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Fermentation of Starchy Potato Waste to Bioethanol by Top Fermenting Brewer's Yeast

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Abstract: Bioethanol can be produced from the wasted potatoes using simple technology and the potato waste residues can ideally produce Bioethanol production. Potato has been used for Bioethanol production because it contains both starch and glucose. The potato waste substrate was collected, cut into small pieces, juice was extracted and subjected to Physical (Steam explosion), Chemical (Acid and Alkali hydrolysis) and Biological methods (Enzymatic hydrolysis) of Pretreatment in order to liberate the fermentable sugars from the complex polysaccharides. From the three Pretreatment methods, Biological pretreatment by Enzymatic hydrolysis using the enzyme source obtained from the organism has liberated more amount of fermentable sugars upto 44.66 %. The yeast *Saccharomyces cerevisiae* was isolated from the extracted potato juice and identified by comparing the characteristics of the *Saccharomyces cerevisiae* Reference strain (MTCC 172). The growth rate of *Saccharomyces cerevisiae* cultures were tested by using different Carbohydrate sugars (Glucose, Sucrose and Fructose) and Nitrogen source (Ammonium sulphate and Urea). Among the Sugars, Glucose supported maximum growth of 0.74 % and in Nitrogen source, maximum cell density 1.47 % was recorded after 48 hours of incubation. The fermentation parameters such as pH, temperature and inoculums size were optimized for enzyme hydrolyzed potato waste residues. It was observed that the pH 5.5, Temperature level of 44°C and Inoculums size of 1 % were ideal for Bioethanol fermentation.

Key words: Bioethanol · Potato Waste · *Saccharomyces cerevisiae* · Pretreatment · Fermentation and Optimization

INTRODUCTION

Ethanol is a clear, colourless, flammable, oxygenated hydrocarbon with the chemical formula C_2H_5OH. Ethanol has been made since ancient times by fermenting sugars. All the ethanol used for fuel and alcoholic drinks and most industries. Fuel ethanol is also known as bioethanol, since it is produced from plant materials by biological processes. Fuel ethanol is the largest market by far, accounting for 60 % of total ethanol production worldwide. Industrial ethanol accounts for 20 % of the market and beverages for about 15 %; both these markets are growing comparatively slowly. The production of ethanol from biomass is progressing in many countries worldwide however the production costs are still relatively high when compared to petrol. The environmental benefits coupled with the social benefits and economic benefits can be seen to have a dollar value offsetting the higher relative cost compared to petrol [1, 2].

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Ethanol was first produced thousands of years ago by fermentation of carbohydrates and in some countries large volumes are still produced by this method. Synthetic alcohol was first produced industrially in the 1930s by indirect catalytic hydration of ethylene but suffered from disadvantages such as corrosion from the large volume of sulphuric acid handled and the energy required for concentration. Fermentation ethanol process based on starch or sugar based feedstock such as corn, potato and sugarcane was being used to meet the demand for ethanol as a fuel. Ethanol fermented from renewable sources for fuel or fuel additives are known as bioethanol. Additionally, the ethanol from biomass based waste materials was considered as bioethanol. Currently, there is a growing interest for ecologically sustainable bio-fuels. The target in the European Union is to increase bioenergy contributions in total energy consumptions from 3 to 12 % by the year 2010 [3].

Ethanol is one of the good sources of liquid energy for automobiles and industries. Ethanol is used as universal solvent. It is also used as fuel. In future, ethanol is going to blend with petrol in high proportion. Among the liquid fuels, ethanol is used as an alternative to petroleum (gasohol) by blending with petrol at the rate of 20 %. To reach the future demand of ethanol, it should be produced in high quantity from the agricultural raw material. The production of ethanol was basically by chemical methods but now, it can be effectively produced using microbial process.

Currently, commercial ethanol production relies on the fermentation of sucrose from cane sugar and molasses or glucose derived from starch based crops such as corn, wheat and cassava and there is a growing need for the industry to improve technology and expand production [4]. Efficient bioethanol production requires a rapid fermentation leading to high ethanol concentrations. An important issue for the efficient ethanol production is to optimize the fermentation step regarding following main parameters temperature, pH, media composition, mixing aeration and elimination of infection [5].

Microorganisms found to be efficient ethanol producers from pure Xylose in well defined growth media will not necessarily ferment the lignocellulosic hydrolysate efficiently. The pre-treated hydrolysate contains various inhibitors of microbial fermentation such as weak organic acids like acetate, furan derivatives and phenolic monomers from sugar and lignin degradation respectively. Sometimes the inaccessibility of the carbohydrates in the hydrolysate to the microorganisms is responsible for the incomplete fermentation and subsequently lower ethanol yields. Furthermore, the microorganisms have to be able to grow and produce ethanol in a pre-treated starch hydrolysate to make them usable in an industrial bioconversion process of lignocellulosic biomass. The most common way of bioethanol production today is by fermentation using the yeast *Saccharomyces cerevisiae* with high ethanol yields from starch based substrates. In the past decades, thermophilic bacteria have gained more attention because of fast growth rates and their ability to degrade a broad variety of both hexoses and pentoses [6, 7, 8]. Although, ethanol tolerance of thermophiles is generally less than those of *Saccharomyces cerevisiae* and the well known mesophilic bacterium *Zymomonas mobilis*, they have several advantages like lower risk of contamination, increased bioconversion rates and product recovery. The present study was aimed to produce bioethanol from the potato waste using *Saccharomyces cerevisiae* and to study the optimization parameters to increase bioethanol production.

MATERIALS AND METHODS

Collection of Starchy Materials: The waste potato sample was collected from the market in Chidambaram. The materials were cut into small pieces and juice was extracted. Then, these samples were used for all the studies of research.

Collection of Reference Strain: The reference strain *Saccharomyces cerevisiae* MTCC-172 was obtained from Microbial Type Culture Collection, Gene Bank, Institute of Microbial Technology (IM Tech), Chandigarh, India and used as reference strain throughout the study. The strains were maintained in Yeast Peptone Dextrose (YPD) agar slant at 30 ±2°C with monthly transfer.

Pretreatment of the Substrate: Pretreatment of the substrate was one of the most important methods in order to process the material to release the fermentable sugars. Here different pretreated methods were employed with the aim of comparing the efficiency of each method to release maximum reducing sugars.

The substrate was subjected to different physical, chemical and biological pretreatment methods.

Physical Method for Pretreatment

Steam Explosion: The substrate was autoclaved at 121°C for 30, 60, 90, 120 and 150 minutes. The contents were then cooled and incubated at room temperature for the estimation of reducing sugar.

Chemical Methods for Pretreatment

Acid Hydrolysis: Twenty five ml of the substrate were taken in 250ml Erlenmeyer flasks. Then it was treated with sulphuric acid at concentrations of 0.1 and 0.2 N and incubated for 12 hours. The acid hydrolysate was centrifuged at 3000 rpm for 15 minutes. The clear supernatant of the hydrolysate from different time intervals *viz*., 2, 4 upto 12 hours was taken for the estimation of reducing sugars.

Alkali Hydrolysis: Twenty five ml of the substrate were taken in 250 ml Erlenmeyer flasks. Then, it was treated with sodium hydroxide at concentrations of 0.5 and 1.0 % and incubated for 12 hours. The alkali hydrolysate was centrifuged at 3000 rpm for 15 minutes. The clear supernatant of the hydrolysate from different time intervals *viz*., 2, 4 upto 12 hours was taken for the estimation of reducing sugars.

Biological Method for Pretreatment

Enzymatic Hydrolysis: For the study of enzymatic hydrolysis, the substrate was taken in 50 ml Erlenmeyer flask, pH adjusted and inoculated with organism. The culture filtrate which was obtained after incubation of 7 days contained enzyme source. The crude enzyme extract were take in different quantities (2 ml and 4 ml) were added in a 250 ml Erlenmeyer flask containing substrates, acetate buffer 0.1 M pH of 4.8, kept on a rotary shaker at a temperature of 50°C for 72 hours. The clear supernatant of the hydrolysate from different time intervals *viz.,* 2, 4 upto 12 hours was taken for the estimation of reducing sugars.

Estimation of Reducing Sugar: The reducing sugar was estimated by Dinitro salicyclic acid (DNSA) method as described by Miller [9]. The representative sample of 0.1 ml from each treatment and replications was taken into boiling test tubes and 0.9 ml of distilled water was added. A reagent blank containing 1ml of distilled was also prepared. Similarly, standards were also included ranging from 100 µg to 1000 µg concentrations of glucose. DNSA reagent 0.5 ml was added to the sample, mixed well and kept on boiling water bath for 5 minutes. The samples were cooled and final volume was made upto 25 ml using volumetric flask. Absorbance in terms of optical density (OD) of the standard and the samples were read at 540 nm using UV spectrophotometer.

Isolation of *Saccharomyces cerevisiae*: Ten ml of potato juice sample was transferred to 90 ml of sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotary shaker (100 rpm) for 30 min at ambient temperature. The well mixed suspension was then diluted appropriately upto 10^{-4} dilution. One ml of the suspension from 10^{-4} dilution was aseptically transferred to sterile petriplates containing 15 - 20 ml of selective Yeast extract Peptone Dextrose (YPD) medium, mixed well with medium by rotating clockwise and anticlockwise direction and allowed to set. Then, the plates were incubated at 28±2°C for 48 hours. After the incubation period, the yeast colonies were selected and transferred to YPD agar slant and maintained at 4°C for further study.

Identification of *Saccharomyces cerevisiae*: The yeast isolate was identified and characterized according to Lodder and Kreger-Rij [10] and Barnett *et al.* [11]. The characteristics of the yeast isolate was compared with *Saccharomyces cerevisiae* Reference strain (MTCC 172) and then confirmed.

Alcohol Fermentation: The pretreated (Acid, Alkali and Enzyme) Potato easte juice was filtered and centrifuged to remove unhydrolysed materials whereas in case of acid hydrolysate, the pH of the supernatant was adjusted to 5.0 with 10 % Ammonium hydroxide solution before inoculation. In the case of Alkali hydrolysate, the pH of the supernatant was adjusted to 5.0 with 10 % Sulphuric acid solution before inoculation. The clear supernatant was then enriched with 0.2 % Urea as Nitrogen source and the fermentation using *Saccharomyces cerevisiae* (4 % w/v) at the Temperature of 40°C and the fermentation carried out for 2 - 3 days.

Distillation of Alcohol: For the distillation of alcohol, 250 ml of ferment wash was taken into 250 ml clean Distillation flask and mixed the ferment wash with 250 ml distilled water. The Distillation flask was then kept on the heating mantle and connects the flask carefully to the condenser. Switched on the mantle to boil the ferment wash and after starting of boiling carefully collected the distilled liquid in clearly washed conical flask. This distilled liquid was then used for alcohol estimation.

Estimation of Ethanol: The ethanol was estimated by Calorimetric method [12]. One ml of the representative samples from each treatment was transferred to 250 ml round bottom distillation flask connected to the condenser and was diluted with 30 ml distilled water. The sample was distilled at 74 to 75°C. The distillate was collected in 25 ml of 0.23 N $K_2Cr_2O_7$ reagents which was kept at the receiving end. The distillate containing alcohol was collected till total volume of 45 ml was obtained.

Similarly, standard (20 to 100 mg ethanol) were mixed with 25 ml of $K_2Cr_2O_7$ separately. The distillate of samples and the standards were heated in water bath at 60°C for 20 minutes and then cooled. The volume was made upto 50 ml with distilled water and the optical density was measured at 600 nm using Spectrophotometer.

Growth Rate of *Saccharomyces cerevisiae* in Different Sugar Solutions: The growth rate of *Saccharomyces cerevisiae* in three different sugar solutions (Glucose, Fructose and Sucrose) was assessed. A volume of 100 ml, each of the sugar solution was taken in 250 ml conical flasks at different concentration of 10, 15, 20 and 25 % (w/v) and sterilized at 121°C for 20 minutes. The flasks were then cooled to room temperature and 1 ml of 24 hours old yeast *Saccharomyces cerevisiae* cultures were inoculated aseptically. The flasks were then incubated at room temperature and fermentation was carried out for 48 hours. Samples were withdrawn at different intervals (12, 24, 36 and 48 hours) for the determination of growth rate by measuring OD at 625 nm in a Spectrophotometer.

Growth Rate of *Saccharomyces cerevisiae* in Different Nitrogen Sources: The requirements of Nitrogen sources for the growth of *Saccharomyces cerevisiae* were determined by using different concentrations of Urea and Ammonium sulphate. In this experiment, 100 ml of 10 % glucose solution was prepared in a 250 ml Erlenmeyer flask and sterilized at 121°C for 20 minutes. After sterilization, it was cooled at a room temperature and 1 ml of 24 hours old yeast *Saccharomyces cerevisiae* was inoculated followed by adding concentration of 500, 1000, 1500 and 2000 mg/1000 ml in Urea and Ammonium sulphate. It was allowed to ferment at room temperature for 48 hours. Samples were withdrawn at different intervals (12, 24, 36 and 48 hours) and the cell density was measured by optical density at 625 nm using Spectrophotometer.

Optimization of Fermentation Process: Parameter optimization are important in any type of fermentation process, because the production may vary with respect to pH, temperature and inoculums size to optimize the fermentation process, the following experiments were carried out.

Effect of pH on Ethanol Production: The pH of the hydrolysate was adjusted to 3.5, 4.5 5.5 and 6.5 with Potassium hydroxide and Concentrated Hydrochloric acid solution. The yeast culture of *Saccharomyces cerevisiae* was inoculated and fermentation was carried upto 7 days at room temperature. The sample was analyzed for Bioethanol yield.

Effect of Temperature on Ethanol Production: The yeast *Saccharomyces cerevisiae* culture at 1 % level was inoculated to fermentation medium, pH was adjusted to 5.0 and incubated at different temperature *viz*., 40, 44, 48°C for 7 days. After fermentation, the samples were analyzed for Bioethanol yield.

Effect of Inoculum Size on Ethanol Production: The extract was inoculated with 0.3, 0.5 and 1.0 % of Inoculums levels of yeast *Saccharomyces cerevisiae* and kept for fermentation at 40°C for 7 days and thereafter samples were analyzed for ethanol yield.

RESULTS AND DISCUSSION

The Potato waste contains sufficient quantities of starch, cellulose, hemicelluloses, lignin and fermentable sugars to warrant use as an ethanol feed stock. Starch is a highly yield feed stock for ethanol production, but its hydrolysis was required to produce ethanol by fermentation. Starch was traditionally hydrolyzed by acids, but the specificity of the enzymes, their inherent mild reaction conditions and the absence of secondary reactions have led to the wide spread use of amylases as catalyst in this process. Starch processing was a technology utilizing enzymatic liquefaction and saccharification which produces a relatively clean glucose stream that was fermented to ethanol by *Saccharomyces* yeasts [13].

Ethanol is a viable transportation fuel and can be used to replace gasoline and fuel diesel in many application, extent energy supplies and provide energy security. The new advanced technologies have resulted in much conversion rate and use of variety of substrates for ethanol production. It has been carried out for obtaining efficient fermentative strains, low cost fermentation substrate and optimum environmental conditions for fermentation to occur. At present ethanol is mainly produced from molasses which is a byproduct of sugar industry. But it is not sufficient to meet the today's energy need. Hence, it is absolutely necessary to search for alternate sources for ethanol production. The potato juice is one of the major substrate which contains relevant amount of sugars which could easily fermented to ethanol. Therefore, the substrate was evaluated for the

efficacy in ethanol production using *Saccharomyces cerevisiae*. Optimization of various parameters including pH, temperature and inoculum size was also carried out in this study. The various results obtained in this study are discussed in this chapter.

Pretreatment Methods

Physical Method

Steam Explosion: The release of fermentable sugars at periodic intervals from steam exploded waste sample was studied and the results are presented in Table 1. The reducing sugar content increased with increase in exploding time. Steam explosion was carried out at different time intervals *viz.,* 0, 30, 60, 90, 120 and 150 minutes. The steam explosion time of 150 minutes released fermentable sugars up to 40.95 %.

Chemical Methods

Acid Hydrolysis: The chemical pretreatment done by Acid hydrolysis showed increase in reducing sugar with both increase in acid concentration and in different periods of incubation. The results are being presented in Table 2. The maximum release of fermentable sugar was released at 0.2 N concentrations for 12 hours treatment.

Alkali Hydrolysis: The release of reducing sugar from the substrate with the concentration of alkali used and on the period of incubation (Table 3). The release of fermentable sugars with alkali treatment was the highest at 1 % concentration of 12 hours treatment. The fermentable sugar released was 43.58 % from the sample. The quantity of fermentable sugars released from the waste was comparable with values recorded at 1 % concentration.

Biological Method

Enzymatic Hydrolysis: The waste substrate was inoculated with culture filtrate. There was increasing the reducing sugar content at different quantity of enzyme source at 2 ml and 4ml and the results obtained are presented in Table 4. In general, 4 ml enzyme source was found to be better than 2 ml enzyme source in the hydrolysis of substrate. Enzymatic hydrolysis released sugars to the level of 44.66 % from the waste substrate over an incubation of 12 hours.

Tasic *et al.* [14] used the cold, concentrated hydrochloric acid to produce glucose contaminated with 6 % of isomaltose within 50 hours. In another process of hydrolysis, the concentrated acid was deposited on an inert mineral sorbent, which was then blended and heated with starch or cereals for potato tuber starch hydrolysis, the optimal reaction conditions such as 1 M hydrochloric acid, the ratio of plant material to acid solution of 1:2 (10/v) 1 hour and 98°C, were established. During the acid hydrolysis, diluted H_2SO_4 was used for reducing the pH of the mixture which was shaken and heated by steam upto 100°C. After 6 hours, about 95 % of the starch chains are transformed into glucose.

Banerjee *et al*. [15] lowered the operating temperatures and pressures during the concentrated acid hydrolysis process. The concentrate acid – hydrolysis involves longer retention times and results in higher ethanol yields than the dilute acid – hydrolysis process. Concentrated acid hydrolysis requires high amount of acid and hence becomes uneconomical acid recycling also entails considerable lost. Alkaline pretreatment consists of two different alkaline reagents were used, aqueous ammonia and dilute NaOH, were used in pretreatment of biomass for delignification and enhancement of digestibility.

Isolation and Identification of *Saccharomyces cerevisiae*: The yeast was isolated from the potato juice and the isolate were identified according to Lodder and Kreger- Rij [16] and Barnett *et al*. [17] (Table 5 to Table 8). The characteristics of the yeast isolate was compared with *Saccharomyces cerevisiae* Reference strain (MTCC 172) and confirmed as *Saccharomyces cerevisiae*. Wai *et al.* [18] identified the yeast on the basis of the above characteristics and it was found at all *Saccharomyces cerevisiae* isolates were belonging to *Saccharomyces* species. Iraj Nahvi *et al.* [19] reported that there dominant role of *Saccharomyces cerevisiae* in inhabited their phyllosphere of fruit trees. Limtong *et al.* [20] reported the ubiquitous *Saccharomyces cerevisiae* in orchard soil the result of the present study clearly revealed that predominant at *Saccharomyces cerevisiae* in the phyllosphere and rhizosphere cashew plants of *Saccharomyces cerevisiae*.

Alcohol Fermentation: The ethanol produced from the pretreated substrate was estimated by calorimetric method and the results are presented in the Table 9. Among the four pretreated method tested, enzymatic hydrolysis showed better yield *viz*., 23.58 g L^{-1} from the potato waste substrate.

Growth Rate of *Saccharomyces cerevisiae* in Different Sugar Solution: Growth rate of *Saccharomyces cerevisiae* in various sugar solutions at different concentration was represented in Table 10.

Table 1: Effect of Steam explosion time on the release of Fermentable sugars from Potato waste

S.No	Time of Steam explosion (min)	Reducing sugar content (%)
1	0	15.20
2	30	27.29
3	60	29.92
4	90	34.12
5	120	40.74
6	150	40.95

Table 2: Effect of Acid hydrolysis on the release of Fermentable sugars from Potato waste

		Fermentable sugar content (%)		
		Acid concentration (H_2SO_4)		
S.No	Incubation period (hours)	Control	0.1 N	0.2 N
1	0	15.26	15.26	15.26
2	2	15.39	19.34	21.13
3	4	15.51	28.41	30.65
4	6	15.72	34.53	35.18
5	8	15.93	37.13	38.69
6	10	16.19	40.69	41.69
7	12	16.38	42.02	42.49

Table 3: Effect of Alkali hydrolysis on the release of Fermentable sugars from Potato waste

		Fermentable sugar content (%)		
		Alkali concentration (NaOH)		
S.No	Incubation period (hours)	Control	0.5 %	1.0 %
1	0	15.26	15.26	15.26
2	2	15.39	20.32	21.36
3	4	15.51	26.25	30.89
4	6	15.72	32.62	36.70
5	8	15.93	36.16	39.56
6	10	16.19	39.82	41.89
7	12	16.38	42.06	43.58

Table 4: Effect of Enzymatic hydrolysis on the release of Fermentable sugars from the Potato waste

		Fermentable sugar released (%)		
		Quality of enzyme source from *Saccharomyces cerevisiae* (ml)		
S.No	Incubation period (hours)	Control	2 ml	4 ml
1	0	15.26	15.26	15.26
2	2	15.39	19.36	21.33
3	4	15.52	25.23	30.85
4	6	15.72	31.68	34.70
5	8	15.93	35.21	39.58
6	10	16.19	37.82	40.82
7	12	16.38	42.08	44.66

Table 5: Colonial appearance of *Saccharomyces cerevisiae* isolates on YMP medium in comparison with Reference strain (MTCC - 172)

S.No	Characteristics	Yeast isolate	Reference strain (MTCC - 172)
1	Colour	Milky white	Milky white
2	Configuration	Round	Round
3	Margin	Smooth	Smooth
4	Elevation	Raised	Raised
5	Consistency	Butyreus	Butyreus

Table 6: Colonial appearance of *Saccharomyces cerevisiae* isolates on YMP medium in comparison with Reference strain (MTCC 172)

S.No	Characteristics	Yeast isolate	Reference strain (MTCC - 172)
1	Shape	Oval to elongate	Oval to elongate
2	Size (µm)	2.85 × 1.35	2.85 × 1.35
3	Multilateral Budding	Present	Present
4	Ring	Present	Present
5	Sediment	Present	Present
6	Pellicle	Absent	Absent
7	Ascospore formation	Present	Present
8	Pseudohyphae formation	Present	Present

Table 7: Fermentation of sugars by *Saccharomyces cerevisiae* isolates in comparison with Reference strain (MTCC 172)

S.No	Sugars	Yeast isolate	Reference strain (MTCC – 172)
1	Glucose	+	+
2	Galactose	+	+
3	Maltose	+	+
4	Lactose	-	-
5	Saccharose	+	+

Table 8: Assimilation of sugars by *Saccharomyces cerevisiae* isolates in comparison with Reference strain (MTCC 172)

S.No	Sugars	Yeast isolate	Reference strain (MTCC – 172)
1	Glucose	+	+
2	Galactose	+	+
3	Maltose	+	+
4	Lactose	-	-
5	Saccharose	+	+

Table 9: Ethanol production from pretreated waste

S.No	Pretreatment methods	Ethanol yield g^{-1}
1	Stem explosion	19.71
2	Acid hydrolysis	20.56
3	Alkali hydrolysis	21.37
4	Enzymatic hydrolysis	23.58

Table 10: Effect of different fermentable sugars at different concentrations on the growth of *Saccharomyces cerevisiae*

		Optical density(OD)			
		Period of incubation(hrs)			
Sugar	Sugar concentrations (%)	12	24	36	48
Glucose	10	0.28	0.42	0.70	0.72
	15	0.36	0.50	0.71	0.74
	20	0.34	0.39	0.45	0.53
	25	0.27	0.33	0.37	0.43
Fructose	10	0.20	0.30	0.45	0.50
	15	0.25	0.39	0.51	0.54
	20	0.22	0.36	0.44	0.48
	25	0.17	0.26	0.31	0.32
Sucrose	10	0.10	0.18	0.23	0.32
	15	0.13	0.24	0.30	0.40
	20	0.07	0.16	0.25	0.30
	25	0.04	0.03	0.16	0.23

Among the different sugar substrates *viz*., glucose, fructose and sucrose used, glucose supported maximum growth of *Saccharomyces cerevisiae*. The results indicated that the glucose was readily fermented in all four concentrations and among the different concentration of sugars, glucose showed maximum growth as evidence from the OD values.

The range of OD values with 15 % sugar concentration was 0.30 to 0.73 from 12 to 48 hours of incubation. The growth increased from 12 to 48 hours and was maximum on 36 and 48 hours. There was an increased in cell mass production with increase in concentration from 10 – 15 % when the Glucose concentration was increased above 15 %, a decreasing trend was observed in cell mass production after 48 hours of incubation. The OD values ranged between 0.34 to 0.53 from 12 to 48 hours of incubation in 20 % Glucose concentration. The corresponding OD values in 25 % Glucose concentration were 0.27 to 0.43 from 12 to 48 hours of incubation.

In the case of fructose as the source of sugar, there was gradual increase in the growth rate from 10 to 15 % sugar concentration and obtained maximum OD values ranging between 0.25 to 0.54 from 12 to 48 hours of incubation in 15 % Fructose concentration. The OD values in the case of 10 % concentration ranged between 0.20 to 0.50 from 12 to 48 hours. There was a fall in the OD values at 25 % concentration. The range was 0.17 to 0.32 from 12 to 48 hours.

In the case of various concentration of sucrose, maximum cell density was observed at 15 % of Sucrose and the OD values ranged from 0.13 to 0.40 from 12 to 48 hours. When the concentration of sugar increased from 20 to 25 % the growth rate was slightly decreased at all sampling periods. With 25 % sugar concentration the OD values ranged between 0.04 to 0.23 from 12 to 48 hours.

Growth Rate of *Saccharomyces cerevisiae* in Different Nitrogen Sources: The effect on nitrogen sources on the growth of *Saccharomyces cerevisiae* was given in Table 11. The result showed that increasing the concentration of nitrogen from 500 to 2000 mg has not increased the cell density. Maximum cell density of 1.47 OD was observed at 48 hours of incubation with 1000 mg ammonium sulphate. The corresponding OD value by supplementation with ammonium sulphate at concentration of 2000 mg was 1.02, when the concentration of nitrogen was increased from 1000 to 2000 mg at 48 hours, the cell mass density significantly decreased from 1.47 to 1.02 OD, respectively. In the case of Urea, maximum cell density of 1.38 OD was obtained at 48 hours when 1000mg was used. The growth rate was slightly decreased as the concentration of urea in the medium was increased. At higher concentration (2000 mg) the growth rate reduced and it was 0.82 at 48 hours.

Saccharomyces cerevisiae was one of the most suitable yeast for producing ethanol even in media with high concentration of glucose [21]. Ethanol fermentation was a conversion of simple sugars to ethyl alcohol microorganisms, especially yeast through a series of enzyme catalyst reaction, primarily by Embden-Mayerhoff and Paranas pathways. The overall net reaction involves the production of two moles each of ethanol, CO_2 and ATP per mole of glucose fermented. Therefore, on a weight basis each gram of glucose can give rise to 0.51 g of alcohol [22].

Optimization of Fermentation Process

Effect of Ph on Ethanol Production: The ethanol yield significantly influenced by different pH levels as indicated in (Table 12). *Saccharomyces cerevisiae* preferred the pH 5.5 for fermenting potato waste substrate residue hydrolysate. The pH level of 3.5, 4.5, 5.5 and 6.5 proportionally increased ethanol. The substrate hydrolysate yielded ethanol of 22.17 g L^{-1} with *Saccharomyces cerevisiae* at pH 5.5.

Effect of Temperature on Ethanol Production: The ethanol yield from enzyme hydrolyzed potato waste residues significantly varied between temperature levels as indicated in (Table 13). The ethanol production from enzyme hydrolysate was studied at temperature levels of 40, 44 and 48°C. The ethanol yield at 44°C gave highest ethanol yield followed was 24.76 g L^{-1} in the substrate.

Effect of Inoculum Size on Ethanol Production: The inoculums size of 0.3, 0.5 and 1 % levels prepared with *Saccharomyces cerevisiae* separately to inoculate the medium containing the hydrolysate of substrate. The inoculums size of 1 % found to be more ideal for fermenting the hydrolysate (Table 14). *Sacchaomyces cerevisiae* at 1 % inoculums size recorded ethanol yield of 24.52 g L^{-1} in the substrate.

Yeasts are generally able to utilize Ammonium ions as the sole source of nitrogen. Ammonium Sulphate is the most widely used nitrogen source in industrial fermentation [23]. Slaughter [24] reported that the Ammonium sulphate can support growth itself or with addition of trace amounts of vitamins.

Table 11: Effect of different nitrogen source at different concentrations on the growth of *Saccharomyces cerevisiae*

		Optical density (OD)			
		Period of incubation (hrs)			
Nitrogen source	Nitrogen source concentrations (mg L^{-1})	12	24	36	48
Ammonium sulphate	500	0.26	0.58	1.06	1.38
	1000	0.34	0.68	1.29	1.47
	1500	0.24	0.55	0.85	1.03
	2000	0.25	0.48	0.66	1.02
Urea	500	0.30	0.76	0.99	1.25
	1000	0.37	0.85	1.03	1.38
	1500	0.22	0.71	0.80	1.04
	2000	0.19	0.59	0.60	0.82

Table 12: Effect of pH on the Ethanol yield from Potato waste

S.No	pH	Ethanol yield g^{-1}
1	3.5	12.25
2	4.5	17.17
3	5.5	22.17
4	6.5	21.23

Table 13: Effect of Temperature levels on the Ethanol yield

S.No	Temperature (°C)	Ethanol yield g^{-1}
1	40	22.86
2	44	24.76
3	48	20.76

Table 14: Effect of inoculum size on the ethanol yield

S.No	Inoculum size (%)	Ethanol yield g^{-1}
1	0.3	14.12
2	0.5	18.52
3	0.1	24.52

Ammonium sulphate was the best nitrogen source as they increased the enzyme activity and cell mass and reduced the generation time as reported by Tangho [25]. Ammonium Sulphate provided some growth needs of the organisms possibly nitrogen and sulphur amongst others [26].

Nimbkar *et al*. [27] studied the effect of different inoculum level *viz*., 2, 4, 6, 8 and 10 % on the ethanol production from unspecialized juice of sweet sorghum and obtained maximum alcohol concentration of 12.45 and 12.23 % (v/v) at 6 and 2 % respectively. They also studied the effect of three different incubation temperatures *viz*., 25, 30 and 35°C on the ethanol production from unsterilized juice of sweet sorghum with *Saccharomyces cerevisiae* and obtained maximum alcohol of 12.45 % (v/v) at 30°C. The effect of different temperatures *viz*., 25, 30 and 40°C, on the ethanol production from Starch and observed that maximum ethanol concentration 21.8 g L^{-1} at optimum temperature of 30°C in 48 hours of fermentation period.

Marakis and Marakis [28] reported maximum alcohol concentration of 5.8 % (v/v) at pH 4.5 from aqueous carob extract after 120 hours of fermentation. Ethanol production was maximum at pH 6 and it was 30 % less in pH 7.7. Ramanathan [29] observed the maximum alcohol yield in 3 days during fermentation of yam to ethanol by *Saccharomyces cerevisiae.* Verma *et al.* [30] studied that the effect of four different fermentation periods *viz*., 24, 48, 72 and 96 hours on ethanol production from starch medium. A maximum ethanol concentration of 24.8 g L^{-1} at 48 hours was achieved as compared to 13.7 and 21.6 g L^{-1} at 24 and 96 hours respectively. Saranraj and Stella [31] studied that the effect of 6 different fermentation period *viz*., 0, 24, 48, 72, 90 and 100 hours on ethanol production from aqueous carob extract and achieved maximum alcohol concentration of 4.75 % (v/v) at 100 hours of fermentation period.

Panesar *et al.* [32] optimized the fermentation parameters *viz*., temperature, pH and inoculum levels as 6 % and 10 % respectively for ethanol production from molasses medium. The foremost parameter, pH of the substrate was tested at various levels to know the optimum level for ethanol production. The maximum ethanol concentration of 79.1 ml/L from cane molasses at operating variables of pH 4.5, temperature 32°C in 5 days operation was obtained.

CONCLUSION

The present research results showed that the potato waste juice was used as a raw material for Bioethanol production. The important criteria needs to be done are to work out the cost benefit ratio. But, the major significant advantage is that the production of Bioethanol from waste potatoes is environment friendly since it uses the non - molasses route. Extensive research has been done on the development of advanced technologies to

prepare more digestible biomass to ease bioconversion of waste materials into ethanol. An ideal cost-effective pretreatment method might have several characteristics. (1) Maximum fermentable carbohydrate recovery; (2) Minimum inhibitors produced from carbohydrate degradation during pretreatment; (3) Low environmental impact; (4) Low demand of post-pretreatment processes such as washing, neutralization and detoxification; (5) Minimum water and chemical use; (6) Low capital cost for reactor; (7) Moderately low energy input; (8) Relatively high treatment rate; and (9) Production of high value-added by-products. Therefore, the future research on pretreatment would be focused on the following areas. First, reduction of water and chemical use; Second, recovery of carbohydrates and value-added by-products to improve the economic feasibility; Third, development of clean delignification yielding benefits of co-fermentation of hexose and pentose sugars with improved economics of pretreatment.

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