Concentration of Exopolysaccharides (EPS) Produced from Fermentation of Hydrolysate Biomass of Tuni Sagoo (Metroxylon sp.) Starch by Lactobacillus plantarum FU-0811 in Semi Pilot Scale using Nanofiltration

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Abstract. Nanofiltration (NF) system is one of the concentration process step to recover exopolysaccharides (EPS) concentrate (retentate) as food additive (thickener, stabilizer and emulsion). Feed was biomass containing EPS as a result of growth of Lactobacillus plantarum FU-0811 culture under 37 °C for 72 hours in semi pilot scale (20 L). Biomass was a growth media containing sagoo sugar as a result of step enzymatic hydrolysis on tuni sagoo (Metroxylon sp.) starch using commercial α–Amylase and Amyloglucosidase (AMG) enzymes and other active compounds. Concentration process was conducted by pump motor frequency of 20 Hz, room temperature, and pressure of 10, 15 and 20 bar for 0, 30, 60, 90 and 120 minutes. Result of experimental work showed that NF system has potential use to concentrate biomass containing higher composition than that permeate. Based on optimum EPS, NF system that results a successfully separation of EPS with rejection of 95.24 % was reached at pressure of 20 bar and 60 minutes to retain EPS in concentrate (retentate) of 2.454 % (dry weight) and freely pass in permeate of 0.0097 % (dry weight) with permeate flux value 37.5 L/m².hour. In this optimum process condition, NF system was able to increase of EPS in retentate of 56.46 % from prior to process (feed) and after process concentration.

1. Introduction
Fermentation process of Lactobacillus plantarum FU-0811 using sagoo (Metroxylon sp.) sugar media as glucose substitution and other active agents in semi pilot (20 L) scale is an initiation effort to get exopolysaccharides (EPS) in production scale. EPS is produced as metabolite of Lactobacillus plantarum FU-0811 in fermentation process at 37 °C for 72 hours [1] and as carrier agent of active compound of oral insulin in pharmaceutical industry [2] and as food additive (thickener, stabilizer and emulsion). EPS is a compound produced by microbes through metabolism process excreted to outer cell [3]. Selection on hydrolysate of tuni sagoo starch obtained through subsequent enzymatic hydrolysis using α-Amylase and Amyloglucosidase (AMG) enzymes caused by hydrolysate recovery containing more specific glucose so that it produces more optimum EPS when compared by hydrolysis using acid (HCl) [4]. Sagoo sugar is use as diversification of carbon source materials, beside glucose, which is utilized to growth of Lactobacillus plantarum FU-0811 or other components, such as tryptone, pepton, yeast extract, etc.).
In semi pilot scale, fermentation is efforted to recover their metabolite and composition with indifferent composition on laboratory scale under optimum condition of Lactobacillus plantarum FU-0811 growth. Nevertheless, larger process scale produces generally different composition with laboratory scale (200 – 300 mL). Factors affecting on product recovery in larger scale are stability of environmental condition (temperature, humidity), growth of Lactobacillus plantarum FU-0811 relating with metabolism and nutritive value, but main factor is ability of microbe adaptation on media in larger volume.

To recover higher EPS concentration from fermentation process in 20 L scale, it is efforted to concentrate biomass through NF membrane in order to separate EPS to retentate with higher purity. NF ability in this process is caused by utilizing NF membrane having pore size range of 1 – 100 nanometer (nm) supported by operation pressure of 7 – 30 bars, so that it is able to pass particles of biomass. NF membrane is able to separate compounds with molecular weight (MW) of 150 Dalton (Da.), such as monovalent anion and di- & multivalent anions, organic compounds and retain particle of sugar solute and multivalent salt, but passes freely a mount of monovalent salt, mineral and water [5]. Membrane separation process has an ability to transport selected components from solution other better components so that component is separated from initial solution. Membrane ability to separate certain components is caused by its presence of driving force, such as pressure, concentration and temperature, and electric potential. In NF-based membrane was used pressure as driving force [6,7].

The goal of this experimental activity was to find out process optimization of EPS concentration as a result of fermentation of hydrolysate of tuni sago starch by Lactobacillus plantarum FU-0811 in 20 L scale through NF membrane on composition of concentration result under fixed condition (pump motor frequency of 20 Hz and room temperature) and different time and operation pressure..

2. Materials and methods

2.1. Materials and Equipments

Materials used in these activities were tuni sago starch (Seram, Maluku), Lactobacillus sp. FU-0811 culture (Pusat Penelitian Biologi – LIPI, Cibinong), α-Amylase and Amyloglucosidase (AMG) (NOVO), chemicals for analysis and preparation of induction medium, and 2 sheets of NF Thin Layer Composite membranes (NF-PE-99, Danish Separation Systems, Denmark) and 2 sheets of MF 0.15 μm [Anonymous, 2000]. Equipments utilized in this work were High Frequency Separation, membrane modul of LabUnit M20 (DSS, Denmark) Fermentor in 20 L scale, equipments used in microbiology process, Instrument of Spectrofotometer UV-1201 and glass ware for chemistry analysis..

2.2. Research Design

This experiment was performed with variation : (1) concentration process time of 0, 30, 60, 90 and 120 minutes and (2) operation pressure of 10, 15 and 20 bar through NF membrane under pump motor frequency of 20 Hz (flow rate 7.5 L/minute) and room temperature. Analysis was conducted on biomass composition, such as total solids (Gravimetric method), EPS (Phenol-Sulphate method), reducing sugar (Somogyi-Nelson method) and Total Acids (Titration method) [8] and NF membrane performance, such as permeate flux value and rejection.

2.2.1. Enzymatic hydrolysis on tuni sago (Metroxylon sp.) starch. A mount of tuni sago starch was subsequent diluted in aquadest, adjusted to pH of 6, heated at 90 – 95 °C, agitated and added α-Amylese (Thermamyl 60 L) enzyme of 0.08 % (v/w, dry starch) for 60 minutes to produce suspension. Suspension produced (dextrin) was then autoclaved at 121 °C for 30 minutes and inactivated at 70 °C and pH 4 for 15 minutes. Suspension was subsequent allowed cool, adjusted pH to 4.5, added with Amyloglucosidase (AMG) enzyme of 0.08 % (v/v), and re-heated at 60 °C for 48 hours to get hydrolysate of tuni sago starch. Hydrolysate was filtered by 200 mesh sieve and introduced to MF system at pump motor frequency of 20 Hz, room temperature, and pressure of 10 bar for 120 minutes to result retentate and permeate. Permeate (filtrate or extract) as sago sugar is raw material of biomass to get EPS through fermentation process by Lactobacillus plantarum FU-0811 culture.

2.2.2. Inoculum preparation and fermentation of Lactobacillus plantarum FU-0811. Preparation of Lactobacillus plantarum FU-0811 inoculum was conducted by inoculating Lactobacillus plantarum FU-0811 isolate in glycerol stock using MRS medium. Grown culture was then re-inoculated in MRS A via spread plate method and incubated aerobic at 37 °C for 48 hours. After single colony is grown, this
culture is then inoculated in induction medium of MRS Broth by modifying formation components consisting of sagoo hydrolysate, peptone, beef extract, Sodium Acetate, yeast extract, Ammonium Citrate, Di-Sodium Hydrogen Phosphate, Magnesium Sulphate, Manganese Sulphate, Tween-80, L-cysteine-HCl and Silicon anti foaming [2]. From this process is reached Lactobacillus plantarum FU-0811 inoculum. Fermentation process is performed by inoculating 2 % (v/v) of inoculum in induction medium (20 L) in fermentor aseptically at 37 ºC for 72 hours with control of biomass of 12 hours.

2.2. Concentration process of biomass through Nanofiltration (NF) membrane. Feed fluid of biomass of sagoo hydrolysate by Lactobacillus sp. FU-0811 culture passing via 200 mesh sieve placed in feed tank of 9 L of capacity were subsequently pumped through 200 μm of sieve, heat exchanger, membrane modul and retentate outlet. Retentate was re-circulated to feed tank. During circulation process, cooling water at 23 – 24 ºC was flown to heat exchanger to stable fluid temperature in tank at room temperature (± 25 ºC). After stable process condition, pump motor frequency was arranged to 20 Hz (flow rate of ~ 7.5 L/minute) and operation pressure was fixed at 10 bar. Fluid passing freely per membrane area unit (permeate) via permeate housing was collected, measured its volume and recorded its time to determine permeate flux value. Investigation and sampling were conducted on permeate and retentate for 0, 30, 45, 60, 90 and 120 minutes. Similar process procedure was employed under pump motor frequency of 20 Hz (flow rate of 7.5 L/minute), room temperature and operation pressures of 15 and 20 Bar.

3. Result and Discussion

3.1. Characteristic of sago starch hydrolysate, biomass and biomass extract as feed

Hydrolysate of tuni sagoo starch was produced through hydrolysis process by α-Amylase and Amyloglucosidase enzymes, which is brown turbid suspension with less thick and sweet taste, as demonstrated in Figures 1.

![Figure 1](image1.png)

Figure 1. Sagoo starch hydrolysate (a), biomass of fermentation result of Lactobacillus plantarum FU-0811 in 20 L scale (b) and biomass as a result of 200 mesh sieve as feed (c).

Feed composition in concentration process through NF membrane indicated that contents of reducing sugar of 1.4871 mg/mL, total solids of 5.1389 %, total acids of 0.033 %, and EPS of 1.0685 % (dry weight). This composition is different with prior to filtration of 200 mesh sieve equipped by High Frequency Separator or after fermentation process (37 ºC for 72 hours). It had been shown, the composition decreases to 10 – 15 % from initial biomass, but this step is carried out to anticipate its occurrence of plugging in NF membrane system and get more optimal process efficiency [Anonymous, 2000]. The process is initial separation to sieve anorganic components contained in biomass of fresh hydrolysate, such as dextrin residues (non-hydrolyzed sagoo component by amyloglucosidase enzyme).

3.2. Effect of concentration process condition on NF membrane performance and composition

**Permeate flux value.** Permeate flux value (J) is a mount of permeate produced per membrane area unit (A) per time unit (t) and expressed as J = V / (A x t). Permeate flux value is one of the most important parameter of membrane performances and becomes a reference of membrane process acting good [9]. Biomass concentration process of Lactobacillus plantarum FU-0811 culture produced from fermentasi in semi pilot (20 L) scale through NF membrane under pump motor frequency of 20 Hz (flow rate of 7.5 L/minute), room temperature and operation pressure of 10, 15 and 20 bar for long time process generated low permeate flux value, as shown in Figure 2.
Figure 2. Relationship of time and permeate flux value in concentration biomass as a result of fermentation of sagoo sugar by \textit{Lactobacillus plantarum} FU-0811 culture.

Concentration process at operation pressure of 20 Bar gave higher value of permeate flux than that of 10 and 15 bar. Decrease of permeate flux value is caused by tangential flow of feed fluid on the top membrane surface, in which tangential flow do not give a chance on solute particles to accumulate on the top membrane surface [6]. It had been shown that pump motor frequency affects generally on flow rate of fluid, while operation pressure affects on permeate flow rate penetrating through membrane, so that high flow rate of feed fluid and permeate flux value becomes more and more high [7]. It had been showed that sharply decrease of permeate flux value is occurred under operation pressures of 15 bar and 20 bar between 0 – 30 minutes, followed by gradually decline between 30 – 60 minutes, and strongly drop to end concentration process. It is predicted that process time of 0 – 30 minutes is not occurred ‘fouling’ on membrane yet.

Fouling is a phenomenon of its occurrence of particle ‘cake’ layer on the top membrane surface, which prevent solutes to pass freely through membrane so that hinders and declines permeate flow rate and permeate flux value [6,7]. After 30 to 120 minutes (end process), permeate flux value drops more gradual because it starts to be happened fouling phenomena causing its prevent of permeate to pass through membrane, but this gradual decrease of permeate flux value is running from 30 to 60 minutes only, and sharp decline to final concentration process because part of components pass already through membrane.

**Exopolysaccharides (EPS) (mg/mL)**. EPS is a polysaccharide produced and secreted out from microbe cells, and get from biomass of microorganism, such as bacteria, micro algae, and fungi [10]. It had been known that \textit{Lactobacillus plantarum} FU-0811 is able to produce EPS through its growth in biomass of sagoo gel get through gelatinization process at 37 °C [2]. In fermentation of sagoo sugar by \textit{Lactobacillus plantarum}-FU 0811 in 20 L scale is generated through enzymatic hydrolysis using \textit{\alpha}-Amylase and re-hydrolyzed using \textit{Amyloglucosidase} (AMG) to glucose alternative. Fermentation process in higher scale produces lower EPS recovery (1.0685 %, dry weight) when compared with fermentation process in 300 – 500 mL of laboratory scale (5.5922 %, dry weight) [4]. Environmental factors affect possibility on EPS recovery, such as process conditions (temperature, time, internal factors of \textit{Lactobacillus plantarum} FU- 0811, such as the growth level of \textit{Lactobacillus plantarum}-FU 0811).

Concentration process by means of NF system showed that NF system demonstrates an EPS increase in retentate (concentrate) relating with long time of concentration under operation pressure of 10, 15 and 20 bar, while in permeate for similar condition tends to be constant. Nevertheless, NF system at pressure of 20 bar increases EPS concentration relating with long time of concentration, as shown in Figure 3.
Figure 3. Relationship between time and EPS contents in retentate and permeate in concentration biomass as a result of fermentation of sago sugar by *Lactobacillus plantarum* FU-0811 culture.

Difference in products are possibility caused by difference in driving force interacting with time. To result optimal EPS concentration, in higher operation pressure will need faster process time. High operation pressure will become more and more high driving force so that it enables to compress and bind EPS particles by adhesi force, such as material components and its deficit of water mass during concentration process.

On membrane performance of NF-PE-99, concentration process of EPS at different pressure for 0 - 120 minutes generated different rejection coefficient of EPS. It had been seen that high operation pressure would become more and more high rejection coefficient of EPS. In other words, selectivity level in NF system at operation pressure of 20 bar was better condition both 15 bar and 10 bar, as shown in Figure 4.

Figure 4. Relationship between time and EPS rejection in concentration biomass as a result of fermentation of sago sugar by *Lactobacillus plantarum* FU-0811 culture.

Rejection of the solute, expressed as rejection of the membrane. Based on membrane performance relating with optimalization of concentration process, the best process condition was reached at operation pressure 20 bar for 30 minutes with the observed EPS rejection of 96 % (EPS concentration of 1.23 %, dry weight). There is a difference in process optimalization based on the highest concentration of EPS. This case is possibility caused by heteropolymer property of EPS, various MW and its present of other components with similar MW, such as organic acids, causing a shearing selectivity or optimum performance of membrane, besides sensitivty of NF membrane.

**Total Solids (%)**. Total solids are all components in biomass consisted of soluble and insoluble components, such as fermentation products, especially lactic acid, mineral, carbohydrate, organic compounds as a hydrolysis product and microbes. Total solids becoming parameter in concentration
process using NF system affects on permeate flow rate. Concentration of EPS produced as shown in Figure 5.

**Figure 5.** Relationship between time and total solids concentration in retentate and permeate in concentration of EPS produced from fermentation of hydrolysate biomass of Tuni sago (Metroxylon sp.) starch by *Lactobacillus plantarum* FU-0811.

Concentration processes, optimum concentration of total solids in retentate at operation pressure of 20 bar for 120 minutes was reached in 8.11 % and 0.79 % in permeate. There is an increase from prior to process as feed 5.1389 % to 8.11 % (36.63 %). This matter indicated that high operation pressure caused its occurrence of high driving force, so that it compacted particles on the top membrane surface, whereas water mass would pass through membrane pores. Using NF-99-PE membrane enabled to raise concentration of solutes due to its specification (material kind, selectivity), in spite of membrane has mainly maximum separation tolerance (30 % of total material) [7].

This optimization level was supported by performance of NF membrane yielding observed rejection value of total solids in range of 78.26 - 85.48 % at operation pressure of 10 bar, 88.15 - 90.5 % at operation pressure of 15 bar, and 90.19 - 99.91 % at operation pressure of 20 bar. In other words, selectivity in NF system at operation pressure of 20 bar was better than 15 bar and 10 bar (20 > 15 > 10 bar), as shown in Figure 6.

**Figure 6.** Relationship between time and total solids rejection in concentration of EPS produced from fermentation of hydrolysate biomass of Tuni sago (Metroxylon sp.) starch by *Lactobacillus plantarum* FU-0811.

It had been shown that this selectivity was tend to lower total solids relating with long concentration time, in which under operation pressure of 20 bar gave the best performance of NF membrane or almost completely observed rejection value (> 90 %) when compared with operation pressures of 15 and 10 bar. Long concentration time in NF system would pass freely solutes in permeate side, so that total
solids in permeate side and retentate side ratio becomes more and more small. This matter would decrease selectivity in NF system, especially for concentration treatment at operation pressures of 10 and 15 bar. This case was possibility caused by internal factors (type of material or feed, membrane material) and external factors (pressure, flow rate, temperature and time).

**Reducing Sugar (mg/mL).** Reducing sugar in biomass of sago sugar by using *Lactobacillus plantarum* FU-0811 culture is all sugars, which has an ability to reduce sugar to carbon source on the growth of *Lactobacillus plantarum* FU-0811 like Lactic Acid Bacteria (LAB). NF system is able to concentrate reducing sugar in retentate under optimum operation pressure and time, in which separation is shown almost completely, so that reducing sugar concentration in retentate is higher compared with in permeate, as shown in Figure 7. High operation pressure and long process time in NF system are able to retain reducing sugar on the top membrane surface, while passed reducing sugar in permeate tends to increase, as well. This case indicated that high driving force of sugar particles will be accumulated on the top membrane surface to form ‘cake’ layer, so that it indicates its occurrence of ‘fouling’.

![Figure 7](image-url)

**Figure 7.** Relationship between time and reducing sugar concentration in retentate and permeate in concentration of EPS produced from fermentation of hydrolysate biomass of Tuni sago (Metroxylon sp.) starch by *Lactobacillus plantarum* FU-0811.

This optimum condition was supported by performance of NF membrane generating observer rejection value of reducing sugar in range of 88.23 - 96.57 % at operation pressure of 10 bar, 90.74 - 94.91 % at operation pressure of 15 bar and 89.45 - 95.89 % at operation pressure of 20 bar, respectively. In other words, selectivity in NF system at operation pressure of 20 bar was better than 15 bar, and operation pressure of 15 bar was better than dan 10 bar (20 > 15 > 10 bar), as shown in Figure 8.

![Figure 8](image-url)

**Figure 8.** Relationship between time and observed rejection value of reducing sugar in concentration of EPS produced from fermentation of hydrolysate biomass of Tuni sago (Metroxylon sp.) starch by *Lactobacillus plantarum* FU-0811.
Observer rejection of reducing sugar fluctuates at operation pressure of 10 bar, in which observer rejection of reducing sugar drops for 30 minutes of process, increases and is optimum for 90 minutes followed by a decrease of observer rejection to end process (120 minutes). While, operation pressures of 15 and 20 bar increase to end process. This condition is possibility caused at initial process (0 - 30 minutes), NF system do not indicate fouling, so that ratio of reducing sugar concentrations in retentate and permeate is not showing in difference yet. Long time of reducing sugar concentration process will become more and more much that is retained and pass. Whereas, thick suspension become more and more deficite due to lack of water. High operation pressure and short time tends to result observer rejection of reducing sugar, so that the whole NF system will become more and more selective to separate sugar particles.

**Total Acids (%)**. Total acids are organic compounds with low MW, such as lactic acid, malic acid as Lactobacillus plantarum FU-0811 metabolite. This fermentation process uses carbohydrate (glucose, maltose, fructose) from sagoo sugar media as a source of carbon and is expressed as titratable total acids. The high concentration of total acids is a parameter, in which its occurrence of homofermentative metabolism process[12]. Long process time at operating pressures of 10, 15 and 20 bar increase lactic acid concentration as titratable total acids in retentate and permeate, as indicated in Figure 9.

![Graph showing the relationship between time and total acids concentration in retentate and permeate in concentration of EPS produced from fermentation of hydrolysate biomass of Tunisago (Metroxylon sp.) starch by Lactobacillus plantarum FU-0811.](image)

**Figure 9.** Relationship between time and total acids concentration in retentate and permeate in concentration of EPS produced from fermentation of hydrolysate biomass of Tunisago (Metroxylon sp.) starch by Lactobacillus plantarum FU-0811.

This optimum condition is not supported by performance of NF system showed by less completely observer rejection or selectivity of NF membrane (smaller than 90 %). (Figure 10). At operation pressures of 10, 15 and 20 bar for 0 - 60 minutes, NF system gave a decline of observer rejection of total acids. In other words, membrane system tends to reject or retain solute of total acids in order to pass membrane, so that ratio of total acids concentration in retentate and total acids concentration in permeate is similar or imperfect observer rejection of total acids.
Figure 10. Relationship between time and observed rejection value of total acids in concentration of EPS produced from fermentation of hydrolysate biomass of Tuni sago (Metroxylon sp.) starch by Lactobacillus plantarum FU-0811.

From the whole concentration process of biomass as a result was reached the best process condition at operation pressure of 20 bar for 60 minutes and gave retentate as thick brown suspension, while permeate was clear liquid like water, as showed in Figure 11. Based on optimization of EPS concentration, the best process condition from the whole concentration process of biomass as a result of fermentation of sago sugar by Lactobacillus plantarum FU-0811 culture in semi pilot scale (20 L) are reached at operation pressure of 20 bar for 60 minutes by resulting retentate as thick brown suspension, whereas permeate is clear liquid like water, as shown in Figure 13a and 13b, respectively.

Figure 11. Retentate (a), permeate (b) as a result of concentration of EPS produced from fermentation of hydrolysate biomass of Tuni sago (Metroxylon sp.) starch by Lactobacillus plantarum FU-0811.

In this condition is reached increasing EPS in retentate of 56.46 % from prior to process (feed) of 1.0685 % to 2.454 % for 60 minutes of concentration process with observer rejection of 95.24 %. Based on investigation on selectivity optimization of NF membrane performance, the best process condition was reached at operation pressure of 20 bar for 30 minutes with observer rejection value of optimum EPS (96 %) by increasing EPS of 13.17 % from prior to process (feed) (1.0685 %) to 1.23 % (dry weight) for 0 - 30 minutes. Based on membrane performance, selection of optimum condition is an important effort to get selected process based on process safety and life time on NF system.

4. Conclusion

Concentration process of biomass as a result of fermentation of sago sugar by Lactobacillus plantarum FU-0811 culture in semi pilot scale (20 L) through NF system gave optimization in process condition on each component based on component property, interaction between operation pressure and time, selectivity aspect or rejection ability and membrane material. Long concentration time would decline permeate flux values for the whole processes and raise EPS concentration, total solids, total acids, and reducing sugar. Operation pressure of 20 bar gave better composition and component rejection than
that operation pressure of 10 and 15 bar. Based on the highest EPS recovery, optimum condition was reached at operation pressure of 20 Bar for 60 minutes shown by raising EPS content in concentrate (retentate) of 56.46 % when compared with prior to process (feed) of 1.0685 % to 2.454 % with observer rejection of 95.24 %. Under this condition was get retentate (concentrate) of biomass with compositions of EPS of 2.454 % (dry weight), total solids of 5.89 %, reducing sugar of 3.148 mg/mL, and total acids of 0.0486 %. Based on NF membrane performance and the highest EPS concentration by various components in biomass (feed), it is occurred a difference in process condition optimalization.

5. References

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