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Short communication

An excellent anaerobic respiration mode for chitin degradation by *Shewanella oneidensis* MR-1 in microbial fuel cells

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ABSTRACT

Shewanella oneidensis MR-1, a model electroactive bacterium, can oxidize a variety of fermentative organics and utilize an electrode as an electron acceptor for anaerobic respiration. Chitin as the main component of food wastes (shrimp and crab shells) is hardly degraded for bioenergy generation. This study demonstrated that *S. oneidensis* MR-1 could degrade chitin in microbial fuel cell (MFC). The metabolites during chitin degradation in MFC were succinate, lactate, acetate, formate, and ethanol. Their concentrations produced in MFC were higher than those in the fermentation system, as well as additional electricity could be recovered in MFC. Furthermore, the degradation of GlcNAc (the intermediate of chitin hydrolysis) by *S. oneidensis* MR-1 was faster than chitin in MFC and fermentation systems. Moreover, the mechanism of enhanced chitin degradation in MFC was speculated. This work might provide a new insight for biomass treatment and energy recovery by *S. oneidensis* MR-1 in MFC.

1. Introduction

Microbial fuel cell (MFC), as the most widely used bioelectrochemical system, can produce electricity from several organic matters for future sustainable energy and chemicals production, in which microorganisms serve as the biocatalyst for oxidizing organic matters [1,2]. Bacterial species identified in MFC are usually called electroactive bacteria, such as *Shewanella putrefaciens*, *Geobacter sulfurreducens*, *Rhodoferax ferrireducens*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Escherichia coli* and so on [3]. Recently, *S. oneidensis* and *G. sulfurreducens* are the species investigated most detailed among the electroactive bacteria.

Electricity can be recycled from wastes such as wastewater, solid wastes, sewage sludge and landfill leachate by MFC [4–6]. Waste biomass is a cheap and relatively abundant source to produce electricity by MFC [7,8]. Chitin is the second most abundant biomass in nature, which is a structural material in many marine invertebrates, such as cuttlefish, shrimp [9,10], crab, and lobster as well as fungi and algae. 10 billion tons of chitin are produced in aquatic environments annually [11]. Chitin is a highly insoluble polymer that consists of *N*-acetyl-β-D-glucosamine (GlcNAc) linked by β-1,4-glycosidic bonds, and can be severed as carbon, nitrogen and energy for a variety of organisms. The survival of some bacteria in an aquatic environment is associated with their ability to utilize chitin as a carbon source. Its biodegradation is also a key step in nutrient recycling processes.

Bacterial chitinases [12] provide environmental organisms the ability to acquire carbon under nutrient limiting conditions. It has been reported that only one chitin hydrolase (chitinase A) is found in *S. oneidensis* [13]. The hydrolysis products of chitin are chito-oligosaccharides and GlcNAc. *S. oneidensis* MR-1 is primarily cultivated on lactate [14], but is also known to oxidize GlcNAc as carbon and energy source [15]. *S. oneidensis* MR-1 could use GlcNAc as a substrate for positive growth in aerobic condition [16,17] and anaerobic growth with fumarate [17]. Yang et al. also investigated the utilization pathway of converting GlcNAc to Fru-6-P by *S. oneidensis* MR-1 [18]. As a popular mode electroactive bacterium [19], *S. oneidensis* MR-1 can convert wastes into bioelectricity and chemicals by using microbial fuel cell technique [7]. But whether chitin could be utilized and accompanied with energy production by *S. oneidensis* MR-1 has not been investigated so far.

Thus, the aim of this study was to explore the potential of chitin degradation by *S. oneidensis* MR-1 in both MFC and fermentation systems, respectively. The degradation mechanism of chitin in MFC was also proposed. This work would be useful for the efficient degradation and sustainable utilization of chitin through the bioelectrochemical technology.

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2. Materials and methods

2.1. Bacterial culture and chitin analysis

S. oneidensis MR-1 (ATCC® number 700550™) was kindly provided by Prof. K. H. Nealson from the University of Southern California [20]. It was cultivated in Luria-Bertani medium, and the dispersed biomass was centrifuged at 5000 *g* for 5 min. The harvest bacteria were washed twice with 30 mM 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and resuspended in 30 mM HEPES. The concentration of the bacterial suspension (OD_{600}) was determine by a spectrophotometer (UV752N, Jingke Co., China). White or light yellow sheet chitin (reagent grade, Sangon Biotech Inc., China) or *N*-acetyl-β-D-glucosamine (GlcNAc, 98%, Aladdin Inc., China) was served as carbon, nitrogen and energy sources. The preparation of suspended chitin was performed as previously described [21]. The contents of C, H, O and N in chitin were analyzed by an elemental analyzer (Vario EL cube, Elementar Co., Germany). The functional groups of chitin were determined by a Fourier Transform Infrared (FTIR) spectrometer (Vertex 70, Bruker Co., Germany).

2.2. MFC installation

The two-chamber MFC was used for chitin or GlcNAc degradation experiments equipped with a 100 mL anode chamber filled with culture medium and a 100 mL cathode chamber filled with 50 mM ferricyanide (pH 7.0 in phosphate buffer). The cation exchange membrane (CMI-7000, Membranes International, Inc., USA) was separated between the anode and cathode chambers, and was immersed in 0.9% NaCl solution for 24 h before use. The carbon felt (3 cm × 3 cm × 3 mm; Beijing Jixing Sheng'an Inc., China) was used as the anode or cathode electrode. A 1000 Ω resistor was connected between the anode and cathode, and the voltages of the resistor were recorded every 10 min. The recovered electrons from chitin or GlcNAc degradation in MFC were calculated through the integration of the output current (*I*) during the entire experiment.

2.3. Chitin degradation experiments

The chitin and GlcNAc degradation experiments were operated at 30 °C in MFC and fermentation systems, respectively. The anode and cathode cells equipped with carbon felt electrodes were autoclaved at 120 °C for 20 min before use. The anodic medium containing mineral salt medium and chitin or GlcNAc was sterilized alone in the erlenmeyer flask. The fermentation experiments were performed in 60 mL serum bottles filled with 30 mL mineral salt medium containing chitin or GlcNAc. After that, the bottles were purged with N_2 for 15 min to create anaerobic conditions, and then sterilized. The components of mineral salt medium (1 L) contained: 1.044 g KCl, 0.225 g K_2HPO_4, 0.225 g KH_2PO_4, 0.001 g $CaCl_2$, 0.42 g NaCl, 0.117 g $MgSO_4{\cdot}7H_2O$, 50 mM HEPES, and 10 mL mineral mix (1 L: 1.5 g NTA, 0.1 g $MnCl_2{\cdot}4H_2O$, 0.3 g $FeSO_4{\cdot}7H_2O$, 0.17 g $CoCl_2{\cdot}6H_2O$, 0.1 g $ZnCl_2$, 0.04 g $CuSO_4{\cdot}5H_2O$, 0.005 g $KAl(SO_4)_2{\cdot}12H_2O$, 0.005 g H_3BO_3, 0.09 g $NaMoO_4$, 0.12 g $NiCl_2$, 0.02 g $NaWO_4{\cdot}2H_2O$, and 0.1 g $NaSeO_4$). The initial concentrations of chitin and GlcNAc were 2 g/L and 4.5 mM respectively, and the OD_{600} of *S. oneidensis* MR-1 was controlled at 0.3.

During the degradation experiments, 0.5 mL sample was taken from the anode chambers or serum bottles every few days. The organic metabolites were determined by a high performance liquid chromatography (HPLC1260, Agilent Technologies, USA) with a refractive index detector. The analytical column was Aminex HPX-87H (300 mm × 7.8 mm; Bio-Rad, USA), and the mobile phase was 5 mM H_2SO_4. The flow rate was 0.5 mL/min. The morphology of the deposit and the carbon felt electrode in the anode chamber were investigated by a scanning electron microscope (SEM) (Sirion200, FEI Co., Netherlands).

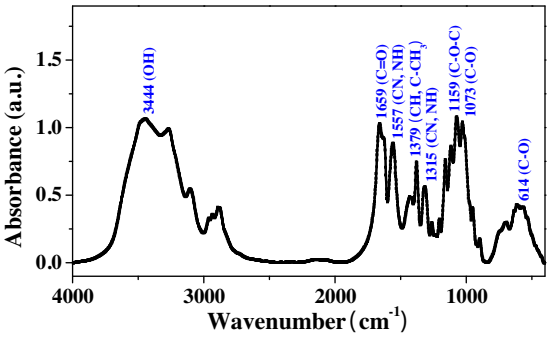

Fig. 1. FTIR spectrum and peak assignment of chitin.

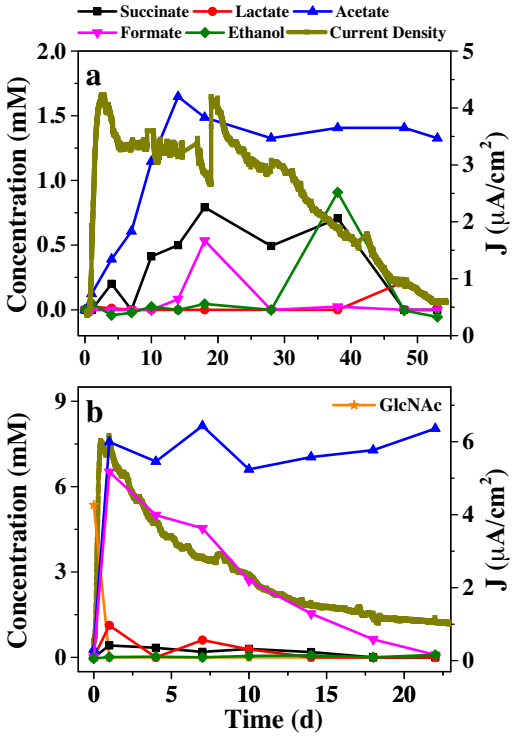

Fig. 2. Metabolite concentrations and output current density produced during (a) chitin and (b) GlcNAc degradation by *S. oneidensis* MR-1 in MFC.

3. Results and discussion

3.1. Chitin or GlcNAc degradation in MFC

The percentage of C, H, O and N elements in chitin were 42.65%, 6.98%, 45.59% and 6.19%, respectively analyzed by the elemental analyzer. The predicted chemical formula of chitin was $C_{8.07}H_{15.86}O_{6.48}N$, which was similar to that of GlcNAc ($C_8H_{15}O_6N$, the monomer of chitin). FTIR of chitin is shown in Fig. 1 and the characteristic peaks were similar to those of chitin reported by the previous study [22]. Fig. 2a shows that the main products of chitin degradation by *S. oneidensis* MR-1 in MFC were succinate, lactate, acetate, formate and ethanol with their maximum concentrations of 0.79, 0.22, 1.65, 0.54 and 0.91 mM, respectively. The output current density reached the maximum value of 4.24 μA/cm^2 at the 2.66th day. This was similar to that of 3.5 μA/cm^2 generated by the MFC cultivated with anaerobic sludge using 1 g/L chitin as the sub-