucose to ethanol by using yeast. The selection of suitable, efficient, effective and tolerant yeast strains for fermentation is the most critical point. The standardized and optimized fermentation conditions for ethanol production such as pH, inoculum size, the concentration of sugar, ethanol concentration, and temperature etc. Moreover, the conversion of potato starch into glucose by enzymes is more cost-effective, and fermentation with baker's yeast *Saccharomyces cerevisiae* yields the maximum amount of ethanol, optimized conditions and evaluated different strains of yeast such as *Saccharomyces cerevisiae* MTCC 170, *S.cerevisiae* MTCC 171, *S.cerevisiae* MTCC 173, *S.cerevisiae* MTCC 174, *S.cerevisiae* MTCC 177 and *S.cerevisiae* MTCC 178.

The substrate was obtained from a fresh saccharification process. The hydrolyzate was rapidly cooled and adjusted to pH 5.0 by adding 3 M KOH. The main part of the hydrolyzate (2 L) was poured into an Erlenmeyer flask (5 L), and the yeast inoculum (prepared by homogenizing 5 g of yeast in 100 mL of the hydrolyzate) was added. The flask was then fixed on a rotary shaker (120 rpm) placed in a thermostated cabinet at 30°C. Samples of the fermentation medium (about 50 mL) were periodically withdrawn and centrifuged (5000 rpm for 10 min). The supernatants were used for the analysis of reducing sugars and ethanol by YSI analyser after proper preparation and dilution (Montesinos and Navarro 2000; Zhang *et al*; 2011; Izmirlioglu and Demirci 2012).

## RESULTS AND DISCUSSION

### Chemical composition of potato mash

The chemical composition of potato mash was analyzed to evaluate the feasibility of using it for ethanol production. As shown in Table 1, starch and dry were the major components of potato pulp, contributing to 70 gm/100gm and 20.2%, w/w of the biomass, respectively.

The dietary fiber consisted mainly of cellulose (1.9% of biomass), pectin (about

**Table 1. Biochemical composition of potato variety Kufri Chipsona 1**

| Moisture (%) | 80 |
|---|---|
| Dry matter (%) | 20.2 |
| Cellulose (%) | 1.9 |
| Protein (mg/100gm FW) | 2.5 |
| Lipid (%) | 0.7 |
| Pectin (%) | 2 |
| Hemicellulose (%) | 0.47 |
| Amylose (%) | 28 |
| Starch %(gm/100gm) | 70 |
| Glucose (mg/100gm FW) | 60 |
| Sucrose (mg/100gm FW) | 104 |

2% of biomass), and very little hemicellulose (<0.5% of biomass). The starch remaining in the potato pulp would adhere to the cell wall, probably due to embedding in fibers. Therefore, amylase should be co-used with the other cell wall degrading enzymes, such as cellulase and pectinase, to achieve a high hydrolysis rate of starch. Moreover, the use of cellulase would result in the conversion of cellulose in the potato pulp to glucose, making the resulting hydrolyzate more suitable for ethanol fermentation. The protein and lipid contents were 2.5% and 0.7% of the biomass, respectively. Glucose, galactose, and galacturonic acid were the main monosaccharides in the potato pulp. The glucose content of the biomass was 60 mg/100 gm FW, and it was primarily derived from cellulose and starch, as this figure was similar to the total quantity of cellulose and starch in the potato pulp. Because the potato pulp contains negligible hemicellulose, the majority of the sugars are most likely generated from pectin. The sugars would be released simultaneously during pectin hydrolysis. The sugars' enzymatic breakdown was not studied in the study due to their limited levels or unavailability to yeasts. Bandana et al. (2016) also analyzed the variations in biochemical parameters among eight different portions of potato cultivars. Kufri Chipsona-1 showed the highest dry matter content (SEC 30.34%) in the stem end cortex, followed by Kufri Frysona (SEC 27.71%). The bud end cortex (BEC 111.3 mg/100 g FW) had the highest mean reducing sugar values (BEC 111.3 mg/100 g FW) while the stem end medulla (SEM 44.05 mg/100 g FW) had the lowest.

In comparison to other lignocellulosic biomass, potato pulp was poor in hemicellulose and lignin, suggesting that it could be saccharified by common yeast, without the need for an engineered yeast capable of fermenting xylose, and it could be treated without delignification process. On the other hand, the use of a complicated enzyme system has to be taken into account to hydrolyze starch, cellulose, and pectin simultaneously. In addition, the presence of protein in potato residue offers a potential economic benefit, indicating the possibility of enhancing the value of the waste (Arapoglou *et al*; 2010).

## Impact of Glucoamylase, α-amylase, β- amylase and cellulose on saccharification of the potato pulp

Since potato pulp contains large amounts of starch, cellulose, and pectin, the effects of α-amylase, cellulase, and pectinase on saccharification of the potato pulp were investigated. The experiments were carried out with 8.5% potato slurry in 5 mL citric acid buffer (0.1 M). The enzyme loadings of α-amylase and glucoamylase were set to 0.1% (v/v) and 0.05% (v/v) respectively, which could be represented by 5 U/g of biomass and 0.1 U/g of biomass in this study. As expected, when the α-amylase with the supplementation of was β- amylase used, glucose was produced, which was derived from starch (Table 2). The potato slurry was treated with different enzyme concentrations 10 U, 15 U and 20 U. The reaction was incubated at 75 °C and pH 5.0. The saccharification step was performed using 10 U of amyloglucosidase at 60 °C, pH 4.5 for 72 h. Samples were withdrawn at different time intervals and the ethanol and consumed sugar assays were carried out by YSI Analyser.

The use of commercial alpha-amylase led to higher reducing sugars released after 72 h (69.21 ± 0.34± 0.32 g/100g) and in the combination of enzymes found the maximum efficiency of saccharification i.e. 81.40 ± 0.56 g/100 g. The results indicated that the enzymatic treatment by commercial

**Table 2. The enzymatic hydrolysis for saccharification**

| Enzymatic hydrolysis yield (%) for saccharification | | | | |
|---|---|---|---|---|
| Enzymes | At the end of the experiment (72 h) | 48 h | 36 h | 24 h |
| Control (Only heat pretreatment) | 20.4 ± 0.45 | 20.34 ± 0.12 | 20.04 ± 0.01 | 19.84 ± 0.17 |
| Cellulase | 41.64 ± 0.22 | 36.44 ± 0.29 | 31.14 ± 0.32 | 29.84 ± 0.12 |
| Alpha-amylase | 69.21 ± 0.34 | 65.57 ± 0.14 | 61.35 ± 0.85 | 55.30 ± 0.74 |
| Beta- amylase | 21.12 ± 0.15 | 20.18 ± 0.05 | 18.62 ± 0.05 | 17.42 ± 0.27 |
| Glucoamylase | 18.12 ± 0.65 | 16.62 ± 0.27 | 15.72 ± 0.05 | 14.02 ± 0.36 |
| Mixture of above | 81.40 ± 0.56 | 77.21 ± 0.28 | 70.61 ± 0.41 | 69.32 ± 0.45 |

enzymes led to higher saccharification efficiency (81.40%) and ethanol yield (0.49 g/g consumed sugars) corresponding to 96% of the maximum theoretical yield of glucose. The combination of cellulose, α-amylase,β-amylase and glucoamylase resulted in significant increases in the concentrations of monosaccharide sugars such as glucose, galactose and galacturonic acid, and provided a half times higher glucose production rate in the first 24 h and a 3.1 times higher concentration after 72 h. This suggests a "multiplier effect" involving the enzymes on starch and cellulose hydrolysis.

Meyer *et al*; (2009) reported the significant decrease in viscosity of the potato slurry was observed when cellulase was combined with amylases and glucoamylase.

Together with galacturonic acid, a large amount of galactose was detected, suggesting that the pectic substrates in the potato residue were probably made up of highly galactose-branched pectin. The formation of galactose that can be converted to ethanol by yeast would lead to a higher conversion rate of the waste to ethanol. Moreover, even though it did not lead to an increase in glucose production, the addition of the pectinase increased glucose production rate; the saccharification of glucan came to its end within 24 h while there was no glucose detected within 24 h in the case of using amylase alone. The improvement in glucose production rate would be due to that the destruction of cell-wall by the pectinase, cellulase led to the leakage of starch, which made enzymes easily attack starch. Besides starch, cellulose is also a main component of the potato pulp and can be converted to glucose (Collado and Corke 1999; Meyer *et al*; 2009; Ben Atitallah *et al*; 2019).

The combination of cellulase and pectinase resulted in significant increases in the concentrations of galactose and galacturonic acid and provided a 1.6 times higher glucose production rate in the first 24 h and a 1.4 times higher concentration of glucose compared with the use of cellulase alone. This suggests a "multiplier effect" involving the 2 enzymes, on cellulose hydrolysis. The multiplier effect can be explained by that due to the hydrolysis of pectin by the pectinase, the viscosity decreased significantly so that cellulose was easily hydrolyzed by cellulase.

## Pretreatment of the potato pulp with steam

Since potato pulp contains cellulose, lignin, little hemicellulose and a large amount of starch, pretreatment under mild conditions would be reasonable. The potato pulp was treated to a 90-minute hydrothermal treatment at 100°C to see if the treatment

was effective. After saccharifying the treated potato pulp with enzymes, the glucose and galactose concentrations were examined. Without adding enzymes, 19.84 g/L glucose was obtained, as shown in Table 1. In that situation, the combination of enzymes yielded the highest saccharification efficiency of 81.40 %. The findings suggested that pretreatment is sufficient for certain enzymatic hydrolysis of potato slurry.

According to Saha and Bothast (1999), a hot water pretreatment at 121°C for 1 hour resulted in inefficient enzymatic hydrolysis of the starch and cellulose part of maize fibre. Starch would be influenced by this preparation in comparison to cellulose. During this pretreatment, starch gelatinization occurred, resulting in the loss of crystalline areas in starch granules, drastically boosting the accessibility of α-amylase to starch.

Zavareze *et al*; (2010) also reported a heat-moisture treatment boosted starch's enzymatic vulnerability and decreased relative crystallinity. The pretreatment in this study consumed less energy since the temperature (121 $^0$C) was lower than that of usual hot-compressed water pretreatments of lignocellulosic biomass (160–200 $^o$C). The hydrolyzate produced had a 3.7-fold greater glucose concentration than the untreated one.

Even though the pretreatment liquefied starch and pectin, it was anticipated that the accompanying enzymes would easily hydrolyze them, and cellulose would be easily attacked by cellulase due to the disintegration of the cell wall. They acquired the predicted result with glucan hydrolysis. In the case of galactan hydrolysis, however, the pretreatment had minimal effect on the galactose yield. The results showed that enzymes could liquefy galactan without the use of pretreatment, and subsequently hydrolyze it. (Cheng *et al*; 2019).

Therefore, for the production of well-balanced enzymes, the study on enzyme production would be focused on how to improve the activity of pectinase in future. It will be highly helpful for enhancing the efficiency in galactan hydrolysis.

## Bioethanol fermentation with the saccharification potato slurry

The YEPD or yeast extract peptone dextrose media, also often abbreviated as YPD, is a complete medium for yeast growth. It contains yeast extract, peptone, double-distilled water, and glucose. YPD medium is rich in protein, free amino acids, and vitamins, and is, therefore, suitable for yeast growth (Zhao and Bai 2009; Lee *et al*; 2012). However, its outrageous cost limits it from being used in industry. Low-cost nutrients and agricultural wastes high in protein have been evaluated as yeast extract and polypeptide alternatives. Hashem and Darwish (2010), for example, found that due to the presence of nitrogen in the substrate, efficient ethanol fermentation by *S. cerevisiae* was possible using a potato residual stream. The potato pulp, in addition to carbohydrates, provides protein (Table 1), which was revealed to be a nutritional source for yeast growth in this research.

As a result, hydrolyzate was examined as a carbon and nutrient source for ethanol fermentation in this study. For the control tests, the nutrient-poor medium and the YPDG medium containing 10 g $L^{-1}$ yeast extract, 20 g $L^{-1}$ peptone, and 20 g $L^{-1}$ dextrose, as well as the hydrolyzate, were utilised. When glucose was ingested from 15% to 5% within 96 hours, as illustrated in Figure. 1, it was consumed from 15% to 5% within 96 h when the hydrolyzate was used without supplementation of nutrients, indicating the feasibility of the hydrolyzate as the nutrient source for ethanol

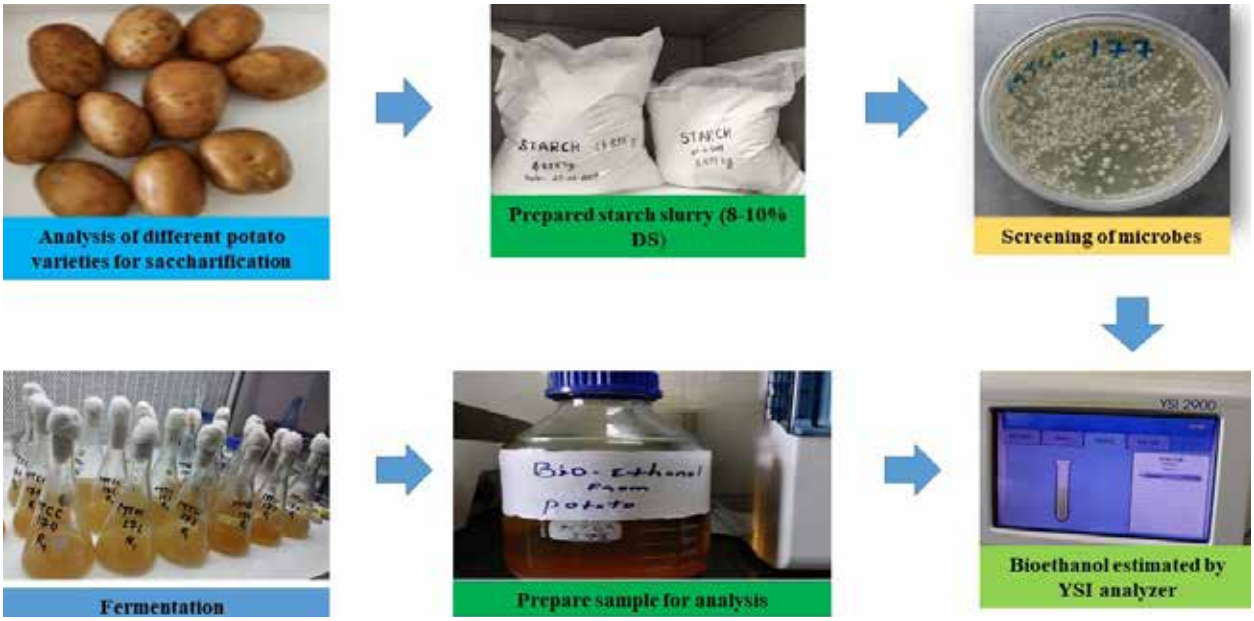


*Figure 1. The bioethanol production process from potato*

fermentation. The concentration of ethanol was very similar to that achieved in the YPDG medium fermentation. Although the ethanol conversion efficiency in the hydrolyzate fermentation seemed to be a little slower.

In the nutrient-poor medium, however, glucose could not be entirely digested during fermentation. The high initial sugar content would have a significant impact on fermentation efficiency. The osmotic effect may be important in glucose ingestion and ethanol generation at high glucose concentrations (Hashem and Darwish 2010).

The cost of potato would be substantially cheaper than that of YE because potato is also a type of agricultural by product. The use of potato pulp as a nutrient would help to reduce the nutrient cost of ethanol fermentation. However, the galactose is not entirely utilised in both the hydrolyzate and the YPDG medium fermentation, most likely due to the inhibiting impact of ethanol. Because galactose is a yeast fermentable sugar, improving galactose to ethanol conversion would increase the total production of ethanol from potato pulp.

In the future, the fermentation variables and strategy should be researched to improve galactose to ethanol conversion rates and, as a result, ethanol yields.

At the end of fermentation, the reducing sugars were 5.53 g $L^{-1}$. Therefore, the sugars consumed were 10 g $L^{-1}$ (Figure 2). In all treatments, the non-fermented sugar at the end of fermentation was very low. The consumed sugar during fermentation was very high (up to 67%), indicating the high ability of *S. cerevisiae* to bioconvert reducing sugars.

Table 3 shows the ethanol production (g $L^{-1}$) and ethanol productivity (g $L^{-1}h^{-1}$) with the use of various strains of *S. cerevisiae*. In all treatments, *S. cerevisiae* MTCC 178 produced high quantities of ethanol (84.194 g $L^{-1}$), with the ethanol productivity (0.877 g $L^{-1}h^{-1}$) and the incubation period was 96 h. In all cases, the product yield corresponded up to 91% of the max theoretical yield. The results indicate

**Table 3. Bioethanol yield and productivity in a different time interval**

| Microorganisms | Ethanol yield and productivity in a different time interval | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 36h | | 48h | | 72h | | 96h | |
| | Ethanol Yield (g/l) | Ethanol productivity (g/l/h) | Ethanol Yield (g/l) | Ethanol productivity (g/l/h) | Ethanol Yield (g/l) | Ethanol productivity (g/l/h) | Ethanol Yield (g/l) | Ethanol productivity (g/l/h) |
| *Saccharomyces cerevisiae* MTCC 170 | 37.300 | 1.036 | 49.633 | 1.034 | 77.200 | 1.072 | 81.710 | 0.851 |
| *Saccharomyces cerevisiae* MTCC 171 | 45.000 | 1.250 | 59.067 | 1.230 | 82.400 | 1.144 | 87.164 | 0.908 |
| *Saccharomyces cerevisiae* MTCC 173 | 28.633 | 0.795 | 43.567 | 0.907 | 67.500 | 0.937 | 76.572 | 0.798 |
| *Saccharomyces cerevisiae* MTCC 174 | 36.971 | 1.027 | 50.514 | 1.052 | 75.700 | 1.051 | 80.500 | 0.838 |
| *Saccharomyces cerevisiae* MTCC 177 | 31.300 | 0.869 | 43.770 | 0.911 | 76.291 | 1.059 | 79.113 | 0.824 |
| *Saccharomyces cerevisiae* MTCC 178 | 39.315 | 1.092 | 48.603 | 1.012 | 79.200 | 1.100 | 84.194 | 0.877 |

LSD (P=0.05) Time = 2.225, Microorganism = 2.225, Time Microorganism =NF

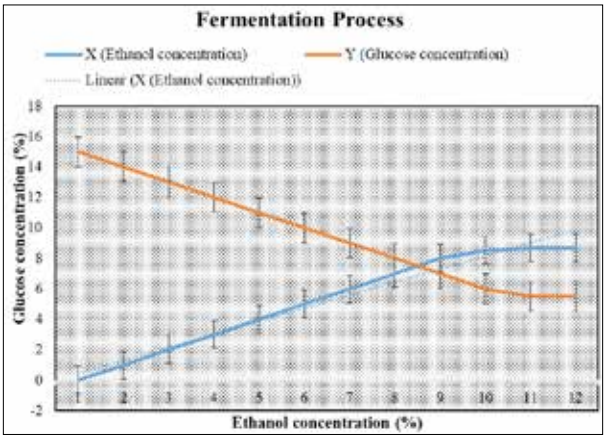


*Figure 2. Bioethanol production of fermentable sugar from enzymatic hydrolysis of potato slurry*

that the critical parameters for ethanol production from PPW were the enzyme combination, the dose and the residence time of hydrolysis. After liquification and saccharification, fermentation by *S. cerevisiae* converted sugars to high yields of ethanol.

## CONCLUSION

In this study, the feasibility of potatoes for ethanol production was investigated. Analytic results of the components showed that the potato was abundant in starch, cellulose, and other carbohydrates. The enzymes are suitable for saccharification of potato pulp due to its high starch, pectin, cellulose and α-amylase activities. A hydrothermal treatment increased the glucose yield by 2 times. The resulting hydrolyzate was efficiently converted to ethanol without nutrient supplementation by various strains of yeast.

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