Waste Management xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

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

# Volatile fatty acids as an added value from biowaste

Emilia den Boer [a,*], Agnieszka Łukaszewska [b], Władysław Kluczkiewicz [c], Daria Lewandowska [a], Kevin King [d], Tero Reijonen [e], Tero Kuhmonen [e], Anssi Suhonen [e], Ari Jääskeläinen [e], Anneli Heitto [d], Reino Laatikainen [f], Elias Hakalehto [d,g,h]

[a] Faculty of Environmental Engineering, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland
[b] Marshall Office of Lower Silesia, Wybrzeże Słowackiego 12-14, 50-411 Wrocław, Poland
[c] Faculty of Mechanical Engineering, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland
[d] Finnoflag Oy, P.O. Box 262, 70101 Kuopio, Finland
[e] Savonia University of Applied Sciences, P.O. Box 6, FI-70201 Kuopio, Finland
[f] Department of Pharmacy, University of Eastern Finland, Kuopio, Finland
[g] Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland
[h] Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland

ARTICLE INFO

*Article history:*
Received 23 April 2016
Revised 2 July 2016
Accepted 5 August 2016
Available online xxxx

*Keywords:*
Volatile fatty acids
Biorefinery
Biowaste
Potato waste
Bioprocess

ABSTRACT

The aim of the present work was to provide proof of concept of employing a co-culture of *K. mobilis* and *E. coli* for producing short and medium chain volatile fatty acids (VFAs) from kitchen biowaste and potato peels. To this aim, experiments were carried out at pilot-scale installation with a bioreactor of 250 L. Different feeding strategies were tested under microaerobic conditions, at pH 6.0–6.5 in order to enhance chain elongation. Acetic acid and ethanol were dominating products in the initial stages of the bioprocess, but in a relatively short time of approx. 20–22 h from the process start accumulation of propionic acid took place followed by a chain elongation to butyric and valeric acids. The highest final products yield of 325 mg/g TS was achieved for the substrate load of 99.1 g TS/L (VS of 91.1 g/L) and pH 6.5, with the productivity of 448 mg/L/h. However, the highest average VFAs chain length (3.77 C) was observed in the process run with the loading of 63.2 g TS/L and pH 6.0. In this study, we demonstrated that the existing symbiosis of the co-culture of *K. mobilis* and *E. coli* favours formation and chain elongation of VFA, induced most likely by the enhanced ethanol formation. Our finding differs from the previous research which focus mostly on anaerobic conditions of VFAs production. The results provide good basis for further optimisation of VFAs production process.



## 1. Introduction

It is generally agreed that the use of fossil resources should be limited, both due to their decreasing reserves as well as $CO_2$ emissions, which are the main contributors to global warming. In fact, an important share of the chemicals and fuels used in the economy could be replaced by adequate substances produced from various biomass sources. One of the main challenges of the bioeconomy sector is to establish viable production processes, such as biorefining (COM, 2012). The following generally agreed definition of biorefining has been developed by the International Energy Agency (IEA) "*Biorefining is the sustainable processing of biomass into a spectrum of marketable products and energy*". In the biorefinery, the value of each stream must be maximized (similar to oil refineries) (Fernando et al., 2006), which may be a key factor of their economic advantage over traditional biofuels production. Using agro-industrial residues or municipal biowaste as biorefineries substrates instead of cultivated biomass promotes further reduction in costs.

According to Agler et al. (2011) three biorefinery platforms exist - the sugar platform, the syngas platform and the third important platform – the carboxylate platform. The goal of the latter is to convert organic feedstocks into short-chain (C2-C4) carboxylates as intermediate feedstock chemicals (mostly volatile fatty acids - VFAs). The carboxylate platform has several attractive features, including feedstock flexibility; minimal feedstock pre-treatment; utilisation of most organic components of the biomass and mixed culture stability (Weimer et al., 2015). Its products can be used either as a hydrogenated mixture of acids or as pure chemicals. Pure VFAs and their derivatives are widely used in food, pharmaceutical, leather, textile and plastics industries (Dishisha et al.,

* Corresponding author.
*E-mail address:* emilia.denboer@pwr.edu.pl (E. den Boer).

http://dx.doi.org/10.1016/j.wasman.2016.08.006
0956-053X/

2013; ElMekawy et al., 2013). Pentanoic (valeric) acid, the first medium chain acid, is primarily used in the form of its esters, ketones and secondary alcohols (Holtzapple and Granda, 2009). Besides, ethanol and hydrogen are obtained during acidogenic digestion as by-products, which can be also used as fuels (Zidwick et al., 2014). However, separation of mixed VFAs into each component is difficult, due to formation of azeotropic mixture with water (Kim et al., 2013). Instead, hydrogenated mixtures of acids and alcohols can be used as substitute for fuel ethanol (Chang et al., 2010) or as substrates for microbial production of *inter alia* biopolymers (polyhydroxyalkanoates and polyhydroxybutyrate), and lipids for biodiesel (Kim et al., 2013).

Most often the carboxylate platform is based on anaerobic fermentation with undefined mixed cultures of microorganisms (Agler et al., 2011; Arslan et al., 2013). Under anaerobic conditions glucose is initially metabolized to pyruvate and pyruvate is subsequently converted to one or more of the following end products: lactate, acetate, ethanol, succinate, formate, $CO_2$ and $H_2$. A conventional anaerobic digestion process - in which a $CH_4$-rich biogas is obtained - can be altered via pH control or the addition of methanogenic inhibitors, upon which short chain VFAs (C2–C4) are extended to medium chain VFAs (C5–C8) (Weimer et al., 2015). The medium chain VFAs can be more easily recovered by extraction or other methods than short chain VFAs, moreover, medium chain VFAs are more energy-dense products than the ones which can be formed from the short chain VFAs (Singhania et al., 2013). Thus, a number of recent studies deal with shifting the VFAs production toward longer chain VFAs (Grootscholten et al., 2013a,b; Liang and Wan, 2015; Weimer et al., 2015). Basically, the production of longer chain acids is achieved by a prolonged operation (De Wever et al., 2012; Kannengiesser et al., 2016). Microorganisms can use VFAs as an electron acceptor and hydrogen or ethanol as electron donors to generate medium chain VFAs such as valerate and caproate (hexanoic acid) (Ding et al., 2010; Steinbusch et al., 2011). These reactions are described as (Agler et al., 2011; De Wever et al., 2012):

$$2\ \text{acetate}^- + H^+ + 2H_2 \rightarrow \text{n-butyrate}^- + 2H_2O \quad \Delta G^\circ r = -47.55\ \text{kJ/mol} \quad (1)$$

$$\text{acetate}^- + \text{ethanol} \rightarrow \text{n-butyrate}^- + H_2O \quad \Delta G^\circ r = -201.68\ \text{kJ/mol} \quad (2)$$

$$\text{propionate}^- + \text{ethanol} \rightarrow \text{n-valerate}^- + H_2O \quad \Delta G^\circ r = -39\ \text{kJ/mol} \quad (3)$$

$$\text{propionate}^- + \text{acetate}^- + H^+ + 2H_2 \rightarrow \text{n-valerate}^- + 2H_2O \quad \Delta G^\circ r = -48\ \text{kJ/mol} \quad (4)$$

$$\text{acetate}^- + 2\ \text{ethanol} \rightarrow 2\ \text{n-caproate}^- + 2H_2O \quad \Delta G^\circ r = -79\ \text{kJ/mol} \quad (5)$$

$$\text{acetate}^- + \text{n-butyrate}^- + H^+ + 2H_2 \rightarrow \text{n} - \text{caproate}^- + 2H_2O \quad \Delta G^\circ r = -48\ \text{kJ/mol} \quad (6)$$

In the recent studies the effects of ethanol addition on acid chain elongation has been tested (Kannengiesser et al., 2016; Weimer et al., 2015).

However, there are some limitations of the carboxylate platform based on anaerobic fermentation. According to Weimer et al. (2015) one of them is the slow rate of VFA chain extension due to lengthy incubation times under anaerobic conditions. On the other hand, Trinh et al. (2011) discovered that most pathways that synthesize advanced biofuels are not necessarily anaerobic and in fact are active under aerobic conditions, even when the host is a facultative anaerobe. Wang et al. (2006) found out that wastewater treatment under microaerobic circumstances lead to the accumulation of propionic acid in the acidogenic reactor at every pH value. According to reactions 3 and 4, propionic acid is a crucial intermediate in the chain elongation to valeric acid. Thus, one option to speed-up the production rates is shifting the bioprocess to aerobic conditions. An advantage of aerobic conditions is a faster biomass growth allowing for a shorter lag phase and more intensive production rates. Preferably facultative anaerobic organisms could be used under such circumstances. *Escherichia coli* (*E. coli*) and *Klebsiella mobilis* (*K. mobilis*) are both facultative anaerobic organisms, which additionally form a symbiotic relationship. Hakalehto et al. (2008) observed that the growth yields of both bacteria in co-culture equalled those of the separate pure cultures indicating mutual benefits of their co-existence. It was suggested that *E. coli* produced a set of organic acids, which lowered the pH, while *K. mobilis* increased the pH up to 2 units; probably due to the acid's conversion to ethanol. Possibly, ethanol formed by the co-culture could be used for VFAs chain elongation, according to reactions 2, 3 or 5.

This paper provides the main findings of an investigation into the production of VFAs from kitchen biowaste and potato peels through the co-culture of two microorganisms - *K. mobilis ATCC 13048* and *E. coli E 17*. The investigations were carried out in a pilot-scale installation, based on a novel biorefinery concept from Finnoflag Oy (Hakalehto et al., 2015). The aim of the research was to substantiate the technology for the VFAs acid chain elongation. To this aim, the presented study tested whether the metabolites produced by the co-culture of *K. mobilis* and *E. coli* favour VFAs formation and their chain elongation under microaerobic conditions. The duration of the testing was two months, during which the viability of the concept was demonstrated and a starting point for further optimisation was established.

## 2. Materials and methods

The tests were performed in a pilot scale installation, consisting of a sequence of tanks: pre-treatment unit, hydrolysis tank, the bioreactor and the separation unit, each with a volume of at least 250 L. The bioreactor type is a fed-batch. Each process run lasted 2–3 days. Results of the two most successful final runs are presented and referred to as run A and run B.

### 2.1. Substrates

Two substrates were used: kitchen biowaste from a restaurant and potato peels from the food processing industry - each was collected separately. Kitchen biowaste was composed of a mixture of fresh and boiled residues of vegetables, meat, fruits, fats etc. Potato peels were delivered as a very homogenous, finely shredded mass. Characteristics of the substrates used in the experiments can be found in Table 1. For comparison, the last column provides mean and standard deviation values for adequate parameters of food waste, based on a statistical analysis of data from 70 scientific papers (Fisgativa et al., 2016). The organics content, very high respiration activity index (AT4) and low heavy metals content rendered waste suitable for the biochemical process, however the levels of some macro- and micronutrients, especially in the potato peels, were significantly lower than the literature values for food waste.

### 2.2. Substrates pre-treatment

Each portion of substrate underwent a sequence of pre-treatment steps: mechanical crushing (only needed for kitchen biowaste), adjusting the total solids (TS) loading by mixing the substrate with water, pasteurization at 80 °C and hydrolysis in the heated hydrolysis tank at 65 °C, pH 5.5 with enzymes

Please cite this article in press as: den Boer, E., et al. Volatile fatty acids as an added value from biowaste. Waste Management (2016), http://dx.doi.org/10.1016/j.wasman.2016.08.006