Fuel 181 (2016) 843–851

Contents lists available at ScienceDirect

Fuel

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

Full Length Article

Enhancement of biogas and methanization of citrus waste via biodegradation pretreatment and subsequent optimized fermentation

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HIGHLIGHTS

- For the first time, using a reinforced biorefining process: biodegradation process.
- Citrus waste's low pH was improved.
- Investigation the balance between ORP, pH, and loading volume.
- Designed systematically optimizing process to improve methane concentration.

ARTICLE INFO

Article history:
Received 23 February 2016
Received in revised form 9 May 2016
Accepted 11 May 2016
Available online 17 May 2016

Keywords:
Citrus waste
Biodegradation pretreatment
Biogas
Anaerobic fermentation

ABSTRACT

In this paper, we explored pretreatment and optimization processes in a type of agricultural feedstock substrate citrus waste (CW), an important pollution source causing surface water to be polluted by excessive acidification. For the first time, we investigated the effects of biodegradation pretreatment (BP), and designed a multi-stage optimization process to increase biogas and methane production in a stepwise manner. The results showed the pH of CW can be significantly raised via biodegradation pretreatment. The most remarkable effects of reducing CW solid waste meanwhile obtaining the highest biogas production were observed using both strains *Phanerochaete chrysosporium* ATCC 20696 and *Aspergillus niger* CCTCC 206113. Further detailed investigation suggested that the increase in biogas is correlated with the relationship between the oxidation–reduction potential (ORP) and pH value, as well as the volume loading, as determined by our detailed, optimized fermentation process, which contains a step-by-step procedure. We identified the parameters necessary to obtain the highest biogas yield of 308.85 mL/g-VS, the highest methane yield of 176.05 mL/g-VS, and the highest methane concentration of 57%, compared to the values before optimization. These results suggest that the environmental pollution problems resulted from CW can be relieved and simultaneously high efficiency bioenergy can be obtained via biodegradation pretreatment which largely decomposes CW solid waste.

1. Introduction

Various kinds of bioconversion programs, including anaerobic digestion/fermentation method, are economically and environmentally favorable methods used to produce biochemicals and biofuels. The majority of biofuel production including bioethanol and biobutanol, however, involves in most cases using starch-based crops [1]. This method is criticized because at present starch is generally derived from food, like corn or cassava. The use of starches to produce biofuels can reduce food supplies and increase food prices, leading to socio-political instability. The use of feedstock that is rich in starch or sugar has also directly increased the price of biofuels, due to the high prices of the raw materials. Digested by fermentation is typically a complicated process due to the diversity of the substrate including proteins, lipids, sugars, and starches. Therefore, new alternative biomass sources are required for economically viable biofuel production. One economically attractive biomass source is the existing surplus of agricul-

Abbreviations: CW, citrus wastes; ORP, oxidation–reduction potential; SMA, starting material of acclimation.

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http://dx.doi.org/10.1016/j.fuel.2016.05.055
0016-2361/

tural waste or by-products rich in lignocellulosic biomass via a process of simplification [2].

Citrus waste (CW) is one kind of abundant agricultural waste. It contains residual by-products, including fruit pulp, fruit kernels, and fruit peels, from fruit juice industry. Currently, a total of five million tons of CW are produced every year in China. With the exception of a small number of citrus peels that are used to extract pectin, which is used in traditional Chinese medicine, and a small amount of CW used as feed, the great majority of CW is abandoned or sent to landfills [3–5]. Largely abandoning or sending to landfills for CW not only is a waste of resources, but also causes serious environmental pollution. How to solve the problem of processing CW scientifically and rationally has become a complicated task for researchers.

It is important to note that, according to reports in literature, a physical or chemical pretreatment process is required to produce biofuels from CW [6–8]. This requirement, however, has a few drawbacks: (i) Physical pretreatment is an energy-intensive process. Existing methods of pretreatment, like the use of steam explosion [9] (generally requiring temperatures at or above 150 °C), are not ideal pretreatments in terms of energy conservation. (ii) Chemical pretreatment, such as acid hydrolysis, can cause reactor corrosion and lead to secondary pollution of environment [10,11]. (iii) Citrus peel waste usually contains 0.8–1.6% D-limonene, which is an inhibitor of yeast fermentation. The presence of this inhibitor leads to low biofuels yield [12]. Therefore, when using CW as a substrate for biofuels production, an additional pretreatment is required to lower the limonene content to below 0.05%, a level at which D-limonene cannot severely inhibit microbial growth [13].

Utilizing CW as the carbon and nitrogen source to produce biogas serves as a locally-provided alternative supplementary fuel for use in citrus orchards or juice processing factories. Therefore, it is necessary to study the process of using CW to produce biogas. However, there are drawbacks due to some natural characteristics of CW, for example, the easily produced byproducts including highly acidic compounds, which lower the pH of fermentation substrates during the natural process of rotting. This, in turn, inhibits the uptake hydrogenase activity [14]. Another drawback is the aforementioned inhibitory effect of some pharmaceutical compounds, like limonene, on microbial growth. These negative factors lead to a very long production time associated with making biogas by anaerobic fermentation, and also result in a low biogas yield.

As an important pretreatment approach, currently, interest in biodegradation pretreatment of biomass has shifted from traditional applications, such as ruminant feed upgrading and biopulping, to biofuel production including bioethanol, biogas, and biobutanol. Biodegradation pretreatment is considered to be a "green" technology for replacing chemical pretreatment as it is performed under ambient conditions without chemical reagent. The main benefits include low energy requirements compared with physical pretreatment, little or no waste stream output and avoiding producing of inhibitors for bacteria growth. It has the potential to be applied to on-farm wet storage for cost-effective biofuels production from lignocellulosic biomass [15].

However, to our knowledge, although some studies have been done on producing biogas, obtaining low concentrations of methane from CW, there is limited research on the application of biodegradation pretreatment approach in CW treatment in this field. Moreover, a comparison between the effectiveness of pretreatments on diversified single fungal species and double fungal species combinations for CW residue has not been presented in the literature.

In order to shorten the fermentation time, increase production of biogas, and avoid physical and chemical pretreatment, in this study, we investigated biogas production using biodegradation pretreatment and established an efficient biogas production process based on a multi-stage fermentation optimization. We investigated the effectiveness of biodegradation pretreatment using different combinations of fungi. We also studied the effects of optimizing hydrolysis and multi-stage fermentation on biogas yield based on biological pretreatment. Our purpose is to reduce environmental pollution from CW meanwhile enhance biogas and methane production via biodegradation pretreatment approach.

2. Materials and methods

2.1. Materials and strains

2.1.1. Determination of citrus waste composition

Fresh citrus waste was provided by Chongqing Citrus Industrialization Development Co. LTD, and ground into a paste for later use. In order to adjust C/N ratio to an appropriate value and obtain a good microbial communities, the pig manure and sewage sludge were used. The pig manure used in the procedure came from local farms. The composition of the pig manure, sewage sludge from sewage treatment plant at Beibei, Chongqing city, and citrus waste were determined (Table 1).

2.1.2. Strains and culture medium

White rot fungi *Phanerochaete chrysosporium* ATCC 20696, mold *Penicillium citrinum* ATCC 10499, *Trichoderma viride* ATCC 32173, *Trichoderma polysporum* ATCC 28044, *Aspergillus oryzae* ATCC 76080, and *Rhizopus stolonifer* ATCC 14037 were purchased from the biological resource center, ATCC, USA. *Aspergillus niger* CCTCC 206113 was purchased from the China Center for Type Culture Collection (CCTCC) at Beijing. Sterile Potato Dextrose Agar Medium (PAD Medium) was used to rejuvenate selected strains. All strains were rejuvenated at constant temperature in an incubator set to 30 °C until each strain grew to fill a whole culture dish with 90 cm diameter. 150 mL sterile Potato Dextrose Medium (PDM), including a nutrient solution composed of 10 g/L ammonium sulfate, 0.5 g/L calcium sulfate, and 0.5 g/L $Ca_3(PO_4)_2$, was used to cultivate the inoculum. Strains were propagated in an incubator

Table 1
The composition of fresh citrus waste and other materials.

Component	Materials used in this study		
	Fresh citrus waste	Fresh pig excrement for acclimation	Sewage sludge acclimation
Water (%)	81.30	84.13	68.63
Total solid (TS, %)	17.90	15.00	28.00
Crude protein (%)	6.62	–	–
Cellulose (%)	12.50	–	–
Crude fat (%)	2.20	–	–
Ash (%)	12.16	12.30	11.40
pH	1.8	7.32	7.15
Total carbon (%)	38.12	8.23	9.87
Total nitrogen (%)	0.85	0.65	0.75
P (g/L)	0.18 ± 0.02	0.42 ± 0.03	0.41 ± 0.14
K (g/L)	0.28 ± 0.03	0.69 ± 0.012	0.78 ± 0.13
Fe (g/L)	0.05 ± 0.01	0.27 ± 0.023	0.38 ± 0.03
Ca (g/L)	0.31 ± 0.04	0.34 ± 0.02	0.42 ± 0.03
Mg (g/L)	0.21 ± 0.02	0.34 ± 0.03	0.38 ± 0.02
C/N	44.85:1	12.66:1	13.16:1