Intensification of a fermentation process for producing lactic acid in a ceramic membrane combined bioreactor system

Rong Fan, Mehrdad Ebrahimi, Matthias Aden, Peter Czermak

INTENSIFICATION OF A FERMENTATION PROCESS FOR PRODUCING LACTIC ACID IN A CERAMIC MEMBRANE COMBINED BIOREACTOR SYSTEM

Rong Fan[1*], Mehrdad Ebrahimi[1], Matthias Aden[2], Peter Czermak[1, 3, 4]

[1]Institute of Bioprocess Engineering and Membrane Technology, University of Applied Sciences Mittelhessen, Giessen, Germany

[2] Faudi Aviation Sensor GmbH, Stadtallendorf, Germany

[3]Department of Chemical Engineering, Kansas State University, Manhattan, Kansas, USA

[4]Faculty of Biology and Chemistry, Justus Liebig University, Giessen, Germany

ABSTRACT

A membrane-based filtration process could efficiently reduce the cost of lactic acid production, which is mainly due to its downstream separation and purification steps. In order to achieve high-volumetric productivity of lactic acid, ceramic micro (MF)- and ultrafiltration (UF) membranes are selected to separate the lactic acid from the fermentation broth. For a successful combination of separation unit and fermentation system, membranes should be characterized separately for the determination of membrane performance and the impact of process parameters on it. In this work, the statistical design of experiment was explored to investigate the effects of the physical properties of fermentation broth (cell density, glucose concentration) and process parameters of filtration (transmembrane pressure (TMP) and tangential flow velocity (CFV)) on membrane performances (flux and membrane fouling). In the meantime, the cell density was tracked with the scattered light sensor (FAUDI Aviation AFGUARD®), which is designed for in situ measurement of particulate matters and firstly developed for online control of biotechnical processes. Under the application of AFGUARD® sensor in the filtration process the cell rejection could be online monitored efficiently and economically. The results indicated that the 50 nm and 100 kDa membranes had the better performances (higher flux and lower fouling) in comparison to the other membranes (0.2 µm and 20 kDa) under the same operating condition. Biomass was successfully remained in retentate and not found in permeate. In the further experiments, the filtration unit will be integrated to the bioreactor system in order to establish a continuous fermentation process in which cell growth kinetics, lactic acid productivity and membrane performance will be studied.

KEYWORDS:

Lactic acid, ceramic membrane, transmembrane pressure, tangential flow velocity, online monitoring

Introduction:

The cost of downstream processing is considered to be the largest part in lactic acid (LA) production [1]. In conventional batch or fed-batch fermentation, lime is added for neutralization of the fermentation broth and avoidance of the product inhibitation. LA is then regenerated from calcium lactate through acidification, precipitation and filtration. A large amount of waste water and gypsum are generated in this process and create a huge economical and environmental burden [2]. As the demand on LA in the world market increases rapidly [1], various new methods, which could enhance the LA productivity and minimize the waste generation, are investigated for replacing the conventional process. In situ LA recovery using membrane technique (micro-, ultra-, nanofiltration and electrodialysis) is a attractive alternative to fulfill this task [3,4]. Due to the high mechanical strength, thermal and chemical resistance, easy cleaning method and permanent use life, ceramic membranes are in comparison with organic membranes more favourable for the integration in membrane bioreactor system [5].

In the continuous LA fermentation using MBR, fermentation broth is continuously removed from fermentation system by filtration while fresh culture medium is added, meanwhile cells are retained in bioreactor for enhancement of the LA productivity. Thereby substrate consumption and LA concentration are influenced by dilution rate, which is controlled by the permeate flux in filtration. Thus, the primary task of this work is selection of a suitable membrane and meanwhile determination of its operating parameter for LA extraction and cell retention. For this purpose, the membrane performances would be evaluated in LA fermentation broth at different operation parameters such as transmembrane pressure (TMP) and cross flow velocity. Furthermore, biomass is another important factor for LA production in MBR system, since the bioconversion is catalyzed by cells and the LA productivity is coupled with cell growth [6]. In the meantime, the filtration efficiency may be influenced by biomass, which could form the covering layer on the membrane surface [7]. Therefore, an innovative optical sensor for the online measurement of biomass concentration is in this work investigated. A successful integration of this equipment could help the process more efficient.

Materials and methods:

Membrane:

Tubular ceramic membranes (mono channel) with nominal pore size/molecular weight cutoff (MWCO) of 0.2 µm, 50 nm, 100 kDa and 20 kDa, respectively, were produced by atech innovations GmbH, Gladbeck, Germany. The membranes have inner and outer diameters of 6 and 10 mm, respectively, and the effective membrane surface was approx. 40 cm^{-2}.

Microorganism and fermentation broth:

Bacillus coagulans PS5, a facultative anaerobic homofermentative L(+) lactic acid producing bacterium used throughout this work was kindly supplied by the company Uhde GmbH. The culture medium was modified MRS medium, which contained 4.0 $g \cdot L^{-1}$ yeast extract, 8.0 $g \cdot L^{-1}$ meat extract, 10.0 $g \cdot L^{-1}$ peptone from casein, 2 $g \cdot L^{-1}$ K_2HPO_4, 0.1 $g \cdot L^{-1}$ $MgSO_4$, 0.03 $g \cdot L^{-1}$ $MnSO_4$,1.0 $g \cdot L^{-1}$ Tween 80 and 50 $g \cdot L^{-1}$ glucose. All the media components were sterilized at 121 °C for 20 min except for glucose that was separately sterilized. The batch fermentations were carried out in the 5 L stirred bioreactor (Biostat® B) at 53 °C with neutralization at pH 7 using 5 M NaOH. The final fermentation broth contained approx. 45 $g \cdot L^{-1}$ lactate and 2.5 $g \cdot L^{-1}$ dry biomass.

Experimental equipment:

The characterization of membranes at different TMPs and flow velocities was carried out using a cross-flow filtration system in the recycle mode of operation, where the permeate returned to feed tank to retain the properties of the feed solution constant. The feed was pumped through the membrane module by a peristaltic pump from a 2 L feed tank. The temperature was kept by magnetic stirrer (Heidolph, Germany) at 53 °C. The TMP and flow velocity were controlled by the peristaltic pump and a stainless steel valve, which mounted on the retentate outlet. Retentate directly returned to the feed tank. Permeate was firstly collected in a 500 mL Erlenmeyer flask, where the mass increase was online monitored by a electronic balance. Once the permeate in flask accumulated to a certain level, which did not cause a distinct concentration and temperature change of the feed solution, it would be recycled back to the feed tank. The flux was calculated after the experiment. TMP was varied between 0.4 and 1.6 bar whereas variation range of flow velocity was 0.8-1.6 $m \cdot s^{-1}$. The TMP was monitored through two manometers located on the inlet and outlet of the membrane module and calculated through the following equation:

$$TMP = \frac{P_1 + P_2}{2}$$

The cross flow velocities ($m \cdot s^{-1}$) were calculated through the quotient of the retentate per inner cross sectional area of the membrane tube. The configuration of the experimental equipment is shown schematically in Fig. 1.

The online measurement of cell density was done by the Afguard® sensor and kindly provided by FAUDI Aviation, Stadtallendorf, Germany. This sensor, which is based on measuring light scattering and absorption simultaneously, is originally applied for the detection of water in fuel used in the aviation industry. The characterization of the sensor was carried out separately in the fermentation broth using a dynamic system as shown in Fig. 2

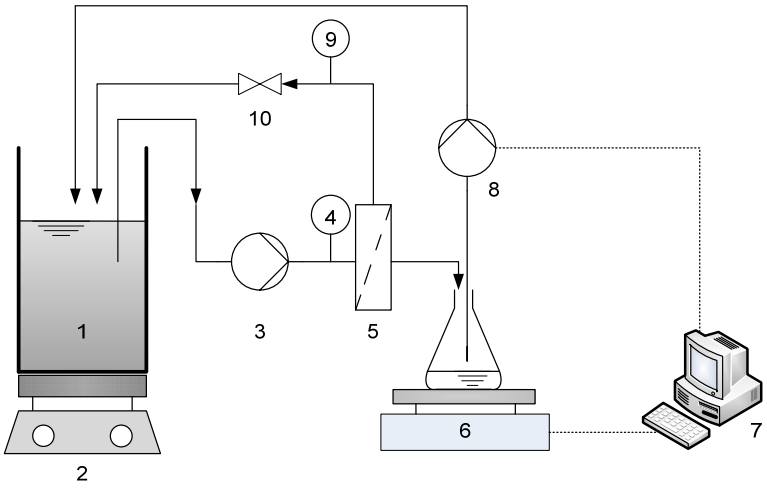

Fig. 1. Schematic diagram of membrane filtration system for extraction of LA. 1. feed tank, 2. magnetic stirrer, 3, 8. peristaltic pump, 4, 9. manometer, 5. membrane module, 6. electronic balance, 7. control system, 10. valve

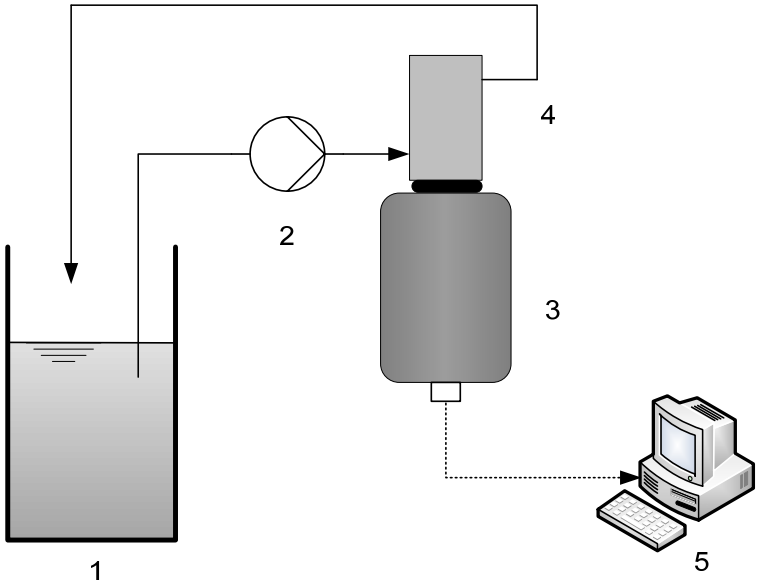

Fig. 2 Schematic diagram of dynamic measurement system for characterization of the optical sensor. 1. cell suspension, 2. peristaltic pump, 3. Afguard® sensor, 4, measurement cell, 5. data receiver

Analytical methods:

10 mL sample from fermentation broth was centrifuged at 4000 rpm for 10 min and the supernatant was collected for the analysis of lactate. The lactate concentration was measured by the enzymatic amperometric biosensor (EKF-diagnostic). The precipitation was transferred in a 1.5 mL centrifugation tube, which has been weighed after drying at 100 °C for 12 h, washed with dist. water and centrifuged at 14000 rpm for 5 min, repeated twice. The biomass was then determined though the weight differential between the empty tube and the tube with cells after drying at 100 °C for 24 h. The optical density (OD) of fermentation broth was determined by the UV/Vis-spectrophotometer (Helio λ, Thermo Fisher Scientific Inc., Waltham, USA) at 600 nm. The particle sizes distribution of biomass in the fermentation broth was determined by the laser scattering particle size analyzer Mastersizer S (Malvern Instruments).

Results and discussion:

Characterization of membranes in pure water and fermentation broth

Water flux of all the membranes, which were found to vary linearly with increasing TMP and increased in the order of 0.2 µm > 50 nm > 100 kDa >20 kDa, were measured before the each run. The membrane permeability in water was compared with the value of new membranes to check whether the membrane was cleaned adequately. After two or three times filtration of fermentation broth, membrane permeability of pure water could not be recovered to the initial level, since a irreversible block of membrane pores occurred. The strongest permeability loss was observed in the filtration using 0.2 µm membrane, thereby the membrane permeability in pure water reduced from 1512 to 1089 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$, i.e. nearly a third of the permeability couldn´t be recovered efficiently through the cleaning process. In contrast, the reductions of membrane permeability in water were not as much as 20% on the other 3 membranes.

Due to the cells or cell fragments, which could form a covering layer on the membrane surface or in some cases block the membrane pores, the permeate flux were much lower than pure water and decreased rapidly after the filtration started. Fig 3 shows the filtration of LA fermentation broth using the 100 kDa membrane at 0.4 bar and with the different cross flow velocities (0.8 and 1.6 $m \cdot s^{-1}$, respectively). After the filtration began, the permeate flux decreased within 30 min and gradually maintain in the steady stage. The results indicate that the higher flux was obtained at 1.6 $m \cdot s^{-1}$ and the similar trend was found at 1.6 bar as well. This is due to the minimization of covering layer formation and concentration polarization through greater convective force [7].

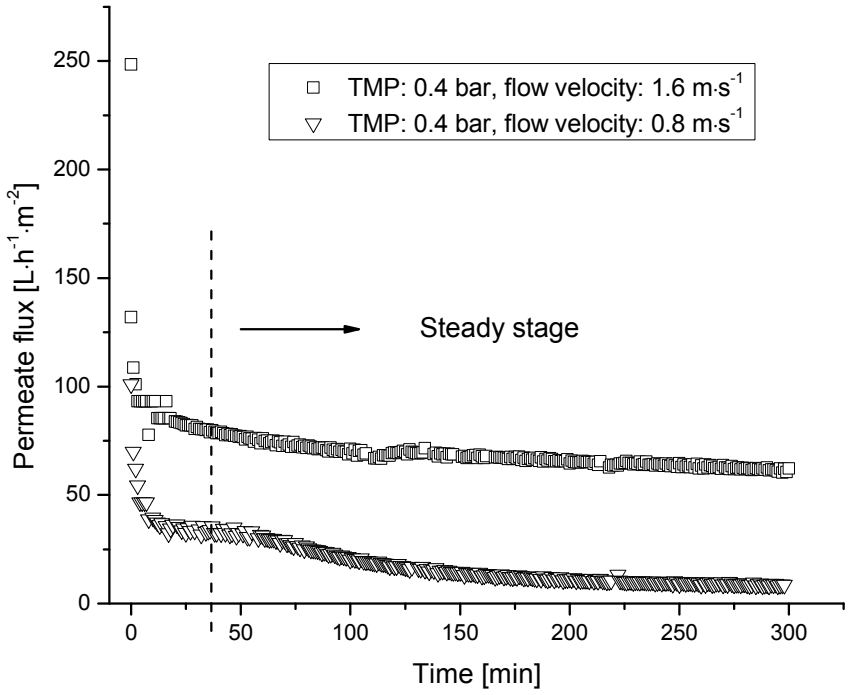

Fig. 3 Filtration of LA fermentation broth using 100 kDa ceramic membrane at different flow velocities. Cell density (*Bacillus coagulans*): ~2.5 g·L^{-1}, lactate concentration: ~45 g·L^{-1}, temperature: 53 °C

However, different from the linearly increasing via rising TMP in pure water, the permeate flux could not be enhanced efficiently through increasing TMP when the filtration of fermentation broth was operated at the constant flow velocity. This is because a compacter filtration cake is preferred to be formed on the membrane surface at the higher TPM and causes a higher transference resistance [7]. Considering the investment and space requirement, the permeate flux in steady stage should maintain permanently as high as possible during the filtration. Thus, through the comparison of the mean permeate flux and its reduction within the last hour (as shown in Tab. 1), the operation with a high flow velocity (1.6 m·s^{-1}) and at a low TMP (0.4 bar) is more favourable for the further investigation.

Tab. 1 Permeate flux and flux reduction after 4 h of running time at different TMP and flow velocity. MWCO: 100 kDa, cell density (*Bacillus coagulans*): ~2.5 g·L^{-1}, lactate concentration: ~45 g·L^{-1}, temperature: 53 °C

Flow velocity [m·s^{-1}]	TMP [bar]	Mean permeate flux within 5. h [L·h^{-1}·m^{-2}]	Permeate flux reduction within 5. h [%]
1.6	0.4	62.7±1.0	6.0
	1.6	44.9±1.2	8.5
0.8	0.4	9.2±0.5	11.9
	1.6	19.2±0.3	4.4

However, cell damage might occur at the too high flow velocity, which causes the high shear force. Therefore, the particle size distribution of biomass was traced in order to observe whether the cells burst while the filtration (Fig. 4). The left peak didn´t move while the right peak shifted to left and diminished gradually during the filtration. It means the single cells kept their shapes while cell agglomerate dissociated of the through the recycling of fermentation broth.

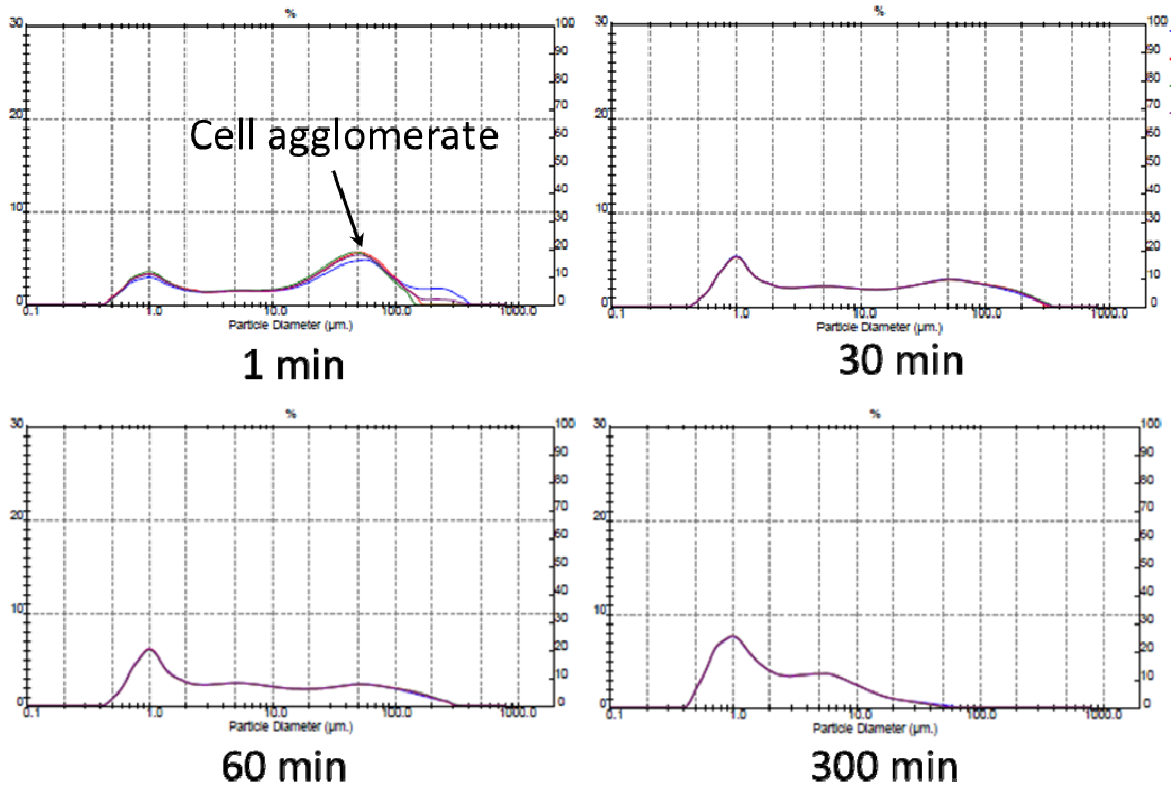

Fig. 4 Particle size distribution of biomass during the filtration. Flow velocity: 1.6 $m \cdot s^{-1}$, biomass concentration (*Bacillus coagulans*): ~ 2.5 $g \cdot L^{-1}$, temperature: 53 °C

Characterization of the optical sensor in fermentation broth

The optical sensor was calibrated in the diluted fermentation broth, in which the cells were already well dispersed after filtration mentioned above. The calibration is compared with the correlation between OD_{600} and cell density as shown in Fig. 5. The turbidity as the output signal of the sensor has a definite linear relationship with the cell concentration and the linear relationship is still valid in the cell suspension with higher concentration (> 1 $g \cdot L^{-1}$), which is already beyond the valid range of photometric method (<0.3 $g \cdot L^{-1}$). The preliminary results indicate that this sensor is advantageous for the further development of the biomass online monitoring.

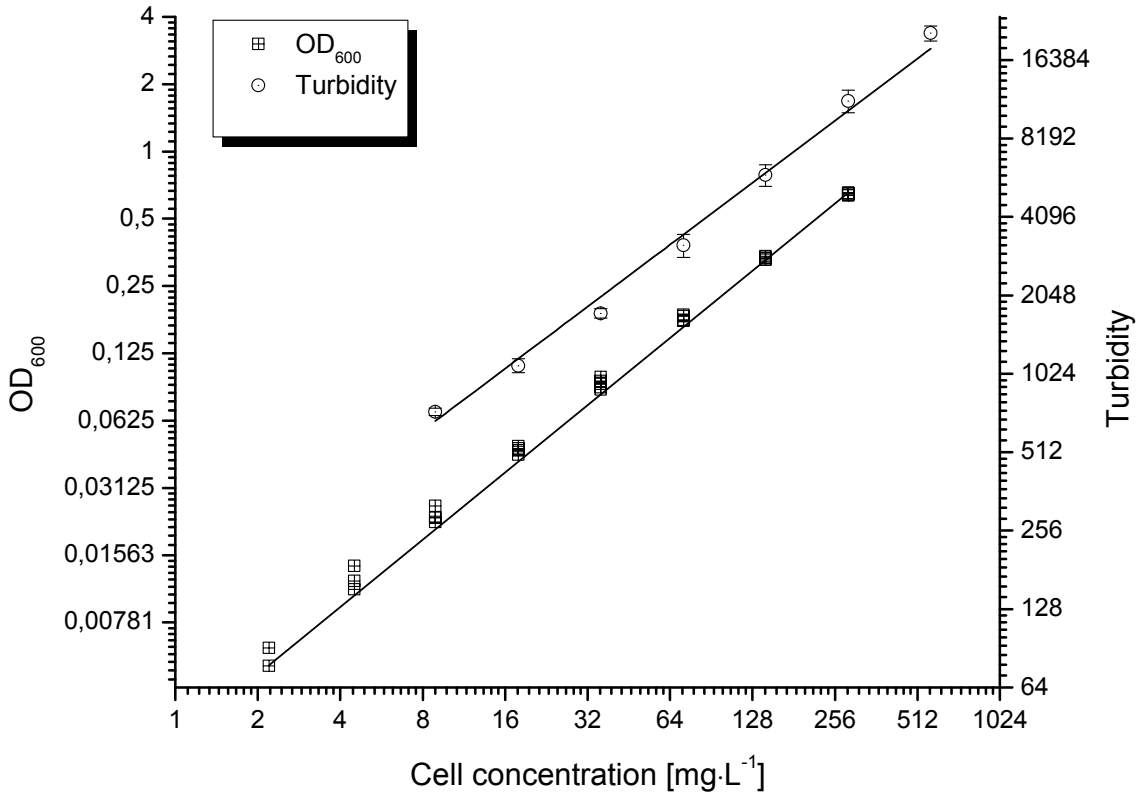

Fig. 5 Linear correlation between the output signal of the Afguard® sensor and cell concentration in comparison with the calibration of cell concentration with OD_{600}

Conclusions and outlook:

In this study, evaluations of the membrane performance in fermentation broth were fulfilled. The characterization of the innovative optical sensor was carried out for the in-situ monitoring of the biomass concentration in the MBR system. The results indicated that LA could be efficiently removed from fermentation broth using UF-membranes at a low TMP and a high flow velocity, while the biomass were retained in fermentation system without significant cell damage. The influences of further parameters, such as biomass and sugar concentration are presently being investigated in details. Furthermore, the implementation of the membrane module in fermenter for continuous LA production, which is monitored by the integrated optical sensor, is going to be conducted in upcoming experiments.

References:

[1] Y.J.Wee, J.N.Kim, H.W.Ryu, Biotechnological production of lactic acid and its recent applications, Food Technol. Biotechnol. 44(2) (2006) 163

[2] C. Müller, A. Fosmer, B. Rush, et al., Lactic acid properties, applications and Production: A review. Trends in Food Sci & Technol., (2012) doi: 10.1016/j.tifs.2012.11.007

[3] P. Pal, J. Sikder, S. Roy, L. Giorno, Process intensification in lactic acid production: A review of membrane based processes, Chem Eng: Process intensifi. 48 (2009) 1549

[4] Z.D. Lu, M. Wei, L. J. Yu, Enhancement of pilot scale production of L(+)-lactic acid by fermentation coupled with separation using membrane bioreactor, Process Biochem. 47 (2012) 410

[5] H. Quitmann, R. Fan, P. Czermak: Acidic organic compounds in beverage, food and feed production, Advances in Biochemical Engineering/Biotechnology (2013) in press

[6] C. Schiraldi, V. Adduci, V. Valli, et al., High cell density cultivation of probiotics and lactic acid production, Biotechnology and Bioengineering, 82 (2) (2001) 213

[7] J. Sikder, S. Chakraborty, P. Pal, et al., Purification of lactic acid from micro filtration broth by cross-flow nanofiltration, Biochemical Engineering Journal, 69 (2012) 130