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Using starchy waste as a promising raw material for bioethanol production with consequence purification using chitosan / sodium alginate polymeric membrane

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Abstract

Current study is concerned by using environmental wastes as raw materials for bio-ethanol production. Alpha amylase enzyme has been used to convert the starch molecules of kitchen waste into simple units of glucose which subsequently fermented into bioethanol. The obtained results showed that 40% substrate and 100 µl of α-amylase were the optimum concentration to produce the highest glucose units at 417.9 and 482.5 milligram/deciliter (mg/dl), respectively. The highest bioethanol production of 423.5 mg/dl was obtained after anaerobic fermentation of free yeast cells at 30^{o}C without shaking. Both of bio-ethanol and 25% ethanol were separated by using amicon cell ultra-filtration system integrated with chitosan or sodium alginate membranes under nitrogen pressure. Each membrane was characterized by Scanning Electron Microscope (SEM) and Ion Exchange Capacity (IEC); while, the hydrophilicity/hydrophobicity was investigated using contact angle. The whole system succeeded to elevate the ethanol concentration in a range of 47-50%, which could be increased with more polymeric modifications.

Keywords: Environmental waste, Chitosan and sodium alginate membranes, Production of bio-ethanol, Bioethanol/water polymeric separation.

1. Introduction

Biofuels produced from renewable biomass have recently received considerable attention. Ethanol can be used as an additive to gasoline fuel or as a transportation fuel. Incorporation of bioethanol into fuels helps to alleviate global warming and environmental pollution. The upcoming depletion and the increasing value of petroleum products have renewed the interest in the production of bioethanol and its use as an alternative fuel or as chemical feedstock (Goshadrou, Karimi, & Taherzadeh, 2013; Mahalakshmi, Angayarkanni, Rajendran, & Rajesh, 2011). Therefore, it was necessary to find dual alternative solutions that focus upon alternative energies with simultaneous reduction of the environmental pollution sources. Some countries depend on agriculture crops such as corn and wheat as a source for production of biofuel (Onuki et al., 2008; Zaldivar, Nielsen, & Olsson, 2001). Such materials cannot be reliable, in the long term as energy sources because they are considered as main sources of human edible foods. Other countries depend on the agricultural wastes as sources of biofuel production. However, the lack of them will harm the animals as these wastes are considered main animal feed (Braide, Kanu, Oranusi, & Adeleye, 2016; Sarkar, Ghosh, Bannerjee, & Aikat, 2012). Recent studies are currently focusing on using food-wastes instead (Kim, Lee, & Pak, 2011; Matsakas & Christakopoulos, 2015). Food waste contain high percent of carbohydrate that can be easily hydrolyzed using enzymatic hydrolysis (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010), the enzyme work on starchy material to yield free glucose units which can be fermented by microbes to produce bio-ethanol (Noufal, Li, & Maalla, 2017; Tanimura et al., 2015). The produced bio-ethanol can be used as a source of energy but after purification process. The most traditional process used at recovery of ethanol is a distillation, which considered as a challenge because of the high costs and energy expenditure required (Vane, 2008). Toward this end, membrane separation processes that applied through the pervaporation technique have been used. The great interest in these processes is mainly because of features such as cost-effectiveness, high energy efficiency and environmental friendliness. Recent technologies that based on membrane separation are normally fulfill the required criteria of energy efficiency and sustainability (Bello et al., 2014; Korelskiy et al., 2013). This study aimed to bioconversion of environmental wastes, which cause serious environmental problems, into beneficial products. The study was concerned by increasing the liberation rates of glucose units using enzymatic treatments followed by anaerobic fermentation of liberated glucose units into bio-ethanol. The study was extended to prepare polymeric membranes that have the ability to enhance the bioethanol/water separation process.

2. MATERIALS AND METHOD

2.1. MATERIALS

The kitchen wastes were collected from different restaurants in different locations in Borg El-Arab, Alexandria, Egypt. Sodium hydroxide (NaOH) pellets, Chitosan (CS) with degree of acetylation of 84% and average M.wt 500000 were obtained from Acros organics, Belgium. Sulfuric acid (H_2SO_4) and HPLC grade absolute Ethanol were purchased from Fisher Scientific UK. Both of the yeast strain (*Saccharomyces cerevisiae*) & amylase enzyme were kindly provided by industrial bioprocess department, GEBRI institute, City of scientific research and technological applications, and potassium dichromate ($K_2Cr_2O_7$) were purchased from Sigma-Aldrich, USA. Glucose kit was purchased from Bio systems, Spain. Sodium alginate (SA), Technical, SLR was purchased from Fisher chemical UK.

2.2. METHODS

2.2.1. Selection of the type of waste

As kitchen wastes include vast number of organic components, the starchy category (especially rice) was chosen as the raw material for the production of bioethanol.

2.2.2. Optimization Parameters

2.2.2.1. Substrate concentration

A wide range of rice concentrations as 10, 20, 30, 40 and 50% were weighted, added to 50 ml dH_2O and

sterilized at 15 psi and 120ºC for 20 min. After sterilization, the glucose concentration was firstly measured in order to check the ability of autoclaving to degrade the bonds of the starch backbone and liberate the glucose units. The flasks were cooled and 50 µl of crude α-amylase enzyme were added, mixed and kept at 30ºC for three hours. The liberated glucose units were then measured and the optimum substrate concentration was determined.

2.2.2.2. Enzyme concentration

After determination of the optimum concentration of the substrate, it was selected and submitted to different concentrations of the amylase enzyme. For more clarification, five conical flasks of optimum weight of rice were prepared and sterilized at 15 psi and 120ºC for 20 min. To each flask, single inoculum of 20, 40, 60, 80 and 100 µl of the crude enzyme was added to each flask individually (where, each microliter of the crude enzyme equals to a concentration of 2.5 µg/ml). After three hours of incubation at 30ºC, the final released glucose concentrations were measured and calculated in mg/dl.

2.2.3. Determination of glucose concentration

The concentration of free glucose units was measured in mg/dl using (Bio systems, Spain) glucose kit according to the manual instructions.

2.2.4. Glucose separation and yeast inoculation

The optimum conditions for the liberation of the highest glucose concentration were applied, and soluble glucose units were separated from the rice debris through centrifugation at 6000 rpm for 10 min. The obtained supernatant was transferred to sterile container and 1ml of free and immobilized overnight cultures of *Saccharomyces cerevisiae* that grew in YPG broth were added under septic conditions.

2.2.5. Immobilization of yeast cells in Ca-alginate

Immobilization of yeast cells in Ca-alginate beads was carried out under septic conditions according to (Taha, Alamri, Mahdy, & Hafez, 2013). Two milliliters of overnight culture were suspended in 5 ml of 3% (w/v) sodium alginate solution. The obtained mixture was dropped through a syringe nozzle into 100 ml of 3.5% (w/v) $CaCl_2$ solution. Alginate drops were cross linked upon their contact with Ca^{+2} ions, forming spherical beads and thus entrapping the yeast cells. The beads were allowed to harden for 30 min and were then washed with a sterile normal saline solution (0.9% NaCl) to remove any excess Ca^{+2} ions and cells.

2.2.6. Fermentation

The free and immobilized yeast cells were inoculated into glucose containing flasks and were then submitted to anaerobic conditions by surface addition of 50 µl mineral oil. The flasks were then incubated statically at 25ºC for three days, and the concentration of the formed bioethanol was measured spectrophotometrically**.**

2.2.7. Spectrophotometric measurement of bioethanol concentration

Potassium dichromate analytical method was used for estimation of produced bio-ethanol according to (Balasubramanian, Ambikapathy, & Panneerselvam, 2011; Hashem, Asseri, Alamri, & Alrumman, 2018; Srivastava, Agrawal, & Rahiman, 2014) with some modifications. After centrifugation of each culture at 1000 rpm for 10 min, 1ml of each ferment was diluted by 4ml of distilled water followed by the addition of 1ml of 2% $K_2Cr_2O_7$. The tubes were kept on ice bath while 1ml of concentrated H_2SO_4 was drop wisely added. After 10 min incubation at room temperature, the absorbance of each sample was measured by spectrophotometry at 660 nm against blank sample contained 1ml dH_2O instead of the ferment. The obtained readings were dropped to ethanol standard curve and the ethanol concentration was calculated.

2.2.8. Polymeric separation of ethanol-water mixture

The Polymeric separation of the produced bio-ethanol was achieved through two steps: first step included the preparation of polymeric (Chitosan and Sodium alginate) membranes. The second step depended on using amicon stirred ultrafiltration cell (USA) integrated with the polymeric membranes. Different nitrogen pressures from 20 to 60 psi were applied and the volume and concentration of each permeate solution were measured each one hour.

2.2.8.1. Membrane preparation

Chitosan membrane (CS) was prepared by adding 2% (W/V) solution of CS in 2% (V/V) aqueous acetic acid, stirred for half an hour then filtered to remove un-dissolved and suspended matter. A bubble-free solution was cast onto a clean glass plate and evaporated to dryness at room temperature for 24h, followed by overnight vacuum drying in an oven at 40^{o}C to remove the present of residual solvent, if any.

Sodium alginate membrane (SA) was prepared by solution casting and solvent evaporation method. A quantity of 3g of SA was dissolved in 100 ml distilled water then stirred to be completely soluble. The solution was cast on a clean acrylic plate petri dish to the desired thickness and dried in atmospheric condition at room temperature, followed by vacuum drying for 5h at 50^{o}C to remove last traces of solvent.

2.2.8.2. Membrane characterization

The morphology of CS and SA membranes were characterized by scanning electron microscope (JEOL, JSM-6360LA, Japan), water contact angle measurements were obtained using contact angle meter VCA 2500 XE equipped with CCD camera and analysis software (AST Products, Billerica, MA**).**

2.2.8.2.1. Ion Exchange Capacity (IEC)

Ion exchange capacity was measured by using acid–base titration according to (Abu-Saied, Fontananova, Drioli, & Eldin, 2013; Abu-Saied et al., 2017) with some modifications. Weighted samples from each membrane before and after separation were placed in 20 ml of 2M NaCl solution at room temperature for 12h. The solution was then titrated with known concentration of NaOH solution, using phenolphthalein as indicator. The IEC can be calculated from the following equation:

$$\mathbf{IEC\ (meq/g)} = \left(\frac{\mathbf{C\ (NaOH)\ \times\ V\ (NaOH)}}{\mathbf{Dry\ weight\ of\ sample}}\right)$$

Where C is the molar concentration of NaOH solution, and V is the volume (ml) of consumed NaOH.

3. Results and Discussion

3.1. Optimization Parameters

3.1.1. The optimum substrate concentration

The obtained results revealed that the autoclaving process was unable to liberate the glucose units from the starch backbone, indicating that other methods are needed. The data obtained after the addition of α-amylase enzyme were much significant, indicating that the enzymatic biodegradation is the most preferable methods. As shown in figure 1, the ability of α-amylase enzyme to liberate glucose units from starch was highly depending on the substrate concentration. At lower concentrations, the activity of the enzyme was quite elevated, which allow the enzyme molecules to freely hang out between the substrate molecules and easily find the suitable degradation position (Saha, Baishnab, Alam, Khan, & Islam, 2014). However, at higher concentrations, the attached and closely linked starch molecules make the pathway of the enzyme quite crowded and hardened the mission accomplishment. These concepts are almost reflected through the results of the concentrations of released glucose units from 10 to 30% of rice. The obtained results showed that these concentrations were able to produce 122-299.2 mg/dl glucose at 10-30% of rice. However, the highest glucose concentration of 417.9 mg/dl was obtained at 40% of rice, which subsequently decreased to 362.5 mg/dl at 50%. These results confirm the principle of lower concentration dependent concept.

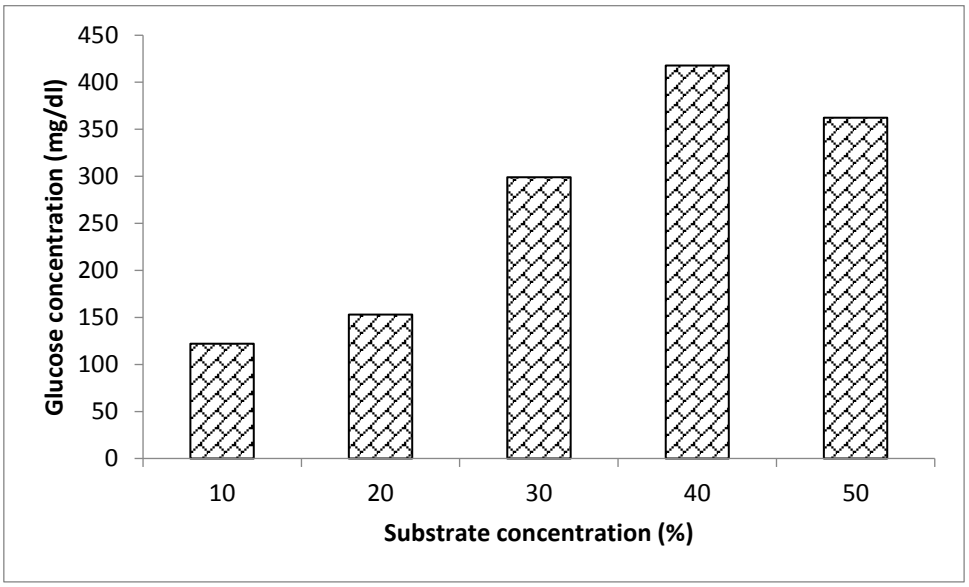

Figure 1: The concentration of released glucose at different substrate concentrations.

3.1.2. The effect of enzyme concentration

The enzyme concentration is considered an important factor for the degradation of complex molecules (figure 2). In current experiment, the obtained data showed how much the enzyme concentration is critical for its activity. As shown in figure 3, the enzyme activity is gradually increased by increasing its concentration. The highest glucose concentration was recorded as 482.5 mg/dl at 100 µl of α-amylase enzyme; however, the concentration of glucose was recorded as 130.4 mg/dl after using 20 µl of the enzyme using the same incubation conditions. The same observation was shown at 60 and 80 µl of the enzyme that showed glucose concentrations of 248.4 and 260.9 mg/dl respectively. These observations are strongly matched with (Nagodawithana & Steinkraus, 1976; Noufal et al., 2017) who reported that the increasing of enzyme concentration is proportional to its activity.

Figure 2: Biodegradation of cooked rice grains by α-amylase enzyme. The left flask is untreated rice grains and the right flask is the treated rice grains.

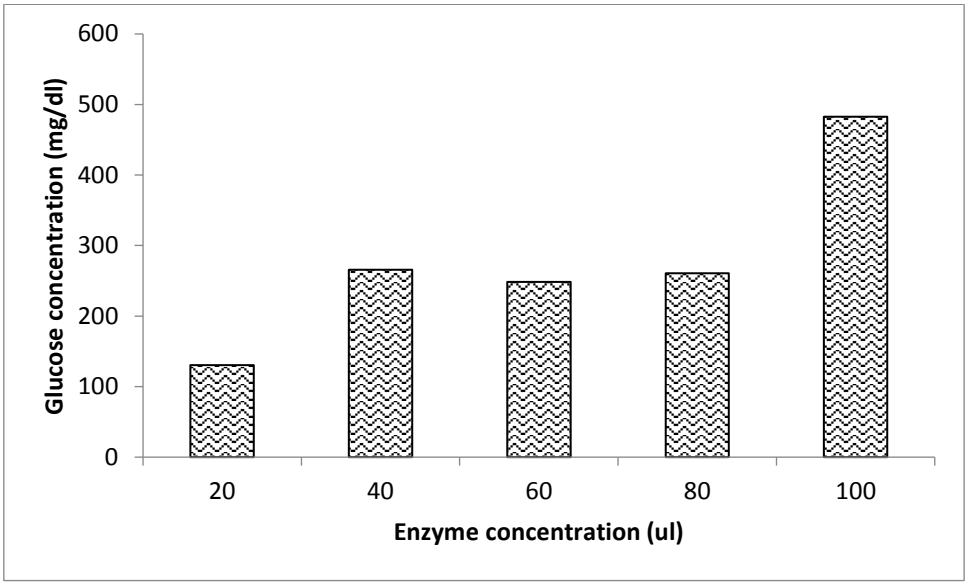

Figure 3: The concentration of released glucose at different α-amylase concentrations.

3.2. Effect of immobilization on bioethanol production

The ability of *Saccharomyces cerevisiae* strain to produce bioethanol at free and immobilized status was recorded. The yeast strain preferred to produce bioethanol when being free rather than being immobilized. The concentration of produced ethanol was 423.5 mg/dl when free cells were used for the fermentation process; whenever, lower concentration of ethanol (319.2 mg/dl) was produced by the immobilized cells.

3.3. Polymeric separation of ethanol/water mixture

3.3.1. Membrane characterization

The morphological appearance of both of CS and SA membranes were characterized by SEM (figure 4). The surface of both membranes appear to have a smooth and homogeneous surface and haven't any cracks (Abu-Saied et al., 2017; Rosi, Iskandar, Abdullah, & Khairurrijal, 2014). Ion-exchange capacity refers to the density of ionizable hydrophilic groups in the membrane matrix, which are responsible for the IC of the membranes, and this is an indirect approximation of the proton conductivity (Abu-Saied et al., 2013; Abu- Saied et al., 2012; Becker & Schmidt- Naake, 2002; M. Eldin et al., 2011; M. M. Eldin et al., 2011). IEC results of CS and SA membranes were reported to be 1.54 and 1.04 (meq/g), respectively. This result illustrates that both membranes have acceptable IEC and suitable for using in the separation application technique. Contact angle was employed to characterize the relative hydrophilicity or hydrophobicity of each membrane surface. CS membrane resulted in contact angle theta (R) 47.80 and theta (L) 46.87; and SA membrane showed theta (R) 48.65 and theta (L) 47.23. These results obtained in the hydrophilic characteristics range, and hence CS and SA are considered hydrophilic materials (Kalyani, Smitha, Sridhar, & Krishnaiah, 2008; Sunitha, Satyanarayana, & Sridhar, 2012).

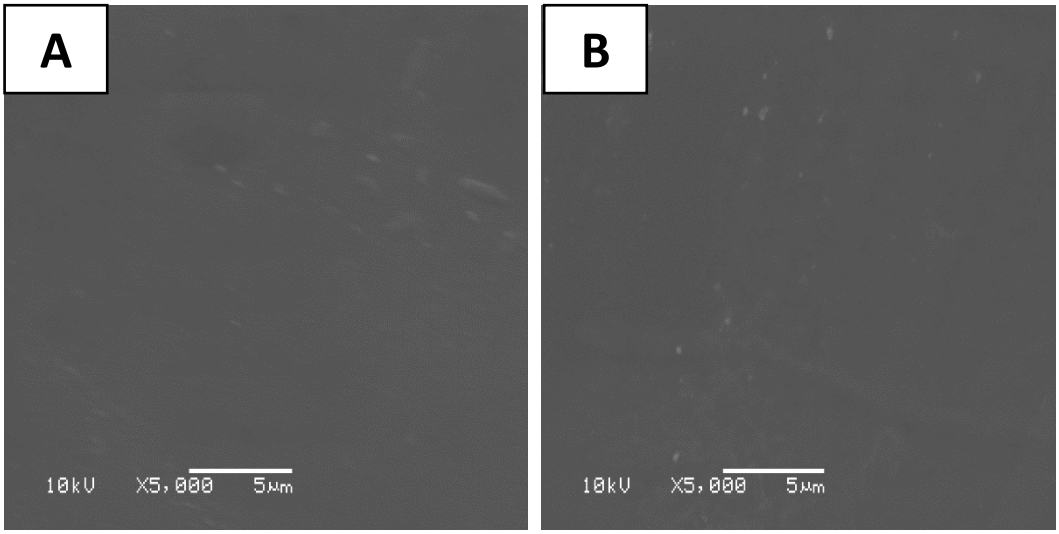

Figure 4: SEM of (a) chitosan membrane and (b) sodium alginate membrane.

3.3.2. Application of the polymeric membrane in separation of ethanol/water mixture.

The separation of ethanol from water that previously reported in many research articles included many separation techniques. In the last few years, the pervaporation technique became the favorable technique for the separation technology (Gongping, Wang, Wanqin, & Nanping, 2012; Sun, Lu, Chen, & Jiang, 2008). The current work did not depend on the same mechanism of pervaporation. It depends on using CS and SA polymeric membranes in separating ethanol/water mixture, and purifying the produced bioethanol from other broth components after the fermentation process. The separation was depending on using amicon cell with different nitrogen pressures as alternative system instead of pervaporation system.

The separation process was performed for two different ethanol/water resources. The first resource was prepared in the laboratory with concentration of 25% chemically prepared ethanol in water. While the second was depending on the separation of 30% produced bioethanol from the other broth components.

- **Separation of 25% chemically prepared ethanol/water mixture**

Different nitrogen pressures from 20 to 60 psi were applied for 12h as shown in table 1. The results showed that the highest permeate volume and concentration was obtained at 30 psi. As shown in figure 5 (A and B) both of permeate volume and permeate concentration were dramatically increased with pressure values from 20 to 30 psi. The permeate volume was increased from 1500 to 3000 µl; while, the permeate concentration was increased from 10.34 to 50.29 mg/ml using CS membrane. In addition, the permeate volume was increased from 1200 to 3300 µl; while, the permeate concentration was increased from 15.22 to 45.66 mg/ml using SA membrane.

As shown in figure 6 (A and B) the highest flux of permeate and separation factor under 30 psi nitrogen pressure value were reported as 49.15 ($mg/m^2.h$) and 80.51 for CS membrane, but it was recorded as 44.16 ($mg/m^2.h$) and 86.56 for SA membrane, respectively. However, increasing of nitrogen pressure from 40 to 60 psi resulted in a dramatic decrease in permeate flux and an increase in the separation factor. It worth mentioning that the flux of permeate and separation factor were calculated by equations reported by (Zou et al., 2012).

Table 1: Separation process of 25% chemically prepared ethanol from water using CS and SA membranes.

Membrane Type	Time (h)	Nitrogen pressure (psi)	Permeate volume (µl)	Permeate concentration of ethanol (mg/ml)	Flux of permeate (mg/m^2.h)	Separation factor
CS	3	20	1500	10.34	25.96	30.62
	4	30	3000	50.29	49.15	80.51
	2	40	1000	34.36	30.4	39.03
	2	50	600	17.11	27.13	40.9
	3	60	400	15.93	18.91	60.52
SA	2	20	1200	15.22	20.23	32.98
	3	30	3300	45.66	44.16	86.56
	3	40	1300	30.79	23.42	37.51
	1	50	700	33.82	30.45	43.87
	3	60	400	20.65	15.95	63.45

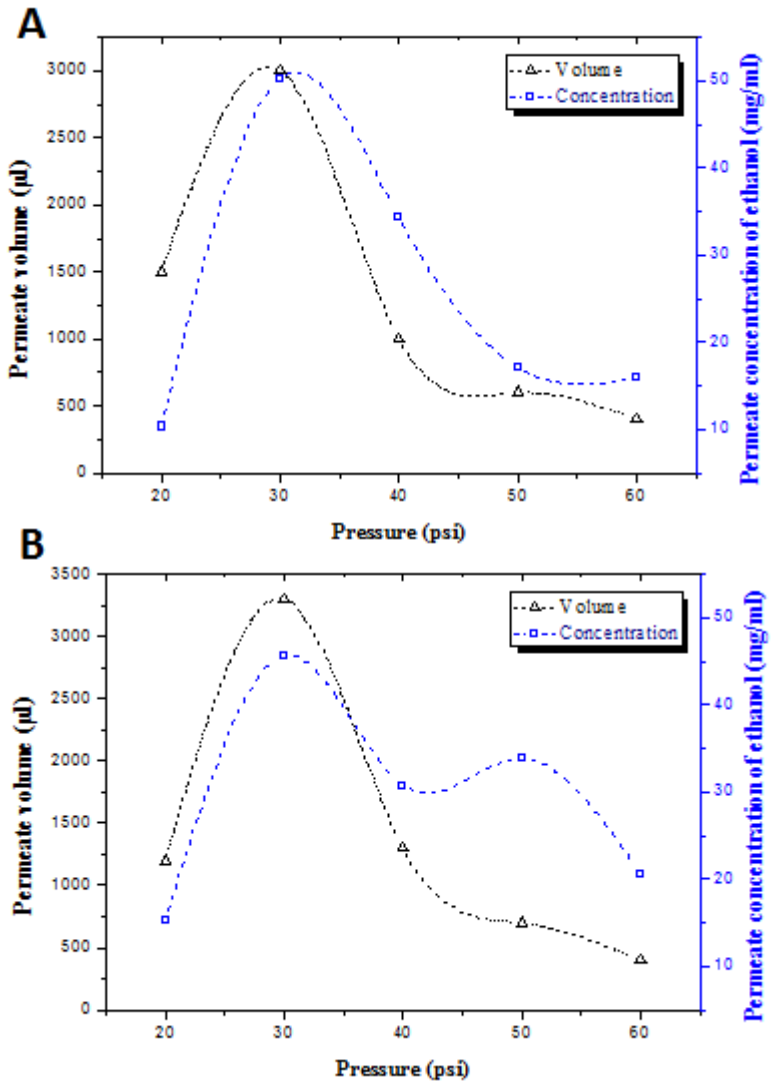

Figure 5: The obtained permeate volume and concentration produced using CS membrane (A) and SA membrane (B) for separation of 25% ethanol/water mixture.

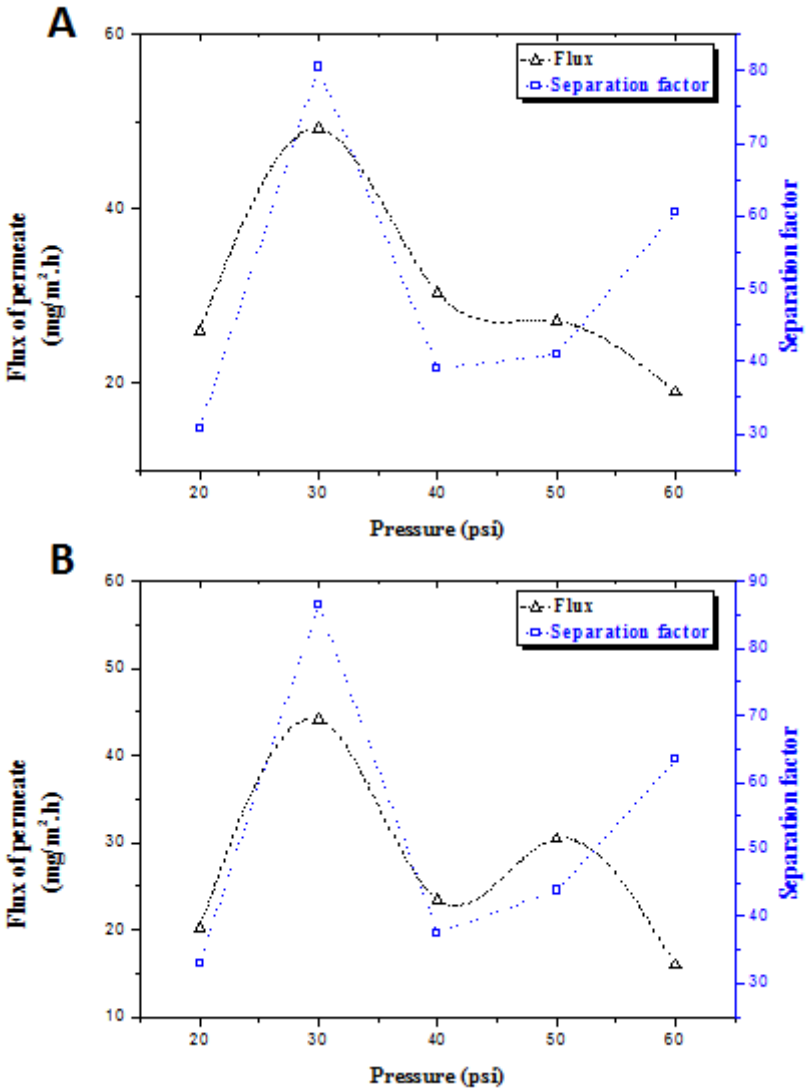

Figure 6: The obtained flux of permeate and separation factor produced using CS membrane (A) and SA membrane (B) for separation of 25% ethanol/water mixture.

- **Separation of 30% bioethanol/culture broth mixture**

The second ethanol resource was obtained biologically as a bio-product of yeast fermentation process of starchy waste. Table 2 demonstrates that the highest permeate volume was recorded at nitrogen pressure 30 psi. As recorded in figure 7 (A and B) the permeate volume were increased from 1100 to 2800 µl for CS membrane and from 1150 to 2750 µl for SA membranes, respectively. However, at nitrogen pressures from 40 to 60 psi, the permeate volume was gradually decreased from 1900 to 800 µl for CS membrane and from 1800 to 800 µl for SA membrane, respectively. Using CS membrane, the highest permeate concentration was recorded as 53.23 mg/ml, which was almost close to the same concentration obtained using SA membrane (55.10 mg/ml) under same pressure (50 psi). On the other hand, 50 psi was recorded as the optimum pressure for obtaining the highest permeate flux using both membranes. However, 30 psi was the optimum one for obtaining the highest separation factor using both membranes. As depicted in figure 8A, the highest permeate flux and separation factor were 40.35 ($mg/m^2.h$) and 65.21 using CS membrane, respectively. While, the highest permeate flux and separation factor were 44.31 ($mg/m^2.h$) and 65.10 using SA membrane, respectively (figure 8B). These results confirm the prolonged stable activity of the prepared polymeric membranes even at the presence of salts and sugars which are present as residues in the fermentation liquor (Chovau, Gaykawad, Straathof, & Van der Bruggen, 2011).

Table 2: Separation process of 30% bioethanol from culture broth using CS and SA membranes.

Membrane Type	Time (h)	Nitrogen pressure (psi)	Permeate volume (µl)	Permeate concentration of ethanol (mg/ml)	Flux of permeate (mg/m^2.h)	Separation factor
CS	2	20	1100	19.53	21.18	29.28
	4	30	2800	35.48	37.44	65.21
	3	40	1900	46.78	33.24	53.48
	2	50	900	53.23	40.35	45.59
	1	60	800	44.21	31.20	38.69
SA	2	20	1150	19.12	21.76	28.97
	3	30	2750	37.94	36.27	65.10
	2	40	1800	48.36	32.84	52.99
	2	50	1000	55.10	44.31	52.55
	3	60	800	42.36	33.86	41.93

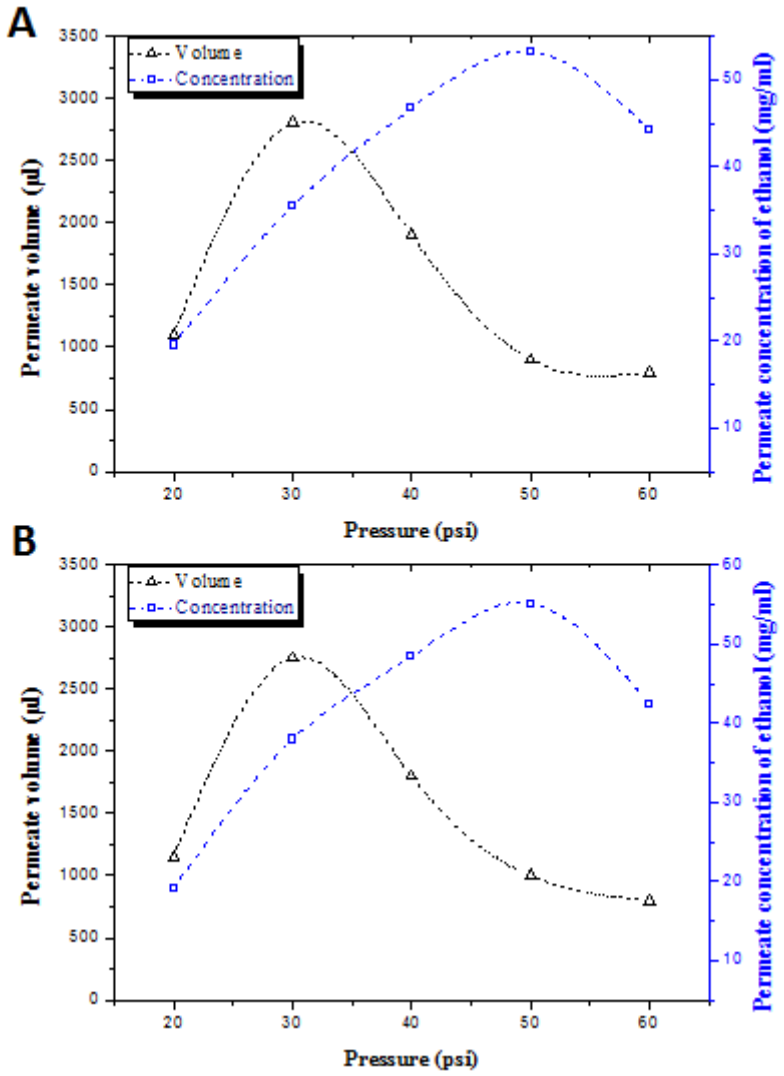

Figure 7: The obtained permeate volume and concentration produced using CS membrane (A) and SA membrane (B) for separation of 30% bioethanol/culture broth mixture.

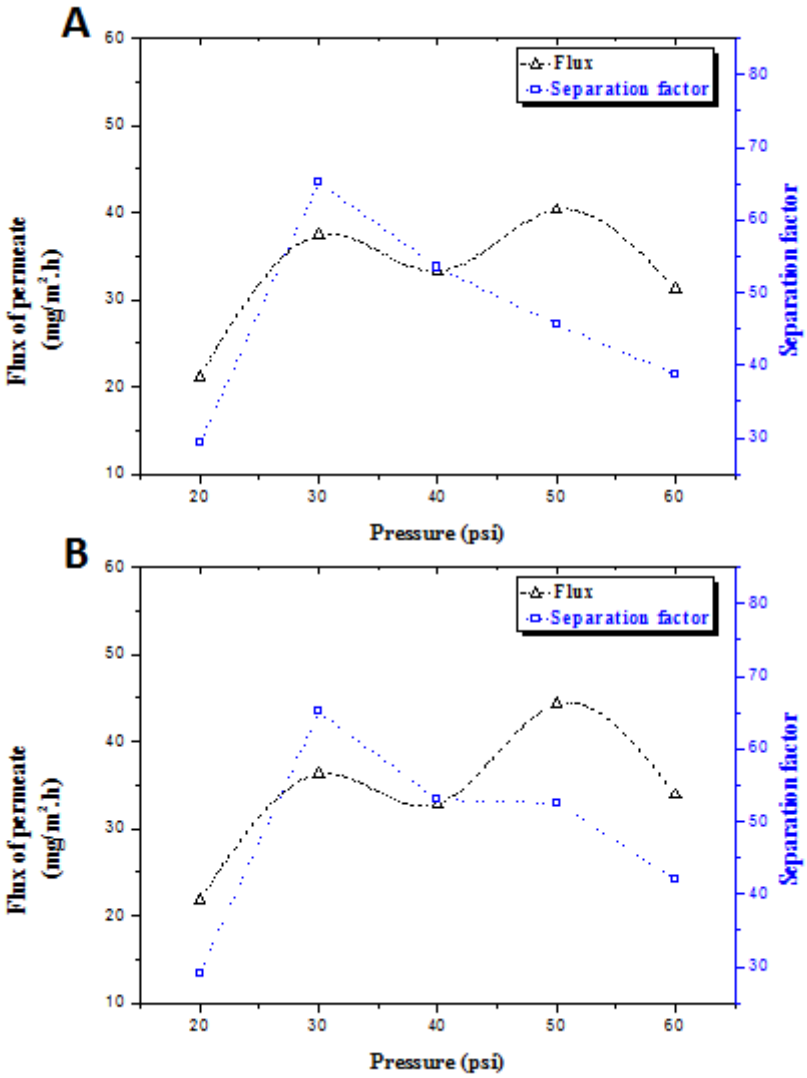

Figure 8: The obtained flux of permeate and separation factor produced using CS membrane (A) and SA membrane (B) for separation of 30% bioethanol/culture broth mixture.

4- Conclusion

The present work deals with the bioconversion of environmental waste (starch) into bio-ethanol. The highest glucose units were liberated using α-amylase enzyme during enzymatic hydrolysis. The highest bioethanol production of 423.5 mg/dl was obtained after anaerobic fermentation of the free yeast cells at 30°C without shaking. CS and SA membranes were prepared and used in separation of bio-ethanol/culture broth process by using amicon cell at different nitrogen pressures with 30 psi as the best optimum one. CS and SA membranes were characterized by SEM, IEC and the hydrophilicity/hydrophobicity of the prepared membranes was also investigated using contact angle. The obtained results confirmed that the prepared polymeric membranes can be used for the separation of different types of ethanol/water mixtures through the pervaporation system or any other systems including our system.

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