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PRODUCTION OF BIO-ETHANOL FROM POTATO STARCH WASTES BY *Saccharomyces cerevisiae*

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ABSTRACT

Bio-ethanol is one of the energy sources that can be produced by renewable sources. Potato starch wastes were chosen as a renewable carbon source for ethanol fermentation because it is relatively inexpensive compared with other feedstock considered as food sources. However, saccharification processes are needed to convert starch of potato into fermentable or reducing sugars before ethanol fermentation. In this study, hydrolysis of potato starch wastes and growth parameters of the ethanol fermentation were optimized to obtain maximum ethanol production by *S. cerevisiae* S288c. The ratio of plant material to acid solution of 1:10 (w/v). Results demonstrated that 0.5% H_2SO_4, 1% H_2SO_4, 2% H_2SO_4 and 3% H_2SO_4 at 121ºC for 20 min by autoclave were enough to hydrolyze all starch contained in the potato starch wastes. The maximum yield of reducing or fermentable sugars was 125.8 mg/g obtained in 0.5% H_2SO_4. The minimum yield was 53 mg/g obtained in 3% H_2SO_4. The yield of bioethanol production by *S. cerevisiae* S288c was (51.37 mg/g) was achieved at pH 5.5, temperature of 30^0C and inoculums size of 10% (v/v) after 72 hours of fermentation.

INTRODUCTION

Increasing industrialization and the population has led to a continuous rise in global energy demand. At present, more than 80% of world energy production of fossil fuel use. However, the depletion of fossil fuels at an alarming rate, the causes of environmental pollution and burned **(Láinez, *et al*., 2019).** Therefore, there is a need for sustainable and renewable energy sources that do not affect the environment and ecosystems. Biofuels have emerged recently fuel tankers are ideal to meet the energy needs in a sustainable manner **(Morais, *et al*., 2019)**. More specifically, can be used as an alternative oil sources bioethanol and has become one of the most dominated biofuels industry because the majority of the emissions of carbon dioxide, which contributed to the transport sector. In addition, ethanol has been renovated high energy oxygen content easily stored **Zhang, *et al*., 2019).**

Agricultural and industrial, such as starchy substrates, waste and high availability has shown, and biological degradation which rich in nutrients. in addition, its use in the production of bio-fuel operations removes waste disposal problems. A variety of agro-industries raw

materials can be used as substrates for biological conversion of ethanol. Waste crop tubers such as potatoes, sweet potatoes and cassava substrates are favorable because they contain enough amounts of starch, which can be hydrolysed to sugars and later fermented to ethanol **(Lin, *et al*., 2010)**. Potatoes are especially suitable because of its high return carbohydrate fermented (**Lantero, *et al*., 2011)**. Furthermore, potatoes are the third most important food crop in the world after rice and wheat, which are the basic crops. Ratio widespread use in the fields of industry and large quantities of waste potato peel (PPW) are created. Manufacturing potatoes produced between 20 and 50% of the waste of raw product **(Rezig, *et al*., 2010)**.

Starchy materials require a reaction of starch with water (hydrolysis) to break down the starch into fermentable sugars (saccharification). Hydrolysis is carried out at high temperature (90 to 110°C); however, at low temperatures, it is also possible and can contribute to energy savings **(Sanchez, *et al*., 2008)**. To convert starch into the fermentable sugars, either acid hydrolysis or enzymatic hydrolysis needs to be performed. Each has their own set of advantages and disadvantages for use. Enzyme hydrolysis is generally chosen even though high cost of enzymes and initial investment because of high conversion yield of glucose **(Tasić, *et al*., 2009)**.

Therefore, this investigation was carried out to study utilization of potato starch waste as a very cheap substrate for the production of Bio-ethanol by *Saccharomyces cerevisiae* S288c.

2. MATERIALS AND METHODS

2.1- Materials:

The potato starch wastes (PSW) were collected from a chips factory for the food industries (Egypt Foods Company, Quesna, Menoufia, Egypt). It was dried at 50°C for 48 hours, ground and sieved to get particles with particle size between 500 and 1000 µm. it was stored at room temperature (25 ± 5°C) until use.

2.2- Chemicals and Reagents:

Chemicals and reagents of the analytical methods used in present study were sulfuric acid, sodium hydroxide, glucose, Benedict's reagent, dinitrosalicylic acid reagent (DNS reagent), sodium sulphite, Rochelle salt (potassium sodium tartrate), phenol, yeast extract, malt extract, sodium chloride, peptone, agar, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, magnesium sulfate, ammonium sulfate, sodium hydroxide and potassium dichromate. They purified and distilled before use. All chemicals were purchased from El- Gamhouria Trading Chemicals and Drugs Company, Egypt.

2.3- Micro-organisms

S. cerevisiae S288c was obtained from Microbial Biotechnology Department, National Research Center (Dokki, Egypt), it was used in this study and maintained on yeast extract-malt extract (YM) agar slant at 4^{o}C.

2.4 - Analytical methods:

2.4.1- Chemical composition of potato starch wastes:

The chemical composition of potato starch wastes (PSW) were determined according to **Zhao *et al.*, (2005)** by Near-Infra Red (NIR) Spectroscopy apparatus, model DA1650, which manufactured by FOSS Corporation. (NIR) spectrometer at wavelength region from (750-2500 nm) of the electromagnetic spectrum, which used to analyze the chemical structure and to take a fingerprint.

2.4.2- Acid Hydrolysis or Saccharification:

The PSW (25g) was dried at 80°C. H_2SO_4 0.5%, 1 %, 2% and 3% (w/v) 1:10 solid-liquid ratio was added to the samples powder, the mixtures were then autoclaved at 121°C for 15 min. Finally, the samples were cooled down and analysed for glucose concentration according to **Sheikh *et al.*, (2016)**.

2.4.3- Determination of reducing sugars:

2.4.3.1- Qualitative analysis of reducing sugars:

A biochemical test to detect reducing sugars in solution, devised by the US chemist **S. R. Benedict (1884–1936)**. Benedict's reagent a mixture of copper (II) sulphate and a filtered mixture of hydrated sodium citrate and hydrated sodium carbonate is added to the test solution and boiled. A high concentration of reducing sugars induces the formation of a red precipitate; a lower concentration produces a yellow precipitate. Benedict's test is a more sensitive alternative to **Fehling's test.**

2.4.3.2- Quantitative analysis of reducing sugars:

Total reducing sugars in the hydrolysate of potato starch wastes were estimated by the dinitrosalicylic acid (DNS) colorimetric method adapted from previous work **(Miller, *et al* 1961 and Ghose, 1987)**.

2.4.3.2.1- Preparation of standard solution

The standard glucose stock solution 10 g/L was prepared by dissolving 0.20 g of D-(+)-Glucose anhydrous ($C_6H_{12}O_6$) in 20 mL of distilled water. Working solutions were daily prepared by appropriate dilution of the stock solution in DI water.

2.4.3.2.2 Preparation of dinitrosalicylic acid reagent

3,5-dinitrosalicylic acid reagent was prepared by dissolving 1 g of 3,5-dinitrosalicylic acid in 20 mL of 2 M NaOH. It was then mixed with potassium sodium tartrate ($C_4H_4KNaO_6$) solution (30 g of $C_4H_4KNaO_6$ in 50 mL of distilled water) on a magnetic stirrer hot plate and diluted to 100 mL with distilled water.

2.4.3.2.3- Calibration curve:

Calibration curve for estimation of reducing sugar yield was obtained by plotting the absorbance (at 520 nm) vs. concentrations of standard glucose in the range of 0.20-1.00 g/L. The concentrations of glucose were daily prepared by dilution of the stock solution.

2.4.3.2.4- Estimation of reducing sugar yield in the hydrolysate of potato starch wastes.

0.50 mL dinitrosalicylic acid was introduced into a test tube containing 0.50 mL of standard glucose or the hydrolysate of cellulose. It was then boiled at 100^{o}C for 10 min and cooled in an ice bath. Afterward, 5 mL distilled water was added, shaken and left for 5 min. The absorbance was measured at 520 nm against reagent blank using a UV-Visible spectrophotometer (Nicolet- evolution300- Thermo Electron Corporation). To calculate the quantitative of reducing sugar yield in the form of g/100 g substrate, the following equation was used:

$$\text{Reducing sugar yield (g/100 g substrate)} = \frac{RC \times V1 \times 100\ g}{1000\ mL \times M1}$$

Where R_C is the reducing sugar concentration (g/L), V_1 is the volume of acid solution (mL), and M_1 is the mass of substrate added (g).

2.5-Fermentation process:

2.5.1- Preparation of inoculums medium:

S. cerevisiae S288c was activated on yeast extract-malt extract (YM) agar plates containing (per L): 3 g yeast extract, 3 g malt extract, 5 g peptone and 10g glucose. It was then incubated at 30^{o}C for 24 h, streaked into YM broth (pH ≈ 5.5) (all ingredients like the Petri dishes of YM agar, except agar powder) and incubated again at 30^{o}C for 24 h, according to **Pridham *et al*., (1957).**

2.5.2- Fermentation medium and conditions:

The acid hydrolysate of the starch under the appropriate conditions was adjusted to pH ≈ 5.5 using 2 M NaOH, supplemented with following additional nutrients (per L): 1 g yeast extract, 1 g $MgSO_4.7H_2O$, 2 g $(NH_4)_2SO_4$ and 5 g KH_2PO_4 **(Akaracharanya, *et al*., 2011)** and then used as an ethanol production medium. This medium with a working volume of 100 mL was transferred into a 250 mL Erlernmeyer flask and sterilized by autoclaving at 121^{o}C, 15 psi for 30 min. Then, an inoculums suspension of *S. cerevisiae* S288c cells was loaded into the sterilized medium (10% v/v). The fermentation was operated at 30^{o}C under static conditions for 72 h. The fermented broth was collected at 6-h time intervals for analysis of ethanol concentration.

2.6.-Estimation of bioethanol:

2.6.1-Qualitative estimation

Bioethanol production was examined by Jones reagent ($K_2Cr_2O_7$+H_2SO_4; **Jones 1953**). One milliliter of $K_2Cr_2O_7$ (2 %), 5 ml of

H_2SO_4 (concentrated) and 3 ml of sample were added to Jones reagent. Ethanol was oxidized into acetic acid with potassium dichromate in the presence of sulfuric acid and gave blue-green color. Green color indicates positive test **(Caputi, *et al*., 1968).**

2.6.2- Quantitative estimation:

Ethanol production was estimated according to **Doelle and Greenfield, (1985)** by detecting the ethanol concentration in each sample after fermentation under all biochemical conditions with Gas chromatography (model 6890, Agilent), equipped with flame ionization detector and nominal capillary column (60 m×530 µm ×5.00 µm). Helium was the carrier gas, flow rate was 25 mL/min. Oven and detector temperature was 300ºC. The theoretical ethanol yield was calculated assuming the conversion of all the hydrolysed sugars at the end of the run to ethanol.

3- RESULTS AND DISCUSSION

3.1- Chemical composition of potato starch wastes:

Data in **Table (1)** shows the chemical composition of potato starch wastes. The moisture content recorded that 14.13%, the fat content 1.60%, the crud protein 1.33%, fiber content 0.18%, ash content 1.21% and the total carbohydrate 82.88%. Results indicate that the potatoes wastes are very rich of carbohydrates, which represent an important source in the production of bioethanol.

Thes**e** results agreement with **Khawla, *et al*., (2014)** mentioned that the characterization of potato peel waste (PPW) contained (on dry basis) proteins (15.1 ± 0.8%, w/w), fat (0.52 ± 0.09%,w/w), moisture (6.78 ± 0.22%, w/w), starch (48.46 ± 1.88%) and ashes (7.2 ± 0.2%, w/w). **Arapoglou, *et al*. (2010)** said that the chemical composition of potato starch wastes were (6.34% ash content، total carbohydrate 68.7%, 8% proteins and fat 2.6%). **Sheikh, *et al*., (2016)** reported that the amount of moisture and ash content of potato peels wastes (PPW) are 7.50 % and 7.71 % respectively. **Liang, *et al*., (2014)** observed chemical composition of potato peel waste (PPW) were carbohydrate 63.2% ± 4.2, starch 34.3% ± 2.7, protein (N_{tot} 6.25) 17.1 % ± 0.3 and lipids 1.2 % ± 0.0. **Rani, *et al*., (2010)** mentioned that the potato flour were 8.12% moisture, 73.0% starch, 10.86% total protein, 1.65% crude fiber, 2.15% ash content, 1.00% total lipids. **Duhan, *et al*., (2013)** reported that the potato flour contained 8.39% moisture, 73.25% starch and 4.86%proteins.

Tab. (1): Chemical composition of potato starch wastes (Average).

Parameters	Dry weight (%)
Moisture Content (%)	14.13
Fat Content (%)	1.60
Crude Protein (%)	1.33
Fiber Content (%)	0.18
Ash Content (%)	1.21
Total Carbohydrate (%)	82.88

3.2- Determination of reducing sugars:

3.2.1- Qualitative analysis of reducing sugars:

Table (2) shows the qualitative analysis of reducing sugars after acid hydrolysis or (scarification) of potato starch wastes by using Benedict's test. The sample A (H_2SO_4 0.5%) a red precipitate appeared, sample B (H_2SO_4 1%) with a red precipitate appeared, sample C (H_2SO_4 2%) it showed up an orange precipitate, sample D (H_2SO_4 2%) an orange precipitate appeared. These results according to Benedict's test.

Tab. (2): Qualitative analysis of reducing sugars by Benedict's test

Samples	H_2SO_4 (%)	Benedict's Reagent	
		Before	after
Sample A	**0.5%**	**Blue color**	**Red precipitate**
Sample B	**1%**	**Blue color**	**Red precipitate**
Sample C	**2%**	**Blue color**	**Orange precipitate**
Sample D	**3%**	**Blue color**	**Orange precipitate**

3.2.2- Quantitative analysis of reducing sugars:

Miller (1959) reported the dinitrosalicylic acid (DNS) was used to test for the present of free carbonyl group (C=O) present in reducing sugars. The aldehyde group in reducing sugars was reduced with 3,5-dinitrosalicylic acid to form 3-amino,5-nitrosalicylic acid, which absorbs light strongly at 520 nm, as presented in Figure (1).

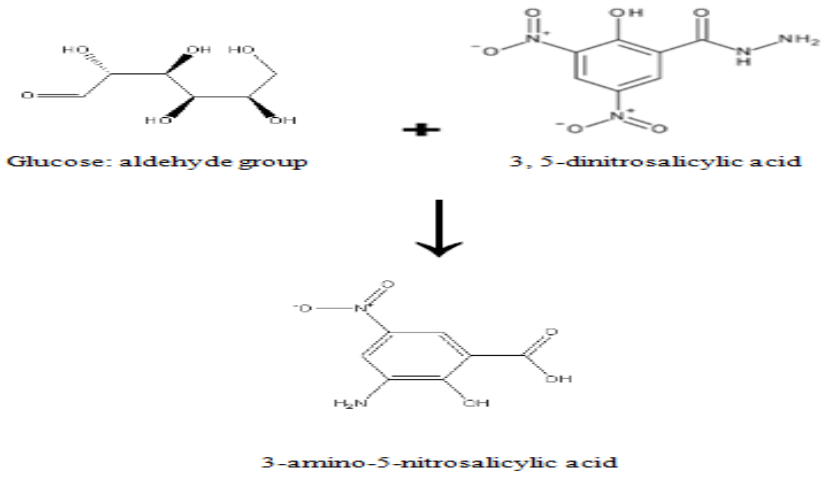

Fig. (1). Chemical reaction of glucose with 3,5-dinitrosalicylic acid **(Miller, 1959)**

It was first introduced as a method to detect reducing substances in urine and has since been widely used, for example, for quantification of carbohydrate levels in blood. It is mainly used in assay of alpha amylase. However, enzymatic methods are usually preferred to DNS due to their specificity.

The determination of reducing sugars by a UV-Visible spectrophotometer by used DNS method for estimation of total reducing sugar yield is presented in **table (3)**.

Tab. (3): estimation of total reducing sugar yield (mg/gm) by a UV-Visible spectrophotometer.

Samples	H_2SO_4 (%)	Total reducing sugar (mg/gm)
Sample A	**0.5**	**125.8**
Sample B	**1**	**91.4**
Sample C	**2**	**67.6**
Sample D	**3**	**53.5**

This table observed the dilute H_2SO_4 break down starch and turns it into reducing sugar in the form of glucose. The results revealed that reducing sugar yield increases from 53.5 to 125.8 mg/gm when H_2SO_4 concentrations decrease. The maximum yield of total reducing sugar yield 125.8 mg/g was obtained in 0.5% H_2SO_4. The minimum yield of total reducing sugar yield 53.5 mg/g was obtained in 3% H_2SO_4, because sulfuric acid in the case of increased concentration removes a molecule of water from glucose and converts it to Hydroxy methyl furfural (HMF) compound. Hydroxymethylfurfural (HMF) is a product of dehydration of hexose sugars such as glucose, fructose and carbohydrates, as presented in **Figure (3)**.

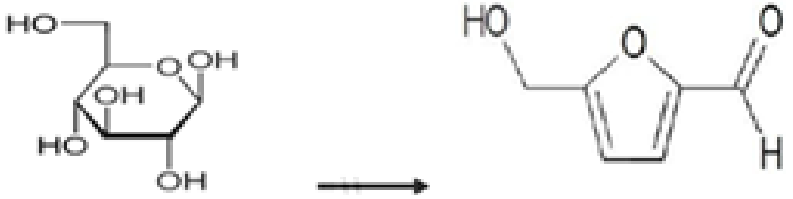

Fig. (2): Formation of HMF from D-glucose **(Tao et al., 2011)**

These results agreements with **Hashem and Darwish (2010)** mentioned that the potato starch residue stream produced during chips manufacturing was used as an economical source for biomass and bioethanol production by *Saccharomyces cerevisiae*. Results

demonstrated that 1% H_2SO_4 at 100 ºC for 1 h was enough to hydrolyze all starch contained in the residue stream.

3.3- Estimation of bioethanol:

3.3.1- Qualitative estimation

After fermentation process bioethanol production was examined by Jones reagent ($K_2Cr_2O_7$+H_2SO_4; **Jones 1953**). One milliliter of $K_2Cr_2O_7$ (2 %), 5 ml of H_2SO_4 (concentrated) and 3 ml of sample were added to Jones reagent. Ethanol was oxidized into acetic acid with potassium dichromate in the presence of sulfuric acid and gave blue-green color. **Table (4)** shows the results of bioethanol reaction with Jones reagent. It showed yellow color with control and sample before fermentation but it was green color with sample after fermentation. **Tiwari, *et al.*, (2015)** mentioned that the bioethanol production was examined by Jones reagent. Ethanol was oxidized into acetic acid with potassium dichromate in the presence of sulfuric acid and gave blue-green color. (**Caputi, *et al.*, 1968)** reported that the ethanol with Jones reagent was green color indicates positive test.

Tab. (4): Qualitative estimation of bioethanol by Jones reagent.

Group	Treatments	Jones reagent
1	Control	-Yellow color
2	Sample before fermentation	-Yellow color
3	Sample after fermentation	+ Green color

3.3.2- Quantitative estimation:

Ethanol yield by GC and productivity obtained by fermentation of the hydrolysate are presented in **Table (5)**. In this study, the hydrolysis of starch was performed using dilute H_2SO_4 further fermented to ethanol using *S. cerevisiae* S288c and it was found that the ethanol yield of 51.37 mg/g substrate corresponded to a productivity ethanol yield of 0.75(mg/g/ h).

These results agreements with **Mahmoodi, *et al.*, (2018)** indicated that the ethanol yield of 44.6 and 44.4 g per 100 g glucose was obtained from hydrolysate and acid treatment liquor, respectively. **Mushimiyimana and Tallapragada (2016)** observed that the alcohol content was 15.34 % and 14.4 % by Gas chromatography by using potato peel and onion peel as substrates. **Tasić, *et al.*, (2009)** showed that the ethanol yield of starch from fresh potato tubers of 31 g/L was obtained in the fermentation of hydrolyzate prepared under the optimal hydrolysis conditions by commercial bakery yeast at 28 ◦C for about 18 h. **Izmirlioglu and Demirci (2012)** they said that the maximum bio-ethanol

production from waste potato mash by using *Saccharomyces Cerevisiae* was obtained at the optimum conditions of 30.99 g/L ethanol.

Tab. (5): Ethanol yield and productivity obtained by fermentation of the starch hydrolysate.

Initial glucose concentration (mg/g substrate)	Optimum fermentation time (h)	Maximum ethanol yield (mg/g substrate)	Ethanol productivity (mg/g/ h)
125.8	72	51.37	0.71

Under anaerobic conditions, the pyruvate is further reduced to ethanol with the release of CO2. Theoretically, the yield is 0.511 for ethanol and 0.489 for CO2 on a mass basis of glucose metabolized, as represented by the following Eq. (**Zhang, *et al*., 2010)**

$$C_6H_{12}O_6 \xrightarrow{\text{Yeast}} 2CH_3CH_2OH + 2\,CO_2$$

Glucose 1g Ethanol 0.51g Carbon dioxide 0.49g

CONCLUSION

The hydrolysis of potato starch wastes by mineral acids was studied. Using 0.5% H_2SO_4, 1% H_2SO_4, 2% H_2SO_4 and 3% H_2SO_4 at 121ºC for 20 min by autoclave the ratio of plant material to acid solution of 1:10 (w/v), the highest fermentable or reducing sugars equivalent of 125.8 mg/g in 0.5% H_2SO_4. The minimum yield was 53 mg/g obtained in 3% H_2SO_4. The yield of bioethanol production by *S. cerevisiae* S288c was (51.37 mg /g) was achieved at pH 5.5, temperature of 30^0C and inoculums size of 10% (v/v) after 72 hours of fermentation.

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إنتاج الإيثانول الحيوي من مخلفات البطاطس النشوية بواسطة *Saccharomyces cerevisiae*

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يعتبر الايثانول الحيوى من أهم مصادر الطاقة الحيوية المتجددة الواعدة فى المستقبل وله فؤائد بيئية واقتصادية، و يمكن إنتاجه من مصادر مختلفة. وقد اختيرت مخلفات البطاطس النشوية كمصدر متجدد للكربون لإنتاج الإيثانول لأنها غير مكلفة نسبياً مقارنة بالمواد الخام الأخرى التي تعتبر مصادر غذائية. وتجرى عمليات التحلل المائي بالأحماض المخففة لتحويل مخلفات البطاطس النشوية إلى سكرات قابلة للتخمير (سكرات مختزلة) قبل عملية التخمر لإنتاج الإيثانول. في هذه الدراسة تم تحسين التحلل المائي لمخلفات البطاطس النشوية ومعدلات النمو للخمائر للحصول على أقصى قدر من إنتاج الإيثانول بواسطة *S. cerevisiae S288c*. وكانت نسبة المواد النباتية إلى محلول الحمض ١: ١٠(و/ح) وأظهرت النتائج أن حمض الكبريتيك تركيز ٠,٥٪ و١٪ و٢٪ و٣٪ عند ١٢١°م لمدة ٢٠دقيقة بواسطة الأوتوكلاف كانت كافية لتحلل جميع النشا الموجود في مخلفات البطاطس النشوية. وكان الحد الأقصى لكمية السكريات القابلة للتخمير أو المختزلة ١٢٥.٨ملجم/جم تم الحصول عليها عند تركيز الحمض ٠,٥٪. وكان الحد الأدنى من السكريات القابلة للتخمير أو المختزلة ٥٣ملجم/جم التي تم الحصول عليها عند تركيز ٣ ٪. و تم تحقيق أعلى إنتاج من الإيثانول الحيوي بواسطة *S. cerevisiae S288c* وهو (٥١,٣٧ جرام / ١٠٠ جم) في درجة حموضة ٥.٥، ودرجة حرارة ٣٠°م وحجم التلقيح ١٠ ٪ (و / و) بعد ٧٢ ساعة من التخمير.