Developing a Sustainable Technology for Clean Production of Lactic Acid

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Abstract—Process intensification in production of high purity and monomer grade lactic acid has been achieved through development of a new membrane-integrated technology. Advantages like, involvement of less processing steps, less energy consumption, less chemical requirement make the system environment-friendly. The particular modular design offers great flexibility in operation of the system which the modern manufacturing sector is seeking desperately in this era of emaciated profit margin. With the optimized involvement of microfiltration and nanofiltration membrane modules in a continuous production system, a reasonably high flux of 76-77 L/m² h⁻¹ was achieved for a greater than 95% pure L (+) lactic acid.

Keywords—Lactic acid, Membrane Technology, Sustainable Development, Fermentation.

I. INTRODUCTION

Interest in the production of monomer grade L (+) lactic acid has dramatically gone up in the recent past following a growing demand for biodegradable polymer (PLA i.e. Poly Lactic Acid), a highly suitable substitute for conventional plastic material. Some major advantages like good heat deflection, ready biodegradability in the environment and sustainability makes PLA even a much better substitute for the petrochemical plastics [1] – [2]. Considering its environment-friendly, thermal, mechanical and chemical nature, PLA can be applied in a wide variety of fields like tissue engineering, controlled drug delivery or in artificial prostheses [3]. Traditional chemical synthesis process for lactic acid production from petroleum resources yields a racemic mixture of D and L-lactic acids instead of pure D or L lactic acid. Conventional fermentation based processes can be suitably modified and operated with selected microbial strain so as to produce only the desired isomer. But existing fermentation-based processes are still in many cases, only batch processes with poor productivity and necessitating quite a number of downstream processing steps which involve not only high energy, equipment, time and labour costs but also harsh chemicals leading to environmental pollution. Thus process intensification in fermentation based lactic acid production is a demand of the industry drawing attention of the researchers across the world. Process Intensification refers to the development of smaller, cleaner, energy efficient and highly flexible technologies to achieve the same and even more production objectives in a compact plant in comparison with traditionally robust process plants. Conventional production processes produce salts of lactic acid instead of direct lactic acid as pH adjustment is a must by addition of alkalis in such batch conventional processes. This adds a an additional 50% cost on account of chemicals as well as additional separation and purification steps separation and purification steps. Such a conventional process dumps large quantity of calcium sulphate as solid waste, produced through the addition of lime and sulphuric acid [4]. Through process intensification, future process industries (chemical and pharmaceutical) must be capable of providing higher production with reduced energy, raw material consumption and reduced waste generation. Through the concept of developing radical technologies for the miniaturization of process plants, future industries will stand up with reduced equipment size as well as plant size with increasing inherent safety. Process intensification is kind of revolutionary approach that has the potential of fostering sustainable growth in chemical and allied process industries. Process intensification will eventually replace old, inefficient plants with new and intensified equipment opening up new opportunities for wide variety of patentable products and processes with scale up potentials [5]. Smaller is safer! Hence, process intensification dramatically increases the intrinsic safety of chemical processes.

Hybrid reactor system fabricated with the suitable combination of cross-flow flat sheet membrane modules with bioreactor system comes up with the achievement of process intensification by performing multiple tasks in a single and compact unit. Fermentation route for L(+)-lactic acid production from renewable resources like sugarcane juice with suitable microorganism has received high acceptance compared to chemical synthesis route to produce optically pure L(+) lactic acid [6, 7]. Continuous fermentation process with membrane cell recycle system is much more economically advantageous than batch process which suffers largely from low volumetric productivities due to end product inhibition and high labour cost due to start up and shut-down procedures [8]-[9]. Uses of NF (nanofiltration) membranes for the separation of undissociated L (+) lactic acid in permeate site is turned out to be much more advantageous than the RO (reverse osmosis) membranes. Development of process intensification through such multifunctional hybrid membrane reactor system by increasing mass transfer rate, productivity, selectivity to achieve desired product by separating other by-products has been found to be promising alternative to the conventional processes.
II. MATERIALS AND METHODS

A. Microorganism And Media Preparation

*Lactobacillus delbrueckii* (NCIM-2025), a homo-fermentative L (+) lactic acid producing bacterium used in our work was brought from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India in lyophilized condition. The culture was maintained in MRS agar slants at 4°C and subcultured subsequently in 50 ml MRS broth in a 100 ml conical flask. Pure sugarcane juice was purchased from local farmers and mainly used as fermentation media. The juice was then pre-filtered to remove unwanted particles like fibres, solids. Pure sugarcane juice collected in the months of March-April contained 132.34 g l\(^{-1}\) sucrose, 7.98 g l\(^{-1}\) glucose, 5.65 g l\(^{-1}\) fructose. The media was supplemented with 13.82 g l\(^{-1}\) yeast extract, 7.69 g l\(^{-1}\) peptone; 0.2 g l\(^{-1}\) MgSO\(_4\).7H\(_2\)O, 0.005 g l\(^{-1}\) MnSO\(_4\).4H\(_2\)O, 1.5 g l\(^{-1}\) sodium acetate, 1.5 g l\(^{-1}\) KH\(_2\)PO\(_4\) and 1.5 g l\(^{-1}\) K\(_2\)HPO\(_4\). All the chemical reagents used were from Sigma Aldrich.

B. Experimental Equipment

The 20 litre pilot plant fermenter made up of stainless still was provided with thermostatic water circulation system, nitrogen gas purging system for ensuring desired constant reactor temperature and anaerobic environment. Feed reservoir was a 10 litre stainless steel tank (Fig. 1). The temperature and agitation were maintained at 41°C and 160 rpm respectively. The fermenter was equipped with cross flow flat sheet membrane modules to which pressure gauges were attached at the inlet and the outlet. A peristaltic pump was used for feed circulation across the microfiltration membrane module(MF).

![Fig.1 Schematic Diagram Of Membrane Integrated Reactor System For Lactic Acid Production](image)

The microfiltration(MF) membranes used in cross flow modules performed cell separation from the feed for recycling. High pressure diaphragm pump(5-40 kgf/cm\(^2\)) was used in nanofiltration membrane modules that helped separation lactic acid from unconverted sugars and other impurities during continuous operation with two stage membranes (microfiltration and nanofiltration). Cross-flow microfiltration experiment was carried out with PVDF laminated membrane with pore size of 0.2 to 0.45 μm (Membrane Solutions, USA). For the nanofiltration step, NF2 membrane (Sepro Membranes, USA) was selected through investigations to separate impurities from lactic acid. Membrane surface area for each module selected for microfiltration as well as for nanofiltration was 0.01 m\(^2\).

C. Analytical Assays

The samples from fermentation broth were taken out at different time interval and the absorbance of those samples were measured by UV spectrophotometer (CECIL, 7000 Series, India) at 620 nm. Samples were then ultra–centrifuged (Sigma Instruments, India) at 12,000 rpm for 15 minutes and supernatants were collected for the analysis of L (+) lactic acid, sucrose, glucose and fructose. L (+) Lactic acid concentration was quantified by Ultron ES-OVM Chiral column (Agilent Technologies, HPLC) with Diode Array Detector (DAD). The measurement of all three carbohydrates (sucrose, glucose and fructose) concentrations were done by RID detector with Agilent Zorbax Carbohydrate Analysis Column. Purity of the nanofiltrated sample was determined through the analysis by peak purity software tool of HPLC (Agilent, series 1200). Protein estimation of the samples were carried out with Lowry’s method. Minerals (Na\(^+\), K\(^+\) and Mg\(^{2+}\)) were quantified with individual electrodes from Thermo SCIENTIFIC, USA.
III. RESULTS AND DISCUSSION

A. Conventional Fermentation-Based Lactic Acid Production Scheme

The synthetic manufacture of lactic acid in a commercial scale started around 1963 in Japan and United States [10]. In this method, Lactonitrile is produced first due to the combination of hydrogen cyanide and acetaldehyde in the presence of base catalyst in liquid phase. The recovered crude lactonitrile is subsequently purified and hydrolyzed into lactic acid by using either concentrated sulphuric acid or hydrochloric acid.

\[
\text{HCN} + \text{CH}_3\text{CHO} \rightarrow \text{CH}_3\text{CH}-(\text{OH})\text{CN}
\]

\[
\text{CH}_3\text{CH}-(\text{OH})\text{CN} + 2\text{H}_2\text{O} + \text{HCL} \rightarrow \text{CH}_3\text{CH}-(\text{OH})\text{COOH} + \text{NH}_4\text{Cl}
\]

Ammonium chloride is produced as a by-product in this production process. Lactic acid is esterified by methanol and as a result it generates methyl lactate. The other steps involved in this process to purify this recovered lactate are distillation and hydrolyzation under acidic condition. There are other chemical synthesis routes for lactic acid production like oxidation of propylene glycol, reaction of acetaldehyde with carbon monoxide and water at elevated temperatures and pressure, hydrolysis of chloropropionic acid and nitric acid oxidation of propylene. But none of these processes were commercialized [11] and those processes being often dependent on other by-product industries are expensive. To cater to the growing demand of pure L (+) lactic acid for production of biodegradable PLA, the fermentative route has been preferred to chemical synthesis route.

Typical conventional fermentation based lactic acid production scheme (Fig. 2) consists of a number of downstream treatment schemes like precipitation, conventional filtration, acidification, carbon adsorption and evaporation. In that production process addition of lime for controlling pH leads to the production of calcium lactate. Calcium lactate is then separated from the microbial cells by filtration and further purified by activated carbon adsorption. In next phase, calcium lactate is evaporated and acidified by sulphuric acid to produce lactic acid. Gypsum (calcium sulphate) is produced as a by-product in the process and is produced at a rate of 1 metric tonne per metric tonne of lactic acid. Thus the conventional production process is associated with a big environmental hazard as gypsum disposal poses a problem. Capital investment cost is naturally very high due to involvement of so many units as shown in the typical schematic diagram (Fig. 2) of such a plant. Thus process intensification is the only natural route of survival and sustainable development of lactic acid manufacturing industry.

B. Batch and Continuous Process With Optimization Of Major Parameters

Design Expert Software (Version 8.0.4) has been successfully applied to optimize lactic acid production in batch process. Response surface methodology (RSM) was chosen in the present investigation, to optimize the operating parameters like temperature, yeast extract concentration as well as peptone concentration during lactic acid production from sugarcane juice.
by *Lactobacillus delbruckii* (NCIM-2025). The experiments were designed through the software by selecting three numeric factors and zero categorical factor with one response. The upper and lower limits of yeast extract, peptone and temperatures were chosen based on the existing literature of lactic acid production. Quadratic Model was suggested by the software to evaluate the results. The Model F-value of 31.18 implies that the model is significant. Value of 'P' was 0.001 and being less than 0.0500 indicates that the model terms are significant. Analysis of variance (ANOVA) has shown the effects of temperature, concentration of yeast extract and concentration of peptone on lactic acid production. It was observed that the concentration of lactic acid initially increased with the increase of temperature up to 35°C but as temperature increased further beyond 41°C, lactic acid concentration started decreasing. At initial temperature, with the increase of yeast extract and peptone concentrations, lactic acid concentration did not vary much but as temperature increased beyond 35°C (upto41°C), the factors were found to have positive impact on lactic acid production. Optimum lactic acid concentration achieved from pure sugarcane juice with the help of RSM model as well as through experiment was 116.28 g L⁻¹ at 41°C temperature, 13.82 g L⁻¹ yeast extract concentration and 7.69 g L⁻¹ peptone concentration. The production yield achieved was 93% with 1.615 g L⁻¹ h⁻¹ productivity. The same production in batch mode was significantly affected by product-inhibition problem and low pH environment resulting in poor productivity.

To improve productivity and to reduce the production cost, continuous production process with membrane cell recycle was adopted. Continuous fermentation can be carried out with one stage membrane separation system or multi stage membrane separation system due to the flexibility of the system. Again number of working modules can be optimized according to the nature of process and desirability of the product quantity. It was only possible due to the super flexibility nature of the hybrid system. We adopted two stage continuous membrane separation system to get pure, polymer grade L (+) lactic acid in industrial production level. Our collected sugarcane juice contained 132.3 g L⁻¹ sucrose, 7.9 g L⁻¹ glucose, 5.6 g L⁻¹ fructose. Total glucose and fructose got consumed within 6 hours of fermentation. After batch fermentation, continuous fermentation with microfiltration cell recycle was started with 6 litres working volume of the reactor. Continuous fermentation was operated with cross flow velocity of 1.23 ms⁻¹ and fresh feed dilution rate of 0.184 h⁻¹. Cell recycling helped main very high cell concentration in the fermentor and thus contributed significantly to enhance production of lactic acid.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Lactic acid Concentration (g L⁻¹)</th>
<th>Product Yield Yps (%)</th>
<th>Productivity (g L⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>116.28</td>
<td>93</td>
<td>1.615 (at 72 hrs)</td>
</tr>
<tr>
<td>Continuous</td>
<td>72.53</td>
<td>92</td>
<td>13.35</td>
</tr>
</tbody>
</table>

By operating four modules in microfiltration cell recycle system and 1 module in nanofiltration system the achieved flux was 76.6 l m⁻² h⁻¹, where complete separation of microbial cells and more than 95% removal of impurities were achieved. The purity of the sample was determined as 95% when sample peak was tested in HPLC peak purity software tool. The results both of batch process as well as continuous process have been summarized in Table I.

**IV. CONCLUSIONS**

Due to the growing demand of L (+) lactic acid for the production of biodegradable plastic (PLA), it has been necessitated to improve conventional fermentation-based lactic acid production process with efficient and sustainable process. Membrane based hybrid reactor system successfully stands in that objective without creating any negative environmental impact. Super flexibility makes the system ideal for the production of L (+) lactic acid in any industrial scale. High productivity and purity achieved in this membrane-integrated fermentation system and in an absolutely environmentally benign process will definitely go in favour of its industrial adoption.

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**REFERENCES**


