Renewable Energy xxx (2017) 1–6

Contents lists available at ScienceDirect

Renewable Energy

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

Inhibition of patchouli oil for anaerobic digestion and enhancement in methane production using reverse membrane bioreactors

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ARTICLE INFO

Article history:
Received 13 January 2017
Received in revised form
26 April 2017
Accepted 28 April 2017
Available online xxx

Keywords:
Methane
Anaerobic digestion
Patchouli biomass
Patchouli oil
Inhibition
Membrane bioreactor

ABSTRACT

Patchouli oil is an essential oil extracted from aromatic crop *Pogostemon cablin* and is widely used in perfumery industry, food industry, and/or even as medicine. The leaves have 4.6% oil that is extracted by steam, but remains an enormous amount of wastes containing ca 0.8% oil. Patchouli waste is an interesting substrate for methane production. However, the oil has been found to have antibacterial activity. The inhibition of patchouli oil on anaerobic digestion was investigated in this study under thermophilic conditions (55 °C). The patchouli oil showed antibacterial effect, where addition of 0.05, 0.5 and 5 g/L patchouli oil reduced biogas production by 16.2%, 27.2% and 100% respectively. As patchouli oil is a lipophilic compound, hydrophilic polyvinylidene difluoride (PVDF) membrane was used to protect the microorganisms against this inhibitor in a reverse membrane bioreactor (rMBR) system. The methane yield of fresh plant and waste were 86 and 179 NmL CH_4/gVS, respectively when using free cells. Although using solely an rMBR did not give significant rise to methane yield, the combination rMBR and free cell strategy to protect part of the digesting microorganisms against this inhibitor considerably enhanced the methane production by 73% for fresh patchouli plant, compared to digestion using free cells.

1. Introduction

Patchouli oil is an essential oil extracted from aromatic crop *Pogostemon cablin*, belonging to the family *Lamiaceae*. Patchouli oil is an intermediate product that is widely used in perfumery industry, food industry or even used as medicine in China [1]. The plant is natively grown in tropic and sub-tropical climate area such as Himalayas, China, Southeast Asia, and Far East [2]. The leaves play an important role in the plant because it has oil glands. In perfumery industry, patchouli oil is considered as best fixative for heavy perfumes, which has impact on strength, character, alluring motes and lasting qualities [3]. In food industry, the oil is used as a flavouring ingredient in major food products including non-−alcoholic and alcoholic beverages, frozen dairy desserts, candy, baked foods, meat and meat products [4]. In Chinese medicine, the decoction of patchouli leaves is used with other drugs to treat nausea, vomiting, diarrhoea, cold and headache [4].

The global market for patchouli oil is 1600 tonnes per annum [5]. The market position of patchouli oil is strong and unique since no synthetic chemical replacement has been found. Commercial extraction of patchouli oil from the shade-dried plant is usually carried out by steam distillation. The oil yield is around 2.6% [4], which means more than 38 kg waste material per kg of the oil is produced. Indonesia, as a tropical country, had 28,200 ha patchouli field in 2014 producing 2100 tonnes of patchouli oil [6]. Using the value of oil yield, the amount of waste generated was ca 80,000 tonnes. In patchouli oil distillery, the waste is usually used as compost or as burning fuel for the steam generator as well as vermicompost [7]. One interesting way to recover energy from degradation of carbon-rich material is through anaerobic digestion that can produce biogas.

Patchouli distillery waste is a lignocellulosic material that can be used as substrate for biogas production. There are many studies on

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http://dx.doi.org/10.1016/j.renene.2017.04.068
0960-1481/

biogas production from lignocellulosic material such as forest residues [8], plantation residue [9] and garden waste [10]. Lignocellulose is a recalcitrant material as it contains crystalline cellulose and lignin, where pretreatment is then usually necessary prior to biogas production. Another challenge of converting patchouli distillery waste into biogas is the presence of patchouli oil residue in the patchouli distillery waste. Patchouli oil is considered as antiseptic substance. Study of rumen fermentation showed that the addition of patchouli oil could suppress methane production during *in vitro* rumen fermentation [11]. Patchouli alcohol, as the main constituent of patchouli oil, is found to have antibacterial effect against several types of bacteria [12]. This effect could make patchouli oil act as an inhibitor against bacteria in anaerobic digestion.

Recently, reverse membrane bioreactors (rMBR) have been developed and applied in fermentation systems in order to prevent cells from coming in direct contact with specific inhibitory compounds in cultivation media. The principle separation in this bioreactors is based on the fact that different chemical compounds have different levels of affinity to hydrophilic/phobic membranes. Having that knowledge, by means of encasing cells in between properly selected membrane cells can be effectively protected from harsh inhibitory media or be less exposed to inhibitory media in comparison to freely suspended cells that all experience same concentrations of toxic compounds [13].

The major difference of this membrane system with conventional membrane bioreactors is that the cells are protected inside the membranes and the nutrients diffuse throughout the membranes due to concentration gradient over the membrane other that pressure difference. Conventional pressure driven membrane bioreactors (MBR) are mainly used for product recovery purposes in order to reduce the number of downstream processing steps and to avoid cell wash-out to achieve high cell concentrations inside the reactor [13]. However, in this regard the advantage of rMBR over the conventional MBRs is that as in rMBRs separation only occurs due to concentration difference over the membrane, favourable nutrients selectively pass through the membrane and reach the cell side where they get involved in metabolic reactions compared to the components which have been repelled by the membrane. On the other hand, rMBR has size limitations in case large particulate substrates *e.g.* cellulose are to pass through the membranes and in order to be available to the microorganisms [13].

rMBRs or encasing cells with membranes were previously used for cultivations in toxic media. Membrane encasing of *Saccaromyces cerevisiae* found to be powerful against the negative effect of the hydrophobic inhibitor limonene in ethanol production from limonene-containing substrates of *e.g.* citrus waste [14]. Membrane encased cells were also investigated for biogas production from citrus waste [15,16]. When a hydrophobic inhibitor such as patchouli oil is considered, the hydrophilic membranes will be effective to let the substrates pass through but not the inhibitors. Polyvinylidene fluoride (PVDF) membrane has shown to be an effective hydrophilic barrier to avoid contact between microorganisms and hydrophobic inhibitors.

Therefore, this study was carried out not only to investigate the inhibition effect of patchouli oil on methane production but also to examine the methane potential of patchouli distillery waste. In addition, the potential of membrane bioreactors to dampen the inhibition effect of patchouli oil was investigated.

2. Material and methods

2.1. Microorganisms

The anaerobic sludge from the 3,000-m^3 thermophilic (55 °C) biogas plant at Borås Energy and Environment AB (Borås, Sweden) was used as inoculum in this study. The sludge was incubated in incubator at 55 °C for 3–5 days after taken from the plant to acclimatize the bacteria to the experimental conditions. Afterwards, it was sieved through 1 mm pore to remove any remaining particles and shaken thoroughly before inoculation into glass digester.

2.2. Substrate

Patchouli leaves (*Pogostemon cablin*) (both sun-dried fresh and de-oiled solid waste from patchouli oil distillation process) and patchouli oil were obtained from local patchouli oil distillery industry in Bantul (Yogyakarta, Indonesia). The fresh leaves were divided into two different sizes *i.e.* cut (±5 mm), and ground then sieved to 40 mesh (0.42 mm). Microcrystalline cellulose (Avicel) was obtained from Sigma-Aldrich and used as control.

2.3. Anaerobic digestion

The inhibition study were carried out according to previous studies about inhibition of hydrophobic compound in anaerobic digestion [17–19] with minor modification in amount but the same inoculum to substrate ratio. The experiments were carried out in a 118-mL glass serum bottle containing 0.3 g VS of substrate (either patchouli biomass or Avicel excluding patchouli oil) and 0.9 g VS of inoculums and added with distilled water until 50 mL working volume. The bottles were sealed tightly with aluminium crimp cap, and the headspace was purged with a gas mixture of 80% N_2 and 20% CO_2 to achieve anaerobic conditions.

Methane production was determined as previously described by Hansen et al. [20]. The measurement of methane production in these batch reactors was based on the measuring gas composition with GC with the same pressure as inside the reactors, releasing the gas from the reactor, and another GC measurement at this lower pressure of the reactor. The syringe used for gas sampling has a valve and, therefore, the gas inside the syringe had the same pressure as the reactor vessel in both of the measurements. The methane gas released was calculated from the difference of peak sizes between two measurements.

In the second experiment, the enhancement of methane production from patchouli biomass was carried out using three different reactor setups. A free cell reactor with 0.3 g VS inoculum, the reverse membrane bioreactor with membrane encased cell (0.3 g VS) and the combination of free cell and membrane bioreactor with half (0.15 g VS) of the inoculum inside and half (0.15 g VS) outside the membrane. 0.1 g VS patchouli biomass was added to each reactor setup outside the membrane. The preparation of membrane encased cells was adopted from previous work about membrane encased cells [16,21–23]. The reactor with only free cells was used as a comparison. The inoculum (the cell) was prepared by centrifugation of anaerobic sludge at 14,000 *g* for 10 min and the pellet was taken as inoculum. The membrane encased cells were prepared by inserting the pellet into a sachet made of PVDF (Durapore®, Thermo Fisher Scientific, Inc., Gothenburg, Sweden) with the size 3 cm × 6 cm.

The reactors used in this experiment were also 118 mL glass serum bottle. The bottles were sealed tightly with aluminium crimp cap, and the headspace was filled with a gas mixture of 80% N_2 and 20% CO_2 to achieve anaerobic conditions. The bottles were incubated at 55 °C for 50 days. During incubation, the bottles were manually shaken once a day. Gas samples were taken from the headspace of the reactors through the septum using a syringe with pressure lock (VICI; A-2 Precision Sampling Inc., Baton Rouge, LA). Samples were taken at 3, 6, 9, 12, 15, 20, 25, 30, 36, 43, and 50 days.

Please cite this article in press as: Lukitawesa, et al., Inhibition of patchouli oil for anaerobic digestion and enhancement in methane production using reverse membrane bioreactors, Renewable Energy (2017), http://dx.doi.org/10.1016/j.renene.2017.04.068