J. Agric. Sci. Mansoura Univ., 34 (8): 8835 - 8848, 2009

PRODUCTION OF BIO-ETHANOL AND ASSOCIATED BY-PRODUCTS FROM POTATO STARCH RESIDUE STREAM BY SACCHAROMYCES CEREVISIAE

Hisham, M.[1] and Soumia M. I. Darwish[2]

[1] Dept. Botany, Faculty of Science, Assiut University 71526, Egypt

[2] Dept. of Food Science and Technology, Fac. of Agric., Assiut Univ.

ABSTRACT

Potato washing residue stream produced during Chips manufacturing was used as an economical source for biomass production of *Saccharomyces cerevisiae* as well as bioethanol production. Results demonstrated that 1% H_2SO_4 at 100 ºC for 1 h was enough to hydrolyze all starch contained in the residue stream. Two strains of *Saccharomyces cerevisiae* (y-1646 and commercial one) were able to utilize and ferment the acid treated residue stream under both aerobic and semianerobic conditions. The maximum yield of ethanol (5.52 gl^{-1}) was achieved at 35 ºC by *Saccharomyces cerevisiae* y-1646 after 36 h after addition of $ZnCl_2$ (0.4 gl^{-1}). Five secondary by-products were found to be associated the ethanol production process. The most important compound was Chlorozotocin that was shown to possess medicinal and pharmaceutical advantages in cancer chemotherapy. Study recommends intensive investigation in this research point to enhance the production of that extremely important compound.

Keywords: Residue stream, bio-ethanol, fermentation, *Saccharomyces cerevisiae*, chlorozotocin**.**

INTRODUCTION

The increase in the prices of petroleum based fuels, strict governmental regulations on exhaust emissions and future depletion of worldwide petroleum reserves encourage studies to search for alternative fuels (Harkin, 2000; Howard, 1994). Alcohols (ethanol and methanol) have been considered as alternative fuels for diesel engines (Ghobadian and Rahimi, 2004).

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 (Wyman, 1999). A dramatic increase in ethanol production using the current corn starch based technology may not be practical because corn production for ethanol will compete for the limited agricultural land needed for food and feed production. A potential source for low cost ethanol production is to utilize lignocellulosic materials such as crop residues, grasses, sawdust, wood chips, solid animal waste and industrial wastes (Prasad *et al*., 2007). The cost of ethanol production from lignocellulosic material is relatively high based on current technologies, and the main challenges are to low yield and high cost of hydrolysis. There is need of process optimization for detoxification and maximize conversion of agro and urban/industrial residues feedstocks for production of ethanol as a cheaper substrate like molasses and other directly fermentable materials (Prasad *et al*., 2007).

There are a number of different urban and industrial wastes such as cotton linters, spent sulfite liquor, cheese whey, wastes from vegetable and fruit industries, coffee waste, etc. These waste materials are presented in the form of solids and liquids and have to be processed for avoiding pollution of the environment. As these wastes can be used for ethanol production, their processing can become profitable (Kosaric *et al*., 1981).

Waste streams from starch processing industries, obtained after the separation of the high-value gluten and the main starch fraction, can provide a low-cost substrate for fuel ethanol production (Wyman, 1996) as well as obviating the need for alternative waste water treatment (Davis *et al*., 2006).

Many industries all over the world utilize potato as raw material. Chips manufacturing is one of the most industries which depends completely on potato. During chips processing, huge quantities of waste water is drained. This water contains some dissolved minerals and solids. The main constituent of drained solids is starch. Starch is very important and abundant natural solid substrate. Essentially, starch is composed of two related polymers in different proportions according to its source: amylose (16–30%) and amylopectin (65–85%). Amylose is a polymer of glucose linked by α-1,4 bonds, mainly in linear chains. Amylopectin is a large highly branched polymer of glucose including also α-1,6 bonds at the branch points.

Starchy materials require a reaction of starch with water (hydrolysis) to break down the starch into fermentable sugars (saccharification). Typically, mixing the starch with water to form slurry, this is then stirred and heated to rupture the cell walls. Specific enzymes that will break the chemical bonds are added at various times during the heating cycle (Badger, 2002). Starchy grains and effluent generated from starch generating unit are the cheap substrates and could be used as potential raw materials for ethanol fermentation (Verma *et al*., 2000).

Saccharomyces cerevisiae is the microorganism of choice and currently enjoys a monopoly in the fuel ethanol industry. Since S. cerevisiae lacks α-amylase and glucoamylase, it not capable to hydrolyze starch but requires previous hydrolyzed starch for fermentation efficiency. Some recent works concern the hydrolysis of the raw (crude or native) starch as it occurs naturally. During the process of gelatinization, starch granules swell when heated in the presence of water, which involves the breaking of hydrogen bonds, especially in the crystalline regions (Raimbault, 1998). Currently, enzyme-catalyzed starch hydrolysis is preferred, as it offers a number of advantages (for instance, milder reaction conditions). However, the high cost of the initial investment and enzymes, as well as the requirements for specialized labor and sophisticated laboratories are factors limiting the use of enzymes (Surmely *et al*. 2004). The well-known disadvantages of acid hydrolysis, such as the possible inhibitory effect of the by-products on yeast growth, the neutralization of hydrolyzates before fermentation, and the expensive constructional material for equipment, are not drawbacks for its industrial use. This process has a number of important advantages including a fast reaction rate, a simple pretreatment for starch feedstocks, a cheap and easily available acid catalyst, and a relatively low reaction temperature with high acid concentration (Tasić *et al*. 2009). Today, hydrolysis with the dilute

sulfuric acid is the pretreatment technology of choice for lignocellulosic ethanol production (Mosier *et al*., 2005). But using acid in pretreatment of lignocellulosic wastes or starch hydrolysis increases the production costs of the overall process.

Study the production of secondary by-products associated with ethanol production which could be effective in medicinal and pharmaceutical fields is of great importance. These by-products in addition to their extreme useful use, will reduce the production cost of the main target process. Optically active compounds, such as methyl-diols and secondary alcohol derivatives were mentioned as by-products produced by yeasts (Fuganit and Grasselli, 1985). For example, chlorozotocin is a cytostatic agent that is used in the investigational treatment of cancers of the stomach, large intestine, pancreas, and lung, melanoma, and multiple myeloma (NTP 1994). Such compound when it is achievable from yeast during fermentation could explore a new important tend in fermentation biotechnology.

The aim of the present study was to use potato starch residue stream as a very cheap substrate for ethanol production for fuel as well as to highlight the productivity of secondary by-products during the target process.

MATERIALS AND METHODS

Raw material

The potato starch residue stream was collected from a Chpis' Factory for Food Industries S. A. E. Assiut, Egypt. Samples were transferred to the laboratory in an ice box, and then kept frozen until use. Concentration of starch in the residue stream samples was 10–20 gl^{-1}.

Acid hydrolysis

Cold and hot acids (HCl and H_2SO_4) were applied to hydrolyze the starch contained in potato residue stream. Serial concentrations of cold acids were added to the samples (1-5% v/v), then, samples were incubated at room temperature for 15 min with hand shaking intervals. Effect to of hot acid and time of heating on hydrolysis of starch were studied by adding 1% of both acid (separately) and boiled for different time intervals. Five ml of starch solution were drown at constant intervals (0, 20, 40, 60 and 80 min.) and subjected to glucose estimation. Glucose resulted from hydrolysis was estimated using method described by Nelson-Somogyi method (Somogyi, 1952). The resulted hydrolyzed starch solution was neutralized with NaOH and prepared as growth or fermentation medium for the yeast.

Yeast strains

Saccharomyces cerevisiae y-1646 was obtained from South Africa (Department of Microbiological, Biochemical and Food Biotechnology, Faculty of Natural and Agricultural Sciences, University of the Free State) and S. cerevisiae (commercial) was obtained from public market in Assiut, Egypt. Yeast strains were propagated and stored on yeast extract- malt extract agar (YMA) slants (3 gl^{-1} of yeast extract, 3 gl^{-1} of malt extract, 5 gl^{-1} of peptone and 10 gl^{-1} of glucose) at 4 ºC. Active cultures for inoculation were prepared by growing the yeast in YM broth on a rotary shaker at 150 rpm for 16 h at 25 ºC (initial pH 3.8 - 4.5).

Biomass and Fermentations under aerobic and semi-anaerobic conditions

Repeated batch cultures were carried out in triplicate using a medium contained potato starch residue stream was hydrolyzed with 1 %, v/v H_2SO_4 at pH 7.0 to estimate the biomass and ethanol production by both yeast strains aerobically. The prepared medium was sterilized at 121°C for 20 min. Experiments were initiated by transferring prepared cell suspension with 10 ml (1.2 x 106 cell/ml) into 150 ml of the medium in 250 ml Erlenmeyer flasks, then shaken in the incubator at 150 rpm at 30°C for 96 h. The experiments were monitored by removing 2 ml samples every 12 h for biomass determination (by absorbance at 500 nm) and ethanol analyses.

The experiment was repeated under semi-anaerobic conditions by replacing conical flasks with 250 bottles fitted with rubber plugs and incubated under the same afore mentioned conditions for 60 h. Incubation temperature was adjusted at the desirable degree (30, 35, 37, 40°C) in case of *Saccharomyces cerevisiae* y-1646 only. By finishing the incubation time, samples were taken and subjected to analysis of associated secondary by-products. Different concentrations of Zn $Cl_2.7H_2O$ (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 0.7, 0.8, 0.9%) and NH_4NO_2 (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5. 4.0, 4.5) were added separately to the hydrolysed potato starch residue stream for testing their effect on biomass production and fermentation process by *Saccharomyces cerevisiae* y-1646.

Analytical method

The chemical composition of raw material and hydrolysed potato starch residue stream fraction was determined with adding of the Analytical Chemistry Laboratory (ACAL) in Chemistry Department, Faculty of Science, Assiut University, Egypt.

Free cell biomass was determined from the absorbance at 500 nm with a UV-2100 UV-visible spectrophotometer. The estimation of starch was carried out using the iodine colorimetric method as described by Tomas and Chamberlain (Tomas & Chamberlain, 1980). Reducing sugars were estimated by the dinitrosalicylic acid method using glucose as the standard (Miller, 1959). Starch and sugars were analyzed by Capillary Electrophoresis (CE instrument, Agilent G1600AX Germany).G1600A capillary. Ethanol and associated secondary by-products were estimated by Gas Chromatography/ Mass Spectrometry (GC/MS) (Agilent 6890 N/5975 B Germany).

Statistical analysis

All experiments were laid out in a completely randomized design. The results were subjected to analysis of variance, and the treatment means were compared using the least significant difference (LSD) values at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Effect of acid pretreatment on potato waste water hydrolysis

Fig. 1 shows that cold HCl was more efficient than H_2SO_4 in potato waste water hydrolysis. The increase in concentration of both acids, the increase in hydrolysis rate expressed in glucose concentration. Addition of

HCl in 4% or 5% produced the maximum hydrolysis, and there was no significant difference between the two concentrations (15.66 and 15.34 gl^{-1}, respectively). This indicates that at 4% of the cold acid, all hydrolysable materials were hydrolyzed and maximum achievable sugars were produced. Our result could be supported by Tasić *et al*. (2009), who investigated the hydrolysis of starch from fresh potato tubers by HCl and H_2SO_4 and they concluded that, the rate of hydrolysis and the maximal dextrose equivalent (DE) rose with increasing acid concentration, probably due to the increase in the activity of hydrogen ions participating in the reaction as catalyst. The same trend was observed when the acid hydrolysis of sweet potato was studied (Kim and Hamdy, 1985). Tasić *et al*. (2009) obtained, using both acids, approximately the same reaction rates and a DE of about 80% after 60min. Some authors preferred using HCl compared to H2SO4. They mentioned that, when HCl is used, fewer alkali hydroxides are needed for neutralization of the final reaction mixture, so the smaller amount of salt, which inhibits yeast growth is formed (Garcia *et al*., 1997; Norbeck and Blomberg, 1997). We used H_2SO_4 in further hydrolysis, because we can get it very cheaply, since it is produced as a waste product from the pesticides manufacturing.

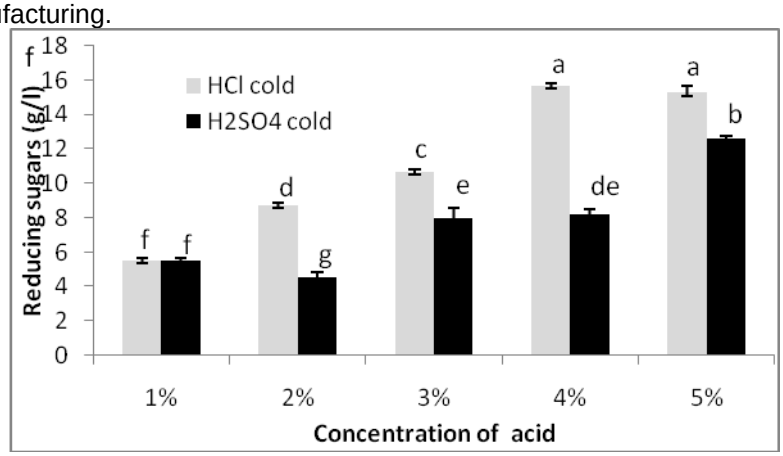

Fig.1. Effect of cold HCl and H_2SO_4 on potato starch residue stream hydrolysis. Columns followed by the same letter(s) are not significantly different at LSD P < 0.05.

When hot acids were applied, a large amount of sugars (15.93-16.2) was produced at low concentration of the two acids (1%) and there was no significant difference between efficiency of both acids at this concentration (Fig. 2). By increasing the concentration of the acids at constant time of heating (66 min), slight increase in sugars production was obtained.

Effect of heating time on acid hydrolysis of starch was studied using the lowest acid concentration (1%). Results indicated that by increasing the time of heating, glucose yield was increased in case of HCl and H_2SO_4 until 60 min, where the maximum yield of sugars was achieved, however after this time there was a light but not significant increase in hydrolysis (18.9%). This indicated that a complete hydrolysis of starch in waste residue was achieved

by using 1% H_2SO_4 after 60 min at 100 C (Fig. 3). This concentration was selected to hydrolyze the waste during all experiments of growth and fermentation of yeast. Our results were closely in agreement with those obtained by Lazić *et al*. (2004) who found the maximal dextrose equivalent achieved in the one hour acid hydrolysis process at 98 ºC by using 1M HCl at the ratio of plant material to acid solution of 1:1 (w/v) (approximately 80%) was greater than that obtained in the two-step enzyme hydrolysis (61%).

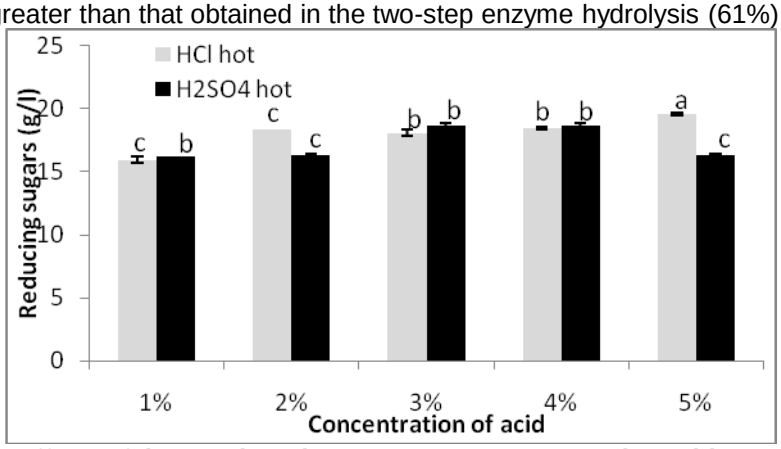

Fig.2. Effect of hot HCl and H_2SO_4 on potato starch residue stream hydrolysis. Columns followed by the same letter are not significantly different at LSD P < 0.05.

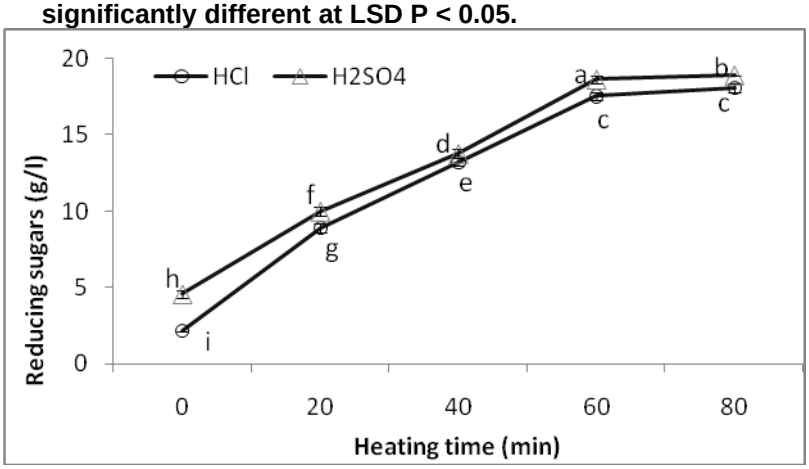

Fig.3. Effect of heating time of 1% of HCl and H_2SO_4 hydrolysis efficiency of potato starch residue stream. Values followed by the same letter are not significantly different at LSD P < 0.05.

Biomass and fermentation of potato waste water hydrolyzate by *S. cerevisiae*

Experiments were conducted to measure the growth rate of tow yeast strains (*S. cerevisiae* y-1646 and *S. cerevisiae* commercial). At 30 ºC, growth curves (measured by OD_{500nm}) of the both two yeasts were nearly the same. They reached the stationary phase at about the same time (Fig. 4).

Growth rate began to decline after 84 h (Fig. 4). During the aerobic growth of the two strains, samples were taken at different intervals and analyzed for their content of ethanol (Fig. 5). Data showed that, the maximum yield of ethanol under aerobic growth, was achieved after 24 h (3.61-3.52 gl^{-1}). Ethanol content was still constant till 36 h, and then began to decline. When we compare the reduction of ethanol production rate after 36 h with the growth rate of the yeast strains which was increase until 48 h, we could assume that after consumption of utilizable sugars in growth media, yeasts potentially utilized the produced ethanol as a carbon source under aerobic conditions.

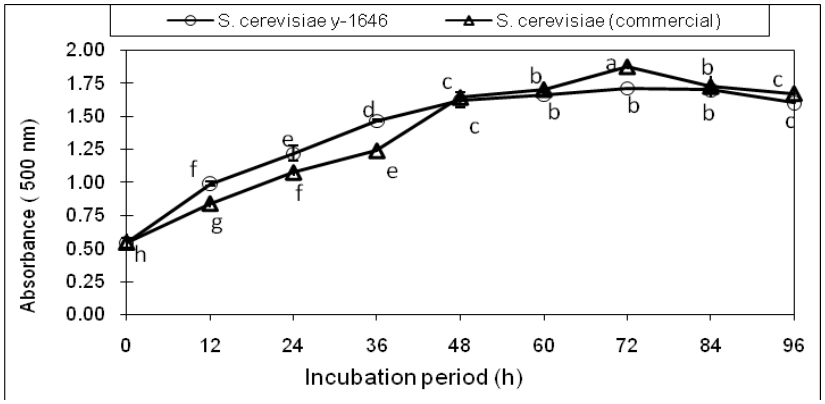

Fig. 4. Growth pattern of yeasts on pretreated starch residue stream measured by absorbance (OD_{500nm}) under aerobic conditions at 30 ºC. Values followed by the same letter are not significantly different at LSD P < 0.05.

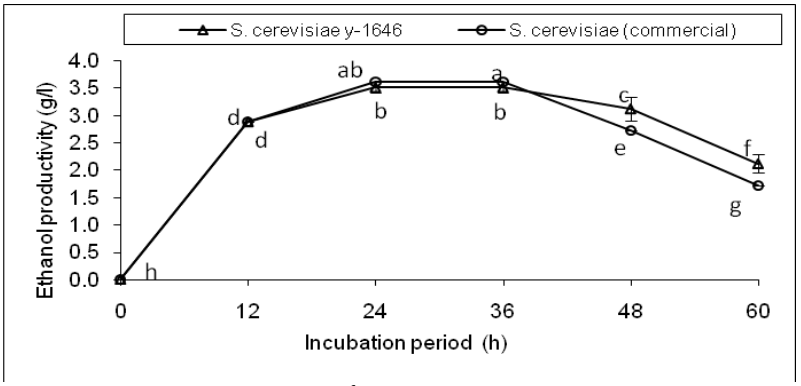

Fig.5. Ethanol productivity (g L^{-1}) by yeasts grown in pretreated potato starch residue stream under aerobic conditions at 30 ºC. Values followed by the same letter(s) are not significantly different at LSD P < 0.05.

Growth pattern and ethanol production by the two yeast strains were studied under semi anaerobic conditions and data was represented in Fig. 6 and 7. There was a close similarity in grow pattern of the two yeasts. Peak of

the growth of the two yeasts was noticed at 24 h. it was estimated as 2.55 and 2.59 (at OD500) in case of *S. cerevisiae* y-1646 and *S. cerevisiae* commercial, respectively. And then growth slightly decreased to 1.74 and 1.65 after 36 h. Fig. 7 showed ethanol productivity of both used yeast strains at 30ºC under semi anaerobic conditions. Obtained results indicated that, maximum ethanol yield from the two strains (3.47- 3.62 gl^{-1}) was achieved after 24 h and still constant 12 h later. After 36 h of incubation, the yield began to decline until the end of the experiment. After 36 h it was noticed that, decreasing in ethanol production rate was accomplished by stability in growth rate. The observation confirmed the hypothesis that yeasts were able to utilize ethanol as a carbon source when sugar is being consumed to be survived. We could assume that after 24 h of fermentation of the waste the batch should be terminated and ethanol should be separated. Growth and ethanol production curve are supposed to be greatly dependant on availability and concentration of fermentable sugars (Patle and Lal, 2008; Altıntaş *et al*, 2002; Tuite and Oliver, 1991; Nellaiah *et al*., 1988).

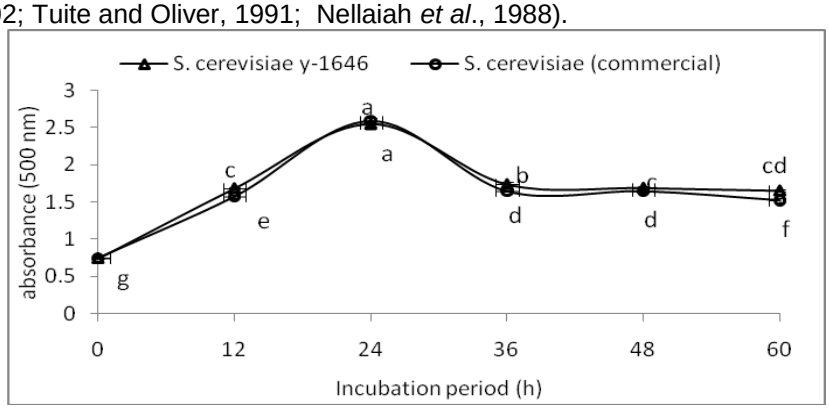

Fig. 6. Growth pattern of yeasts on pretreated potato starch residue stream measured by absorbance (OD_{500nm}) under semi-anaerobic conditions at 30 ºC. Values followed by the same letter(s) are not significantly different at LSD P < 0.05.

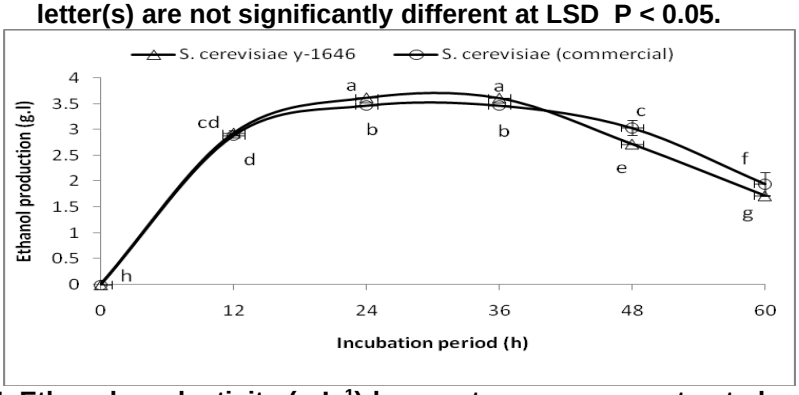

Fig.7. Ethanol productivity (g L^{-1}) by yeasts grown on pretreated potato starch residue stream under semi-anaerobic conditions at 30 ºC. Values followed by the same letter(s) are not significantly different at LSD P <0.05.

Effect of temperature on biomass and ethanol productivity

Effect of temperature on growth of *S. cerevisiae* y-1646 and ethanol production was studied. Data proved that 30 ºC was the most appropriate temperature for yeast growth. At this temperature biomass was estimated as 2.47 (OD_{500nm}), while, 35 ºC and 37 ºC reduced the growth significantly (Fig. 8). Conversely, production of ethanol by *S. cerevisiae* y-1646 favored by temperature of 35 ºC and reached its maximum as 5.29 gl^{-1} after 36 h. At 37 ºC, ethanol production was reduced to 4.38gl^{-1}. The other two temperature (30 and 40 ºC) had great negative impact on ethanol production and reduced it to 3.52 and 3.07 gl^{-1}, respectively (Fig. 9). Thermostability of a yeast strain is more likely genetically controlled. Variation in thermal requirements for biomass and ethanol production stimulate the suggestion that enzymes involved ethanol fermentation vary in their thermal optima than those are involved in biomass synthesis.

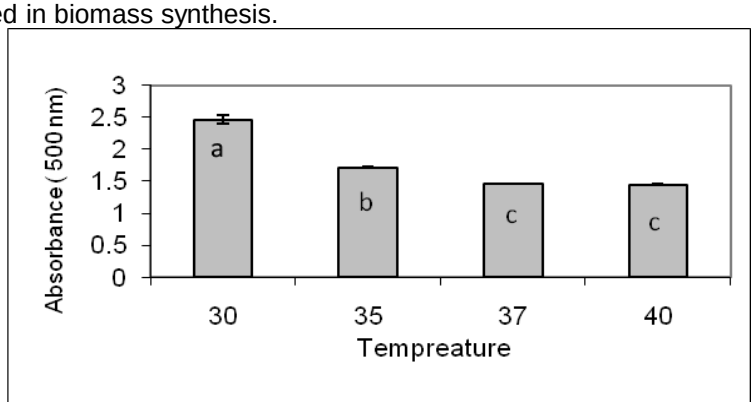

Fig. 8. Effect of temperature on growth of *S. cerevisiae* y-1646 on pretreated potato starch residue stream under semi-anaerobic conditions. Columns followed by the same letter are not significantly different at LSD P < 0.05.

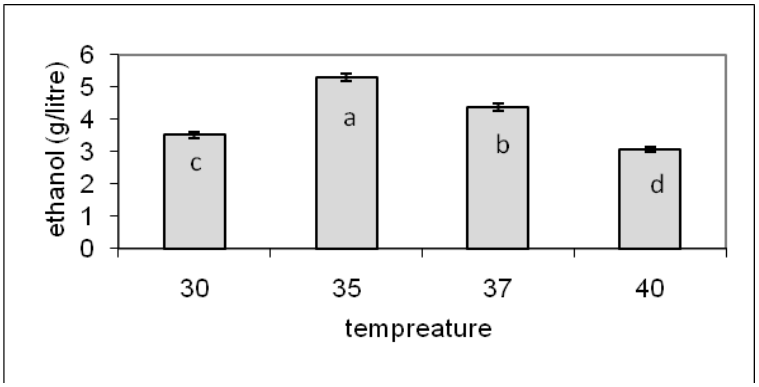

Fig.9. Effect of temperature on ethanol production by *S. cerevisiae* y-1646 on pretreated potato starch residue stream under semi-anaerobic conditions. Columns followed by the same letter are not significantly different at LSD P < 0.05.

Effect of nutrients supplementation in potato water hyrolyzate on biomass and ethanol production

Addition of NH_4NO_3 (0-4.5 gl^{-1}) as a source of nitrogen did not significantly affect neither growth nor ethanol production by S. cerevisiae y-1646. A slight increase in growth and ethanol yield after addition of 4 gl^{-1}, but this increase was not significant (Table 1). This result could be attained because, substrate (potato waste) contains some nitrogenous organic metabolites adequate for growth of yeast and other metabolic process, therefore, yeast do not need more of external nitrogen. Siqueira *et al*. (2008) showed that the addition of NH4NO3 (3.5 gl^{-1}) to the soybean molasses medium did not improve ethanol production and process yield. They mentioned that soybean molasses is rich in mineral salts and contains a considerable amount of proteins that can be used as nitrogen source by the yeast. However, for other substrates such as beet molasses, addition of nitrogen source (ammonium sulfate 0.2 gl^{-1}) can improve ethanol production in about 10% (Nahvi *et al*., 2002). Because of the digestible nitrogen deficiency of sugar beet molasses, ammonium phosphate, ammonium dihydrogen phosphate and ammonium sulfate are usually added to the fermentation medium for better productivity (Ergun and Mutlu, 2000).

Table 1: Effect of addition of some nutrients on growth and ethanol production by *S. cerevisiae* y-1646 on pretreated potato starch residue stream under semi-anaerobic conditions at 35ºC after 36h.

Concentration (g^{-l})	Growth (absorbance at 500 nm)	Ethanol (g^{-l})
NH_4NO_3		
0.0	1.69 ± 0.01	3.25 ± 0.11
0.5	1.63 ± 0.11	2.75 ± 0.21
1	1.71 ± 0.01	2.17 ± 0.21
1.5	1.70 ± 0.00	2.17 ± 0.21
2	1.67 ± 0.05	2.46 ± 0.21
2.5	1.68 ± 0.02	3.18 ± 0.00
3	1.65 ± 0.07	2.88 ± 0.02
3.5	1.64 ± 0.00	3.98 ± 0.11
4	1.72 ± 0.02	3.98 ± 0.11
4.5	1.69 ± 0.02	2.17 ± 0.21
$ZnCl_2.7H_2O$		
0.0	1.69 ± 0.01 de	3.25 ± 0.11 e
0.1	1.70 ± 0.04 de	3.62 ± 0.21 cd
0.2	1.69 ± 0.02 de	3.33 ± 0.21 de
0.3	1.98 ± 0.03 ab	3.02 ± 0.00 e
0.4	2.22 ± 0.17 a	5.62 ± 0.21 a
0.5	2.12 ± 0.01 ab	5.52 ± 0.10 a
0.6	1.93 ± 0.01 bc	3.91 ± 0.21 bc
0.7	1.81 ± 0.09 cd	4.00 ± 0.07 b
0.8	1.73 ± 0.04 d	3.98 ± 0.11 b
0.9	1.58 ± 0.01 e	4.12 ± 0.10 b

Values followed by the same letter(s) are not significantly different at LSD P < 0.05.

Addition of $ZnC_{l2}.7H_2O$ (0-0.9 gl^{-1}) as a source of Zn had a significant effect on both growth of the yeast and ethanol production. The most effective concentration was 0.4 and 0.5 gl^{-1}. At this concentration, maximum values of

growth was 1.72 (OD_{500}) and yield of ethanol was 4.77 gl^{-1}. Zinc is seem to be one of the most effective elements in yeast's metabolic pathways, so, addition of this element as additional source have a good impact on growth and ethanol production.

Secondary metabolites of bioethanol production

GC/MS detected seven other secondary by-products associated with acid hydrolysis of waste material and during ethanol production under semi anaerobic conditions (Table 2). After acid hydrolysis five by-products were detected, among them, 3-propyl-1-heptanol was completely removed after fermentation. After fermentation, 3 new by-products were determined in addition to increase or decrease in concentration of the other four. Among these by-products 3-Octanol (1.276 mg/ml) and 2-Methyl-1-propanol (0.41 mg/ml) are of some importance, that, they could also used as fuels in addition to ethanol. A new very important by-product namely as Chlorozotocin was detected (0.061 mg/m) after fermentation. This compound was mentioned as a very important medicinal and pharmaceutical by-product which is used as cancer chemotherapy. Chlorozotocin is used in the investigational treatment of cancers of the stomach, large intestine, pancreas, and lung, melanoma, and multiple myeloma. It has been given intravenously at doses of 100 to 225 mg/m2 (IARC 1990, Arnold *et al*., 2005).

Table 2. Detectable by-products (mg g^{-1})associated with ethanol production by *S. cerevisiae* y- 1646 on pretreated potato starch residue stream under semi-anaerobic conditions at 35 ºC after 36 h.

Products	Before fermentation	After fermentation
6-Nirt-1,2,3,4-tetrahydrocarbazol-2-ol	0.000	0.033
2-Methyl-1-propanol	0.829	0.410
Chlorozotocin	0.000	0.061
3-Octanol	0.903	1.276
3-Valeraldehydesemicarbozone	0.211	0.149
2,3-Dihydro-2,8-dimethyl benz[b]1,4-oxazepine-4(5H)-thione	0.000	0.076
2-Mercapto ethanol	$8x10^{-5}$	0.001

Conclusion

Our results conclude the possibilty of using potato washing residue stream as an econmical sourece for yeast biomass and bioethanol production for fuel after appropoiate hyrolysis. This subsanace do not need any other additional organic adative, but some minerals such Zn in small doseas could have a good impact on ethanol yield. A very important medicinal compound "Chlorozotocin" was found to be synthesized during ethanol production from the target source. Production of such medicinal active compund should be confirmed in further resaech and its optimal contitions should be optimized.

Acknowledgements

The authors thank Assuit University's administration for support the research.

REFERENCES

Altıntaş M; M. K.Ülgen, B. Kırdar; Z. Önsan; and S.G. Oliver (2002) Improvement of ethanol production from starch by recombinant yeast through manipulation of environmental factors. Enzyme and Microbial Technology 31: 640–47.

Arnold R.; A. Rinke; C. Schmidt; and L. C. Hofbauer (2005) Best Practice and Research Clinical Gastroenterology. 19(4): 649–56.

Badger P.C. (2002)Ethanol from cellulose: a general review. In: Janick J, Whipkey A, editors. Trends in new crops and new uses. Alexandria, VA: ASHS Press; p. 17–21.

Davisa L.; P. Rogersb; J. Pearcec; and P. Peirisa (2006) Evaluation of Zymomonas-based ethanol production from a hydrolysed waste starch stream. Biomass and Bioenergy. 30: 809–14.

Ergun M.; and S. F. Mutlu (2000) Application of a statistical technique to the production of ethanol from sugar beet molasses by Saccharomyces cerevisiae. Biores Technol. 73: 251–55.

Fuganti C.; and P. Grasselli (1985) Stereochemistry and synthetic application of products of fermentation of α, β-unsaturated aromatic aldehydes by baker's yeast. In Enzymes in organic synthesis. Ciba Foundation Symposium 111: 112-25.

Garcia M. J.; G. Rios, and R. Serrano (1997) Comparative physiology of salt tolerance in Candida tropicalis and Saccharomyces cerevisiae. Microbiology 143: 1125–31.

Ghobadian B.; and H. Rahimi (2004) Biofuel: Past, Present and Future Perspective. International Iran and Russian Congress of Agricultural and Natural Science. Shahre- Kord University, Shahre Kord, Iran;.

Harkin T.(2000) Petruoleum and Ethanol Fuels: Tax Incentives and Related G. A. O. Works. United States General Office. Washington; 2000.

Howard L. (1994). Biodiesel vs. Other Alternative Fuels. Bio-state Development Agency.

IARC (1990) Pharmaceutical Drugs. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 50. Lyon, France: International Agency for Research on Cancer; p. 415.

Kim K.; M.K. Hamdy (1985) Acid hydrolysis of sweet potato for ethanol production, Biotechnol Bioeng. 27: 316–20.

Kim S.; B.E. Dale (2004) Global potential bioethanol production from wasted crops and crop residues. Biomass Bioenergy 26:361–75.

Kosaric N.; Z. Duvnjak; and G.G Stewart (1981) Fuel ethanol from biomass: production, economics and energy. Adv Biochem Eng 20:119–51.

Lazić M.L.; S. Rašković; M.Z. Stanković; and V.B. Veljković (2004) Enzymatic hydrolysis of potato starch and ethanol production. Chem Ind 58: 322–26.

Miller G.L. (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31 (3): 426–28.

Mosier N.; C. Wyman; B. Dale; R. Elander; Y.Y. Lee; and M. Holtzapple (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96:673–86.

Nahvi I; G. Emtiazi; and L. Alkabi (2002) Isolation of a flocculating *Saccharomyces cerevisiae* and investigation of its performance in the fermentation of beet molasses to ethanol. Biomass Bioenergy. 23: 481–86.

Nellaiah H.; T. Karunakaran; and P. Gunasekaran (1988) Ethanol fermentation of cassava starch by Zymomonas mobilis NRRL B-4286. Biomass. 15:201–7.

Norbeck J.; and A. Blomberg (1997) Metabolic and regulatory changes associated with growth of *Saccharomyces cerevisiae* in 1.4 M NaCl—Evidence for osmotic induction of glycerol dissimilation via the dihydroxyacetone pathway. J Biol. Chem. 272: 5544–54.

NTP (1994) Report on Carcinogens, 7th ed. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program.

Patle S.; and B. Lal; (2008) Investigation of the potential of agro-industrial material as low cost substrate for ethanol production by using Candida tropicalis and Zymomonas mobilis Biomass Bioenergy. 32:596 – 602.

Prasad S.; A. Singh; and H. C. Joshi (2007) Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resources, Conservation and Recycling. 50: 1–39.

Raimbault M. (1998) General and microbiological aspects of solid substrate fermentation. Electr J Biotechnol 1(3):174–88.

Siqueira P. F.; S.G. Karp; J. C. Carvalho; W. Sturm; J. A. Rodríguez-Leon.; J-L Tholozan; R. R. Singhania; A. Pandey; and C.R. Soccol (2008) Production of bio-ethanol from soybean molasses by *Saccharomyces cerevisiae* at laboratory, pilot and industrial scales. Bioresource Technology. 99: 8156–63.

Somogyi M. (1952) Notes on sugar determination. Journal of Biological Chemistry. 195:19–23.

Surmely R.; H., Alvarez; M. P. Cereda; O.F. (2004) Vilpoux Hydrolysis of starch, in collections, in: Cereda, M.P., Vilpoux, O.F. (Eds.), Latin American starchy tubers, Book 3: Technology, use and potentialities of Latin American starchy tubers. NGO Raizes and Cargill Foundation, Sao Paulo, Brazilp. 389–69.

Tasić M. B.; B. V. Konstantinović; M.L. Lazić; and V.B. Veljković (2009) The acid hydrolysis of potato tuber mash in bioethanol production Biochemical Engineering Journal. 43: 208–11.

Tomas L. C.; and G. J. Chamberlain (1980) Colorimetric Chemical Analytical Methods, Tintometer Ltd., Salisbury, UK. p 3–5.

Tuite, M. F.; and Oliver, S.G. (1991) Expression of heterologous genes. In: Saccharomyces. New York: Plenum Press. p. 178–80.

Verma G.; P. Nigam; and D.Singh; K. (2000) Chaudhary Bioconversion of starch to ethanol in a single-step by co-culture of amylolytic yeasts and *Saccharomyces cerevisiae* 21. Bioresour Technol. 72:261–6.

Wyman C. E. (1999) Biomass ethanol: technical progress, opportunities, and commercial challenges. Annual Review of Energy and the Environment. 24:189–226.

Wyman C.E. (1996) Handbook on bioethanol: production and utilisation. Applied energy technology series. London: Taylor and Francis. p. 1–16.

إنتاج الإيثانول الحيوي والنواتج الأخرى المصاحبة له من النشا الناتج من مخلف ماء البطاطس بواسطة *Saccharomyces cerevisiae*

محمد هشام[١] و سوميه محمد أبراهيم درويش[٢]

١- قسم النبات - كلية العلوم - جامعة أسيوط – مصر

٢- قسم علوم وتكنولوجيا الأغذية - كلية الزراعة - جامعة أسيوط - مصر

الماء الناتج من عملية غسيل البطاطس أثناء صناعة الشبسى يمكن أستخدمه من الناحية الاقتصادية كمادة لتنمية *Saccharomyces cerevisiae* لإنتاج الايثانول الحيوي . وقد أثبتت نتائج البحث أن ١ % من H_2SO_4 على ١٠٠ °م لمدة ساعة كانت كافية لتحليل المحتوى الكلى للنشا في ماء غسيل البطاطس وذلك باستخدام سلالتين من *Saccharomyces cerevisiae* وهما (y- 1646 – والتجارية) فكانت قادرة على تخمير الحامض المعالج في ماء الغسيل تحت الظروف الهوائية وشحيحة الاحتياج للهواء. و كانت اعلي نسبة لإنتاج الايثانول (٥,٥٢ جم / لتر) وذلك على ٣٥ ° م بواسطة *Saccharomyces cerevisiae* y-1646 بعد إضافة $ZnCl_2$ (٠,٤ جم / لتر) لمدة ٣٦ ساعة. كما وجدت خمس نواتج ثانوية أثناء عملية إنتاج الإثانول وكان من أهمها Chlorozotocin والذي له أهمية صيدليه في علاج السرطان .

وتوصى الدراسة بتكثيف البحث في هذه النقطة لزيادة إنتاج هذا المركب الهام .