Journal of Cleaner Production 166 (2017) 706–716

Contents lists available at ScienceDirect

Journal of Cleaner Production

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

Development of a citrus peel-based biorefinery strategy for the production of succinic acid

Maria Patsalou [a], Kristia Karolina Menikea [a], Eftychia Makri [a], Marlen I. Vasquez [a], Chryssoula Drouza [b], Michalis Koutinas [a, *]

[a] Department of Environmental Science & Technology, Cyprus University of Technology, 30 Archbishop Kyprianou Str., 3036, Limassol, Cyprus
[b] Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 30 Archbishop Kyprianou Str., 3036, Limassol, Cyprus

ARTICLE INFO

Article history:
Received 4 March 2017
Received in revised form
23 June 2017
Accepted 5 August 2017
Available online 5 August 2017

Keywords:
Citrus peel waste
Actinobacillus succinogenes
Succinic acid
Bioprocess
Biorefinery

ABSTRACT

A preliminary study has been performed for the valorization of citrus peel waste (CPW) through the biorefinery platform aiming to produce succinic acid. Following extraction of essential oils and pectin, different conditions of dilute acid hydrolysis were evaluated based on estimation of the sugars liberated and subsequent fermentation of hydrolyzates for production of succinic acid by *Actinobacillus succinogenes*. The most suitable pretreatment conditions involved 116 °C for 10 min using 5% (w/v) of dry raw material (drm). Thus, a total sugar (ts) yield of 0.21 $g_{ts}\ g_{drm}^{-1}$ and a succinic acid (sa) yield via microbial fermentations of 0.77 $g_{sa}\ g_{tsc}^{-1}$ was achieved, while the use of lower solid contents resulted in higher sugar yields. The residues from dilute acid hydrolysis were applied for subsequent enzyme hydrolysis using commercial enzymes and the most suitable combination of enzyme units included 30 IU cellulases and 25 BGL β-glucosidases achieving a yield of 0.58 $g_{ts}\ g_{drm}^{-1}$. Moreover, elemental analysis in hydrolyzates obtained from dilute acid hydrolysis and a combination of acid and enzyme hydrolysis indicated that during the combined treatment, high concentrations of Mg^{2+} and Ca^{2+} ions are liberated as compared to dilute acid hydrolysis, while the concentration of hydroxymethylfurfural was 0.038 g L^{-1} demonstrating low formation of inhibitors. The hydrolyzate generated through the combined pretreatment proposed was applied as feedstock for the production of succinic acid achieving a yield of 0.70 $g_{sa}\ g_{tsc}^{-1}$. However, although the combined hydrolysis approach could approximately double the sugars released in the hydrolyzate, the economic analysis performed confirmed that the use of the enzymatic treatment could not be competitive. The developed bioprocess constitutes a valuable alternative to the application of energy intensive chemical technologies for succinic acid production.

1. Introduction

Food waste (FW) constitutes a global environmental, economic and societal problem, that should be addressed by a combination of prevention and valorization approaches (Turon et al., 2014). The food manufacturing sector is generating 38% of the 90 million tons of FW produced by the European Union (Pfaltzgraff et al., 2013), while vegetables and fruits usually comprise the most-utilized items (Kosseva, 2013). Thus, the citrus worldwide production constitutes over 121 × 10^6 tons per year resulting in industrial generation of CPW that exceeds 25 × 10^6 tons (FAO, 2016). CPW formed during the processing of citrus for juice extraction, consist of peels, seeds and segment membranes, accounting for 50% of the whole fruit (Marín et al., 2007; Wilkins, 2009), while traditional management practices include the use of CPW as animal feed or disposal in landfills (Angel Siles López et al., 2010).

The need to replace the use of petroleum with new renewable resources for the production of fuels and chemicals and to identify novel practices for the reduction of biodegradable waste has led to the application of food waste as a feedstock for biorefineries (Lin et al., 2013). The valuable composition of the peel renders CPW a promising feedstock for biotechnological production of added-value commodities through the biorefinery platform. Specifically, CPW comprise a high content of cellulose, hemicellulose, soluble sugars, and pectin (Angel Siles López et al., 2010). Furthermore, CPW include 0.5% g $g_{wet\ mass}^{-1}$ of essential oils, consisting 90% of D-

* Corresponding author.
E-mail address: michail.koutinas@cut.ac.cy (M. Koutinas).

http://dx.doi.org/10.1016/j.jclepro.2017.08.039
0959-6526/

limonene (Li et al., 2010b) known to act as antimicrobial agent with numerous applications in the food and medical industries (Martín et al., 2010).

Pretreatment of CPW prior to the bioprocess usually requires the removal of essential oils due to the antimicrobial properties of D-limonene that may cause inhibition of the biosystem. However, pectin extraction is equally important comprising multiple functions in foods (jams, frozen foods, sugar replacer), pharmaceuticals (reduction of blood cholesterol levels, gastrointestinal disorders) and other applications such as paper substitute, edible films, foams and plasticizers (Thakur et al., 1997). Previous studies demonstrated that the generation of a hydrolyzate rich in carbohydrates through acid or enzymatic hydrolysis of CPW may serve as a valuable fermentation feedstock for the production of biofuels (e.g. bioethanol, biomethane), single cell protein or other products (Wilkins, 2009; Martín et al., 2010; Pourbafrani et al., 2010; Ruiz and Flotats, 2016; Koutinas et al., 2016). Thus, CPW could be employed through the biorefinery platform for the fermentative production of succinic acid, which is predicted to be one of the most important bio-based platform chemicals used for the manufacture of various added-value products. Succinic acid ($C_4H_6O_4$) is a dicarboxylic acid produced mainly by chemical routes such as catalytic hydrogenation, paraffin oxidation and electrolytic reduction of maleic acid or anhydride. Its conventional industrial applications include the production of polyester polyols, polybutylene succinate-terephthalate, resins, coatings and pigments as well as use in the pharmaceutical industry and in the food industry as flavorant and sweetener (Pateraki et al., 2016). However, the bio-based production of succinic acid entails a series of advantages, compared to its chemical production due to high theoretical yield and environmental friendly impact.

Various microorganisms such as *Mannheimia succiniciproducens* (Kim et al., 2004; Song et al., 2007) *Anaerobiospirillum succiniciproducens* (Lee et al., 1999, 2003), *Basfia succiniciproducens* (Scholten et al., 2009) and *Actinobacillus succinogenes* (Jiang et al., 2014; Li et al., 2010a) have been tested for succinic acid production in previous studies. Among the strains examined, *Actinobacillus succinogenes* is predicted to be one of the most promising industrial succinic acid-producing microorganisms based on the ability to utilize CO_2 and to produce high concentrations of succinic acid. *Actinobacillus succinogenes*, isolated from bovine rumen, is a capnophilic and mesophilic bacterium which is capable of valorizing monosaccharides under anaerobic conditions (Jiang et al., 2014).

The aim of the current work was to conduct a preliminary study for the development of a CPW-based biorefinery, as depicted on Fig. 1. The CPW biorefinery proposed was applied for valorization of the waste using *Actinobacillus succinogenes*. Specifically, the bioprocess presented targets estimating suitable dilute acid and enzyme hydrolysis conditions for enhancing the release of fermentable sugars from CPW in the hydrolyzate generated, following the removal of essential oils and pectin. The hydrolyzates formed were applied as fermentation feedstocks for the production of succinic acid, while the release of metal ions and fermentation inhibitors in the different hydrolysis approaches followed has been also evaluated.

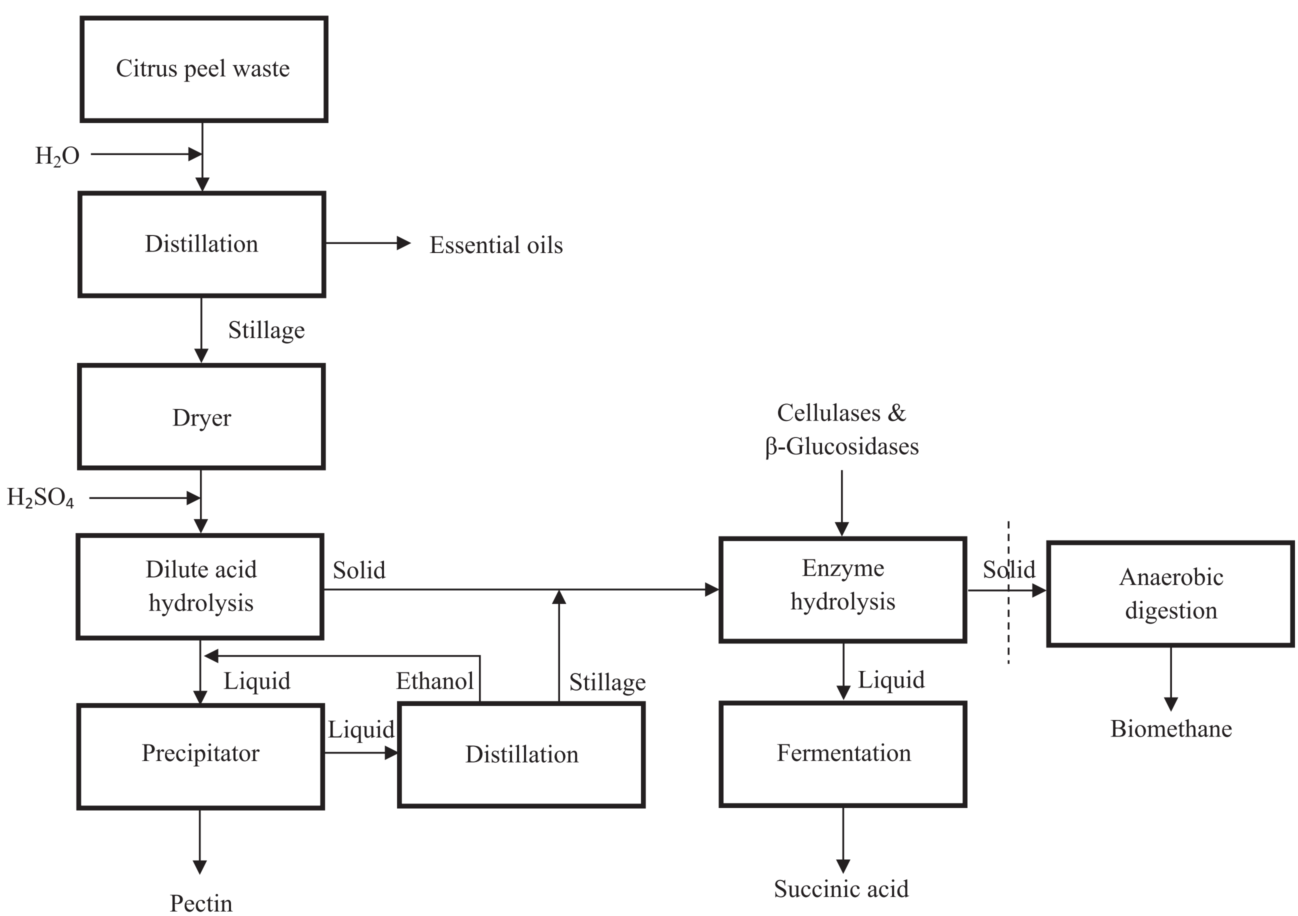

Fig. 1. Process flow sheet of the biorefinery used for CPW valorization. The dashed line denotes that anaerobic digestion was not examined in the present study.