Journal of the Taiwan Institute of Chemical Engineers 000 (2018) 1–9

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

journal homepage: www.elsevier.com/locate/jtice

Fermentative production of gluconic acid: A membrane-integrated Green process

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ARTICLE INFO

Article history:
Received 5 August 2017
Revised 20 January 2018
Accepted 23 January 2018
Available online xxx

Keywords:
Gluconic acid
Membrane-integrated system
Clean production
Continuous fermentation
Green process
Sustainable technology

ABSTRACT

On integrating downstream membrane based separation modules with conventional fermentation unit, gluconic acid representing a class of industrially important organic acids could be produced from fermentation of sugar cane juice—a renewable cheap carbon source in an environmentally benign process. Using *Gluconobacter oxydans as* microbial agents in the bioconversion, the membrane-integrated hybrid fermentation system succeeded in producing 93 g/L gluconic acid of 98% purity with yield of 0.94 g/g and productivity of 6.7 g/L/h. The system performance parameters establish superiority of this novel technology over the existing ones. Against the overwhelmingly practiced batch or semi-batch processes involving multiple energy-intensive unit operations, this continuous production scheme ensures efficient bioconversion without substrate–product inhibitions in a quite simplified plant characterized by high degree of process intensification.

1. Introduction

In moving toward sustainable clean technology regime, chemical and biochemical process industries are gradually adopting bio-based production strategies rather than pure chemical technologies. However, bio-based production alone can't ensure eco-friendliness of a process technology as downstream separation–purification of the products often involves quite a few energy-intensive steps that may require harsh chemicals also. This is where membrane-based purification schemes are stepping in [1-3]. In this context, development of a clean technology for production of gluconic acid assumes significance. Being a multifunctional organic acid, the gluconic acid (GA) has wide applications in food, pharmaceutical and chemical industries. The gluconic acid salts of sodium, calcium, iron, copper, zinc are consumed in chemical, pharmaceuticals, food (acidulant), beverages, and dairy and construction industries. These properties are attributing to its increase in annual market demand at the rate of 9%. There is huge potential for growth of gluconic acid manufacturing industries and the same could be tapped fully provided cost of production is brought down. Current production cost is high (US$ 1.2–8.5/Kg) largely because of use of glucose (being a finished carbon source) as carbon source and involvement of multiple steps in conventional production schemes [4,5]. It has been found that 50–80% of the total cost of production is attributed to separation and purification expenses of the desired product from the fermentation broth when organic acids are synthesized by fermentation [6].

Gluconic acid mainly produced industrially by *Aspergillus niger* and *Gluconobacter suboxydans*. However, *G. suboxydans* is preferred over *A. niger* for continuous fermentation as chemostat cultivation is not possible in later case [7]. *G. oxydans* can't oxidize sugars completely into CO_2 and H_2O but intermediary industrially useful products like D-gluconic acid due to absence of glycolysis and incomplete set of enzymes required for tri-carboxylic acid cycle [8,9].

Conventional downstream process for recovery of a fermentative product like GA involves a series of additional steps that are not only complex, energy sensitive and expensive but also release a large amount of waste water which in turn degrades the environment and demands additional manpower cost [10]. A series of processing steps such as centrifugation, evaporation, product precipitation, liquid–liquid extraction, conventional electrolysis or adsorption (with anion-exchange resin, active charcoal or zeolite) [11] are involved in removal of the remnant microbial cells, water and volatile compounds. Some unit operations are also required to convert organic acid salts to its pure form such as acidification by cation-exchange resins, bipolar electrodialysis or thermal methods. The conventional purification by electrodialysis needs frequent pH changes in the feed solution. The current batch processes suffer from low productivity and high labor cost due to the need for

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https://doi.org/10.1016/j.jtice.2018.01.030
1876-1070/

Table 1
Characteristics of thin-film composite polyamide nanofiltration membranes (165 µm thick) used in our work.

Characteristics	NF membranes				
	NF-1	NF-2	NF-3	NF-20	NFX
Average MWCO (kDa)	0.15–0.3	0.15–0.3	0.15–0.3	0.15–0.3	0.15–0.3
pH range	3–10	3–10	3–10	3–10	3–10
Solute rejection (%) at 10 bar pressure with solute concentration 2 g/L					
MgSO4	(%)	99	97	98	98
NaCl	(%)	90	50	60	35
Maximum operating pressure (bar)	83	83	83	83	83
Maximum operating temperature (°C)	50	50	50	50	50
Surface area used (m^2)	0.01	0.01	0.01	0.01	0.01
Pore radius (nm)	0.53	0.57	0.55	0.54	–

Table 2
Characteristics of microfiltration membranes used in the present study.

Parameters	PVDF 0.45 µm	Nylon 0.22 µm
Type of membrane	Flat-sheet	Flat-sheet
Material	Poly (vinylidene fluoride)	Nylon
Membrane surface area used (m^{-2})	0.01	0.01
Membrane thickness (µm)	110–160	110–150
Nature of filtration	Micro-filtration	Micro-filtration
Average pore size (µm)	0.45	0.22
Maximum process temperature (°C)	80	50
pH resistance	2–11	2–11
Molecular weight cut-off (g/mol)	5000–500,000	5000–100,000
Maximum operating pressure (bar)	5	5

frequent shut-down and start-up of the system. Such limitations can be overcome by integrating membrane system with traditional fermenter through provisions for cell recycle to maintain high cell density and continuous removal of products. Pressure-driven membrane filtration system may be operated in different modes like cross-flow mode or dead-end mode [12,13].

Though quite a few studies [14-18] have been reported on downstream purification of some organic acids/amino acids using nanofiltration (NF) membrane, gluconic acid production schemes remain the same [19]. These studies [20,21], however, indicate that integration of multi-stage micro and nanofiltration steps in appropriate and judicious membrane modules with fermentative production units can bring about substantial process intensification in organic and amino acid manufacture and for gluconic acid production, switching over to such a green technology is a must for ensuring sustainability in operation and business. Thus membrane based system has the advantages of lower energy consumption, sustainable processing, simpler operation and relatively easy scale-up provisions. Being selective in nature, NF membrane is the most prominent candidate for recovery of GA due to their negative charge and low molecular weight where both Donnan and steric effects are likely to be exploited [22]. When well-screened NF membrane of proper selectivity and permeability is integrated with a conventional fermenter, a compact system evolves permitting simultaneous production and purification in the same unit [23]. This study, therefore, develops a membrane-based green technology for gluconic acid production where multi-stage membrane integration is done with basic fermentative production unit.

2. Materials and methods

2.1. Inoculum and culture medium preparation

Gluconobacter oxydans (NCIM 2095), was brought from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (Pune, India). A loopful of *G. oxydans* (NCIM 2095) from fresh agar plate (activation medium) was used to inoculate into 1000 mL Erlenmeyer flask with 250 mL of medium for seed culture, having composition (g/L): yeast extract, 20; sorbitol, 50; $(NH_4)_2SO_4$, 2; $MgSO_4{\cdot}H_2O$, 1; $FeSO_4$, 0.01; KH_2PO_4, 3.5; Sodium citrate$\cdot 2H_2O$, 0.2. The pH was adjusted to 6.5 by 1 M NaOH/0.1 N HCl and autoclaved at 15 psi pressure for 15 min. This flask was incubated on an orbital shaker (Digitech System, Kolkata, India) at 250 rpm and 30 °C for 15 h. All chemical were of reagent and supplied by Merck India Limited, Loba Chemie and Sigma-Aldrich. The production medium was having same composition with aqueous medium of pretreated sugarcane juice (SCJ) containing glucose as carbon source.

The raw sugar cane juice obtained from local farmer contained 10.4% (w/v) of total sugar which include fructose, 10.1 g/L; glucose, 25.3 g/L and sucrose, 68.6 g/L. Sucrose was further degraded into glucose and fructose by thermal hydrolysis at pH 3.0 (maintained by addition of measured small dose of concentrated nitric) at 80 °C in oven for 40 min [24]. The pH of hydrolyzed sugar cane juice was adjusted to 6.5 by 2 M NaOH and micro-filtered 0.45 µm PVDF MF membrane to remove the suspended particles and sludge formed before using in the production medium as carbon source containing 61.4 g/L final glucose concentration.

2.2. Membranes

Five thin film composite polyamide membranes, namely, NF1, NF2, NF3, NF20 (Sepro Inc. USA) and NFX (Synder, USA) were procured and tested for product separation and concentration. The microfiltration of fermentation broth was done to recycle the biomass by two different MF membranes, 0.45 µm polyvinylidene fluoride (PVDF, Membrane Solutions, USA) and Nylon 0.22 µm (Membrane Solutions, USA) hydrophilic. The characteristics of these membranes are presented in Tables 1 and 2.

2.3. Experimental equipment

Lab scale 30 L capacity mechanically stirred (250 rpm) fermenter made-up of stainless steel (SS-316) with sterile air/O_2 gas purging system and pH, temperature, DO probes was used (Fig. 1). A peristaltic pump (Enertech, India) and high pressure diaphragm pump (Hydra-cell pump, 2.2 kW, Minneapolis, USA) were used to circulate the feed to the MF and NF membrane modules via fermenter. The whole system was disinfected by circulating 2% potassium meta-bisulfite solution for 1 h followed by proper rinsing with sterilized deionized water till restoration of neutral pH level. The whole experimental system was sterilized periodically by purging with steam.

2.4. Fermentation and experimental procedure

Total 20 L sterile fermentation media was added including 10% (v/v) inoculum (seed culture) to the fermenter. At the start of the

Please cite this article as: S. Banerjee et al., Fermentative production of gluconic acid: A membrane-integrated Green process, Journal of the Taiwan Institute of Chemical Engineers (2018), https://doi.org/10.1016/j.jtice.2018.01.030