



# Computational and experimental analysis of organic degradation positively regulated by bioelectrochemistry in an anaerobic bioreactor system



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## ABSTRACT

Methane production was tested in membrane-less microbial electrolysis cells (MECs) under closed-circuit ( $R_{CC}$ ) and open-circuit ( $R_{OC}$ ) conditions, using glucose as a substrate, to understand the regulatory effects of bioelectrochemistry in anaerobic digestion systems. A dynamic model was built to simulate methane productions and microbial dynamics of functional populations, which were colonized in groups  $R_{CC}$  and  $R_{OC}$  during the start-up stage. The experiment results showed significantly greater methane production in  $R_{CC}$  than  $R_{OC}$ , the average methane production of  $R_{CC}$  was  $0.131 \text{ m}^3/\text{m}^3/\text{d}$ , which was 1.4 times higher than that of  $R_{OC}$  ( $0.055 \text{ m}^3/\text{m}^3/\text{d}$ ). The simulation results revealed that bioelectrochemistry had a significant influence on the abundance of microorganisms involved in acidogenesis and methanogenesis. The abundance of glucose-uptaking microorganisms was 87% of the total biomass in  $R_{OC}$  without applied voltage, which was 20% higher than that in  $R_{CC}$  (67%) when external voltages were applied between the anode and cathode. The abundance of hydrogenotrophic methanogens in  $R_{CC}$  was 6% higher than that in  $R_{OC}$ . The simulation results were verified through 16S rDNA high-throughput sequencing analysis. An electron balance analysis revealed that alteration of the acidogenesis type led to more acetate and hydrogen production from glucose fermentation, compared with the situation without bioelectrochemistry. An additional pathway from acetate to hydrogen was introduced by bioelectrolysis. These two factors resulted in significant enhancement of methane production in  $R_{CC}$ . Bioelectrolysis process directly contributed to 26% of the total methane production after the start-up stage. When the applied voltages were cut down or decreased,  $R_{CC}$  could maintain considerable methane productions, because the microbial communities and electron transfer pathways were already formed. Starting-up with high voltage, but operating under low voltage, could be an energy-favorable strategy for accelerating biogas production in bioelectro-anaerobic bioreactors.

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## 1. Introduction

Anaerobic digestion (AD) technologies can reduce organic

pollution from agricultural and industrial operations, while simultaneously offsetting the use of fossil fuels (Chen et al., 2008). AD can be divided into four major steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. With the cooperation of hydrolytic, fermentative bacteria and methanogens, complex organics are degraded and biogases (methane and carbon dioxide) are finally formed (Batstone and Jensen, 2011). AD technology has numerous advantages, such as low sludge production, low overall cost, and energy recovery (vanStarkenburg, 1997), however, poor operational stability caused by inhibitory factors prevents AD from being widely commercialized (Chen et al., 2008; Zhang and

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Nomenclature			
AD	Anaerobic digestion	$f_{H_2}$	The proportion of hydrogen produced from glucose degradation
ARB	Anode respiration bacteria	MEC	Microbial electrolysis cell
ADM1	Anaerobic Digestion Model No.1	BES	Bioelectrochemical systems
PCR	Polymerase Chain Reaction	MFC	Microbial fuel cell
FID	Flame ionization detector	PBS	Phosphate buffer solution
$X_{su}$	The concentration of glucose-uptaking microorganisms	COD	Chemical oxygen demand
$X_{pro}$	The concentration of propionate-uptaking microorganisms	$X_{bu}$	The concentration of butyrate-uptaking microorganisms
$X_{H_2}$	The concentration of hydrogenotrophic methanogens	$X_{ac}$	The concentration of acetotrophic methanogens
$S_{su}$	The concentration of soluble glucose	$X_e$	The concentration of electricigenic microorganisms
$S_{pro}$	The concentration of propionate	$S_{bu}$	The concentration of butyrate
$S_{H_2}$	The concentration of hydrogen	$S_{ac}$	The concentration of acetate
$S_{CO_2}$	The concentration of soluble carbon dioxide	$S_{CH_4}$	The concentration of soluble methane
$M_{red}$	The concentration of reduced-form intracellular mediator in electricigenic microorganism	$M_{ox}$	The concentration of oxidized-form intracellular mediator in electricigenic microorganism
$V_{H_2}$	The volume of gaseous hydrogen	$V_{CH_4}$	The volume of gaseous methane
$X_{max}$	The maximum concentration of attainable biomass	$V_{CO_2}$	The volume of gaseous carbon dioxide
$f_{bu}$	The proportion of butyrate produced from glucose degradation	$f_{ac}$	The proportion of acetate produced from glucose degradation
		$f_{pro}$	The proportion of propionate produced from glucose degradation

Angelidaki, 2015a; 2015b, c). In an anaerobic bioreactor system, a sophisticated microbial structure and electron transfer paths spontaneously form in cells, which lack direct and effective regulatory approaches. Failure to maintain the balance among microorganisms is the primary cause of AD reactor instability (Demirel and Yenigün, 2002).

Microbial electrochemical cells (MECs) are promising technologies for anaerobic wastewater treatment and energy recovery (Zhang and Angelidaki, 2014). In MECs, electrochemical active bacteria oxidize the substrate and extracellularly transfer electrons to the anode. With the drive of a small voltage (0.2–0.8 V), electrons travel through an external circuit to the cathode, which can be used for hydrogen production (Liu et al., 2005). The bioelectrochemical process is more easily controllable than typical AD processes. External voltage acts like a “pump”, which can adjust the direction and velocity of electron flows to achieve optimal energy efficiency. Introducing bioelectrochemistry into an AD system as a special metabolic pathway may regulate the establishment of microbial structures and electron transfer paths, and increase overall energy efficiency and stability. Currently, attempts to introduce MEC electrodes into AD reactors have been reported to be practically effective in promoting the efficiency and stability of the entire system (Cerrillo et al., 2016; Gajaraj et al., 2017; Liu et al., 2016; Zhang et al., 2013), however, the role of bioelectrochemistry in an anaerobic bioreactor system has not been thoroughly investigated, and it is essential for the further development of MEC-AD coupling processes.

Methane-production in an AD system relies on coexistence and cooperation between various anaerobic microbial populations, while bioelectrochemical processes are closely related to the metabolism of electrochemically active bacteria (Liu et al., 2012). When electrochemical conditions are applied in an AD system, cooperation and competition between anaerobic-digestion populations and electrochemically active bacteria will be key factors affecting overall efficiency. The start-up stage is the key period for the establishment of microbial structures (Ruiz et al., 2014),

therefore, this study will focus on the effect of bioelectrochemical processes on the enrichment of functional populations during the start-up stage.

Tracking population enrichment dynamics or analyzing electron flow in a complex system requires an effective tool, such as dynamic simulation. A few studies have reported on the modeling of bioelectrochemical systems (BESs). Wang et al. simulated CO<sub>2</sub> utilization and fuel production in a microfluidic electrochemical cell (Wang et al., 2013). Pinto et al. proposed a multi-population MEC model (Pinto et al., 2011a) and a unified dynamic model describing MFC and MEC in 2011 (Pinto et al., 2011b), but existing models have failed in representing anaerobic digestion processes in an MEC system. It is, therefore, necessary to modify MEC models with comprehensive AD models, such as the Anaerobic Digestion Model No.1 (ADM1), which has been widely recognized as a platform for anaerobic digestion simulation since it was first published by the International Water Association (IWA) in 2001 (Batstone et al., 2002).

In this study, membrane-less MEC reactors fed with glucose were started up under both closed- and open-circuit conditions ( $R_{CC}$  and  $R_{OC}$ ) to represent the mode of anaerobic bioreactors with or without bioelectrochemical regulation. A multi-population dynamic model for the glucose digestion process was built to simulate the start-up processes in  $R_{CC}$  and  $R_{OC}$ . The effects of bioelectrochemistry on the colonization of the main functional microbial populations, particularly those related with acidogenesis fermentation, were analyzed based on the simulation results and 16S rDNA high-throughput sequencing results of biomass samples. Electron balance analysis was used to evaluate the regulatory effect of bioelectrochemistry on electron transfer paths in anaerobic bioreactor systems. The contribution of the bioelectrolysis process to methane production was evaluated through simulation and verified by decreasing or cutting down applied voltage. The perspective of introducing bioelectrochemistry to an anaerobic system for enhancing methane production in practice was also discussed.

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