



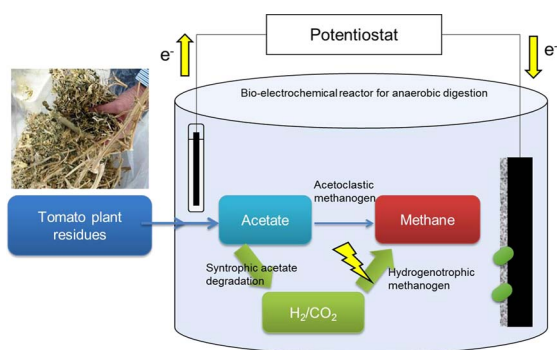
Analysis of a bio-electrochemical reactor containing carbon fiber textiles for the anaerobic digestion of tomato plant residues



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GRAPHICAL ABSTRACT



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ABSTRACT

A bio-electrochemical system packed with supporting material can promote anaerobic digestion for several types of organic waste. To expand the target organic matters of a BES, tomato plant residues (TPRs), generated year-round as agricultural and cellulosic waste, were treated using three methanogenic reactors: a continuous stirred tank reactor (CSTR), a carbon fiber textile (CFT) reactor, and a bio-electrochemical reactor (BER) including CFT with electrochemical regulation (BER + CFT). CFT had positive effects on methane fermentation and methanogen abundance. The microbial population stimulated by electrochemical regulation, including hydrogenotrophic methanogens, cellulose-degrading bacteria, and acetate-degrading bacteria, suppressed acetate accumulation, as evidenced by the low acetate concentration in the suspended fraction in the BER + CFT. These results indicated that the microbial community in the BER + CFT facilitated the efficient decomposition of TPR and its intermediates such as acetate to methane.

1. Introduction

A bio-electrochemical system (BES) is a type of bioreactor in which both biological and electrochemical processes can take place to

generate electricity, hydrogen, or other products (Lu and Ren, 2016; Wang et al., 2015b). Electro-fermentation (EF), a term first proposed by Rabaey, is a new BES method that has recently been applied to the traditional fermentation process (Moscoviz et al., 2016; Roy et al.,

Abbreviations: BER, bio-electrochemical reactor; BES, bioelectrical system; CFT, carbon fiber textile; CSTR, continuous stirred tank reactor; DGGE, denaturing gradient gel electrophoresis; EF, electro-fermentation; HRT, hydraulic retention time; OLR, organic loading rate; ORP, oxidation reduction potential; qPCR, quantitative polymerase chain reaction; SS, suspended solid; TPR, tomato plant residue; VFA, volatile fatty acid

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2016). Electrochemical oxidizing or reducing reactions on electrodes in a BES can modify the oxidation reduction potential of a medium (extracellular ORP), which subsequently affects the intracellular ORP via the reduced/oxidized NAD (NADH/NAD⁺) balance, impacting the metabolic process (Berríos-Rivera et al., 2002). In EF, redox environments are controlled to stabilize fermentation and obtain high yields of target products via electrochemical reactions on electrodes (Moscoviz et al., 2016). Unlike other types of BES, EF does not require high current densities because the electric current is a source of reducing or oxidizing power to optimize extracellular and intracellular redox conditions, and is not the main energy source for microbial reactions. To take advantage of this property, the method has been applied to the fermentative production of energy products such as ethanol, butanol, and energy-rich biogas.

Anaerobic digestion is a popular industrial fermentation process for the treatment of biodegradable solid waste (e.g., food waste, sewage sludge, and agricultural plant residues) and for renewable energy production. Improving the performance of anaerobic digestion is a key challenge for increasing the energetic and economic performance of these processes. The EF method was previously applied to anaerobic digestion with the goal of improving energy recovery from organic waste, including garbage slurry, sewage sludge, and filter paper as model cellulose substrates (Sasaki et al., 2011a,b, 2013b). In all of these studies, an applied reductive potential of -0.6 V or -0.8 V (vs. Ag/AgCl) in the bio-electrochemical reactor (BER) successfully stabilized methane gas production at a higher organic loading rate (OLR) and increased energy yield (methane) in comparison with those obtained in a control experiment without electrolysis. Although a correlation between total methane fermentation activity and applied potential on a working electrode has been suggested, the microbes that are influenced by the electrochemical potential in the BER and the mechanism by which methane fermentation is improved remains unclear.

In this study, to evaluate the effect of electrochemical regulation on microbial communities in anaerobic digestion as well as the mechanism explaining previous electrochemical methane fermentation results, a fed-batch BER with an attached carbon fiber textile (CFT) on a working electrode for anaerobic digestion was developed. Attachment of the CFT to the working electrode was previously shown to result in high reactor performance by retaining a high cell density (Sasaki et al., 2011b). Moreover, since application of the EF method to anaerobic digestion has not been examined using actual cellulosic waste to date, in this study, agricultural tomato plant residue (TPR) was used as a cellulosic feedstock of anaerobic digestion. TPR is an agricultural cellulosic waste that is targeted for effective utilization because it is discharged year-round from greenhouses and is usually landfilled in nearby production sites or combusted as a garbage. Although there has been no investigation on the use of EF for agricultural waste such as TPR waste, EF has shown potential for such application with a model cellulose substrate (Sasaki et al., 2011a, 2013a). During the stable anaerobic digestion of TPR in BERs, the gas production rate was measured and microbial communities were analyzed by both DNA- and RNA-based denaturing gradient gel electrophoresis (DGGE) and real-time PCR to detect actively growing microbes.

2. Materials and methods

2.1. Feed material and inoculum

Agricultural TPRs, including stalks, leaves, and residual tomatoes, were obtained from a plant factory (with a closed growing system for the year-round production of vegetables) in Abiko, Japan. Collected TPRs were air-dried and shredded to a particle size of less than 2 mm using a food processor and kitchen blender. The TPR feedstock for anaerobic digestion was composed of 10% TPR and medium, including (p liter) 0.8 g of KH₂PO₄, 1.6 g of K₂HPO₄, 1.0 g of NH₄Cl, 2.0 g of NaHCO₃, 0.1 g of MgCl₂·6H₂O, 0.2 g of CaCl₂·6H₂O, and 0.8 g of NaCl,

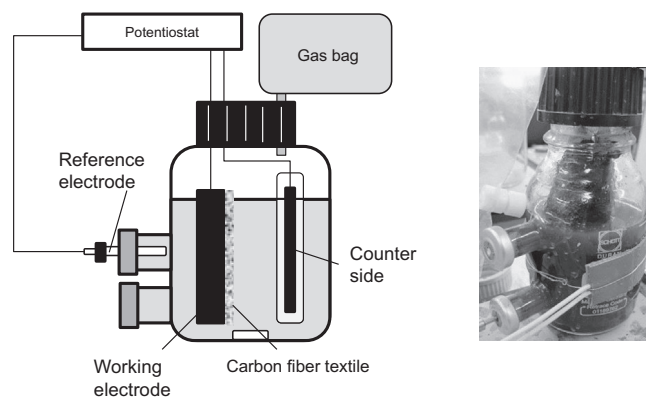


Fig. 1. Schematic diagram of the bio-electrochemical reactor (BER) containing carbon fiber textiles (CFT). The same configuration was used for the control reactor without electrochemical regulation (CFT reactors). The electrodes (working, counter, and reference electrode) were not included in the continuous stirred tank reactor (CSTR).

along with 10 mL of DSMZ medium 131 trace element solution and DSMZ medium 141 vitamin solution. Anthraquinone 2,6-disulfonate was added to the medium as an electron mediator to facilitate the electrochemical redox regulation, as in previous reports (Sasaki et al., 2011a; Hirano et al., 2013; Chen et al., 2016). The TPR feedstock was autoclaved at 121 °C for 30 min.

2.2. BERs containing carbon fiber textile (CFT) for electrochemical methane fermentation

All electrochemical methane fermentation experiments were performed using an apparatus comprising a bottle-type single chamber. A three-electrode system, which included a working electrode with a CFT, a reference electrode, and a counter electrode, was used (BER + CFT, Fig. 1). The anodic bag was formed using a proton exchange membrane (Nafion 117; DuPont Co., Wilmington, DE, USA) and inserted in the cathodic working chamber. A carbon plate (25 × 75 × 1 mm) was used as the working electrode and a smaller carbon plate (10 × 65 × 1 mm) was used as the counter electrode. On the working electrode, CFT (25 × 75 × 2.4 mm) was attached to the side of the reference electrode using Pt wire, as previously described (Sasaki et al., 2011b). An Ag/AgCl reference electrode was inserted into the cathodic working chamber. A gas bag (Aluminum Bag; GL Sciences, Tokyo, Japan) was connected to each chamber via a silicon cap on the top of reactors for the collection of gas produced during the operation. The cathodic chamber and the anodic bag were filled with 250 mL of medium and 5 mL of 100 mM NaCl, respectively. In the BER, three electrodes were connected with a potentiostat (PS-08; Tohogiken, Yokohama, Japan). Since a positive effect of regulated potential at -0.8 V on stable methane production and decomposition of cellulosic substrate was previously observed (Sasaki et al., 2011a, 2013a), the potential of the working electrode was electrochemically regulated to -0.8 V (vs. Ag/AgCl) in the present study. All reported potentials on the working electrode pertain to the Ag/AgCl reference electrode (type: saturated KCl). BER containing CFT was prepared in triplicate. Three reactors containing CFT in which the potential at the working electrode was not electrochemically regulated were prepared as control 1 (CFT reactors). In addition, three CSTRs without CFT and electrochemical regulation were operated as control 2. The contents of reactors were thoroughly mixed using a magnetic stirrer.

2.3. Operation of reactors

First, the same volume (250 mL) of sludge from the methane fermentation (55 °C) of artificial solid garbage slurry (Sasaki et al., 2011a,b) was injected into each working chamber after it was filled

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