Direct production of lactic acid from source-sorted organic household waste: focusing on bio-augmentation application

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Lactic acid (LA) is an important platform molecule and widely applied in food and chemical industry. Particularly, it attracts more attention for the application in the production of biodegradable poly-lactic acid (PLA) polymer, alternative to conventional plastic (Bruno et al., 2018). Nowadays, microbial fermentation is the main method for LA production due to high optical purity and low energy consumption (Li et al., 2015). However, high cost of raw material is still the obstacle for industrial LA production.

As an inescapable issue, a large amount of household waste was generated, approximately 250 million tons every year in European Union (EU) (Eurostat, 2018). Furthermore, mandatory source separation of organic household waste has set as targeted in EU by the year of 2020 (European Parliament, 2015). Therefore, it supplies the convenience to develop cost-effective bio-industrial process to treat the household waste and produce bio-based molecules. To this respect, the sugar content in dry source-sorted household waste (SSOHW) can reach 30-40 % w/w, settling it as a promising candidate for industrial LA production.

LA production by pure lactic acid bacteria (LAB) culture is not always feasible due to the high cost of sterilization for substrate and equipment, and limited capability for hydrolytic decomposition of complex materials in SSHOW (Alves et al., 2018). Fermentation of SSHOW with indigenous microbes is usually resulting in low LA yields and mix optical isomer due to presence of various LAB taxa, as well as other acidogenic bacteria competing for same substrate leading to alternative products than LA. In the present study, bio-augmentation with LAB was applied as a mean to address these challenges with non-sterile SSOHW.

The SSHOW was collected form the HCS A/S Transport & Spedition (Glostrup, Denmark) in the form of biopulp. Two LABs were used as bio-augmentation strains: *Lactobacillus delbrueckii DSM 20074* and *Pediococcus acidilactici NCIMB 702925*. The de Man, Rogosa and Sharpe (MRS) medium was used to culture the bacterial strains. Initially, batch experiments were conducted in 235 mL serum bottles with 90 mL substrate and 10 mL inoculum. After sparged with N_2 for 10 min, the bottles were placed in a mesophilic incubator (37 °C). Subsequently, the LA production was examined with sterilized (121 °C for 20 min) and pasteurized (70 °C for 30 min) SSHOW. The impact of indigenous microbes was investigated and the fermentation tests were conducted at the same conditions as the first batch. The LA and sugar content were measured using high performance liquid chromatography (HPLC) and volatile fatty acids (VFAs) were evaluated by gas chromatograph.

Table.1 shows the LA and VFAs changes during fermentation process with *L. delbrueckii* and *P. acidilactici* at 37 ˚C, initial pH of 6.25 and total solid loading of 70 g/kg. A continuous increased trend was observed during the first 4 days with *L. delbrueckii* and abiotic augmentation (control). In contrast, LA concentration rapidly increased at the first day of fermentation process and the highest LA titer (20.7 ± 0.3 g/L) was achieved at second day with addition of *P. acidilactici*. However, there was a slight decrease of LA concentration over the last 3 days due to the degradation of LA by H_2 and acetic acid producing bacteria. The LA yield of *P. acidilactici* and *L. delbrueckii* were 0.73, 0.60 g/g-sugar, which were 33.3% and 15.7% higher compared to control ($p<0.05$). The main optical isomer changed from D-LA to L-LA after all fermentation tests and the content of L-LA were 70.1 ± 3.1%, 62.0 ± 1.8% and 52.1 ± 1.2% for *P. acidilactici, L. delbrueckii* and abiotic treatment, respectively.

VFAs as the by-products were also produced during the fermentation process. Acetic acid and ethanol were the main by-product. The concentration of ethanol rapidly increased from 1.0 ± 0.1 to 2.4 ± 0.2, 3.2 ± 0.4 and 1.8 ± 0.02 g/L with *P. acidilactici*, *L. delbrueckii* and abiotic treatment at the first 2 days, respectively. In contrast, it was almost unchanged after the 2nd day. Regarding for acetic acid production, the same trend was obtained with *L. delbrueckii* and abiotic treatment. However, there was an obvious increase of acetic acid over the last of 2 days for *P. acidilactici* and LA concentration slightly decreased. In this study, mix culture was used and the acetic acid bacteria could degrade LA to acetic acid (Wu et al., 2015). Therefore, the LA degradation made contribution to acetic acid increase. Meanwhile, 1-propanol concentration gradually increased during fermentation process.

Table.1 Lactic acid and VFAs production with bio-augmented *Lactobacillus delbrueckii* and *Pediococcus acidilactici*

	L. delbrueckii				*P. acidilactici*				control			
	LA	Ethanol	Acetic acid	1-propanol	LA	Ethanol	Acetic acid	1-propanol	LA	Ethanol	Acetic acid	1-propanol
0	4.1±0.02	0.8±0.2	1.0±0.2	0.0±0.0	4.1±0.02	0.8±0.2	1.0±0.2	0.0±0.0	4.1±0.02	0.8±0.2	1.0±0.2	0.0±0.0
1	13.7±0.1	1.7±0.02	2.1±0.1	0.05±0.0	19.1±0.5	3.1±0.02	2.5±0.1	0.08±0.0	12.7±0.1	1.7±0.06	1.5±0.04	0.05±0.0
2	15.2±0.2	1.9±0.2	2.4±0.2	0.16±0.0	20.7±0.1	3.3±0.2	3.2±0.4	0.28±0.0	14.5±0.1	1.8±0.03	1.8±0.03	0.21±0.0
3	15.8±0.1	2.0±0.01	2.5±0.03	0.31±0.0	20.2±0.2	3.3±0.04	3.3±0.1	0.51±0.01	14.7±0.1	2.0±0.03	2.2±0.02	0.41±0.01
4	16.8±0.1	2.2±0.1	2.8±0.1	0.42±0.02	20.2±0.6	3.7±0.2	4.2±0.1	0.75±0.01	15.2±0.2	2.1±0.02	2.5±0.05	0.53±0.01
5	16.6±0.4	2.2±0.1	2.7±0.03	0.45±0.01	19.2±0.6	3.4±0.03	4.4±0.2	0.88±0.08	14.6±0.3	2.1±0.04	2.8±0.1	0.64±0.03

Concerning the sterilization and pasteurization tests, LA was only produced at first day, achieving the same LA concentration (~ 13 g/L) at both treatments (Fig. 2a). On the contrary, the LA concentration reached 29.1 ± 1.2 g/L at the control operation. Meanwhile, soluble glucose and xylose were not detected after the first day with sterilized and pasteurized SSHOW. In addition, the by-product of VFAs with sterilized and pasteurized SSHOW were always lower than control (Fig. 2b).

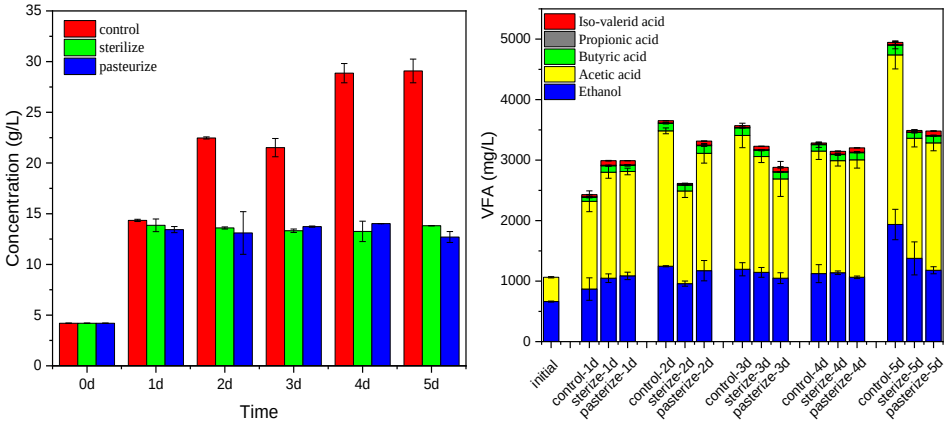

Fig. 2 Lactic acid (a) and VFAs (b) production from sterilized and pasteurized SSHOW

In conclusion, bio-augmentation with LAB could improve the LA production. More specifically, the superiority of *P. acidilactici* as bio-augmentation inoculum was revealed compared to *L. delbrueckii*. Meanwhile, indigenous microbes had contribution for sugar degradation and LA production. Inoculated LAB and indigenous community build a collaborative community with desired functionality during the fermentation process.

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