



## Production of Bioethanol from Waste Potatoes

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### Abstract

In this research, production of ethanol from waste potatoes fermentation was studied using *Saccharomyces cerevisiae*. Potato Flour was prepared from potato tubers after cooking and drying at 85°C. Homogenous slurry of potato flour was prepared in water at solid liquid ratio 1:10. Liquefaction of potato flour slurry with  $\alpha$ -amylase at 80°C for 40 min followed by saccharification with glucoamylase at 65°C for 2 hr. Fermentation of hydrolysate with *Saccharomyces cerevisiae* at 35°C for two days resulted in production of 33 g/l ethanol.

The parameters studied were; temperature, time of fermentation and pH. It was found that Saccharification process is affected by enzyme Amylo 300 concentration and concentration of 1000 $\mu$ l/100ml gives efficient effect of the process. The best temperature for fermentation process was found to be about 35°C. Also it was noticed that ethanol production was increased as time of fermentation increased but after 48 hr further increase in fermentation time did not have appreciable effect. Finally, the optimal value of pH for fermentation process was about 5 to 6.

**Keywords:** Fermentation, *Saccharomyces cerevisiae*, saccharification, bioethanol.

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### 1. Introduction

Bioethanol as an alternative source of energy has received special attention world over due to depletion of fossil fuels. From the 18<sup>th</sup> century to the beginning of this century, major discoveries about the biology and chemistry of fermentation and distillation made it possible to produce cheaper ethanol from variety of organic materials [1, 2].

To produce ethanol from biomass two key processes are followed, first the starch or hemicelluloses and cellulose portions of the biomass are broken down into simple sugars through a process called saccharification. Second, the sugars are fermented to produce ethanol. [3, 4]

The sugary substrates available are comparatively expensive than molasses but can be easily used for ethanol production with some modification in the process. On the other hand cellulosic materials are cheaper and available in plenty but their conversion to ethanol involves many steps and is expensive. The starchy substrates are promising due to their economic viability and availability. Starchy crops like corn, barley, wheat, rice and tuber crops viz. potato, sweet potatoes are being exploited for the production of bioethanol world wide since it is rich in starch, which makes it a cheap substrate for ethanol production. The problems associated with its processing will also be less than in other grains. It is also semi-perishable food which can be stored for considerable period without

spoilage. Good quality alcohol can be produced from potato which can be used for fuel. The purpose of this research was to explore the parameters and operating conditions that give the best conversion of potato starch into ethanol. [4, 5, 6].

## 2. Experimental Work

### 2.1. Material and Methods

**2.1.1. Potato preparation:** Waste of potato was brought from chips factory and restaurants. 1 kg waste potatoes were cooked with two liters water in a pressure cooker at 85 °C for one hour then it dried overnight in oven at 70°C and mashed to a fine powder (0.3mm).

**2.1.2. Preparation of potato slurry:** Slurries were prepared from potato powder mixed with water at ratio (1:20 w/w). The slurry was treated with amylase enzyme (obtained by Himedia) (1000µl/100ml) at 80 °C for 40 min under shaking conditions.

**2.1.3. Analyses of waste potatoes:** Waste of potatoes contained 77% moisture, 18.2% starch, 2.4 % proteins and 0.6% total sugars while the respective hydrolysate of potato was 13.2, 70, 10.8, 1.5 and 4.5%, (Table 1).

**Table 1,**  
**Composition of waste potato and potato hydrolysate** [6].

Component	Composition (%)	
	Waste potato	Potato hydrolysate
Moisture	77	13.2
Starch	18.2	70
Proteins	2.4	10.8
Total sugar	0.6	1.5
Others	1.8	4.5

**2.1.4. Saccharification of slurry:** Saccharification of slurry was carried out at 65°C for 2 hr using Amylo-300 enzyme (obtained by

Himedia) of concentration (1000 µl/100ml). The reaction was monitored by the yield of total reducing sugars estimated by dinitrosalicylic acid method [7].

**2.1.5. Effect of enzyme concentration:** The liquefied potato flour was saccharified with different concentrations of Enzyme Amylo-300 (600, 800, 1000 µl/100ml) at 65°C. The reaction was monitored by the yield of total reducing sugars which was estimated by dinitrosalicylic acid method [8].

**2.1.6. Yeast culture:** A fast fermentation strain of *Saccharomyces cerevisiae* was maintained on yeast extract peptone dextrose agar medium containing in grams per 100ml ( potato starch 10; peptone 0.1; malt extract 0.1 ; yeast extract 0.2; magnesium chloride 6 H<sub>2</sub>O 0.1; calcium carbonate 0.2 ; ammonium phosphate 0.2 ; and ferrous sulfate .7H<sub>2</sub>O 0.001). Yeast cells were grown in inoculums medium at 35°C for 20 hr under shaking condition (100 rpm) and centrifuged at 8000 rpm for 20 min. For testing the effect of pH on fermentation few drops of 1N HCl or 1N NaOH were added to this medium to obtain the desired initial pH [8].

**2.1.7. Fermentation of potato's hydrolysate:** The hydrolysate of potato was inoculated with *Saccharomyces cerevisiae* of 0.8% concentration (w/v) at 35°C. Ammonium sulphate of 0.2% concentration (w/v) was added as source of nitrogen. Flasks were incubated at 35°C under stationary condition for 96hr and ethanol content was measured at an interval of 24 h by gas liquid chromatography (GC 8200) using capillary column and flame ionization detector.

## 3. Results and Discussion

### 3.1. Effect of Concentration

Fig.(1) shows that as enzyme concentration increased the total reducing sugar was also increased and concentration of 1000 µl/100ml gave efficient effect in saccharification. This behavior is caused by the higher growth rate of microorganisms at high values of inoculums concentrations which lead to higher rate of organic matters degradation in the process. This finding is in agreement with that found by Nagoda T [9].

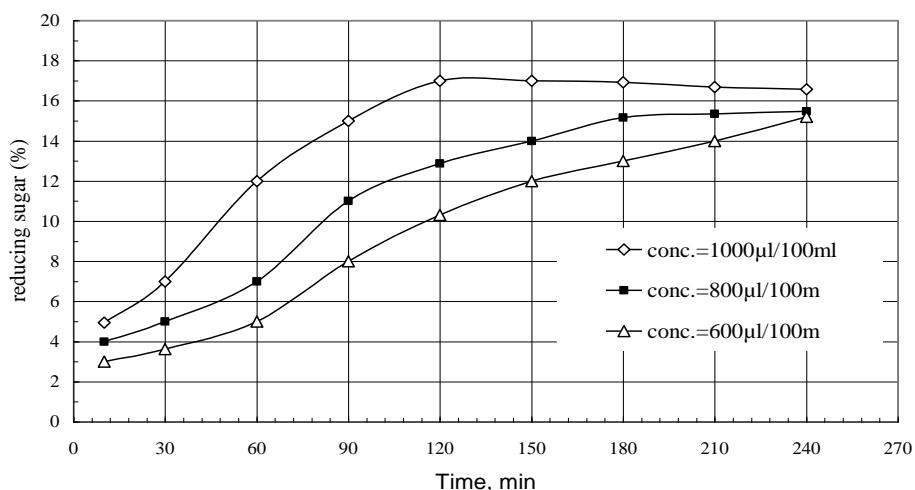


Fig. 1. Effect of Amylo concentration on Saccharification on hydrolysate at 65 °C.

Fermentation of 30% slurry of potato hydrolysate was carried out at different temperatures (25, 30, 35, 40 °C) under stationary conditions up to 48 hr. Fig.(2) shows that fermentation at 35°C gives maximum content of ethanol of 32g/l. This is due to the effect of temperature on the activity of the microorganisms.

It is well known that temperature above 40°C affects the membrane composition of microorganisms, e.g. the phospholipids fatty acid composition changes with temperature and hence affect the enzymatic system of the microorganisms [10]. This leads in turn to decreasing the rate of fermentation process.

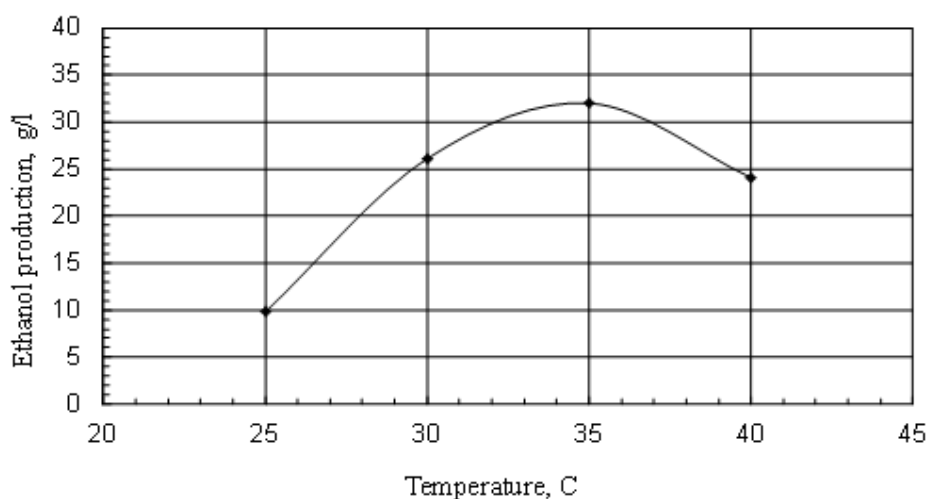


Fig. 2. Effect of temperature on ethanol production (Enzyme Conc. =1000µl/100ml, Time of fermentation = 48 hr, pH=5.5).

### 3.2. Effect of Time Fermentation

Fermentation of potato hydrolysate was carried out at 35 °C for different time intervals using enzyme concentration (1000µl/100ml) of Amylo-300 containing amyloglucosidase. Fig.(3) shows

that ethanol production was increased as time of fermentation increased from 24 to 48 hr so it reached to 32g/liter at 48hr ;however further increase in fermentation time did not have appreciable effect. This result is in agreement with that mentioned by Wang F [11] and Oner[12].

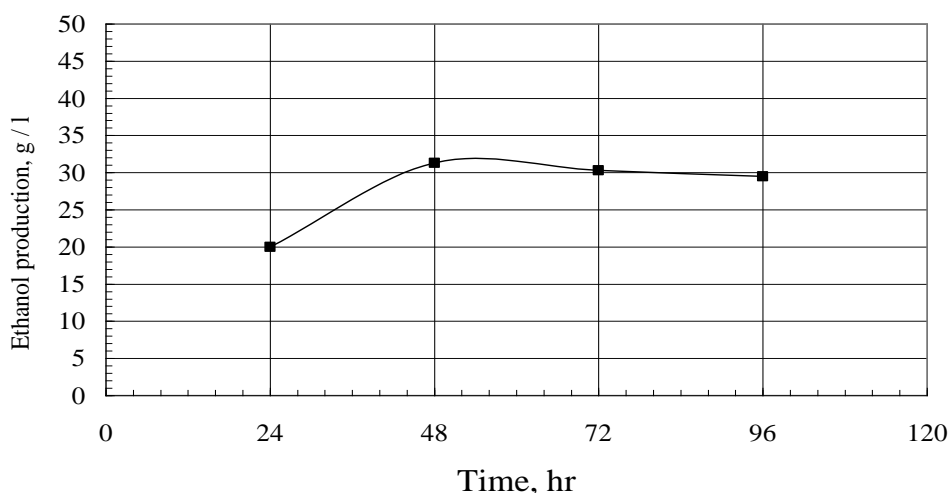


Fig. 3. Effect of fermentation time on ethanol production (Enzyme Conc. =1000 $\mu$ l/100ml, Temp =35°C, pH =5.5).

### 3.3. Effect of pH

Figure (4) shows that the production of ethanol is increased with increasing pH from 3 to 6. The optimal production of ethanol was 32g/l which occurs at pH 6. After that the production began to

decrease with increasing pH and reached to 22 g/l at pH 8. This behaviors can be interpreted by the effect of pH on the activity of  $\alpha$  amylo enzyme, Since the activity of this enzyme is severely effected by the value of pH specially after value of pH=6 [8].

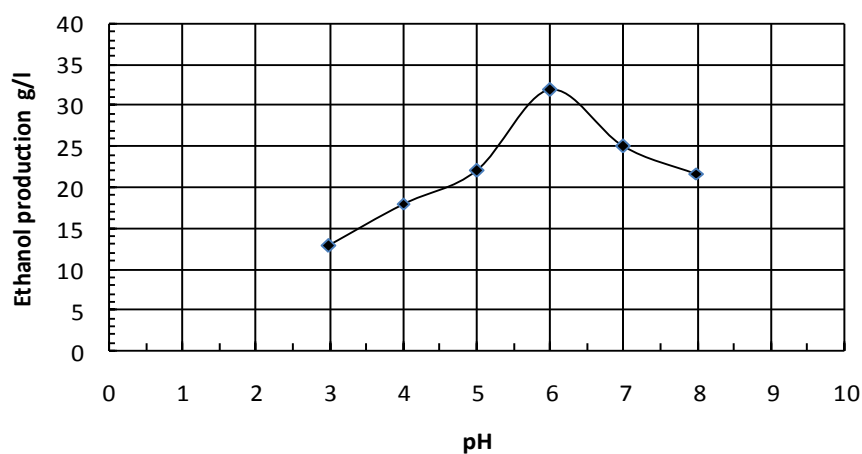


Fig. 4. Effect of pH on ethanol production(Enzyme Conc.=1000 $\mu$ l/100ml, Temp.=35°C, time of fermentation=48 hr.)

#### 4. Conclusions

1. Saccharification process is affected by enzyme Amylo 300 concentration and concentration of 1000µl/100ml gives efficient effect on the process.
2. The best temperature for fermentation of potato starch to produce ethanol using *Saccharomyces cerevisiae* is about 35°C.
3. Ethanol production was increased as time of fermentation increased from 24 to 48 hr; however further increase in fermentation time did not have appreciable effect.
4. The optimal value of pH for fermentation process to produce ethanol production was about 6.

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## انتاج الايثانول الحيوي من نفايات البطاطا

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### الخلاصة

في هذا البحث تمت دراسة انتاج الايثانول من تخمير نفايات البطاطا باستخدام خميرة الخبز سكرومايسيس سيرفيسيا. كما تم تحضير مسحوق البطاطا وذلك بطبخ درنات البطاطا وتجفيفها عند درجة حرارة ٨٥ م<sup>٠</sup>. وتم كذلك تحضير مهورس البطاطا في الماء بنسبة ١:١٠ صلب- سائل ومعاملة المهورس مع انزيم الفا اميليز عند درجة حرارة ٨٠ م<sup>٠</sup> لمدة ٤٠ دقيقة وبعدها معاملة الناتج مع انزيم كلوكواميليز عند درجة ٦٥ م<sup>٠</sup> لمدة ساعتين. تمت عملية التخمير باستخدام خميرة سكرومايسيس سرفيسيا عند درجة ٣٥ م<sup>٠</sup> لمدة ٤٨ ساعة، تم الحصول على الايثانول بتركيز ٣٣ غم/ لتر .  
العوامل التي تمت دراستها في هذا البحث هي : درجة الحرارة، زمن التخمير، والدالة الحامضية (pH). ووجد ان عملية تحويل النشا الى سكر تتاثر بتركيز انزيم الاميلو ٣٠٠ و ان افضل تركيز للانزيم هو ١٠٠٠ مايكرو لتر/ ١٠٠ مللتر. كما وجد ان تركيز الايثانول يزداد بزيادة زمن التخمير لحد ٤٨ ساعة ولم يلاحظ بعد هذا الزمن اي تأثير على الإنتاجية وكانت أفضل درجة حرارة لعملية التخمير هي ٣٥ م<sup>٠</sup> عند مدى (pH) من ٥ الى ٦ .