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Production of Lactic Acid from Microalgal Biomass *Chlorella vulgar* ESP-31 as a feedstock using PVA Immobilized Bacteria *L. Plantarum* 23

Atika Nandini [1] *, Dilirani Nagarajan [2], Jo-Shu Chang [2,3]

[1]Department of Chemical Engineering, Universitas Pembangunan Nasional "Veteran" Surabaya, East Java, Indonesia
[2]Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan
[3]Research Center for Energy Technology and Strategy, National Cheng Kung University, Tainan, Taiwan

**Corresponding author:*
E-mail:
atika.nandini.tk@upnjatim.ac.id

ABSTRACT

Lactic acid is a valuable industrial chemical that is mostly used in the food and non-food industries such as the pharmaceutical industry. Production of lactic acid from renewable materials can be an alternative method to reduce the high production cost associated with raw material acquirement. In this study, polyvinyl alcohol (PVA) immobilized *L. plantarum 23* was used. To obtain high lactic acid productivity and yield from renewable feedstock, the optimal fermentation conditions were determined in both batch and continuous mode. The renewable feedstock used was microalgal biomass *Chlorella vulgaris* ESP-31. The optimal conditions for this fermentation are pH 5.5, temperature 30°C, PVA particle loading 12.5%, PVA concentration 5.25g cell/L, HRT: 2-4 hrs, carbon source concentration 40 g/L. The feedstock was pretreated and hydrolyzed appropriately and the reducing sugars obtained were used. With microalgal sugars as a feedstock in continuous fermentation mode, the maximum lactic acid productivity of 12.59 g/L/h was achieved, compared to glucose (7.39 g/L/h). The highest yield achieved in this study (0,98 g/g) was obtained when using pure glucose as the feedstock. Considering high productivity as the most important parameter, microalgal biomass seems to be the best feedstock for lactic acid production in continuous fermentation, giving high productivity and yield of 12.59 g/L/h and 0.91 g/g, respectively.

Keywords: L. plantarum 23, lactic acid fermentation, renewable feedstock, *C. vulgaris* ESP-31

Introduction

Lactic acid (LA) was one of the most important industrial products that were mostly used in many industries including cosmetic, food, chemical, and pharmaceutical industries. It is also used for the production of biodegradable plastics (PLA), which is a promising biocompatible and environmentally friendly alternative for fossil fuel-derived plastics. The demand for LA has been estimated to grow yearly at 5-8% (Yadav, Chaudhari, & Kothari, 2011). LA can be obtained by chemical synthesis or by microbial fermentation. Almost 90% of all LA production is achieved by microbial fermentation (Zhou *et al.,* 2006). Despite that, the major challenge in LA production is the high primary production cost which is associated with the acquirement of raw materials, expensive nitrogen and carbon source, the downstream recovery and purification process.

The important parameters for efficient and economic LA production are higher production of LA and inexpensive raw material. To achieve high productivity and yield for LA production, two fermentation strategies (batch and continuous mode) were investigated. Although batch fermentation is the most used in LA production, it also has several disadvantages including low productivity due to long fermentation times and low cell concentrations. To solve this problem, another

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method such as continuous fermentation was investigated. However, these methods have some disadvantages and need to develop these processes to achieve efficient LA production. The fermentation using immobilization of microorganism in polymeric matrices have been reported to be efficient (Abdel-Rahman, Tashiro, & Sonomoto, 2011). The continuous fermentation using PVA immobilization of cells can achieve high LA productivity and yield.

In addition to reducing the cost of raw materials, renewable feedstock was used as an alternative feedstock to produce LA. The demand for LA has increased considerably due to its wide range of applications, but the high cost of the raw materials which accounts for the highest portion of the production cost is known as one of the most serious problems for LA fermentation (Datta *et al.,* 1995). Renewable feedstock such as microalgae biomass has been considered as a potential source because it has high sugar and carbohydrate content.

Research Method

Bacterial strain and media

The bacterial strain used in this study is *L. plantarum 23*. The condition of pre-culture for *L. plantarum* 23 at 30°C. It was cultured in modified MRS (deMan, Rogosa and Sharpe) medium contains peptone, 10 g/L; beef extract, 10 g/L; Tween 80, 1 mL/L; dipotassium phosphate, 2 g/L; sodium acetate, 5 g/L; ammonium citrate, 2 g/L; magnesium sulphate, 0.1 g/L; manganese sulphate, 0.05 g/L. The glucose concentration in the medium was varied according to the experimental requirements. For pre-culture, the *L. plantarum* 23 was cultured in 100 mL modified MRS medium with 20 g/L glucose in a 500 mL flask at 30 ℃with 200 rpm agitation for 14 h.

Immobilization of bacterial cells

L. plantarum 23 was cultured in 100 ml modified MRS (Man, Rogosa, Sharpe) medium for 12-14 hours. The fermentation broth was centrifuged at 10000 rpm for 5 minutes at 25℃. The supernatant was discarded, cells were washed once with sterile normal saline (0.9% w/v NaCl), and resuspended in 20 ml sterile normal saline. A 20 ml of sterile normal saline with the cells was mixed with 80 ml of 10.5% (w/v) polyvinyl alcohol. After thorough mixing, the immobilized cell beads were formed by dropping the mixture in a buffer solution composed of 0.1M $Na_2HPO_4 \cdot 12H_2O$, 0.4M $NaH_2PO_4 \cdot 2H_2O$, and 1.13M H_3BO_3 for 8 hours. The beads were rinsed using 4℃ ice water and were stored in sterile ice-cold water for 2 days.

Batch and continuous fermentation

A bead of PVA immobilized *L. plantarum* 23 (with a cell concentration of 5.25 g/L) was reactivated in 200 ml modified MRS medium containing 20 g/l glucose in a 500 ml flask at 200 rpm and 30℃ for 15 h. The reactivated PVA immobilized beads were transferred to a 0.25 L reactor containing a fresh modified MRS medium with a glucose concentration of 40 g/L to form the 0.2 L of final working volume. The PVA particle loading of 12.5% was used for the fermentation. Argon was used to replace the air in the fermenter and 1 ml of L-cysteine HCl (100 g/l) was added into the medium. The fermentation was conducted under anaerobic conditions at 30 °C, pH 5.5, and 200 rpm.

The continuous fermentation was initiated as a batch culture with an initial glucose concentration of 40 g/L. The conditions of fermentation were at 30 °C, pH 5.5, 200 rpm agitation, and anaerobic condition. The culture conditions were maintained at a pH of 5.5 by 5 N NaOH solutions. The fresh nutrition modified MRS medium including 40 g/l glucose and 0.5 g/L L-cysteine HCl was fed into the reactor after the batch mode of 14 hrs. The varying HRT (hydraulic retention time) of 4, 3, and 2 hrs were used in this fermentation. To carry out the LA production from renewable materials as a feedstock, the glucose concentration of 40 g/L as a carbon source was replaced by microalgal hydrolysate.

Renewable feedstock (Chlorella vulgaris ESP-31)

Microalgae (*Chlorella vulgaris* ESP-31) biomass was consist of carbohydrate, 46.88%; protein, 13%; lipid, 20.55% and ash, 12.11% (on a dry weight basis). The dry microalgae biomass needs to be hydrolyzed to release the simple sugar component. The acid pretreatment was used for hydrolysis of the algal biomass (sulfuric acid 4%, 120ºC, 29 min). The hydrolysate was neutralized with calcium carbonate, centrifuged, filtered and the composition was analyzed. The total reducing sugar concentration was 25.8 g/L (glucose, 21.76 g/L; xylose, 2.66 g/L and arabinose, 1.38 g/L). A 0.56 g/L of HMF and 0.12 g/L of furfural were present in very low concentration as fermentation inhibitors.

Analytical methods

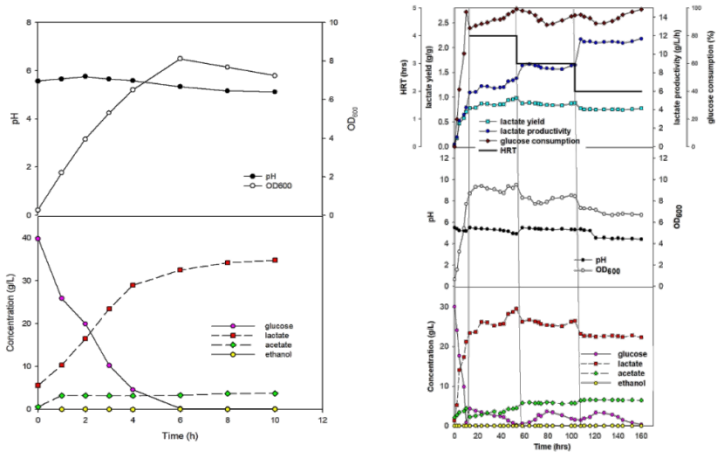
The biomass concentration of *L. plantarum* 23 was determined by the absorbance wavelength at 600 nm using UV/Vis spectrophotometer (Model U-2001, Hitachi, Japan). The concentration of sugar, lactic acid and other by-products (acetic acid, ethanol) were determined by HPLC equipped with ICSep ICE-COREGEL 87H3 Column and refractive index detector (RID). The mobile phase used was 0.008 N H2SO4 at the flow rate of 0.4 mL/min. The column temperature was maintained at 70 ℃and the injection volume was 20 µL. The concentration of sugar, lactic acid, and by-products (acetic acid, ethanol) were calculated from the standard calibration curve.

Results and Discussion

LA production using pure glucose as a carbon source

As shown in Figure 1(a), LA concentration, yield, and productivity from batch fermentation using PVA immobilized *L. plantarum* 23 were 34.81 g/L, 0.87 g/g, and 3.48 g/L/h, respectively. A 3.73 g/L of acetic acid was produced as a by-product during batch fermentation.

Compared to batch fermentation, Figure 1(b) shows in continuous fermentation can achieve a maximum lactate concentration of 29.56 g/L with yield and productivity of 0.98 g/g and 7.39 g/L/h, respectively. Continuous LA fermentation is attractive in terms of overcoming the by-product inhibition that occurs in batch fermentation by diluting the product in the fermentation broth with fresh medium (Wee & Ryu, 2009). Considering productivity, yield and production, continuous fermentation is more effective compared to batch fermentation, with high productivity and yield.



A B

Figure 1. Lactic acid fermentation using glucose as the carbon source with immobilized cell (a) batch fermentation, (b) continuous fermentation

LA production using microalgae biomass as a feedstock

The microalgae biomass of *Chlorella vulgaris* ESP-31as a feedstock for LA production and the result shown in Fig. 2. The initial sugar concentration after microalgae hydrolysate mixed with modified MRS medium was 46.88 g/L (glucose, 40.35 g/L; xylose, 3.66 g/L, and arabinose, 2.87 g/L). In batch fermentation, LA concentration of 40.30 g/L was attained with a maximum productivity of 6.72 g/L/h and a yield of 0.97 g/g (Figure. 2(a)). The hexose (glucose) was utilized first to produce LA. After glucose was completely consumed, bacteria consumed xylose and arabinose, and convert it to LA. Acetic acid concentration as a by-product increased to 5.55 g/L due to the metabolism of pentose. Figure 2(b) showed the performance of continuous LA production. the performance of continuous LA production. A maximum lactate concentration of 40.58 g/L was achieved with a high yield of 0.98 g/g when using reducing sugar concentration of 40 g/L with PVA immobilized cell and HRT of 4 hours. The highest productivity of 16.17 g/L/h was obtained in HRT 2 hours. A higher acetic concentration of 5.63 g/ L was produced during fermentation in HRT 2 hours as a by-product. Lactate concentration of 37.76 g/L was achieved with yield and productivity of 0.91 g/g and 12.59 g/L/h when using reducing sugar concentration of 40 g/L with PVA immobilized cell and HRT of 3 hours. The inhibitor (acetic) concentration of 5.53 g/L was produced during this fermentation as a by-product.

Table 1 showed that using continuous fermentation was better than using batch fermentation. Considering the yield, productivity and LA production, continuous fermentation using HRT 3 hours seem to be the optimal condition compared to the HRT 4 and 2 hours. Microalgae are typically rich in proteins, and the biomass used in this fermentation has a protein content of 13%. Also, microalgae are a natural source of vitamins, minerals, and other essential nutrients. When microalgal hydrolysate was used as a carbon source, the organic nitrogen content of the medium increased from 6.12 g/L (glucose-MRS medium) to 8.52 g/L. Amino acid analysis indicated that the predominant amino acids present in the hydrolysate were serine, methionine, histidine, glutamic acid, aspartic acid, cysteine, phenylalanine, and tryptophan. Of these, glutamic acid, histidine, serine, and aspartic acid were preferentially utilized by *L. plantarum* 23.

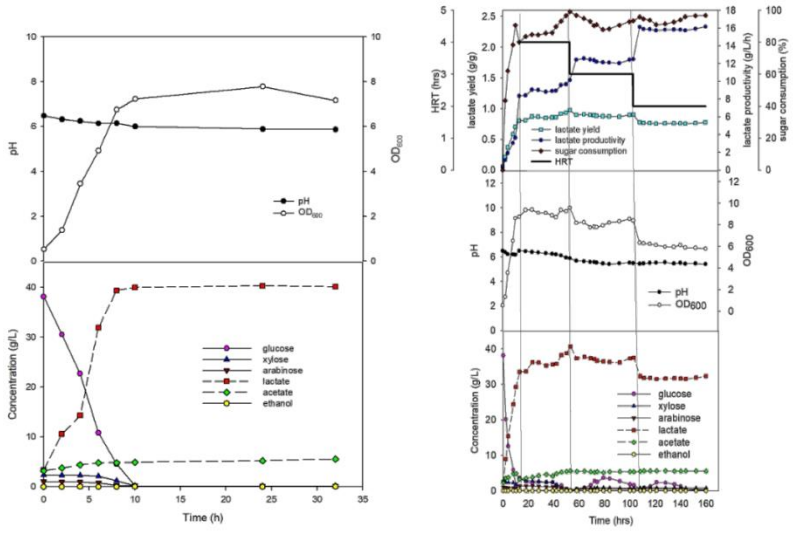


Figure 2. Lactic acid fermentation using microalgae hydrolysate as the carbon source with immobilized cell (a)batch fermentation, (b) continuous fermentation

Table 1. LA production using different fermentation strategies using microalga hydrolysate as a feedstock

Fermentation mode	LA concentration (g/L)	LA yield (g/g)	LA productivity (g/L/h)	Sugar consumption (%)	Acetate concentration (g/L)
Batch	40.30	0.97	6.72	99.11	5.55
Continuous					
HRT 4 hrs	40.58	0.98	10.15	99.22	5.6
HRT 3 hrs	37.76	0.91	12.59	96.90	5.53
HRT 2 hrs	32.33	0.78	16.17	96.80	5.63

Conclusion

Increasing LA productivity by using PVA immobilized *L.plantarum* 23 and microalgae biomass as a renewable feedstock was determined. Microalgal biomass seems to be a promising low-cost feedstock for lactic acid production on continuous fermentation mode, giving high productivity and yield obtained of 12.59 g/L/h and 0.91 g/g, respectively. The microalgal biomass (*C. vulgaris* ESP-31) has high sugar content (glucose, xylose, and arabinose) and also rich in other essential nutrients like proteins, vitamins, and minerals. So it can simultaneously provide carbon source and nitrogen source. This leads to marked enhancement in lactic acid production.

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References

Abdel-Rahman, M. A., Tashiro, Y., & Sonomoto, K. (2011). Lactic acid production from lignocellulose-derived sugars using lactic acid bacteria: Overview and limits. *Journal of Biotechnology, 156*(4), 286-301

Datta, R., Tsai, S.-P., Bonsignore, P., Moon, S.-H., & Frank, J. R. (1995). Technological and economic potential of poly (lactic acid) and lactic acid derivatives. *FEMS Microbiology Reviews, 16*(2-3), 221-231.

Wee, Y. J., & Ryu, H. W. (2009). Lactic acid production by Lactobacillus sp. RKY2 in a cell-recycle continuous fermentation using lignocellulosic hydrolyzates as inexpensive raw materials. *Bioresource Technology, 100*(18), 4262-4270.

Yadav, A. K., Chaudhari, A. B., & Kothari, R. M. (2011). Bioconversion of renewable resources into lactic acid: an industrial view. *Critical Reviews in Biotechnology, 31*(1), 1-19.

Zhou, S., Shanmugam, K. T., Yomano, L. P., Grabar, T. B., & Ingram, L. O. (2006). Fermentation of 12% (w/v) glucose to 1.2 M lactate by Escherichia coli strain SZ194 using mineral salts medium. *Biotechnology Letters, 28*(9), 663–670.