
BIO-ETHANOL PRODUCTION FROM ENVIRONMENTAL WASTE FOLLOWED BY POLYMERIC SEPARATION OF FORMED ETHANOL/WATER MIXTURE

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ABSTRACT

Recent studies are concerned by future energy shortage that projected to occur as a result of fossil fuel depletions. Our study was interested to use the environmental wastes as a raw material for bio-ethanol production. Kitchen wastes are one of the most distributed wastes all over the world. Starchy ingredients in the form of rice mainly are the major component of such wastes. Crude alpha amylase enzyme has been applied to convert the starch molecules into simple units of glucose. *Saccharomyces cerevisiae* has been subsequently used to ferment the produced glucose units into bioethanol anaerobically. The obtained results showed that 40% rice substrate is the optimum concentration to produce the highest glucose units at 417.9 mg/dl. However, the higher concentration of the substrate (rice) was recorded as a blocking agent for glucose production. On the other hand, the higher percentage of alpha amylase (100 µl) was recorded as the most preferable one to produce the most elevated glucose concentration of approximately 482.5 mg /dl. The highest bioethanol production of 423.5 mg/dl was obtained after anaerobic fermentation of the free yeast cells at 30°C without shaking. The produced bio-ethanol compared with standard 25% ethanol was separated by using amicon cell ultra-filtration at different nitrogen pressures. Chitosan and sodium alginate membranes were prepared to be used in the bio-ethanol/water separation process. Chitosan and sodium alginate membranes were characterized by SEM and IEC. The hydrophilicity/hydrophobicity of the prepared membranes were investigated using contact angle.

Keywords: Environmental waste, Chitosan and sodium alginate membranes, production of bio-ethanol, separation process by amicon cell system.

1. INTRODUCTION

Bio-ethanol produced from renewable biomass has received considerable attention in current years. Ethanol can be used as a gasoline fuel additive and transportation fuel. This helps to alleviate global warming and environmental pollution. The decreasing reserves and increasing value of petrochemicals have renewed the interest in the production of bioethanol and its use as fuel and chemical feedstock [1,2]. 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 wheat and corn as a source for production of biofuel [3,4]. 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 [5,6]. Recent studies have been focused on using food-wastes [7,8]. Food waste contain high percent of carbohydrate which can be easily hydrolyzed using enzymatic hydrolysis [9], the enzyme work on starchy material to yield monomeric

glucose units which can be subsequently fermented to produced bio-ethanol [10, 11]. 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, but is a challenge because of the high costs and energy expenditure required [12]. Toward this end, membrane separation processes such as pervaporation have been used. The great interest in these processes is mainly because of features such as cost-effectiveness, high energy efficiency and environmental friendliness. Membrane based separation technologies normally fulfill the criteria for sustainability and energy efficiency [13,14]. 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 preparing polymeric membranes to enhance the ethanol/water separation process.

1. MATERIALS AND METHOD

1.1. MATERIALS

The kitchen wastes were collected from different restaurants in different locations in Borg El-Arab city, 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 Ethanol absolute HPLC grade were purchased from Fisher Scientific UK. Both of the yeast strain (*Saccharomyces cerevisiae*) and the 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.

1.2. METHOD

2.2.1. Selection of the type of waste

As kitchen wastes include vast number of organic materials, 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 and added to 50 ml d H_2O and sterilized at 1.5 psi at $120^\circ C$ 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 alpha amylase enzyme were added, mixed and kept at $30^\circ C$ for three hours. The liberated glucose units were then measured and the optimum substrate concentration was determined.

2.2.2.2. Enzyme concentration

The optimum concentration of substrate, this concentration 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 1.5 psi at $120^\circ C$ for 20 min. to each flask, single inoculum of 20, 40, 60, 80 and 100 μl of the crude enzyme was added to the flasks individually. After three hours of incubation at $30^\circ C$, the final released glucose concentrations were determined and calculated in mg/dl.

2.2.3. Determination of glucose concentration

The concentration of glucose 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 1 ml of free and immobilized overnight cultures of *Saccharomyces cerevisiae* in YPG broth were added under septic conditions.

2.2.5. Immobilization of yeast cells in calcium alginate

Immobilization of yeast cells in alginate capsules was carried out under sterile conditions according to [15]. Two milliliters of overnight culture was 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 solidified upon contact with CaCl_2 , forming capsules and thus entrapping the yeast cells. The capsules 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 inoculated flasks were then submitted to anaerobic conditions by surface addition of 50 μl mineral oil. The flasks were then incubated statically at 25°C 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 bio-produced ethanol according to [16-18] with some modifications. After centrifugation of each culture at 1000 rpm for 10 min, 1 ml of each ferment was diluted by 4 ml of distilled water followed by the addition of 1 ml 2% $\text{K}_2\text{Cr}_2\text{O}_7$. The tubes were kept at ice bath and 1 ml of H_2SO_4 drop was wisely added. After 10 min incubation at room temperature, the absorbance of each sample was measured by spectrophotometry at 660 nm. 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. Second step using Amicon stirred ultrafiltration cell (USA) integrated with the polymeric membranes under different nitrogen pressures from 20 to 60 psi with different time intervals where the volume and concentration of permeate solutions were measured every hour.

2.2.8.1. Membrane preparation

Chitosan membrane was prepared by adding 2% (W/V) solution of chitosan in 2% (V/V) aqueous acetic acid, stirred for a period of half an hour then filtered to remove undissolved matter. A bubble-free solution was cast onto a clean petri dish of glass plate and evaporate to dryness in atmosphere at room temperature for 24h, followed by vacuum drying in an oven at 40 °C overnight to remove the present of residual solvent.

Sodium alginate membrane was prepared by solution casting and solvent evaporation method, 3g of sodium alginate was dissolved in 100 ml distilled water then stirred to 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 °C to remove last traces of solvent.

2.2.8.2. Membrane characterization

The morphology of chitosan and sodium alginate membranes were characterized by scanning electron microscope (JEOL, JSM-6360LA, Japan), Water Contact angle measurements were done 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

Ion Exchange capacity can be measured by using acid–base titration according to [19, 20] with some modifications. Weighted samples from membrane before and after separation were placed in 20 ml of 2 M NaCl solution at room temperature for 12h. The solution was then titrated with NaOH solution of known concentration, using phenolphthalein as indicator. The IEC can be calculated from the following equation:

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

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 alpha amylase enzyme were much significant, indicating that the biodegradation is most preferable than chemical or physical methods. As shown in figure 1, the ability of 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 hang out between the substrate molecules and easily find the suitable degradation position [21]. 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 482.5 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.

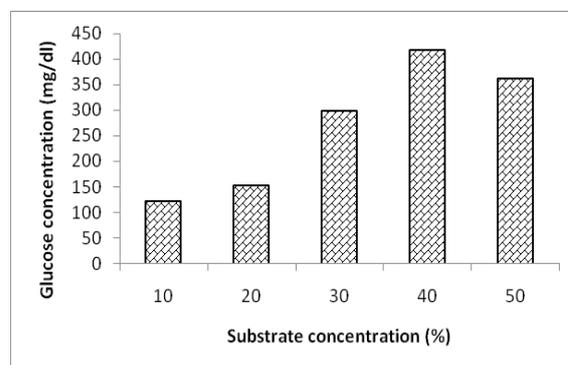


Figure 1: The concentration of released glucose at different substrate concentrations.

3.1.2. The effect of enzyme concentration

The enzyme concentration is considered an important factor for the degradation of specific 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 alpha 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 matched with [11, 22] who reported that the increasing of enzyme concentration is proportional to its activity.

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.



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

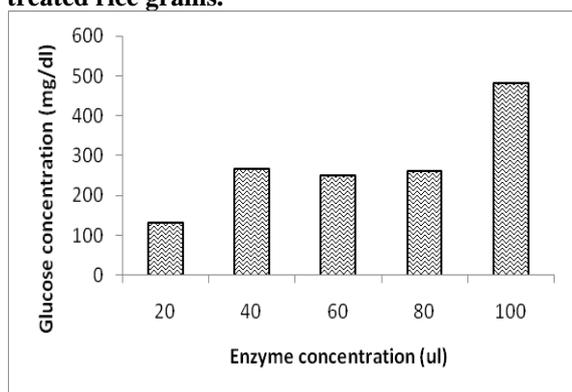


Figure 3: The concentration of released glucose at different alpha amylase concentrations.

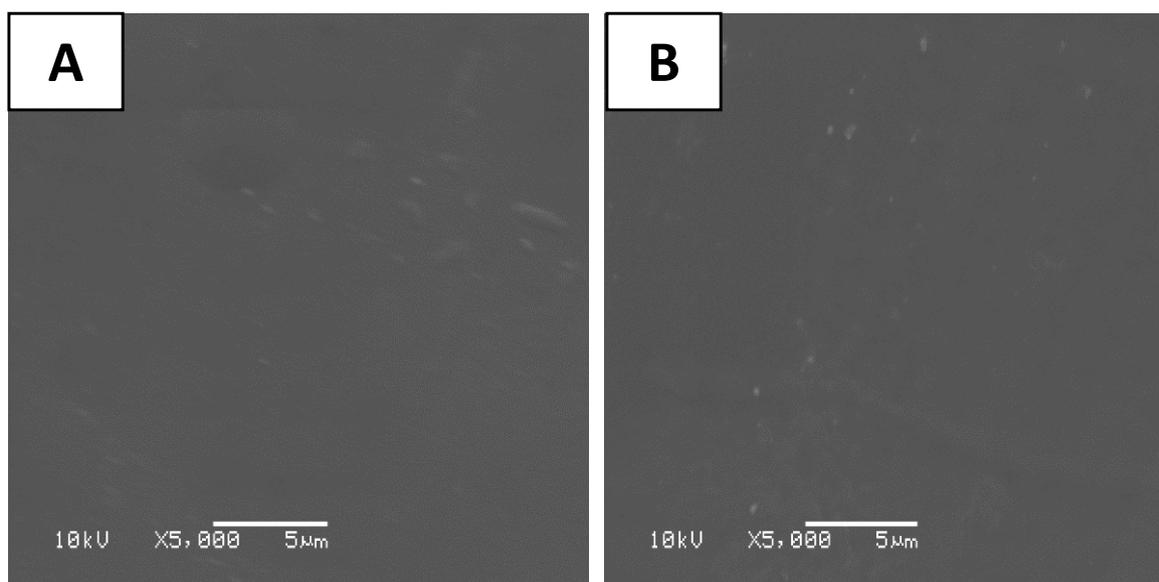


Figure 4: SEM of (a) chitosan membrane and (b) sodium alginate membrane.

3.3. Polymeric separation of ethanol-water mixture

3.3.1. Membrane characterization

The morphology of chitosan and sodium alginate membranes were characterized by SEM. The surface of both CS and SA membranes appear to have a smooth and homogeneous surface and haven't any cracks [19,23]. Ion-exchange capacity indicates 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 [20, 24-27]. IEC results of CS and SA membranes were reported to be 1.54 and 1.04 (meq/g), respectively. This result showed that the membranes have good IEC and suitable for using in the separation application technique. Contact angle was employed to characterize the relative hydrophilicity or hydrophobicity of the membrane surface. CS membrane resulted in contact angle theta (R) 47.80 and theta (L) 46.87; and SA membrane theta (R) 48.65 and theta (L) 47.23 this result obtained in range hydrophilic characteristics hence CS and SA are hydrophilic material [28, 29].

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

The separation of ethanol from water, reported at many researches, included many separation techniques. In the last few years, the pervaporation technique became the favorable technique for the separation technology [30, 31]. The current work depends on the same mechanism of pervaporation by using CS and SA polymeric membranes in separating ethanol-water mixture and purifying bio-ethanol 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% ethanol in water. Different nitrogen pressures from 20 to 60 psi were applied for 12h as showed in table (1, 2). The obtained results showed the highest permeate volume and concentration at 30 psi. As shown in figure (5, 6) both of permeate volume and permeate concentration were dramatically increased with pressure values from 20 to 30 psi in case CS and SA membranes.

As shown in figure (7, 8) the highest flux of permeate and separation factor under nitrogen pressure values 30 psi were reported 49.15,

44.16(mg/m².h) and 80.51, 86.56 for CS and SA membranes respectively. It worth mentioning that the flux of permeate and separation factor were calculated by equations reported by [32].

On the other hand, the second ethanol resource was obtained as a bio-product of yeast fermentation process rice waste. Table (3,4) demonstrates that the highest permeate volume was recorded at nitrogen pressure 30 psi. As shown in figure (9, 10) the permeate volume were increased from 1100 to 2800 and 1150 to 2750 μ l for CS and SA membranes respectively. However, at nitrogen pressures from 50 to 70 psi, both of them were decreased from 453.54 to 105.63 mg/ml and from 600 to 200 μ l, respectively, while the highest permeate concentration was recorded at nitrogen pressure 50 psi 53.23 and 55.10 mg/ml for CS and SA membranes respectively. While the flux of permeate and separation factor for CS and SA membranes as showed in figure (11, 12) observed the difference at nitrogen pressures, for CS membrane the highest flux of permeate 40.35 (mg/m².h) at nitrogen pressure 50 psi while the highest separation factor 65.21 at nitrogen pressure 30 psi. Also SA membrane the highest flux of permeate 44.31 (mg/m².h) at nitrogen pressure 50 psi while the highest separation factor 65.10 at nitrogen pressure 30 psi. 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 [33].

Table 1: Separation process of 25% ethanol from water feed under different nitrogen pressures using CS membrane.

Time (h)	Pressure (psi)	Permeate volume (μ l)	Permeate concentration of ethanol (mg/ml)	Flux of permeate (mg/m ² .h)	Separation factor
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

Table 2: Separation process of 25% ethanol from water feed under different nitrogen pressures using SA membrane.

Time (h)	Pressure (psi)	Permeate volume (μ l)	Permeate concentration of ethanol (mg/ml)	Flux of permeate (mg/m ² .h)	Separation factor
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

Table 3: Separation process of broth 30% feed under different nitrogen pressures using CS membrane.

Time (h)	Pressure (psi)	Permeate volume (µl)	Permeate concentration of ethanol (mg/ml)	Flux of permeate (mg/m ² .h)	Separation factor
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

Table 4: Separation process of broth 30% feed under different nitrogen pressures using SA membrane.

Time (h)	Pressure (psi)	Permeate volume (µl)	Permeate concentration of ethanol (mg/ml)	Flux of permeate (mg/m ² .h)	Separation factor
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

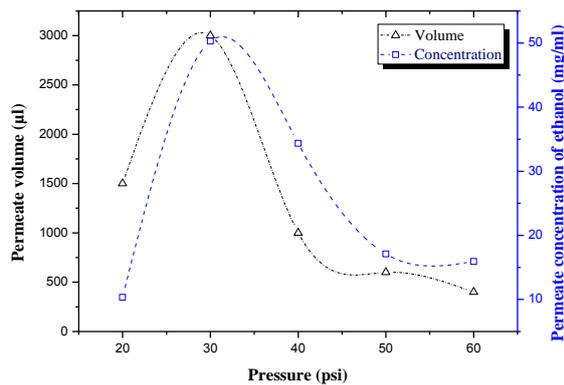


Figure 5: Separation process at 25% ethanol to water under different nitrogen pressures using CS membrane.

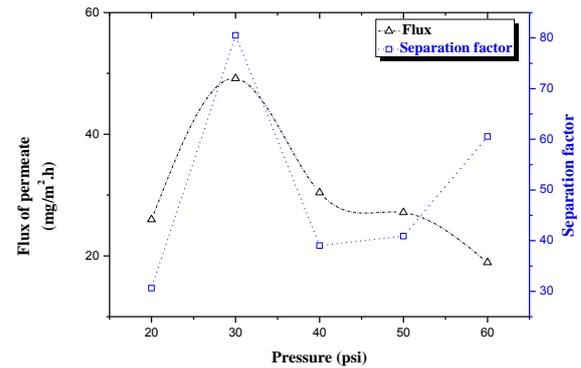


Figure 7: Flux of permeate and separation factor of Separation process at 25% ethanol from water under different nitrogen pressures using CS membrane.

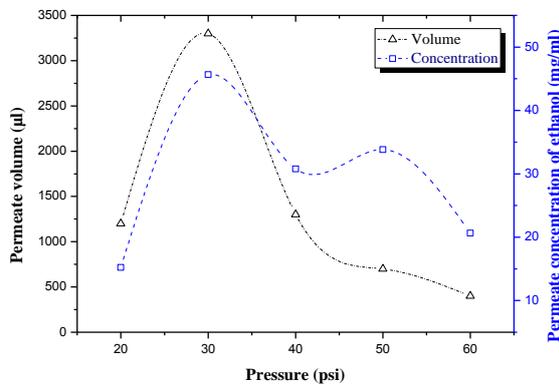


Figure 6: Separation process at 25% ethanol to water under different nitrogen pressures using SA membrane.

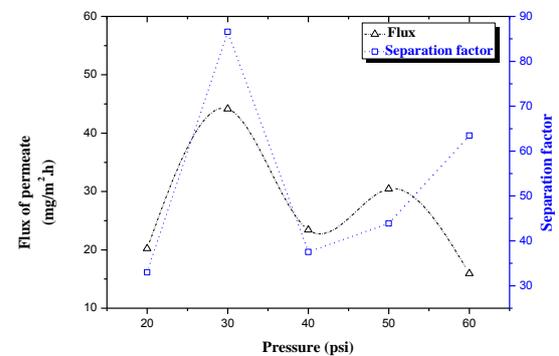


Figure 8: Flux of permeate and separation factor of Separation process at 25% ethanol from water under different nitrogen pressures using SA membrane.

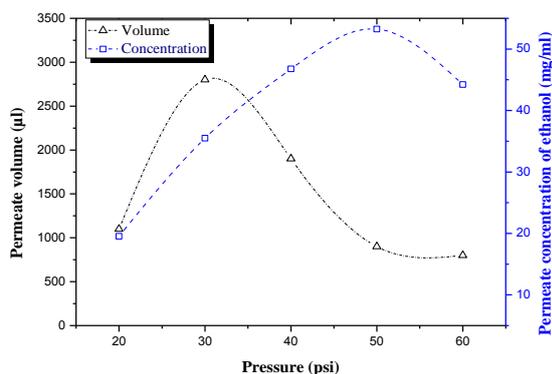


Figure 9: Separation process at 30% ethanol to cultivation broth under different nitrogen pressures using CS membrane.

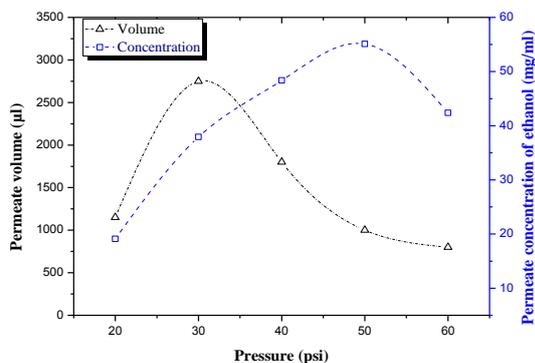


Figure 10: Separation process at 30% ethanol to cultivation broth under different nitrogen pressures using SA membrane.

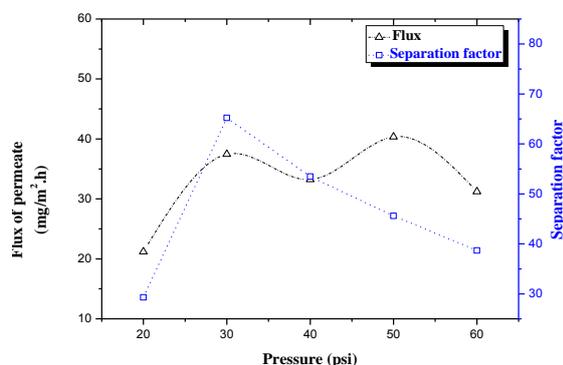


Figure 11: Flux of permeate and the separation factor of the separation process at 30% ethanol to cultivation broth under different nitrogen pressures using CS membrane.

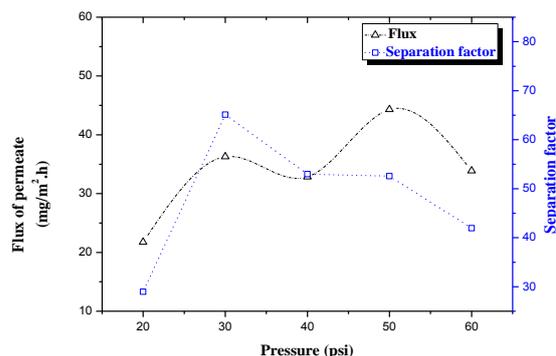


Figure 12: Flux of permeate and the separation factor of the separation process at 30% ethanol to cultivation broth under different nitrogen pressures using SA membrane.

4- CONCLUSION

The present work deals with the bioconversion of environmental waste (starchy) into bio-ethanol. The higher glucose units' was liberated using alpha 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/water 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. The hydrophilicity of the prepared membrane was also investigated using contact angle. The obtained results confirmed that membranes can be used for the separation technology of ethanol-water mixture according to the pervaporation system or any other systems including our system.

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الملخص العربي

إن تناقص موارد الطاقة المتوقع حدوثه، دفع العديد من الدول إلى البحث عن مصادر بديلة للوقود وإيجاد الحلول لتجاوز مشكلة عدم الوفرة المتوقعة في الوقود الحفري؛ لذلك قد يكون الوقود الحيوي هو البديل الأمثل، إذ يعرف بأنه هو الطاقة المستدامة من الكائنات الحية سواءً الحيوانية منها أو النباتية.

وهو يعتبر من أهم مصادر الطاقة المتجددة على خلاف غيرها من الموارد الطبيعية، مثل النفط وكافة أنواع الوقود الأحفوري والوقود النووي، لذلك سعت الكثير من الدول لزراعة أنواع معينة من النباتات خصيصاً لاستخدامها في مجال الوقود الحيوي، ومنها الذرة وفول الصويا في الولايات المتحدة الأمريكية، وقصب السكر في البرازيل، وزيت النخيل في شرق آسيا..

كما تم الحصول على الوقود الحيوي من التحليل الصناعي للمزروعات والفضلات، وبقايا الحيوانات التي يمكن إعادة استخدامها. مثل القش والخشب والسماد إضافة إلى تحلل النفايات ومخلفات الأغذية والأطعمة، التي يمكن تحويلها إلى الغاز الحيوي.

وتستخدم الدول الوقود الحيوي لتقليص اعتمادها على المصدر الأساسي للوقود ألا وهو النفط، ففي الولايات المتحدة الأمريكية مثلاً تسعى إلى تقليص اعتمادها على النفط بنسبة ٢٠% في العام ٢٠١٧ وتعويضه باستخدام الوقود الحيوي.