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Protein Separation from fermentation Broth (*E. coli*) using polyethersulfone UF and MF membranes

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Abstract

In this paper, the intracellular *E.coli* fermentation broth was developed in fermentor. The broths were separated using 0.2µm microfiltration polyethersulfone membranes. The Cells of *E.coli* was retained in microfiltration membrane, were broken in high pressure homogenization. The cells debris and protein were separated using polyethersulfone 0.2µm PES Microfiltration membranes. In microfiltration, cells of *E.coli* were rejected and proteins collected in permeate sides. After microfiltration, 30KD Ultra filtration membrane was used to separate proteins. About 91.01 % of the proteins were separated by the ultra filtration polyethersulfone membrane.

Keywords: Ultrafiltration; Microfiltration; Polyethersulfone; Fermentation; High pressure homogenization

Introduction

Synthetic polymers such as polysulfone, polyethersulfone, polycarbonate, polyamide, Cellulose acetate etc. are widely used for the preparation of membranes. Polyethersulfone (PES) is a favorable material for membranes as it has properties like resistance to oxidation, acids and alkalis, and excellent biocompatibility. PES has better solubility as compared with polysulfone [1-4]. The wettability of PES can be improved and its biocompatibility enhanced by adding poly (vinyl pyrrolidone) (PVP). This also increased the diffusive transport properties of solute through the membrane. *E. coli* are commonly used hosts for recombinant product expression because of their ability to produce large quantities of protein quickly and economically. During the past twenty-five years, regulatory requirements have directed industry to use modern technology to improve process purity and control. In the same period, the protein industry has become focused on process efficiency, speed, yield and cost. Centrifugation has remained, essentially unchanged since the mid1950s; however recent advances in membrane technologies have facilitated improvements in process purity, control, efficiency, speed, yield and cost.

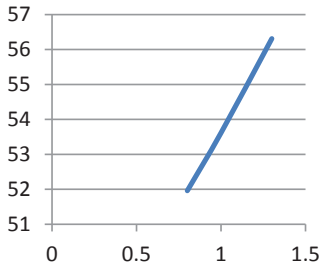

Figure 1: Trans membrane pressure Vs concentrate flux.

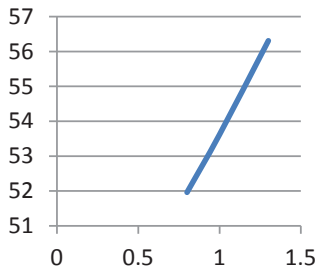

Figure 2: TMP Vs permeate flux.

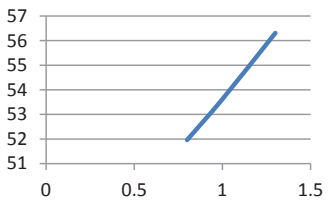

Figure 3: TMP Vs Permeate Recovery.

Cell harvesting, clarification of broth, recycling of cells and separation of cell debris from extracellular products represent important potential applications of membrane technology in the emerging field of biotechnology [l-5]. Membrane separation is a very advanced separation technique as compared to convectional separation method such as rotary filter, sedimentation and centrifugation etc. The importance of the membrane process is due to less energy requirement & workability at ambient temperature & pressure, clean technology with ease operation, produce high quality products, greater flexibility in designing system. The types of membrane operation like UF, MF have wide variety of application in fermentation downstream operation treatment in biopharmaceuticals industries for recovery & separation of valuable components. Typically, a separation specialist takes on the challenge of designing steps to separate the various components

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of a complex fermentation broth that the fermentation-process designers included to maximize fermentation performance. Hence, if the required separation becomes complex and costly, the most efficient fermentation may not necessarily yield the optimum overall process. Typically, 50-70% of the total production cost in classical processes is due to downstream processing, whereas in fermentation that employs recombinant DNA, the fraction can reach up to 80-90%. This large percentage is often due to separation and purification of the fermentation product.

Escherichia coli are widely used for the expression of mammalian and bacterial proteins. Over expression of these recombinant proteins in *E. coli* often results in the formation of insoluble protein inclusion bodies (1-7). The first step in the recovery of the intracellular recombinant protein, whether soluble or insoluble, typically involves cell disruption. On a large scale, cell disruption is usually done with either a high pressure homogenizer or a bead mill [1-12]. Increasing the number of passes through a high pressure homogenizer causes greater protein release.

In this paper, E coli fermentation broth is separated using 0.2µm microfiltration polyethersulfone membranes. Cells of *E. coli* separated from microfiltration passes through high pressure homogenizer. Again 0.2µm microfiltration membranes used to separate cells debris in retained and permeate are feed to 30KD UF membrane to separate proteins.

Materials and Methods

Polyethersulfone membrane of molecular weight cut off 30KD, membrane assembly, fermented broth of *E.coli*, alkaline sodium carbonate solution (20g/lit Na2CO3 in 0.1 mol/lit .Copper sulphate-sodium potassium tartrate solution (5g/lit CuSO4.5H2Oin 10 g/lit Na, K tartrate). Folin- ciocalteau reagent. Standard protein (Albumin solution 0.2 mg/ml)

Experimental work

Fermented broth (E-coli) was used as a feed for (MF/UF) cross flow membrane system. Recovery of protein from the E-coli (fermented broth) involved a number of steps:

1) Microfiltration 2) Homogenization 3) Microfiltration 4) Ultra filtration

Seven liter intracellular *E.coli* fermentation broth was feed to 0.2µm polyethersulfone microfiltration membrane to concentrate cells of *E. coli*. Concentrated cells were disrupted in homogenizer to separate out intracellular protein. Cell debris and protein were again separated using polyethersulfone 0.2µm MF membranes. Cell debris as retained and proteins in permeate sides of the membranes. Finally proteins were separated in 30KD ultra filtration polyethersulfone membranes. The concentration of proteins are determined by Lowry's method.

Microfiltration

Microfiltration: Membrane polyethesulfone 0.2µm (Table 1).

Microfiltration: Feed (cell debris + protein) =2.635L (Table 2).

Ultra filtration: Feed 2.185L (Table 1).

Result and Discussion

Polyethersulfone microfiltration 0.2µm membrane was used for concentrating and separating cells of *E-coli* -fermented broth. Seven liters *E-coli* -fermented broth [7L] were used as a feed for microfiltration membrane, where cells of E-coli were concentrated (635ml) and 6.325 L as permeated. The concentrated (0.635 L) cells were passed in high pressure Homogenizer, where intracellular proteins given out from the cells. After Homogenization, cell debris and proteins were separated in 0.2µm microfiltration membranes.2.185L protein were collected in permeate sides and 0.410L cells debris collected in retained sides of microfiltration membranes. The permeate which were collected in microfiltration were passed to 30KD PES ultra filtration membranes. The total proteins concentration in permeate were estimated by using Folin-Lowry method of protein assay .The proteins concentration of feed to 30KD UF membranes were found 845µg/ml and in permeated sides 75µg/ml.The total proteins rejected was found out to be 91.01 percentages in 30 KD polyethersulfone ultra filtration membranes.

The Figure. 1 shows that, when transmembrane pressure increased concentrate flow rate decreased, whereas in Figure 2, showed that when transmembrane pressure increased permeate flow rate also increased continuously .After some times, it was found that permeate flow rate decreased with increase in transmembranes pressure. The resistance creates due to fouling and plugging problems of the membrane and permeates flux decreased (Figure 3).

SR.No	Feed flux rate(L/m^{2}min)	Permeate flux rate(L/m^{2}min)	Concentrated flux rate(L/minm^2)	Inlet press(Bar)	Outlet press(Bar)	TMP	%Recovery
01	126.25	63.25	62.5	1.2	0.6	0.9	50.49
02	129.47	69.479	60	1.4	0.8	1.2	52.66
03	126.354	71.35	55	1.7	01	1.35	56.47

Table 1:

Sr.no	Feed flow rate(L/m^{2}min)	Permeate flow rate(L/minm^2)	Concentrated flow rate(L/m^{2}min)	Inlet press(Bar)	Outlet press(Bar)	TMP	%Recovery
01	127.08	65.83	61.25	1.2	0.6	0.9	51.80
02	128.64	71.145	57.5	1.4	0.8	1.1	55.30
03	127.5	75	52.5	1.7	1	1.35	58.82

Table 2:

Sr.No	Feed flow rate(L/minm^2)	Permeate flow rate(L/minm^2)	Concentrated flow rate(L/m^{2}min)	Inlet press(Bar)	Outlet press(Bar)	TMP	%Recovery
01	130.104	67.5	62.5	1.2	0.4	0.8	51.96
02	132.08	70.83	61.25	1.4	0.6	01	53.62
03	128.75	72.5	56.25	1.7	1.3	1.3	56.31

Table 3:

Conclusion

E.coli fermentation broth was developed and separated using 0.2µm polyethersulfone microfiltration membranes. The concentrated cells were passed to high pressure homogenizer where intracellular proteins separated out. The cell debris separated using 0.2µm PES microfiltration membranes as retained and proteins collected as permeate side. The proteins were separated in 30KD PES ultra filtration membrane. The total 90.01% proteins separated using polyethersulfones UF membranes. Ultra filtration can be successfully used to separate protein and bacteria cells from *E.coli* fermentation broth. Cells and proteins were retained by the ultra filtration membrane with MWCO of 30,000 Daltons. Increased transmembrane pressures caused higher permeate flux.

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