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End-product inhibition and acidification limit biowaste fermentation efficiency

Maraike Probst [a,*], Andreas Walter [a], Gilbert Dreschke [a], Flavio Fornasier [b], Thomas Pümpel [a], Janette Walde [c], Heribert Insam [a]

[a] Institute of Microbiology, University of Innsbruck, Technikerstraße 25d, 6020 Innsbruck, Austria
[b] Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per lo Studio delle Relazioni tra Pianta e Suolo, Via Trieste 23, 34170 Gorizia, Italy
[c] Department of Statistics, University of Innsbruck, Universitätsstraße 15, 6020 Innsbruck, Austria

HIGHLIGHTS

- Cascading approach of biowaste utilization offers optimized biowaste treatment.
- Solid–liquid separation increases biomethane potential of fermentation residues.
- Acid extraction counteracting end-product inhibition increases process efficiency.
- pH control counteracting acidification increases process efficiency.
- Bacterial community is influenced by treatment (and time) rather than by substrate.

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ABSTRACT

Converting waste to resource may mitigate environmental pollution and global resource limitation. The platform chemical lactic acid can be produced from biowaste and its liquid fraction after solid–liquid separation. A fermentation step for lactic acid production prior to the conversion of biowaste to methane and organic fertilizer would increase the biowaste's value. Despite the huge potential and promising results of the treatment procedure, the reasons for efficiency loss observed previously need to be addressed in order to pave the way for an up-scaling of the fermentation process. Therefore, biowaste was fermented applying pH control, acid extraction and glucose addition in order to counteract reasons such as acidification, end-product inhibition and carbon limitation, respectively. The fermentation was competitive compared to other renewable lactic acid production substrates and reached a maximum productivity of >5 g $C_{lactic\ acid}$ g^{-1} C h^{-1} and a concentration exceeding 30 g L^{-1}. A combination of acidification and end-product inhibition was identified as major obstacle. *Lactobacillus crispatus* and its closest relatives were identified as key lactic acid producers within the process using Miseq Illumina sequencing.

1. Introduction

Lactic acid (LA) is a platform chemical with wide application in the food and non-food industry. Currently it is produced from a variety of resources. For the production of biodegradable plastics made from LA, alternative substrates such as molasses (Wang et al., 2010) that do not compete with the food-industry are desirable (Dusselier et al., 2013). Also biowaste can serve as substrate for LA (Dreschke et al., 2015; Probst et al., 2015a, b). No further inoculum is needed for efficient LA production due to Lactobacilli dominating the bacterial community in biowaste, provided the process conditions are appropriate.

In many European countries organic waste from kitchens and households is collected source-separated and termed municipal biowaste. Currently it is treated either by composting or anaerobic digestion (AD). Although the production of energy is efficient a LA production step prior to AD would exploit more of the biowastés potential without significant losses in methane yield (Dreschke et al., 2015; Schneider et al., 2013). Quite the contrary, the fermentation step could optimize the initial AD phase. The typical microbial community increasing metabolic diversity and the low pH of the LA fermentation may both contribute to the depolymerization of macromolecules.

* Corresponding author. Tel.: +43 512 507 51325; fax: +43 512 507 2938.
E-mail address: Maraike.Probst@uibk.ac.at (M. Probst).

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The separation of liquid and solid fractions of the biowaste could further enhance overall productivity: The solid fraction, enriched in macromolecules and nutrients not easy to access, could be used to produce biomethane via AD. The liquid fraction, predominantly containing easily degradable nutrients, could be used for LA production prior to AD. However, Probst et al. (2015a, b) observed productivity loss, LA degradation and acetic acid (AA) accumulation during semi-continuous fermentation of both the total biowaste and its liquid fraction after 24–72 h of efficient production.

Consequently, the aim of this study was to improve the fermentation efficiency of liquid and total biowaste in a semi-continuous fermentation mode. The likely reasons for efficiency loss – acidification, end-product inhibition, and carbon limitation – were counteracted by pH control, dialysis and glucose addition, respectively. The efficiency of fermentation was analyzed according to physicochemical fermentation characteristics, such as carbon balance of the process, as well as LA and by-product productivity, product concentrations and enzymatic activities. The fermentation efficiency was evaluated depending on the three experimental factors substrate (liquid and total biowaste), treatment (pH control, dialysis and glucose addition) and time. Furthermore, the microbial community present in the fermentation substrate and the biomethane potential of fermentation residues were considered for the overall evaluation. This information shall pave the way for an upscaling of the process and justify an evaluation of socioeconomic implications.

2. Methods

2.1. Sampling, pre-treatment and fermentation conditions

This study compared the fermentation characteristics of municipal biowaste as a whole and its liquid fraction, collected in Tyrol, Austria, from municipal biowaste treatment plants in summer 2013. The biowaste was mechanically milled for homogenization at the facility. Prior to fermentation it was diluted 2:3 with deionized water to achieve a suitable viscosity (B_{total}). The liquid fraction, B_{liquid}, was obtained by sieve-pressing of the biowaste (6.3 mm mesh; 2.94 kPa pressure) after facility internal washing and homogenization.

Both sample types collected can be considered representative for source separate collected biowaste and its liquid phase as common in Austria and match samples of prior studies (Probst et al., 2013; Dreschke et al., 2015; Probst et al., 2015a, b).

Semi-continuous fermentations of liquid and total biowaste were conducted in 500 mL Erlenmeyer flasks with a working volume of 300 mL at 37 °C. Substrate was removed and fed twice a day. Removed substrate was analyzed regarding physicochemical characteristics (see Section 2.2), microbial community composition (Sections 2.3 and 2.4) and enzymatic activity (Sections 2.5 and 2.6). Flasks were orbitally shaken at 150 rpm to assure homogeneity within the reactors. In order to prevent oxygen intake, flasks were covered with plastic films. A hydraulic retention time of 2 d was applied, as was found best in a pre-experiment (data not shown). Semi-continuous fermentations were performed for 336 h (2 weeks) for B_{liquid} and 504 h (3 weeks) for B_{total}, respectively. Assuming an ideal mixture of the fermentation broth a retention time of 2 d ensures an equilibration of over 90% within these observation periods. In previous studies, we reported a productivity loss after 1–2 days, attributed to acidification, end-product inhibition, and nutrient limitation (Probst et al., 2015a, b). Excessive acidification was counteracted by daily pH adjustment to 5 using NaOH (5 M). End-product inhibition was counteracted by extraction of volatile fatty acids (VFA) via dialysis tubings (cut-off: 10,000 D; 100 mL volume – equivalent to a 1:4 dilution) changed once per day. To prevent nutrient limitations, glucose (10 g L^{-1}) was added daily. An overview of the factor 'treatment' in the fermentation experiments is presented in Fig. 1. All fermentations were carried out simultaneously and in three parallel reactors to allow for full comparisons and statistical analyses, respectively. With this experimental setup, single effects and two- and three way interaction effects can be evaluated (Fig. 1).

2.2. Chemical and physical parameters

Electrical conductivity, pH, and redox potential were analyzed using standard probes.

Dry matter (DM) was determined by drying and weighting 30–50 g of fermentation broth in glass petri dishes at 105 °C overnight. Organic dry matter was calculated from loss of weight after igniting DM in a muffle furnace (550 °C, 5 h). Total C and N

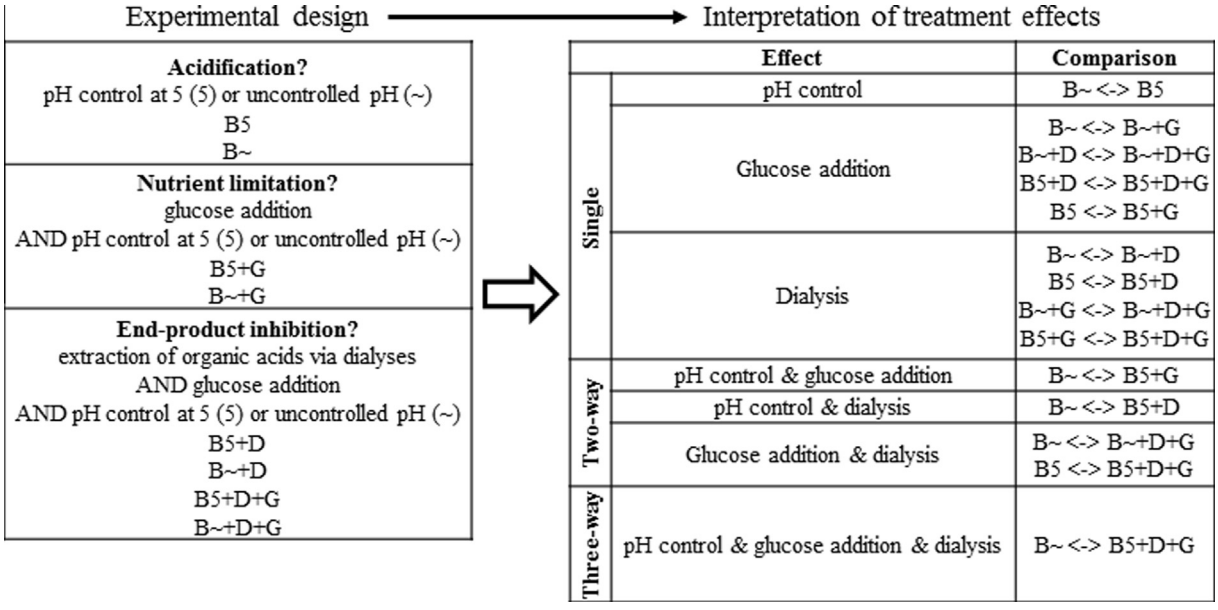

Fig. 1. Experimental design of the study and interpretation overview of the factor 'treatment' = experimental conditions.