Continuous Production of Lactic Acid in a Two Stage Process Using Immobilized *Lactobacillus casei* MTCC 1423 Cells

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***Abstract*—Experimental investigations has been carried to obtain high productivity of lactic acid by *Lactobacillus (Lb.) casei* MTCC 1423 cells immobilized in sodium alginate which is characterized by long term stability and high cell densities maintained in fermentation media. Influence of sugar concentration and dilution rate on lactic acid production in a continuous two stage process consists of constant stirred tank reactor and a packed bed column by immobilized *Lb. casei* cells has been investigated. After the 1st stage reactor (CSTR), with the dilution rate enhancement (0.05 - 0.3h^{-1}), lactic acid production had decreased from 106±3 to 65.36±3 g/L, from 68±2.6 to 43.64±2.5 g/L and 36.34±2.2 to 27.12±2.0 g/L at initial molasses sugar concentration of 175, 100 and 50 gm/L respectively. During 2nd stage, at the low dilution rate (=0.05 h^{-1}), an enhancement of approximately 10±2%, 8±1.5% & 5±1.5% in lactic acid production was observed at high initial sugar content of 175 g/L,100 g/L & 50 g/L respectively after 1st stage. Negligible/very low additional lactic acid production/sugar consumption was observed in the 2nd stage reactor (plug flow reactor) for dilution rate ≥0.1 h^{-1}.**

***Index Terms*—constant stirred reactor, packed bed reactor, immobilized cells, *Lactobacillus casei*, dilution rate, sugar concentration**

I. Introduction

The major applications of lactic acid are in food sector as a food acidulant, preservative, emulsifying agents etc., pharmaceutical & cosmetic industries and moreover as a precursor for biodegradable polylactic acid production [1]. The most preferred economical and environmentally benign method for the lactic acid production is via fermentation route. Since the fermentive production of lactic acid must be cost-competitive with chemical synthesis, hence till date, various efforts have been undertaken by researchers, for achieving high concentrations as well as high productivity of lactic acid. The productivity and the concentration of the fermentation product generally influence very closely the economics of the process. Batch or fed batch type lactic acid fermentation leads to reasonably higher lactic acid concentration, whereas continuous fermentation provides higher productivity [2]. The product quality and efficiency of the process can be significantly enhanced by continuous fermentation process and it also provides the environmental advantages. The product losses and operating cost can be reduced by the continuous fermentation process [3]. The residual substrate during a continuous fermentation process became the effluent and the dilution as well as the production of the inhibiting products can be constantly and simultaneously achieved. Moreover higher yield and productivity at steady state during continuous fermentation can be obtained due to consistent growth rate and regular maintenance of cells at its physiological state [4].

The continuous fermentation process productivities depend upon the dilution rate/residence time and at high dilution rate, 2-3 times higher productivities can be achieved in comparison to batch and fed-batch process [5]. Lactic acid productivity can be enhanced in a continuous fermentation process by integrating it to a cell recycling system [6]. As compared to conventional continuous and batch fermentation, 1.8 times higher lactic acid productivity (8.0 g/L-h) by *Lb. plantarum* from cassava starch was reported to be obtained during continuous cell recycling [5]. At higher dilution rates, there is always a risk of washout of the cells which can be avoided by utilizing either immobilized cell method or attaching a filtration module like microfiltration and ultrafiltration with bioreactor as it provides an opportunity to retain the cells within the bioreactor at sufficiently high density. Practically the earlier technique is easier and more economical in comparison to the latter

Manuscript received February 27, 2018; revised May 20, 2018.

doi: 10.18178/ijfe.4.3.216-222

one [7]. Moreover it shows efficient cell viability since immobilized beads provides protective layer to retain the biomass inside the reactor [8].

Each type of the reactor has its own advantages. The continuous stirred tank reactor (CSTR) is advantageous being easy to operate and stirring facilitates uniform suspension of substrates and nutrients. It provides larger surface area for better mass transfer of substrates and nutrients across the beads containing microbes as stirring avoids agglomeration of beads in the reactor. Hence production efficiency of microbes got enhanced [9]. Two major types of the continuous process technological arrangements viz: single stage and multistage can be made [10]. The direct integration of the two reactors is desirable from economic point of view and for reduction in operation complexity and for maintaining the continuous characteristics of the process. Moreover the two stage fermentation process facilitates greater process stability and better acidification control as compared to single stage process [11]. Two reactors connected in series had been utilized for continuous as well as sequential lactic acid and xylitol production [1].

Hence present study was undertaken in a two stage process for continuous production of lactic acid by utilizing immobilized *Lb. casei* MTCC 1423 by directly integrating a CSTR with an upflow packed bed reactor in series using industrial waste/byproduct, molasses and corn steep liquor as low value substrate and nitrogen source respectively.

II. Materials and Methods

A. Procurement of Microbial Cultures and Cell Culture Preparation

Lb. casei MTCC 1423, the microbial strain used during this study had been procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Sterilzed MRS (de Mann Rogosa Sharpe) broth has been utilized for culturing the freeze-dried microbes (1%, w/v) for 20 h at 37 ℃. Then sub-culturing (37 ℃, 20h) of obtained culture was again carried out twice using inoculums size of l% (w/v) in sterile MRS broth.

B. Molasses, Corn Steep Liquor and Chemicals

A byproduct, sugarcane molasses having total sugar: 52.27 ±1.03% (w/w) and reducing sugar: 36.50 ±1.54% (w/w) was obtained from Bhagwanpura Sugar Mill Limited Dhuri, Punjab, India and molasses was diluted with deionized water to obtain the desired concentration of molasses sugar. Corn steep liquor (CSL), as nitrogen source was collected from Sukhjeet Industries, Phagwara, Punjab, India. Both sugarcane molasses and corn steep liquor were utilized without pretreatment and were stored at 4 ℃. All the chemicals except for HPLC analysis employed during this study were of analytical grade. For HPLC analysis, chemicals were of HPLC grade.

C. Immobilization Lb. casei MTCC 1423 Cells

The immobilization of *Lb. casei* MTCC 1423 cells has been carried out by following the method of Idris and Suzana [12] and Kaleem *et al.* [13]. The double coated beads (ACA) were prepared with the help of sterilized syringe by mixing the *Lb. casei* MTCC 1423 cells with alginate solution (2%, w/v). The sodium alginate (2%, w/v) solution was sterilized at 121 ℃ for 15 min and the cells were mixed with this solution. The resultant mixture was added drop wise with the help of sterilized syringe into a sterile solution of calcium chloride (0.2 M). Na^{2+} ions of sodium alginate were replaced by Ca^{+2} ions of calcium chloride solution. The beads after hardening for 30 min were rinsed with sodium chloride solution (0.85%, w/v) and stored at 4 ℃.The alginate beads (A) thus obtained were coated with chitosan/alginate (double layer) as described by Klinkenberg *et al.* [14] to obtain beads of 2.5 mm size. The alginate beads containing *Lb. casei* cells were coated with chitosan (AC) and consecutively with alginate to obtain chitosan/alginate coated beads (ACA). A chitosan (0.4%, w/v) solution was prepared by dissolving appropriate quantity of chitosan in acetic acid solution (1%, v/v) using magnetic stirrer and a pH of 5.6 was adjusted for coating the beads with chitosan. This solution was sterilized and then cooled. The alginate beads were immersed in it and stirred to apply a chitosan coat. After 45 min, the beads were washed with sterile distilled water after sieving off from the chitosan solution. To apply another additional alginate layer, these chitosan coated beads were transferred to a sterilized solution of sodium chloride (0.2 M) and calcium chloride (0.05 M) and stirred for 15 min. The beads were then immersed in a sodium alginate solution (0.5%, w/v) and washed with sterile distilled water after stirring in this solution for 10 min. Afterwards these beads were put back into a solution of sodium chloride (0.2 M) and calcium chloride (0.05 M). These bead coating processes were accomplished under ambient temperatures and finished beads had been stored in peptone solution (0.75%, w/v) until utilized.

D. Fermentation Media and Conditions

Fermentation medium (3L) containing diluted molasses (50-175 g/L sugar content), CSL (2.75 %, v/v), $MnSO_4$ (20 mg/L) $CaCO_3$ (0.25 g/g of molasses sugar) was sterilized along with bioreactor and incubated with the immobilized *Lb. casei* MTCC 1423 cells. *Lb. casei* MTCC 1423 cells for immobilization were cultivated in MRS broth for 24 h at a controlled temperature of 37 ℃. Cells were harvested aseptically by centrifuging for 12 min at 6700 rpm and 4 ℃ and rinsed with phosphate buffer (0.1 [M] & pH 7.0) twice. The double coated beads (ACA) were prepared with the help of sterilized syringe by mixing the *Lb. casei* MTCC 1423 cells (20.0 gm, CDW) harvested from MRS broth with 550 mL alginate solution (2%, w/v) and coated with chitosan/alginate (double layer) to obtain beads of 2.5 mm size. The beads were incubated (37.5 ℃) with the fermentation media at initial pH of 6.5. The pH (6.5) was maintained in the CSTR with the help of NaOH (4.0 N).

E. Experimental Setup

The experimental setup for the two stage continuous lactic acid production process is shown in Fig. 1. The

experimental setup was composed of two main units: the constant stirred tank reactor vessel (New Brunswick Scientific, USA) with a 5 L vessel (working volume of 3 L) and the packed bed column (inner diameter 2.0 cm, total height 40 cm); and attached to each other via autoclavable tubing (Fig. 1).

The CSTR vessel was equipped with a four blade agitator and the temperature (37.5 ℃) was controlled automatically by built in heating jacket along with circulating cold water combination, while pH was controlled by automatic addition of 4.0 N NaOH. The temperature in the water jacketed packed bed reactor (PBR) at 37.5 ℃ was controlled by using temperature controlled circulating water bath. The packed bed reactor (60% of the volume) was packed with beads. The feed and the effluent collection glass vessels (5L each) were connected with CSTR and PBR respectively.

Figure 1. Experimental setup for continuous lactic acid production in a two stage process by immobilized *Lb. casei* MTCC 1423 cells

F. Experimental Procedure

The beads containing immobilized *Lb. casei* MTCC 1423 cells were transferred to the CSTR containing sterilized media (3L) and to the packed bed reactor aseptically. The agitation speed (150 rpm) in the CSTR was maintained automatically by control unit of the reactor. Since the two reactors were connected in series, these were allowed to run on total recycle for 60 h. No additional substrate had been added to the reactors during this period. After 60 h these were allowed to operate in continuous manner by transferring the fresh medium continuously to the reactor maintaining the dilution rate (0.05 h^{-1}) with respect to the first reactor (CSTR) for next 48 h. After that increased dilution rate (0.1 h^{-1}) was maintained for next 36 h. After operating a dilution rate of 0.2 h^{-1}, dilution rate was enhanced to 0.2 h^{-1} and continued for 24 h. Afterwards the dilution rate was kept at 0.3h^{-1} for next 24 h. The spent broth was removed after the end of the fermentation cycle with one sugar concentration and a new cycle of immobilization (new sugar concentration) was commenced by aseptic addition of medium to the CSTR before drying of cells and matrix.

G. Estimation of Total Sugar Lactic Acid Concentration and pH

The total molasses sugar concentration has been determined as per the standard phenol sulfuric acid method [15]. The concentration lactic acid produced was measured under the conditions described by Kishore *et al.* [16] using a high performance liquid chromatograph at 210 nm outfitted with C18 reversed phase chromatographic column, a dual wavelength ultraviolet detector and low pressure quaternary gradient pump (Shimadzu LC 2010 CHT, Shimadzu Corporation, Kyoto, Japan). Lactic acid produced and sugar consumed in the two reactors was determined separately after drawing the samples from each reactor using outflow tube provided after PBR prior to product stream reservoir and the sampling port of CSTR. The filtration of samples before analysis was achieved by using 0.22 µm membrane micro filter. Eutech pH 5+ was employed for *pH* measurement.

H. Calculations of Fermentative Parameters

Product yield per gram of consumed substrate ($Y_{P/S}$, g/g) was estimated as

$$Y_{X/S} = \frac{\Delta P}{\Delta S} \quad (1)$$

where, ΔP and ΔS represents lactic acid concentration change (g/L) and total sugar concentration (g/L) respectively. Lactic acid productivity (Q_p, g/L-h) was estimated by calculating the product of the final concentration of LA produced and dilution rate of the process.

III. Results and Discussion

Preliminary experiments were conducted for optimizing the operational conditions for lactic acid from sugar molasses by immobilized cells of *Lb. casei* MTCC 1423. High lactic acid productivity was observed during the preliminary experiments which were characterized by high yield in the fermentation media and long term stability and reusability of immobilized beads.

A. Effect on Dilution Rate on Lactic Acid Production during 1st Stage

The investigation had been carried out for observing the effect of dilution rate (D) and sugar concentration on lactic acid production in a continuous two stage process. The effect of dilution rate and initial sugar concentration on lactic acid production as well as sugar consumption rate during the first stage (CSTR) by immobilized *Lb. casei* MTCC 1423 cells has been illustrated in Fig. 2 and Fig. 3 respectively. As evident from the Fig. 2, with the enhancement in dilution rate, a decrement in the lactic acid production had been observed.

With the increase in initial sugar concentration, lactic acid production as well as the sugar consumption rate has enhanced. With the increament in the dilution rate (0.05-0.3 h^{-1}), the lactic acid production had decreased from 31.34±1.2 to 27.78±1.5g/L, from 68±2.0 to 43.84±2.3 g/L and from 106±2.5 to 65.36±2.8g/L, for 50, 100 and 175 g/L sugar concentration respectively. The decrement in lactic acid production with the increase in dilution rate was more at high initial sugar concentration (S_0=175 g/L) in comparison to low initial sugar concentration. Maximum lactic acid production (106±2.5 g/L) in 1st stage was obtained for sugar concentration (S_0) of 175 g/L at dilution rate of 0.05 h^{-1}.

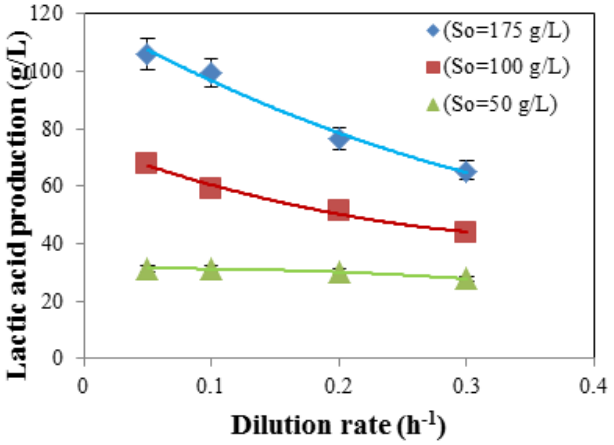

Figure 2. Effect of dilution rate and sugar concentration on lactic acid production after 1st stage (Experimental conditions: fermentation broth: 3L; CSL: 2.75% (v/v); $MnSO_4$: 20 mg/L; $CaCO_3$: 0.25 g/g of molasses sugar; pH: 6.5; incubation temperature: 37.5 ℃)

B. *Effect on Dilution Rate on Sugar Consumption during 1st Stage*

The sugar utilization during the 1st stage has been found to be decreased with the increase in dilution rate irrespective of the initial sugar concentration (S_0) (Fig. 3). The sugar consumption decrement is more pronounced at higher initial concentration in comparison to lower initial sugar concentration. For S_0=50g/L, the sugar consumption has decreased from 32.97±1.5 to 29.45 ±1.55 g/L; 72.69±1.7 to 53.62±1.56 g/L & 115.07±2.2 to 80.93±2.0 g/L for S_0= 50; 100 & 175 g/L respectively with the increase in dilution rate (0.05 h^{-1}-0.3 h^{-1}). A substrate conversion of 100% at D=0.22 h^{-1} was reported in stirred tank reactor by Krischke *et al.* [17] during lactic acid production by *Lb. casei* cells immobilized onto a porous sintered glass beads.

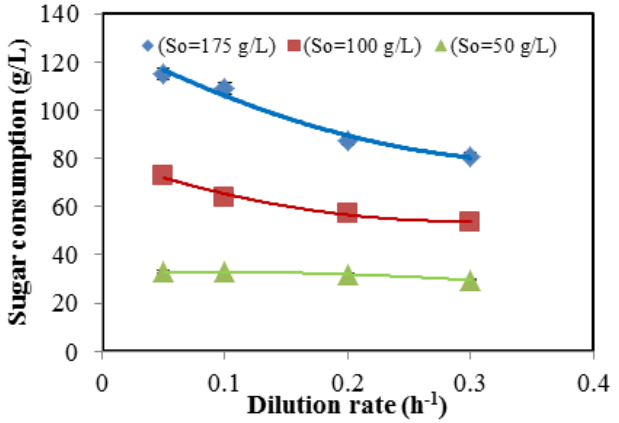

Figure 3. Effect of dilution rate and sugar concentration on sugar consumption after 1st stage (Experimental conditions: fermentation broth: 3L; CSL: 2.75% (v/v); $MnSO_4$: 20 mg/L; $CaCO_3$: 0.25 g/g of molasses sugar; pH: 6.5; incubation temperature: 37.5 ℃)

C. *Effect on Dilution Rate on Lactic Acid Productivity During 1st Stage*

The lactic acid productivity has been noticed to be increased with the dilution rate enhancement irrespective of sugar concentration (Fig. 4). The productivity tends to reach a plateau at 0.3 h^{-1} dilution rate. The peak productivity of 19.61±0.84 g/L-h was observed for dilution rate of 0.3 h^{-1} at S_0=175 g/L. The productivity has increased from 1.57±0.069 to 8.33±0.54 g/L-h; 3.4±0.09 to 13.15±0.7 g/L-h & 5.3±0.012g/L-h to 19.61±0.84 g/L-h for S_0= 50 g/L; 100 g/L & 175 g/L as dilution rate has increased from 0.05 h^{-1} to 0.3h^{-1}. The volumetric lactic acid productivity of 3.1 g/L-h for 0.1 h^{-1} dilution rate in a CSTR (1L) by *Lb. casei* immobilized in *k-carrageenan* by grouping with reversibly soluble-auto precipitating amylase had been reported by Hoshino *et al.* [18].

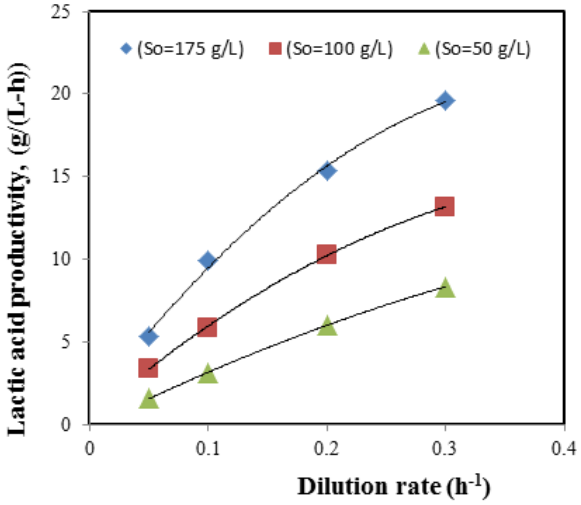

Figure 4. Effect of dilution rate and sugar concentration on lactic acid productivity after 1st stage (Experimental conditions: fermentation broth: 3L; CSL: 2.75% (v/v); $MnSO_4$: 20 mg/L; $CaCO_3$: 0.25 g/g of molasses sugar; pH: 6.5; incubation temperature: 37.5 ℃)

For 1.21 h^{-1} dilution rate, 28.5 g/L-h of lactic acid productivity in a CSTR has been observed during the fermentation of from whey permeate by *Lb. helveticus* immobilized in the gel beads of *K-carrageenan*+locust bean gum using yeast extract as nitrogen source [19]. In stirred tank reactor for dilution rate of 0.22 h^{-1}, volumetric lactic acid productivity of 5.5 g/L-h was reported [17].

D. *Effect of Dilution Rate on Yield during 1st Stage*

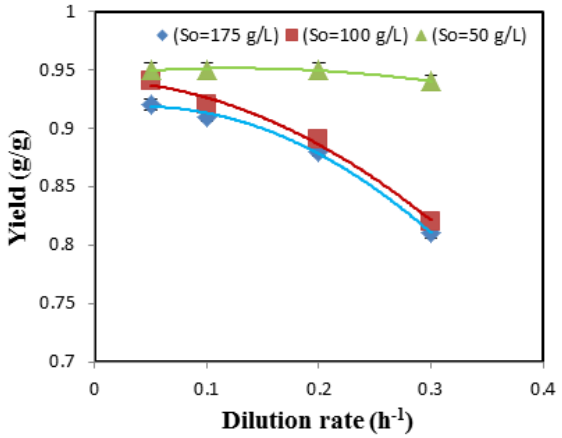

Figure 5. Effect of dilution rate and sugar concentration on yield after 1st stage (Experimental conditions: fermentation broth: 3L; CSL: 2.75% (v/v); $MnSO_4$: 20 mg/L; $CaCO_3$: 0.25 g/g of molasses sugar; pH: 6.5; incubation temperature: 37.5 ℃)

Higher yield (g/g) was observed at low dilution rates irrespective of S_0 (Fig. 5). The yield was found to be highest (0.95±0.008) at lowest initial sugar concentration (S_0=50g/L) for dilution rate of 0.05 h^{-1}. The yield was observed to be decreased with the increase in S_0 for all the dilution rates. The decrease in yield with the increase in initial sugar concentration was more at high dilution rate in comparison to low dilution rate. The yield has decreased from 0.95±0.008 to 94±0.003 for S_0=50 g/L with the increase in dilution rate (0.05-0.3 h^{-1}). While the yield had decreased from 0.92±0.0052 to 0.81±0.0083 & 0.94±0.0012 to 0.84±0.0175 for S_0=175 g/L & 100g/L respectively with increase in dilution rate (0.05-0.3 h^{-1}) after first stage.

E. Effect of Initial Sugar Concentration on Sugar Consumption after 2nd Stage

Negligible/very low additional lactic acid production/sugar consumption was observed in the 2nd stage reactor (packed bed reactor) for dilution rate ≥0.05. This might due to the reason that the dilution rate for PBR (0.6 h^{-1}) was much higher for the small volume of PBR in comparison to that of CSTR (0.05 h^{-1}). Hence the continuous lactic acid fermentation in second stage was limited to the dilution rate of 0.05 h^{-1}.

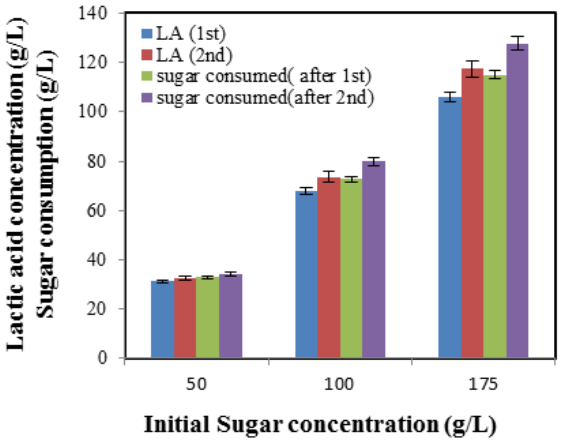

Figure 6. Effect of initial sugar concentration on sugar consumption after 1st stage and 2nd stage (Experimental conditions: fermentation broth: 3L; CSL: 2.75% (v/v); $MnSO_4$: 20 mg/L; $CaCO_3$: 0.25 g/g of molasses sugar; pH: 6.5; incubation temperature: 37.5 ℃; dilution rate: 0.05 h^{-1} (CSTR), 0.6 h^{-1}(PBR))

However at the low dilution rate (=0.05), approximately 10.7±0.7% & 8.2±0.5% increase in the lactic acid production at initial sugar concentration of 175 g/L & 100 g/L respectively and approximately 3.5±0.16% increase in lactic acid production at low initial sugar concentration (50 gm/L) had been observed.

The comparison for lactic acid production and sugar consumption after 1st stage (CSTR) and after 2nd stage (PBR) has been presented in Fig. 6. The lactic acid concentration exiting the 2nd stage of the reactors in series was 117.34±2 g/L; 73.58±1.8 g/L; & 34.54±1.55 g/L while that of after the 1st stage was 106±2.5 g/L; 68±2 g/L & 32.84±1.6 g/L for S_0= 175 g/L; 100g/L and 50 g/L respectively at dilution rate of 0.05 h^{-1}. 14.8 g/L lactic acid was obtained in a packed bed of *Lb. Casei* immobilized on wood chips at 0.2h^{-1} dilution rate [20].

At the same flow rate with the initial sugar concentration of 175g/L, 100g/L and 50g/L in the multistage system, the sugar consumed was 127.77±2g/L, 77.79±1.9g/L, & 34.24±1.6 while that of in the single stage (CSTR) 115.07±2.2g/L,72.69 ±1.7g/L & 32.99±1.55g/L was consumed respectively. 97% conversion at D = 0.47 h^{-1} for feed concentration of 25 g/L by immobilization of *Lb. casei* on carbon fibres in a fibrous bed bioreactor was reported by Afolabi *et al.* [21].

The highest lactic acid production of 117.34±2 g/L was obtained for S_0= 175 g/L and dilution rate of 0.05 h^{-1} in two stage reactor system (CSTR & PBR) connected in series and lactic acid productivity at these condition was 1.76 g/L-h (Fig. 6). Lactic acid production of 125 g/L was reported to be obtained during the continuous fermentation of glucose (S_0= 150g/L) in a packed column by *Lb. casei* immobilized in calcium alginate at dilution rate of 0.0075 h^{-1}[22].

F. Effect of Initial Sugar Concentration on Lactic Acid Productivity and Yield After 2nd Stage

The lactic acid productivities (g/L-h) and yield (g/g) in fermentations conducted in multistage system has been depicted in Fig. 7. It is evident from the figure that the lactic acid productivity has got enhanced in the 2nd stage (PBR) The yield after 2nd stage has slightly decreased may be owing to the reason that the immobilized cells had not experienced optimal *pH* and a *pH* gradient may have generated across the PBR. According to Fig. 6, the yield in second stage was 0.9477±0.004 (g/g) for S_0=50 g/L. The yield in the 2nd stage has decreased to 0.9224±0.0005 and 0.9184±0.0012 (g/g) at S_0=100 g/L & 175 g/L respectively.

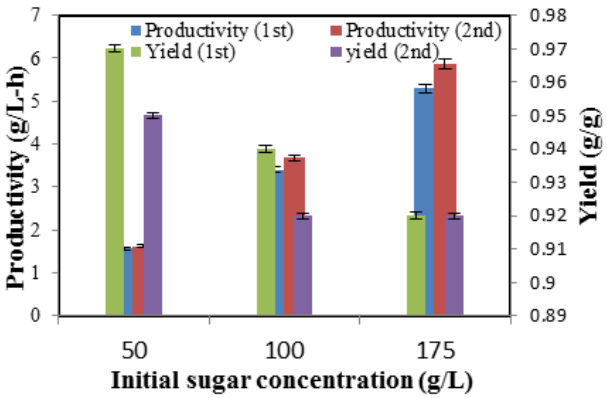

Figure 7. Effect of initial sugar concentration on lactic acid productivity and yield after 1st stage and 2nd stage (Experimental conditions: fermentation broth: 3L; CSL: 2.75% (v/v); $MnSO_4$: 20 mg/L; $CaCO_3$: 0.25 g/g of molasses sugar; pH: 6.5; incubation temperature: 37.5 ℃; dilution rate: 0.05 h^{-1} (CSTR), 0.6 h^{-1}(PBR))

The lactic acid productivity after the 2nd stage has enhanced from 1.57±0.069 to 1.62±0.072 g/L-h; 3.4±0.09 to 3.68±0.081 g/L-h & 5.3±0.012 to 5.87±0.067 g/L-h at S_0=50; 100 & 175g/L respectively with respect to the productivity after 1st stage. Lactic acid productivity of 3.90 g/L-h and 0.96 g/g yield has been reported during the continuous fermentation of whey by immobilized *Lb. helveticus* cells in a PBR after the 18 h of retention time [23]. During the continuous fermentation of sucrose, 5

g/L-h lactic acid volumetric productivity has been achieved in a dual reactor system consists of CSTR and PBR in series by immobilized *Lb. delbrueckii* NCIM 2365 cells [24]. Productivity of 23.3g/L-h for S_0=150 g/L was reported at dilution rate of 0.0075 h^{-1} during the continuous production of lactic acid in a column packed with immobilized *Lb. casei* [22].

IV. Conclusion

The effect of sugar concentration and dilution rate on the L(+)-lactic acid production in a continuous two stage process (CSTR and PBR in series) by immobilized *Lb. casei* MTCC 1423 cells was investigated and has indicated that at high sugar concentration (175gm/L), the L(+)-lactic acid production had decreased from 106±3 to 65.36±3g/L, at low sugar concentration (50gm/L), the L(+)-lactic acid production decreased from 36.34±2.2 to 27.12±2.0g/L and at sugar concentration of 100 gm/L, it had decreased from 68±2.6 to 43.84±2.5g/L, as the dilution rate had been increased from 0.05 to 0.3 h^{-1}. There was very low additional L(+)-lactic acid production/sugar consumption was observed in the 2nd stage reactor (packed bed reactor) for dilution rate ≥0.1h^{-1} (1st stage) since dilution rate has become was too high for 2nd stage. However at the low dilution rate (=0.05 h^{-1}), approximately 10±2% & 8±1.5% increase in the lactic acid production for the initial sugar concentration of 175, 100 and 50 gm/L respectively and approximately 5±1.5%increase in lactic acid production at low initial sugar concentration (50 gm/L) has been observed in addition to the lactic acid production from 1st reactor.

Acknowledgment

Authors are thankful to Sant Longowal Institute of Engineering and Technology, Longowal, India for providing the lab facilities and all necessary help to perform this research work

References

[1] J. M. Salgado, N. Rodr ́guez, S. Cort és, and J. M. Dom ́nguez, "Coupling two sizes of CSTR-type bioreactors for sequential lactic acid and xylitol production from hemicellulosic hydrolysates of vineshoot trimmings," *New Biotechnology*, vol. 29, no. 3, pp. 421-42, 2012.

[2] S. Moon, Y. Wee, and G. Choi, "A novel lactic acid bacterium for the production of high purity l-lactic acid, *Lactobacillus paracasei subsp. paracasei* CHB2121," *Journal of Bioscience and Bioengineering*, vol. 114, no. 2, pp. 155-159, 2012.

[3] S. Kumar, S. P. Singh, I. M. Mishra, and D. K. Aadhikari, "Continuous ethanol production by *Kluyveromyces sp.* IIPE453 immobilized on bagasse chips in packed bed reactor," *Journal Petroleum Technology and Alternative Fuels*, vol. 2 no. 1, pp. 1-6, Jan 2011.

[4] T. Gao and K. Ho, "l-Lactic acid production by *Bacillus subtilis* MUR1 in continuous culture," *Journal of Biotechnology*, vol. 168, no. 4, pp. 646-651, 2013.

[5] W. Bomrungnok, K. Sonomoto, S. Pinitglang, and A.Wongwicharn, "Single step lactic acid production from cassava starch by *Lactobacillus plantarum* SW14 in conventional continuous and continuous with high cell density," *APCBEE Procedia*, vol. 2, pp. 97-103, 2012.

[6] Y. Wang, Y. Tashiro, and K. Sonomoto, "Fermentative production of lactic acid from renewable materials: Recent achievements, prospects, and limits," *Journal of Bioscience and Bioengineering*, vol. 119, pp. 1 10-18, Jan. 2015.

[7] T. Dishisha, M. T. Alvarez, and R. Hatti-Kaul, "Batch- and continuous propionic acid production from glycerol using free and immobilized cells of *Propionibacterium acidipropionici*," *Bioresource Technology*, vol. 118, pp. 553-562, 2012.

[8] P. Sivagurunathan, P. Anburajan, J. H. Park, G. Kumar, H. D. Park, and S. H. Kim, "Mesophilic biogenic H_2 production using galactose in a fixed bed reactor," *International Journal of Hydrogen Energy*, vol. 42, pp. 3658-3666, 2017.

[9] A. Reungsang, C. Sreela-or, and P. Plangklang, "Non-sterile bio-hydrogen fermentation from food waste in a continuous stirred tank reactor (CSTR): Performance and population analysis," *International Journal of Hydrogen Energy*, vol. 38, no. 35, pp. 15630-15637, 2013.

[10] D. P. Bayrock and W. M. Ingledew, "Ethanol production in multistage continuous, single stage continuous, *Lactobacillus*-contaminated continuous, and batch fermentations," *World Journal of Microbiology and Biotechnology*, vol. 21, no. 1, pp. 83-88, 2005.

[11] C. Nualsri, P. Kongjan, and A. Reungsang, "Direct integration of CSTR-UASB reactors for two-stage hydrogen and methane production from sugarcane syrup," *International Journal of Hydrogen Energy*, vol. 41, no. 40, pp. 17884-17895, 2016.

[12] A. Idris and W. Suzana, "Effect of sodium alginate concentration, bead diameter, initial pH and temperature on lactic acid production from pineapple waste using immobilized *Lactobacillus delbrueckii*," *Process Biochemistry*, vol. 41, no. 5, pp. 1117-1123, 2006.

[13] I. Kaleem, H. Shen, B. Lv, B. Wei, A. Rasool, and C. Li, "Efficient biosynthesis of glycyrrhetic acid 3-O-mono-β-d-glucuronide (GAMG) in water-miscible ionic liquid by immobilized whole cells of *Penicillium purpurogenum* Li-3 in alginate gel," *Chemical Engineering Science*, vol. 106, pp. 136-143, 2014.

[14] G. Klinkenberg, K. Lystad, D. Levine, and N. Dyrset, "Cell release from alginate immobilized *Lactococcus lactis ssp. lactis* in chitosan and alginate coated beads," *Journal of Dairy Science*, vol. 84, no. 5, pp. 1118-1127, 2001.

[15] M. DuBois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith," Colorimetric method for determination of sugars and related substances," *Analytical Chemistry*, vol. 28, no. 3, pp. 350-356, 1956.

[16] G. Kishore, A. Karthik, S. V. Gopal, A. R. Kumar, M. Bhat, and N. Udupa, "Development of RP-HPLC method for simultaneous estimation of Lactic acid and glycolic acid," *Der Pharma Chemica*., vol. 5, no. 4, pp. 335-340, 2013.

[17] W. Krischke, M. Schrfider, and W. Trfisch, "Continuous production ofl-lactic acid from whey permeate by immobilized *Lactobacillus casei subsp. Casei*," *Applied Microbiology and Biotechnology*, vol. 34, no. 5, pp. 573-578, 1991.

[18] K. Hoshino, M. Taniguchi, H. Marumoto, K. Shimizu, and M. Fujii, "Continuous lactic acid production from raw starch in a fermentation system using a reversibly soluble-autoprecipitating amylase and immobilized cells of *Lactobacillus casei*," *Agricultural and Biological Chemistry*, vol. 55, no. 2, pp. 479-485, 1991.

[19] S. Norton, C. Lacroix, and J. Vuillemard, "Kinetic study of continuous whey permeate fermentation by immobilized *Lactobacillus helveticus* for lactic acid production," *Enzyme and Microbial Technology*, vol. 16, no. 6, pp. 457-466, 1994.

[20] A. Kazemi and P. Baniardalan, "Production of lactic acid from whey by immobilized cells" *Scientia Iranica*, vol. 8, no. 3, pp. 218-222, 2002.

[21] A. S. Afolabi, O. S. Mobee, and A. S. Abdulkareem, "Production of lactic acid from cassava starch hydrolysate using immobilized *Lactobacillus Casei* in a fibrous bed bioreactor," *Proceedings of the World Congress on Engineering*, U.K.: London, vol. 3, July 4 - 6, 2012.

[22] V. Arasarathnam and K. Alasuramaniam, "Viability of calcium alginate entrapped *lactobacillus casei* during continuous lactic acid production," *Journal of Natn Science foundation Sri Lanka*, vol. 28, no. 3, pp. 155-163, 2000.

[23] M. S. A. Tango and A. E. Ghaly, "A continuous lactic acid production system using an immobilized packed bed of

Lactobacillus helveticus," *Applied Microbiology and Biotechnology*, vol. 58, pp. 712-720, 2002.
[24] V. Rangaswamy and S. Ramakrishna, "Lactic acid production by *Lactobacillus delbrueckii* in a dual reactor system using packed bed biofilm reactor," *Letters in Applied Microbiology*, vol. 46, no. 6, pp. 661-666, 2008.

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