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Determination of Microbial Growth by Protein Assay in an Air-Cathode Single Chamber Microbial Fuel Cell

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Microbial fuel cells (MFCs) have gathered attention as a novel bioenergy technology to simultaneously treat wastewater with less sludge production than the conventional activated sludge system. In two different operations of the MFC and aerobic process, microbial growth was determined by the protein assay method and their biomass yields using real wastewater were compared. The biomass yield on the anode electrode of the MFC was 0.02 g-COD-cell/g-COD-substrate and the anolyte planktonic biomass was 0.14 g-COD-cell/g-COD-substrate. An MFC without anode electrode resulted in the biomass yield of 0.07 ± 0.03 g-COD-cell/g-COD-substrate, suggesting that oxygen diffusion from the cathode possibly supported the microbial growth. In a comparative test, the biomass yield under aerobic environment was 0.46 ± 0.07 g-COD-cell/g-COD-substrate, which was about 3 times higher than the total biomass value in the MFC operation.

Keywords: Microbial fuel cell, aerobic process, biomass yield, microbial growth, wastewater

Introduction

Wastewater treatment has been successfully carried out by conventional technologies such as activated sludge and trickling filters to remove organic compounds efficiently. However, the excess sludge production in wastewater treatment requires substantial energy input and efforts to treat and dispose of the sludge. About 25–65% of the total operational cost of a wastewater treatment plant can be accounted for sludge treatment [7]. In addition, energy costs for aeration will typically be 45–75% of all energy consumption in conventional bioreactor operation [4]. As a result, a novel technology needs to be developed to treat wastewater with less consumption of energy and sludge formation.

Microbial fuel cells (MFCs) have been suggested as an innovative and environmentally friendly technology that can treat wastewater with simultaneous electricity generation [10]. Additionally, this MFC technology is considered to have low sludge production compared with an aerobic process. In general, the biomass yield under aerobic condition is around 0.4 to 0.64 g-COD-cell/g-COD-substrate, and in anaerobic condition, it is about 0.04 to 0.25 g-COD-cell/g-COD-substrate [3, 4, 8]. In MFC operation, the biomass yield was reported to range from 0.07 to 0.22 g-COD-cell/g-COD-glucose in the suspended solids [12]. However, the previous studies obtained the MFC biomass yield using a defined substrate such as acetate and glucose instead of real wastewater, and also direct comparative determination of the biomass yield between MFC and aerobic processes was not attempted.

In this study, comparative biomass production was investigated using real domestic wastewater in an air-cathode single chamber MFC and aerobic process. Protein concentration was determined using a protein assay kit for accurate calculation of active biomass [15]. The protein assay kit is considered as a more precise measurement of the total amount of bacteria, even at low concentration, compared with conventional volatile suspended solid (VSS) measurement where some cells are lost through a porous glass fiber filter [2]. Substrate degradation during microbial growth was obtained with soluble chemical oxygen demand (SCOD), and the biomass yield was calculated based on the protein and SCOD. The biomass

yield in the MFC was determined from both an anode electrode and anodic solution separately. An MFC control test without an anode electrode was carried out to see the difference in biomass production between with and without electricity generation measurements.

Materials and Methods

Inoculation and Media

Domestic wastewater was collected from the Giheung Respia Wastewater Treatment Plant (Yongin-si, Korea), and it was used as the inoculum and medium. The collected wastewater samples were purged with pure nitrogen gas and then stored at 4°C until needed.

Activated sludge was also collected from the Giheung Respia Wastewater Treatment Plant and used for determining the relation between protein and VSS concentration. Different concentrations of activated sludge were prepared with dilution using distilled water at 5, 12.5, 25, 50, and 100 times.

Reactor Configuration and Operation

A single chambered MFC with air cathode was used to produce electricity from wastewater, and the reactor volume was 280 ml with a working volume of 260 ml. Plain carbon paper (projected surface area = 40.6 cm^2) was used as the anode electrode, and 10% platinum-coated carbon cloth (14 cm^2; Fuel Cell Earth, USA) was used as the cathode electrode. Copper wire was used to make electrical connections, and an external resistance of 1,000 Ω was loaded between the anode and cathode electrodes. Pretreated Nafion-117 was used as a proton-exchange membrane, and the membrane pretreatment was carried out as previously described [6, 11]. The MFC was fed with 260 ml of wastewater without any substrate, purged with pure nitrogen gas, and sealed with silicones. For protein assay, the anode electrode was taken out by cutting a piece of it at certain intervals, and bacteria were extracted from the anode electrode. The total extraction of biomass from the anode electrode was done in an anaerobic glove box, which was purged continuously with pure nitrogen gas. At the same time, the anolyte suspended biomass (planktonic biomass) was collected and measured using a protein assay to determine the biomass yield.

In the aerobic process, a bottle-type bioreactor was used, having a working volume of 260 ml (total reactor volume = 280 ml). The bioreactor fed with wastewater was continuously aerated mechanically at an air flow rate of 90–100 ml/min. Dissolved oxygen (DO) concentration was maintained at approximately 6–7 mg/l. The amount of biomass in the aerobic bioreactor was analyzed by protein assay as done in MFC operation.

For the MFC control test, the MFC configuration except for an anode electrode was used to determine biomass generation without current generation, and also purged with nitrogen gas. The biomass yield was determined by collecting the anolyte samples and measuring the biomass by protein assay. All the reactors were continuously stirred at 160 rpm using a magnetic stirrer (DAIHAN Scientific Co., Ltd, Korea) in a temperature-controlled incubator (30 ± 1°C; Vision, Korea).

Analyses

Volatile suspended solid was measured by standard methods for the examination of water and wastewater. The 25 ml of original activated sludge and 30 ml of each diluted activated sludge samples (5, 12.5, 25, 50, and 100 times) were used in this study.

The protein concentrations of biomass on the anode and in the anodic solution were measured by the bicinchoninic acid (BCA) method, which uses a bovine serum albumin standard in 0.1 N NaOH with reagents from Thermo Scientific (Pierce BCA Protein Assay kit; USA). The bacterial biomass was extracted from the anode electrode, and the anolyte biomass was collected by centrifuging the anodic solution for measurement of protein. The extraction of protein from the bacterial cell was carried out as described previously [1, 5]. For biomass collection from the anode electrode, a piece of anode electrode (2.9 × 1.5 cm) was cut using a sterilized scissor and placed into a conical tube containing 15 ml of 0.2 N NaOH. Then this conical tube was incubated at 4°C for about 1 h and mixed well for every 15 min interval, using a vortex shaker. The anode piece was further rinsed with ultrapure water (15 ml) to collect more biomass on the anode electrode. For protein extraction from the biomass, 30 ml of biomass solution at 0.1 N NaOH was treated with the freeze-thaw method by freezing at -20°C and then thawing at 90°C for 10 min. This freeze-thaw method was repeated 3 times to extract the complete protein from the bacterial biomass. In the same way, the planktonic biomass in the anodic medium was obtained by centrifuging a 5 ml sample at 4,500 rpm for 10 min. Once the biomass was obtained, it was combined with 1 ml of 0.1 N NaOH, and then the freeze-thaw method was carried out as done for protein extraction from biomass on the anode electrode.

The SCOD concentration of samples filtered by using a 0.20 μm syringe filter (Minisart RC 25, Germany) was measured by a HUMAS COD-M kit (50–1,500 mg/l).

Voltage across a fixed external resistance was measured every 5 min using a data acquisition system (National Instruments 9205, USA).

Calculations

Biomass yield (Y) was calculated by the following equation:

$$Y = \Delta X / \Delta S$$

where ΔX is the biomass (g-COD-cell) produced over time, which was calculated using the protein concentration. The protein content was assumed to be around 50% of the cell mass, and for obtaining the theoretical COD value from the cell mass, the ratio of 1.25 g-COD-cell/g-cell was used for MFC operation, and 1.42 g-COD-cell/g-cell was used for aerobic processes [8]. ΔS is the

substrate (g-COD-substrate) removed with time by analyzing the SCOD concentration [9].

Results and Discussion

Protein Measurement of Activated Sludge Using BCA Method

The amount of biomass in activated sludge was conventionally determined using VSS measurement [2]. The activated sludge collected from the wastewater treatment plant was diluted with distilled water to obtain different VSS concentrations ranging from 22 to 1,347 mg/l. Protein concentrations of different VSS samples were measured using the BCA method, and the protein concentration ranged from 21 to 883 mg/l. In Fig. 1, the relationship between protein and VSS concentration could be described with a good linear regression (R^2 = 0.9982). This result suggests that the BCA method can properly determine the biomass concentration and also represent the increase in protein concentration according to biomass increase. In addition, protein measurement can be used to determine a quite low biomass density either on the MFC anode or in the anolyte. In other studies, the protein assay was also used to determine the biomass concentration on the anode electrode in MFC operation [1, 5]. Moreover, if there are other organic matter rather than bacteria present in solution or on the electrode, the protein assay method needs to be used for a more accurate measurement of bacterial cells [15].

Voltage Generation and Biomass Yield in MFC Operation

After an 11-day operation of startup period, the MFC produced voltage, and the average voltage generation was 57 ± 9.5 mV. The repeated loadings were performed to get a stable voltage generation during about 6 months, and then the anode electrode was cut to obtain the initial protein concentration. The MFC quickly produced voltage as fresh wastewater was fed to the anodic chamber, and a maximum voltage of 53 mV with 1,000 Ω resistance was obtained within 1.3 h (Fig. 2A). Then, the cell voltage decreased gradually with time until the end of operation. During this period of current generation in the MFC, the protein concentration of biomass from the anolyte was measured at 0, 20, and 30 h of operation, and the SCOD concentration was measured at the same time (Fig. 2B). The protein concentration of the biomass on the anode electrode was measured at the beginning and ending times of the cycle when the MFC voltage was dropped. At the beginning point of the MFC voltage cycle, the measured protein extracted from biomass on the anode was 0.31 ± 0.01 mg of protein/cm^2 (0 h). After around 30-h operation, the protein concentration increased to 0.33 ± 0.008 mg of protein/cm^2 (30 h). The protein concentrations of biomass in the anodic solution were 28.6 ± 1.06, 31.2 ± 0.94, and 33.2

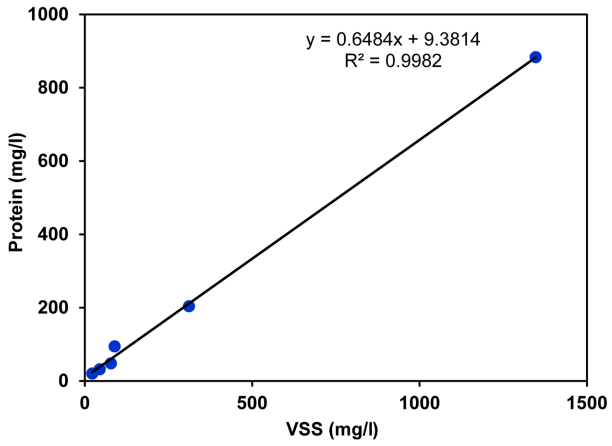

Fig. 1. Protein concentration *vs*. volatile suspended solid (VSS) in activated sludge (dilutions: 5, 12.5, 25, 50, and 100×).

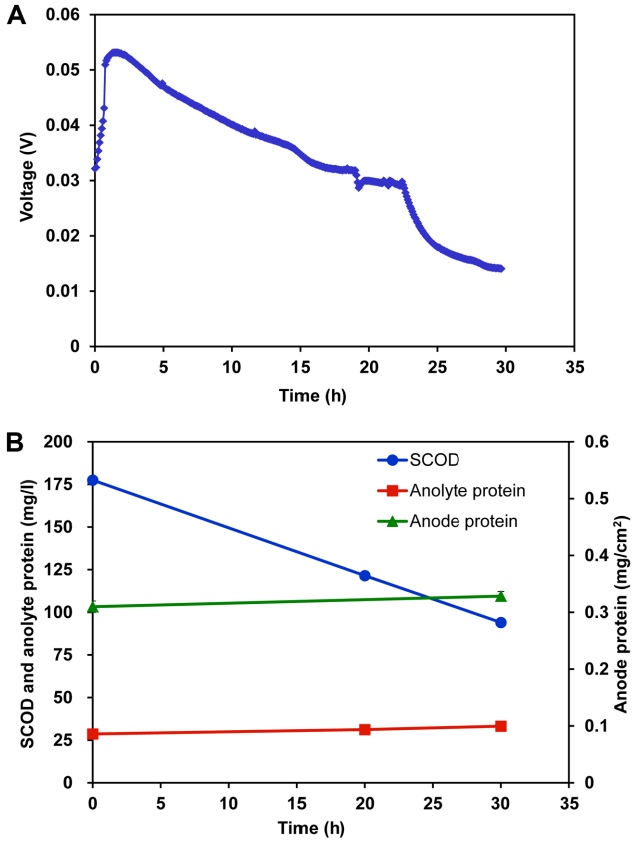

Fig. 2. Voltage generation (**A**) and protein and SCOD concentrations (**B**) as a function of time in the MFC.

± 0.47 mg/l at 0, 20, and 30 h, respectively. The initial SCOD concentration was 178 ± 0.7 mg/l (0 h), and during this 30 h cycle, the SCOD value decreased to 122 ± 0.7 mg/l (20 h) and 94 ± 1.4 mg/l (30 h). During 20 h operation, 32% of SCOD was removed, and for 30 h operation, 47% of the SCOD was removed. With anodic biomass, the cell yield was 0.12 g-COD-cell/g-COD-substrate based on protein and SCOD concentrations between 0 and 20 h. The biomass yield on the anode electrode based on protein and SCOD concentrations for 30 h MFC operation was 0.02 g-COD-cell/g-COD-substrate, and the anodic biomass yield was 0.14 g-COD-cell/g-COD-substrate, so the total biomass yield in MFC operation for 30 h was 0.16 g-COD-cell/g-COD-substrate. The total biomass yield of 0.16 was in the reported value range from 0.07 to 0.22 g-COD-cell/g-COD-substrate in other studies [9, 12, 13]. Moreover, it was about 60% smaller than the typical value (0.4 g-COD-cell/g-COD-substrate) of aerobic operation.

Protein and SCOD Concentration Changes in Aerobic Process

Protein and SCOD changes with time in aerobic condition were investigated three times using three individual reactors, and each sample was measured in duplicate (Fig. 3). In one of the aerobic tests, the initial protein concentration of planktonic biomass in the aerobic process was 42.4 ± 0.71 mg/l, and it was increased to 57.7 ± 1.65 mg/l after 18 h, and then to 57.4 ± 0.94 mg/l at 21 h of operation. The SCOD concentration at 0 h was 157 ± 7.6 mg/l, and at 18 and 21 h of operation the SCOD value was dropped to 74 ± 6.7 mg/l (53% of SCOD removal) and 50 ± 2.7 mg/l (68% of SCOD removal). The other tests showed a similar result to the previous one in SCOD and protein concentrations. Based on the SCOD and protein data from all three reactors, the average biomass yield for the aerobic process was 0.46 ± 0.07 g-COD-cell/g-COD-substrate, which was within the typical range of 0.4 to 0.64 g-COD-cell/g-COD-substrate for the aerobic process. This biomass yield under aerobic condition was almost 23 times higher than the value with biomass on the anode electrode, and it was about 3 times higher than the total biomass yield (0.16 g-COD-cell/g-COD-substrate) in the MFC operation. This result suggests that microorganisms could get much more energy for their cell growth using oxygen as the final electron acceptors. However, in the MFC operation, relatively small energy was used for cell growth with the electrode as the final electron acceptor, and with oxygen diffused from the cathode, the planktonic bacteria could grow with oxidation of organic matter [14]. This result suggests that the MFC operation in real wastewater treatment will produce less sludge, which is a benefit to reduce operational and sludge removal costs.

Protein and SCOD Concentration Changes in MFC Control

In the MFC operation, microorganisms will grow with two different final electron acceptors of either anode electrode or oxygen being diffused from the cathode side. In this test, the biomass yield without anode electrode in two identical MFC reactors was determined based on the measurements of planktonic biomass amount and SCOD removal over time (Fig. 4). In Fig. 4, the protein amounts of suspended biomass and SCOD from one reactor are presented. At the initial time, the measured protein concentration was 52.6 ± 0.35 mg/l, and after 9 h of operation the protein concentration

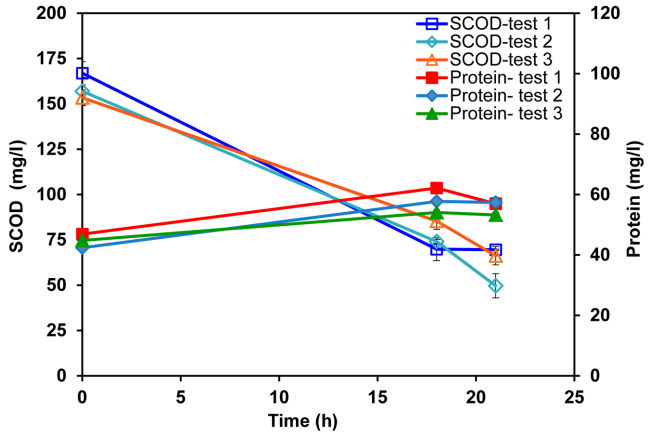

Fig. 3. Protein and SCOD concentration changes as a function of time in three separate operations under aerobic condition.

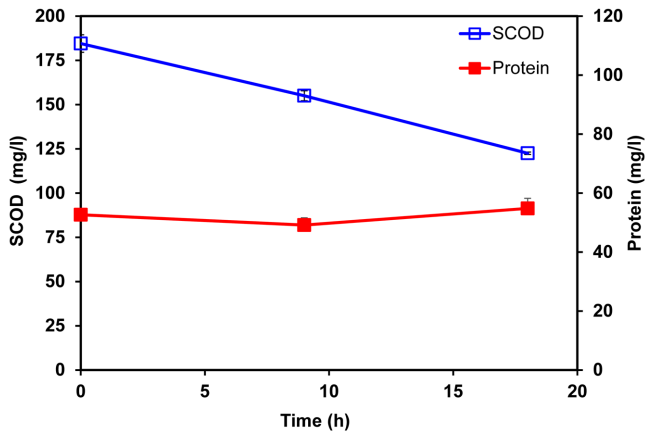

Fig. 4. Protein and SCOD concentrations as a function of time in MFC reactors without anode (MFC control).

decreased slightly to 49.1 ± 2.47 mg/l. After 18 h of operation, the protein concentration increased to 54.8 ± 3.42 mg/l. The SCOD concentration was initially 185 ± 4.9 mg/l and decreased to 155 ± 2.8 mg/l after 9 h (16% removal). At 18 h, this SCOD amount decreased further to 123 ± 0.7 mg/l, in which 34% of SCOD removal was obtained. The biomass yield based on the data from the two reactors was 0.07 ± 0.03 g-COD-cell/g-COD-substrate (0−18 h), which was about 44% of the total biomass yield (0.16 g-COD-cell/g-COD-substrate) in the MFC operation with current generation. This significant biomass growth observed without an anode electrode (no current generation) was possibly due to diffusion of molecular oxygen through the membrane and cathode electrode. This result suggests that controlling oxygen diffusion from the cathode part will be important to reduce the biomass growth without much sludge production in the MFC operation.

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