Bioaugmentation with Lactobacillus delbrueckii to enhance the production of lactic acid from municipal biopulp

Alvarado-Morales, Merlin; Tsapekos, Panagiotis; Bosma, Elleke Fenna; Baladi, Samin; Angelidaki, Irini

Publication date:
2019

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):
Alvarado-Morales, M., Tsapekos, P., Bosma, E. F., Baladi, S., & Angelidaki, I. (2019). *Bioaugmentation with Lactobacillus delbrueckii to enhance the production of lactic acid from municipal biopulp*. Abstract from 16th World Congress on Anaerobic Digestion, Delft, Netherlands.

Bioaugmentation with *Lactobacillus delbrueckii* to enhance the production of lactic acid from municipal biopulp

Merlin Alvarado-Morales[1], Panagiotis Tsapekos[1], Elleke F. Bosma[2], Samin Baladi[1] and Irini Angelidaki[1]

[1]Department of Environmental Engineering, Technical University of Denmark, Kgs. Lyngby, DK-2800, Denmark
[2]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kgs. Lyngby, DK-2800, Denmark
(E-mail*: meal@env.dtu.dk; ptsa@env.dtu.dk; elfebo@biosustain.dtu.dk; sambal@env.dtu.dk; iria@env.dtu.dk*)

Abstract
Municipal biopulp originated from the organic fraction of source separated municipal waste can be used as a feedstock for the production of lactic acid (LA) due to the high content of fermentable carbohydrates and its indigenous content lactic acid producing bacteria. In this study, the fermentation of biopulp with *Lactobacillus delbrueckii* as lactic acid bacteria (LAB) was examined. Additional parameters (i.e. pH adjustment, autoclavation) were also examined to define the optimal conditions to achieve high LA yield. Results showed that inoculation with 10% v/v of *L. delbrueckii* adjusting the pH at 6.2 was connected with the highest LA yield (0.65 g/g_{Carbs}). However, pH adjustment without bioaugmentation was also associated with high yield (0.57 g/g_{Carbs}) due to the presence of homo- and hetero-fermentative LAB in the indigenous microcosmos. Overall, the results showed that biopulp could be a suitable substrate for LA production.

Keywords
Municipal biopulp; lactic acid; *Lactobacillus delbrueckii*

Session – *Bio-polymer production using anaerobic system*

INTRODUCTION

Valorisation of municipal biowaste can stand as an alternative for lactic acid (LA) production. LA is used as platform chemical to produce biodegradable polymers (i.e. poly-lactic acid (PLA)), chemicals, etc. Members of the *Lactobacillaceae* family are known for their high tolerance to acidic pH, high salt and ethanol concentrations (Bosma et al., 2017). These properties can be helpful for the fermentative LA production from biopulp; as biopulp can present the aforementioned adverse characteristics. Among *Lactobacillaceae*, the homofermenter *Lactobacillus delbrueckii* is commonly used in industrial processes as it can produce D-lactate of 100% optical purity. However, municipal biowastes are rather intact after collection and thus, efficient pretreatments are needed to make available fermentable carbohydrates for LAB. Biopulping is reported as cost-effective method to pretreat recalcitrant municipal biowastes (Khoshnevisan et al., 2018). Hence, it is worth to investigate the use of municipal biopulp after pulping treatment for LA production. The aim of this work was to evaluate the fermentative production of LA from municipal biopulp and *L. delbrueckii* as bioaugmentation inoculum. Abiotic augmentation was investigated to define the effect of indigenous microcosmos. Conditions (e.g. pH, autoclavation) leading to highest LA yield were revealed in batch operation.

MATERIALS AND METHODS

Substrate, inoculum, and fermentation experiments

Pretreated biopulp was provided by HCS A/S Transport & Shipping, Copenhagen. *Lactobacillus delbrueckii* DSM 20074 was used as bioaugmentation inoculum. Inoculum was initially grown at 37 °C for 24 h in 20 mL flasks at a concentration of 10% (v/v) along with 90% (v/v) of sterilized MRS medium and pH was adjusted to 6.2. Batch fermentations were conducted in serum bottles (SB´s) with total and working volume of 250 and 100 mL, respectively. All SB´s were filled with 90% (v/v) biopulp and sealed with rubber stoppers and aluminium crimps and flushed with N_2 for

five minutes to ensure anaerobic conditions. The SB´s were inoculated with 10% (v/v) of exponentially growing bacteria at 37 °C. SB´s assays were prepared to investigate the effect of pH adjustment (i.e. 6.2), the influence of bioaugmentation with *L. delbrueckii*, and the impact of autoclavation of biopulp before fermentation. All experiments were conducted in duplicates.

Analytical methods

Dry and organic matter and pH were measured according to Standard Methods (APHA, 2005). Volatile fatty acids (VFA) concentrations in biopulp and fermentation broth were determined with a GC-TRACE 1300. Soluble carbohydrates, ethanol, succinic, lactic and formic acids, were quantified by HPLC.

RESULTS AND DISCUSSION

Fermentation of municipal biopulp to lactic acid

Abiotic augmentation experiments led to a LA titer of 13.88 g/L in the final broth (Table 1). Furthermore, 43% of the available sugars were fermented to LA during the abiotic augmentation. Thus, the presence of indigenous populations of fermentative LAB in the municipal biopulp is clearly indicated. Thus, the natural microcosmos could naturally produce LA. However, the initial pH of the abiotic experiment was low (pH=4.9) due to acidity of biopulp. In contrast, the LA titer was significantly boosted by 17% when the pH was adjusted to 6.2.

Table 1. Experimental design and results of LA fermentation using municipal biopulp.

Bioaugmentation, *L. delbrueckii*	pH adjustment	Autoclavation (121 °C at 100 kPa for 20 min)	Final titer, g/L	Yield, g/g_{Carbs}	Productivity, g/L/h
✗	✗	✗	13.88	0.43	1.91
✗	✓	✗	16.27	0.57	2.47
✓	✗	✗	14.64	0.47	2.07
✓	✓	✗	18.41	0.65	2.85
✓	✗	✓	8.20	0.16	0.72
✓	✓	✓	9.26	0.22	0.95

When inoculation with *L. delbrueckii* was performed without pH adjustment, an insignificant impact compared to the abiotic test was observed. Most probably *L. delbrueckii* could not compete with the indigenous microcosmos already adapted to the more acidic conditions. In the bioaugmentation tests with pH adjustment, the highest LA titer (18.41 g/L) was recorded. At optimal pH with *L. delbrueckii* inoculation the LA titer was improved by 32% compared to the abiotic augmentation. Contrariwise, autoclavation had a negative impact. Autoclavation was applied to boost the release of monosaccharides and make them available to LAB. However, both autoclaved samples had significantly lower final titers, yields, and productivities (Table 1). Ethanol (4.5 g/L) and acetic acid (5.5 g/L) were the major by-products. VFA were increased in the absence or presence of *L. delbrueckii* showing that the natural microcosmos was still active also in the bioaugmentation tests.

CONCLUSIONS

This study demonstrated that LA production can be achieved using municipal biopulp. Specifically, 0.65 g/g_{Carbs} were produced when *Lactobacillus delbrueckii* was used as bioaugmentation inoculum

reaching a final titer of more than 18 g/L at a productivity of 2.85 g/L/h. In addition, semi-controlled natural fermentation by pH adjustment leaded to comparable values.

ACKNOWLEDGEMENTS

Authors would like to acknowledge financial support to Københavns Kommune and the project "CPH-Biopulp-Upgrading, Biowaste from Copenhagen municipality as a useful resource: Investigating possibilities for valorising the biowaste from households"

REFERENCES

Bosma, E.F., Forster, J., Nielsen, A.T., 2017. Lactobacilli and pediococci as versatile cell factories – Evaluation of strain properties and genetic tools, Biotechnol Adv. 35, 419-442.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, Washington, DC, USA, American Public Health Association

Khoshnevisan, B., Tsapekos, P., Alvarado-Morales, M., Rafiee, S., Tabatabaei, M., Angelidaki, I., 2018. Life cycle assessment of different strategies for energy and nutrient recovery from source sorted organic fraction of household waste. J. Clean. Prod. 180, 360–374.