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Fermentative production of poly (γ-glutamic acid) from renewable carbon source and downstream purification through a continuous membrane-integrated hybrid process

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HIGHLIGHTS

- Poly (γ-glutamic acid) produced in a fully membrane-integrated bioreactor.
- The process ensures high degree of purity, yield and concentration.
- Complete separation and recycle of cells and residual carbon source ensured.
- This continuous system represents high process intensification.

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ABSTRACT

Experimental investigations were carried out on continuous and direct production of poly-(γ-glutamic acid) in a hybrid reactor system that integrated conventional fermentative production step with membrane-based downstream separation and purification. Novelty of the integrated system lies in high degree of purity, conversion, yield and productivity of poly-(γ-glutamic acid) through elimination of substrate–product inhibitions of traditional batch production system. This new system is compact, flexible, eco-friendly and largely fouling-free ensuring steady and continuous production of poly-(γ-glutamic acid) directly from a renewable carbon source at the rate of 0.91 g/L/h. Cross-flow microfiltration membrane modules ensured almost complete separation and recycle of cells without much fouling problem. Well-screened ultrafiltration membrane module helped to concentrate poly-(γ-glutamic acid) while ensuring recovery and recycle of 96% unconverted carbon source resulting in yield of 0.6 g/g along with high product purity.

1. Introduction

Poly amino acid like poly-γ-glutamic acid (PGA) is a naturally occurring homo-polyamide that consists of D- and L-glutamic acid (GA) units linked through amide bonds catalyzed by some simple enzymes (Bajaj and Singhal, 2011). Ivanovics and Bruckner discovered PGA as a capsule of *Bacillus anthracis* which was released into the medium on autoclaving or on aging and autolysis of the cells (Shih et al., 2005). A Japanese traditional food "natto" (fermented soybeans) which contains a mixture of PGA and fructan is produced by *Bacillus natto* Sawamura (Fujii, 1963). Microbially produced PGA can have molecular weights ranging from 100,000 to over 1,000,000 Da depending on factors like fermentation time and hydrolytic enzymes (Goto and Kunioka, 1992). PGA is easily bio-degradable, nontoxic, non-immunogenic and is assimilated *in vivo*. So, PGA and its derivatives can be used in a wide range of products such as food stuff, cosmetics, medicines, thickener, humectant, bitterness relieving agent, cryoprotectant, sustained released material, curable biological adhesive, drug carrier, biopolymer flocculants and heavy metal absorber (Shih and Van, 2001).

Wide range of application potential of PGA has drawn attention of many research groups to its production and recovery from various carbon sources. The PGA-producing bacteria are classified into two major groups namely GA-dependent bacteria and GA-independent bacteria on the basis of nutrient requirements (Cao et al., 2011). In the past, various strategies for the production of PGA by bacterial fermentation have been developed and new sources are still being explored (Berekaa and Al-otaibi, 2013; Wei et al., 2014). In order to enhance the PGA productivity, researchers have

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isolated new and improved strains and optimized their nutrient requirements as the nutrient requirements vary according to the strains (Jeong et al., 2010; Xu et al., 2005). However, most of the studies have been conducted on either batch or fed-batch systems and hardly any downstream purification of PGA from fermentation broth (Yoon et al., 2000) using membrane has been investigated.

For fermentation of PGA, the most widely used carbon sources have been carbohydrates ranging from glucose and starch to glycerol (Du et al., 2005). Carbon from renewable resources like untreated cane molasses and GA from wastewater containing monosodium glutamate are some of the low cost materials that have been explored for the economical production of PGA (Zhang et al., 2012). However, materials like molasses, corn starch and whey are considered cheap carbon sources and have been frequently investigated in several studies. These substrates, however, demand additional treatments to avoid membrane fouling (Nayak and Pal, 2013). On the other hand, a very promising carbon source like sugarcane juice has been tried very little though it could be a very promising source being clean, relatively cheap and renewable (Kumar et al., 2014). In some major sugarcane growing countries like India and Brazil, sugarcane juice is easily available throughout the year (Sikder et al., 2012). Unfortunately, the refinement of PGA from fermentation broths is quite expensive, owning to the low concentration and presence of other molecules, such as unfermented sugar (Torăng et al., 1999). To separate PGA from fermentation broth, properties of product such as solubility, molecular size, affinity to adsorbent and charge characteristics may be exploited (Timmer et al., 1998). To produce pure PGA, efficient separation of other impurities from fermentation broth is essential. A number of downstream steps such as precipitation, filtration/centrifugation, acidification, extraction, neutralization, crystallization and evaporation are involved in conventional purification scheme. PGA is normally precipitated out by using ethanol (82% recovery) or by divalent copper ion (85% recovery) from cell free fermentation broth (Manocha and Margaritis, 2010). The major disadvantage of ethanol precipitation is that along with PGA, 48% proteins present in the fermentation broth also gets precipitated out. Moreover, conventional schemes require frequent centrifugation for separation of cells from the fermentation broth and this adds to the cost of the process. Few authors have tried to immobilize the whole microbial cells in bagasse during the batch/fed-batch production of PGA in fibrous-bed bioreactor (Xu et al., 2014). Ion-exchange process has also been used for the purification of PGA involving substantial amount of acid and base for regeneration of ion exchange resin. In addition to that, conventional batch fermentation also suffers from low volumetric productivity due to both substrate and product inhibitions. Such production scheme is also laborious due to requirement of frequent shutdown and start-up of batch process. The membrane-integrated cells recycle bioreactors with continuous fermentation, separation and recycle of unconverted carbon source and cells can ensure high cell density, much higher productivity, yield and high PGA concentration in a continuous process. Steady state operation with prolonged exponential growth phase along with proper cell bleeding are absolutely essential to high productivity and yield (Bouchoux et al., 2006). Membrane separation is expected to be one of the most promising tools in successfully separating target PGA from large number of other impurities present in the fermentation medium (Li et al., 2003). Sweeping flow of the fermentation broth over the membrane surface in cross-flow membrane module can ensure largely fouling-free operation over long duration. Membranes have been hardly used for the downstream purification of PGA from its fermentation broth. However, several attempts have been made in this direction over last two decades for integration of traditional fermenter with membrane based separation and purification of other organic acid like lactic acid (Giorno et al., 2002; Tong et al., 1998; Xu et al., 2006). In most of the cases, authors have used only a single stage membrane separation integrated with fermentor. In these studies, membrane module often suffered from serious fouling problems after some time of operation. Two-stage membrane separation has been attempted in very few cases. Ultrafiltration (UF) is pressure-driven membrane separation technique for dissolved and suspended species based on size and molecular weight (Delgado Diaz et al., 2012). It has been widely used in various chemical and biochemical processes since it minimized the physical damage of biomolecules from shear effect, minimal denaturation, high recovery yield and avoidance of re-solubilization problems because the solutes can be retained in the solution phase, high throughput and cost effectiveness. Being modular in design, membrane-based processes offer great flexibility in scale of production depending on market demand. Such a process permits simultaneous production and purification resulting in a compact design of low capital cost. Separation and purification in this case can be carried out at significantly reduced energy consumption as it involves no phase change (barring pervaporation). Through this study, a continuous and eco-friendly production process for polyglutamic acid (PGA) is developed that consists of a two stage membrane integrated system. The process is capable of producing pure polyglutamic acid with quite high productivity.

To achieve this target, a judicious combination of microfiltration (MF) membrane in the first stage for cell recycle followed by UF membrane in the second stage in flat sheet cross-flow membrane module with provision of instant separation of PGA from fermentation media and a cheap, clean and renewable carbon source

Table 1
Characteristics of membranes used in the present investigation.

Parameters	PVDF 0.45	PES-5	PAN 10
Membrane type	Flat-sheet	Flat-sheet	Flat-sheet
Material	Poly (vinylidene fluoride)	Polyethersulfone	Polyacrylonitrile
Membrane surface area (m^2)	0.01	0.01	0.01
Membrane thickness (μm)	110–160	165	165
Nature of filtration	Microfiltration	Ultrafiltration	Ultrafiltration
Pore size (μm)	0.45	0.001	0.001
Maximum process temperature (°C)	50	80	80
pH resistance	2–11	2–11	2–11
Molecular weight cut-off (d)	5000–500,000	6000	20,000
Contact angles[a]	49.6 ± 4.9°	73.1 ± 5°	65.7 ± 4.5°
Normalized water flux (L/(m^2 h))/bar[b]	360	57	68
pH resistance range	2–11	2–10	2–10
Temperature (°C)	80	50	50
Maximum operating pressure (bar)	5	10	10

[a] Płatkowska-Siwiec and Bodzek (2012).
[b] Obtained in the present work.