Bioresource Technology 175 (2015) 142–151

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# A closed loop for municipal organic solid waste by lactic acid fermentation

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## HIGHLIGHTS

- Efficient lactic acid production from unsterilized municipal organic solid waste.
- Fermentation at pH 5 or uncontrolled pH at 37 °C was highly productive.
- Selective enrichment of Lactobacilli during the fermentation process.
- *Lactobacillus acidophilus* was the main producer of lactic acid.
- Development of a *momentum* resulted in community shift after several days.

## ARTICLE INFO

*Article history:*
Received 4 September 2014
Received in revised form 6 October 2014
Accepted 8 October 2014
Available online 19 October 2014

*Keywords:*
Lactic acid bacteria
Unsterilized biowaste
Organic residues
*Lactobacillus*
Fermentation

## ABSTRACT

In order to investigate the feasibility of producing lactic acid from municipal organic solid waste different pH values (4–7) and temperatures (37 °C and 55 °C) were tested. For the evaluation of fermentation conditions the chemical, physical, and microbial characters were monitored over a period of 7 days. Quantitative real time PCR, PCR–DGGE, and next generation sequencing of a 16S rRNA gene library were applied to identify the key players of the lactic acid production and their association. *Lactobacillus acidophilus* and its closest relatives were found to be efficient lactic acid producers (>300 mM) under most suitable fermentation conditions tested in this study: 37 °C with either uncontrolled pH or at a pH of 5. These data provide the first step in the realization of the idea "reuse, reduce, and recycle" of municipal organic solid waste.



## 1. Introduction

Due to their non-pathogenic nature, applicability in the food and other industries, and often probiotic properties, the importance of lactic acid bacteria (LAB) has been continuously increasing during recent decades. All LAB predominantly ferment single sugars to their main metabolic end product lactic acid (LA). Depending on whether they produce LA alone or by-products such as acetic acid and $CO_2$, they are defined as either homofermentative or heterofermentative.

In addition to possible by-products, different optical isomers of LA are produced by LAB, depending on the bacterial species or family. Optical purity of LA increases application possibilities: poly-lactic acid (PLA) e.g., a biologically degradable plastic with high potential, only comes with high mechanical and thermal stability if one single isomer is polymerized (Dusselier et al., 2013).

Lactic acid is a widely employed platform chemical that is produced either by microbial fermentation or by chemical synthesis. Several hundred thousand tons are produced annually worldwide (Dusselier et al., 2013) – with increasing demand due to growing application options, notably its polymerization to PLA. In order to deal with the increasing need and the resulting decreasing price, growing production costs, and lessening availability of resources, current production procedures need to be optimized and new technologies are necessary. Many substrates, like glucose and other sugars, are unfavourable, because of their high costs and, more importantly, their alternative applicability in the food-industry. Therefore, a number of cheaper alternative substrates, not utilized in the food-industry, such as molasses (Wang et al., 2010), potato waste (Pagana et al., 2014), cafeteria garbage (Zhao et al., 2009) or *Curcuma longa* waste (Nguyen et al., 2013), have been evaluated.

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http://dx.doi.org/10.1016/j.biortech.2014.10.034
0960-8524/

An alternative idea is the utilization of municipal organic solid waste (MOSW) for LA production (Probst et al., 2013). Municipal organic solid waste is an organic waste stream consisting of kitchen and garden residues separately collected in many European countries. Currently, it is treated either mechanically or biologically by composting or anaerobic digestion (Eurostat, 2011). Because of the high energetic and nutritional potential which is not exploited in current treatment technologies, the application of MOSW as a resource for the production of organic acids, such as LA, is considered highly attractive from the viewpoint of waste management. Although a considerable potential of MOSW for volatile fatty acid (VFA) production has been exploited (Silva et al., 2013; Yesil et al., 2014) the integration of a LA production step into current European waste treatment technology has not been investigated until now. Despite many studies targeting its resource capacity and alternate application, e.g., the production of biohydrogen (Boni et al., 2013; Escamilla-Alvarado et al., 2013), its inherent microbial communities have not been identified in detail. The utilization of MOSW as a fermentation substrate with its indigenous bacteria could reduce LA production costs and the total amounts of wastes generated, provide an environmentally friendly and economically efficient waste treatment procedure, and save resources which are needed elsewhere.

If one develops this idea further, applying the biorefinery concept, the fermentation of MOSW to LA could take place prior to current treatment technologies, thus increasing the value of the MOSW. The LA produced in the fermentation could be processed to PLA and further utilized in the form of bags for MOSW collection, thus closing the waste production and treatment circle by recycling.

The aim of the study was to investigate if the indigenous microbiota of MOSW would allow for a fermentative production of LA. In addition to chemical and physical analyses to evaluate conditions of highest productivity, LA concentration, by-product formation and other parameters, special focus was given to the (LA) bacterial community to investigate microbial community dynamics. To our knowledge this is the first study targeting LA production in semi-continuous fermentation mode using unsterilized MOSW with only its indigenous microbiota including detailed community analyses.

## 2. Methods

### 2.1. Sampling, pre-treatment and fermentation conditions

Fresh, mechanically milled MOSW, collected separately from municipal areas, was taken from Höpperger GmbH Pfaffenhofen, Austria, in July 2012. The MOSW is collected weekly and subsequently stored in a tank. The samples taken may be considered representative. They also match samples of prior studies (Probst et al., 2013). To achieve a fermentable consistency, samples were homogenized using a blender and subsequently diluted 2:3 (w/w) with deionized water.

Fermentation was conducted in 150 ml volumes as a fed-batch process. Fermentation was conducted at a standard temperature of 37 °C and a thermophile temperature of 55 °C. To cover the broad pH range of LAB four different pH values 4, 5, 6, and 7 were tested at 37 °C. Fermentation at uncontrolled pH served as control. Previous studies reported enrichment of *Bacillus coagulans* in Asian kitchen waste at 55 °C and a pH of 6 allowing the production of optically pure L-LA (Tashiro et al., 2013). Therefore pH at 55 °C was set either to 6 or left uncontrolled. A shaking speed of 150 rpm was applied to ensure homogenous substrate distribution in the fermenters. All fermentations were conducted in triplicate to allow statistical analyses. Observation period was 168 h (7 d). Assuming ideal mixture of the fermentation broth a hydraulic retention time of 5 d was supposed to ensure an equilibration of approximately 90% within the fermenters.

Prior to measuring protein, reducing sugar, and ionic concentrations samples were homogenized using an ultra turrax (Heidolf DIAX 600), diluted and subsequently filtered. Samples were diluted in 0.1% $HNO_3$ for ionic concentration measurements.

### 2.2. Chemical and physical parameters

Redox potential, electrical conductivity and pH were measured directly using standard measuring probes. Amounts of reducing sugars were defined using a phenol–sulphuric acid method (Dubois et al., 1956). A dilution series of glucose (0–100 mg $l^{-1}$) was used as a standard. Protein concentration was determined using the Bradford method (Bradford, 1976). Bovine serum albumin in concentrations of 0–100 mg $l^{-1}$ was used as a standard. Concentration of Na was analyzed using atomic absorption spectrometry (contra 700, AnalytikJena, Germany) with the operating software Aspect CS 1.5.5.0. A sodium solution (in 0.1% $HNO_3$) with concentrations of 0.1, 0.2, 0.5, 1.0, and 2.0 mg $l^{-1}$ was used as a standard. Concentration of volatile fatty acids (VFA) and the optical ratio of LA were investigated using HPLC by analyzing dialysate collected after 24 h. VFA analysis was carried out on a BioRad HPX-87H column as described by Wagner et al. (2012). D-lactate and L-lactate were separated on a Chirex 3126 (Phenomenex, Germany) as described before (Probst et al., 2013).

Total solids (TS) were determined by weighing 30–50 g fresh sample into glass petri dishes. After drying at 105 °C overnight, TS were calculated from weight loss. Organic dry matter was calculated as loss of weight after igniting 1–2 g of TS in a muffle furnace (550 °C). Due to the reduction of organic matter in the fermentation broth (OM) providing information about fermentation efficiency, the amount of organic matter excluding the fraction made up by LA was calculated via subtraction of LA weight proportion from organic matter content ($OM_{-LA}$). These data allowed differentiating between fermentation efficiency producing VFAs and fermentation efficiency producing LA. A separate analysis was performed confirming that the amount of LA in the sample persisted during the drying and igniting process and was measured in the TS and organic dry matter (data not shown).

### 2.3. DNA extraction

One ml fermentation broth from each parallel reactor was collected at different time points and centrifuged at 10,000*g* for 10 min. DNA was extracted from the pellet using a "Genomic DNA from soil" kit (Macherey Nagel, Germany).

### 2.4. PCR–DGGE

The 16S rRNA gene in DNA extracts of all parallel reactors at the time points 0, 24, 72, 120, and 168 h was amplified for using primers specific for the *Lactobacillus* group (Lac 1 and Lac 2-GC), which targets the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Weissella* (Walter et al., 2001) and the *Streptococcus* group (Lac 3 and Lac 2-GC) which targets the genera *Lactococcus*, *Enterococcus*, *Tetragenococcus* and *Vagococcus* (Endo and Okada, 2005). After quantification using a PicoGreen® dsDNA quantitation kit (Molecular Probes Inc., Oregon, USA) and a fmax Fluorescence Microplate Reader (Molecular Devices, CA, USA), 60 ng of PCR products were separated by denaturing gradient gel electrophoresis (DGGE) as described by Muyzer et al. (1993) using an Ingeny PhorU2 system (Ingeny International BV, Netherlands). DGGE conditions for Lactobacilli and Streptococci were 8% Acrylamide and a denaturing Formamide and Urea gradient of 32–50% and 40–60%, respectively.