Halomonas venusta Coupled Operation of Nitrogen Removal and Electricity Generation under High Salt

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Abstract. In this paper, the moderately halophilic bacterium Halomonas venusta DSM 4743T was selected to optimize the N removal conditions and the electricity generation conditions in the MFC, and conduct the coupled operation of the electricity generation and denitrification of MFC high-salt wastewater. The results showed when sodium succinate was used as carbon source, C/N was 5:1, pH was 9, and NaCl was 30 g/L, 93.22% N removal rate was obtained at 168 h. The maximum output voltage of MFC is 346 mV under the conditions of 6 g/L NaAc, 50 μM riboflavin, pH=8 and 30 g/L NaCl. H. venusta DSM 4743T MFC generates electric denitrification coupled operation, with the maximum output voltage up to 224 mV and the denitrification rate of 53.5%.

1. Introduction

The food processing industry, the leather industry and the petroleum industry are the main sources of high-salt wastewater. High-salt wastewater usually contains organic matter, nitrogen, phosphorus and other substances. Biological denitrification has the advantages of high efficiency, low cost and no secondary pollution. However, high salinity reduces microbial denitrification capacity, and when salinity increases from 0% to 6%, NH₄⁺-N removal efficiency decreases from 96% to 39%[1]. High salt can inhibit the growth and metabolism of microorganisms. When the NaCl concentration exceeds 35.5 g/L, the respiration inhibition rate of general microbial cells reaches 81%[2].

Microbial fuel cell (MFC) is a new wastewater treatment technology that integrates wastewater resource utilization, sludge reduction, and water purification[3]. High salt can help proton transfer, thereby reducing the internal resistance of the system, which is generally considered to be beneficial to MFC power generation. However, high salt will inhibit the activity of anode electrogenic bacteria, which is not conducive to electricity production[4]. The antagonistic effect of high salt on MFC electricity generation can be solved by finding electrogenic bacteria that can tolerate high salt.

In this paper, a moderately halophilic bacterium Halomonas venusta DSM 4743T was selected to study its nitrogen removal properties under high-salt conditions and its power generation performance in MFC, and conduct the combined operation of MFC high-salt wastewater generation and N removal.

2. Materials and Methods

2.1. Strain

Halomonas venusta DSM 4743T: Purchased from DSMZ (German Collection of Microorganisms and Cell Cultures).
2.2. Medium

**N removal medium (g/L):** The carbon source is determined according to the experimental conditions, \((\text{NH}_4)_2\text{SO}_4 2, (\text{NH}_4^-\text{N} \text{ concentration} 545 \text{ mg/L}), \text{KH}_2\text{PO}_4 0.1, \text{K}_2\text{HPO}_4•3\text{H}_2\text{O} 0.3, \text{MgSO}_4•7\text{H}_2\text{O} 0.4, \text{MnSO}_4•\text{H}_2\text{O} 0.01, \text{NaCl} 30, \text{trace mineral solution} 2 \text{ mL/L}, \text{pH} 7.2.

The MFC electricity generation substrate (g/L): sodium acetate 4, L-monosodium glutamate 2, \text{K}_2\text{HPO}_4•3\text{H}_2\text{O} 11.47, \text{KH}_2\text{PO}_4 2.75, (\text{NH}_4)_2\text{SO}_4 1, \text{mineral} 12.5 \text{ mL/L}, (the NaCl concentration was determined according to the experimental conditions), \text{pH} 7.2.

**Substrate for electricity generation and N removal (g/L):** sodium acetate 6, glucose 20, sodium glutamate 2, \text{Na}_2\text{C}_6\text{H}_5\text{O}_7•2\text{H}_2\text{O} 30, (\text{NH}_4)_2\text{SO}_4 8, \text{yeast extract} 0.5, \text{K}_2\text{HPO}_4 9, \text{KH}_2\text{PO}_4•3\text{H}_2\text{O} 3, \text{MgSO}_4•7\text{H}_2\text{O} 0.4, \text{MnSO}_4•\text{H}_2\text{O} 0.01, \text{NaCl} 30, \text{trace mineral solution} 2 \text{ mL/L}, \text{pH} 7.2.

LB, growth medium, trace mineral solution and mineral were prepared according to the literature method[5]. All media, agents, and substrate, except the trace mineral solution and mineral, were sterilized by autoclaving at 121 °C for 20 min. The trace mineral solution was sterilized by filtration (0.22 μm pore size filters).

2.3. **SND method and ammonia nitrogen concentration determination method**

**SND method:** The strain was cultivated in 5 mL LB medium containing 30 g/L NaCl at 30 °C and 120 rpm in a rotary shaker for 24 h. Then 1% of the cultures were inoculated in 300 mL shake flasks containing 30 mL N removal medium at 30 °C and 120 rpm in a rotary shaker[5]. The N removal rate is defined as the percentage reduction in ammonia nitrogen in the system.

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\text{N removal rate (\%)} = \frac{(\text{TN}_0-\Delta\text{CN}-\text{TN}_t)}{(\text{TN}_0-\Delta\text{CN})} \times 100\%
\]

Among them, \(\text{TN}_0\) is the total ammonia nitrogen \((\text{NH}_4^-\text{N})\) at the beginning of N removal, \(\text{TN}_t\) is the \(\text{NH}_4^-\text{N}\) concentration at a certain moment in the N removal process, and \(\Delta\text{CN}\) is the increase in cellular nitrogen. \(\text{NH}_4^-\text{N}\) was determined by Nessler’s reagent method. Determination method of cell total N (CN) was determined by Kjeldahl method[5]. CN with different cell dry weight (CDW) was determined. The relationship between CDW and CN was calculated. CN was calculated by determining the CDW in experiment.

2.4. **Construction and operation of single-chamber MFC**

A single-chamber air cathode MFC was constructed with an effective volume of 28 mL. Carbon fiber brush was used as anode, and the cathode used air cathode[3]. The anode and cathode were connected with an external resistance of 1,000 Ω. *Halomonas venusta* DSM 4743T were cultured with LB medium for 20 h, then inoculated into the growth medium (30 mL / 300 mL Erlenmeyer flask) at 1% inoculation volume, and cultured at 30 °C for 48 h in a 120 rpm shaker. Afterwards, culture medium of *H. venusta* DSM 4743T was transferred into the electricity generation substrate of MFC. The MFC was operated in a sequencing batch mode and stored in a 30 °C constant temperature incubator. The voltage is measured with a digital multimeter.

3. Results and discussion

3.1. **Characteristics of N removal by *Halomonas venusta* DSM 4743T**

3.1.1. Effect of organic C sources and C/N ratio

Glucose, sucrose, starch, sodium acetate (NaAc), sodium succinate and trisodium citrate were set as carbon sources (C concentration 5.30 g/L) for N removal to investigate the influence of carbon sources on N removal, and the results were shown in Figure 1a. When NaAc, sodium succinate and sodium citrate were used as carbon sources, the N removal rate was above 80%, and sodium succinate as carbon source obtained the highest N removal rate of 90.79%.

The carbon source of the N removal medium was sodium succinate, and C/N was set as 0:1, 2.5:1, 5:1, 7.5:1 and 10:1. The effect of C/N on the N removal of *H. venusta* DSM 4743T was investigated, and the results were shown in Figure 1b. When the C/N was 5:1, the maximum N removal rate was
84.82%.

Figure 1. N removal characteristics of *H. venusta* DSM 4743T under different conditions. Abbreviations: Glc, Glucose; Suc, sucrose; Sta, starch; NaAc, sodium acetate; AcsNa, sodium succinate; CitNa, trisodium citrate.

3.1.2. Effects of pH and NaCl concentration
Sodium succinate was used as carbon source and C/N was 5:1. The pH value of the N removal medium was set as 7, 8, 9 and 10 by using 50 mM Tris-Hcl and Borax-NaOH buffer solution. The effect of pH on N removal medium of *H. venusta* DSM 4743T was investigated, and the results were shown in Figure 1c. The N removal rate of the strain was maintained at a high level in the range of pH 7 to 10, and the maximum N removal rate was obtained at a pH of 9 of 89.99%.

The carbon source of N removal medium was sodium succinate, C/N was 5:1, and pH was 9. NaCl concentration was set as 0 g/L, 30 g/L, 60 g/L, 90 g/L and 120g/L to investigate the influence of NaCl concentration on N removal rate and the result are shown in Figure 1d. The maximum N removal rate was 92.33% when the concentration of NaCl was 30 g/L.

3.1.3. N removal process of *H. venusta* DSM 4743T under optimized conditions
The optimal N removal conditions were: sodium succinate as carbon source, C/N at 5:1, pH=9, NaCl concentration at 30 g/L. The N removal process of *H. venusta* DSM 4743T under optimized conditions was shown in Figure 2. Under these conditions, 93.22% N removal rate was obtained at 168 h.

Figure 2. N removal process of *H. venusta* DSM 4743T under optimized conditions.
3.2. Optimization of Halomonas venusta DSM 4743T electricity generation condition in MFC

3.2.1. Effects of carbon source and concentration of substrate on electricity generation

The influence of carbon source in the substrate on the electricity generation of MFC was investigated by testing four carbon sources (C concentration 1.17g/L) of NaAc, sodium succinate, sodium citrate and glucose. The results were shown in Figure 3a. The average voltage of MFCs during the stable period (2-24 h) was 88 ± 4 mV, 88 ± 3 mV, 84 ± 4 mV, and 28 ± 6 mV respectively. The following study used NaAc as the carbon source of the MFC matrix.

Figure 3. Effects of carbon source type and concentration of MFC substrate on electricity generation.

The influence of NaAc concentration of 2 g/L, 4 g/L, 6 g/L, 8 g/L and 10 g/L on the power generation of MFC was studied. Figure 3b showed when the NaAc concentration was 6 g/L, the average voltage output of MFC in the stable period reached 108 ± 9 mV. As the concentration of NaAc further increased to 8 g/L and 10 g/L, the voltage output of MFC did not change significantly.

3.2.2. Effect of riboflavin concentration on MFC electricity production

Riboflavin is a common type of electronic medium. The influence of riboflavin concentration on the electricity generation of H. venusta DSM 4743T was investigated. Concentrations of riboflavin were set at 0 μM, 10 μM, 50 μM, 100 μM, 250 μM, and 500 μM. The voltages of MFCs are shown in Figure 4. The average steady-state voltage of 50 μM riboflavin was 208 ± 12 mV. Compared with the same condition without riboflavin, the voltage output of MFC increased by 92%.

Figure 4. Effect of riboflavin concentration on MFC electricity production.

3.2.3. Effect of initial pH of substrate on electricity generation

The effect of pH on the MFC electrical performance was investigated. The buffer solutions were used to adjust pH of the substrate to 6, 7, 8, and 9, respectively. The results (Figure 5) showed that the MFCs output voltage increased with the increase of pH. When the substrate pH=8, the average output voltage in the stable state reached a maximum value of 281 ± 36 mV.
3.2.4. Effect of NaCl concentration in the substrate on electricity generation
The effect of NaCl concentration of the substrate on the electricity generation was studied at the NaCl concentrations of 15 g/L, 30 g/L, 45 g/L, and 60 g/L. The voltage output results (Figure 6) showed that MFCs was able to generate electricity when the NaCl concentration of the substrate was in the range of 15 g/L ~ 60 g/L. The average voltage at the stable state was 280 ± 36 mV at 30 g/L NaCl.

3.2.5. Electricity generation process under the optimal conditions
The optimized electricity generation conditions of the \textit{H. venusta} DSM 4743\textsuperscript{T} strain were: 6 g/L NaAc, 50 \(\mu\)M riboflavin, pH=8, 30 g/L NaCl. Under this condition, the electricity generation process of MFC was investigated, and the results were shown in Figure 7. The MFC produced the highest output voltage of 346 mV in three power generation cycles, the average output voltage during the stable period was 286 ± 34 mV, and the voltage output changed periodically and steadily.

3.3. \textit{Halomonas venusta} DSM 4743\textsuperscript{T} operated coupled with denitrification and electricity generation under high salinity
The effect of \textit{H. venusta} DSM 4743\textsuperscript{T} on N removal of high-salt wastewater in MFC was investigated. Figure 8 showed that the maximum output voltage of MFC is up to 224 mV under the condition of high-salt wastewater (30 g/L NaCl). The highest N removal rate was 53.5% at 132 h.

4. Conclusion
The optimal carbon source, C/N, pH and NaCl concentrations were sodium succinate, 5:1, 9 and 30g/L, respectively. Under these conditions, 93.22% N removal rate was obtained at 168 h. Under the conditions of 6 g/L NaAc, 50 \(\mu\)M riboflavin, pH=8 and 30 g/L NaCl, the highest output voltage was 346 mV, which was 2.68 times as much as the original 94 mV before optimization. The coupling operation process of electricity generation and N removal of \textit{H. venusta} DSM 4743\textsuperscript{T} MFC under 30 g/L NaCl is described. The maximum output voltage of MFC is 224 mV, and the maximum N removal rate is 53.5%.
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References
[1] Uygur, A., Kargi, F. (2004) Salt inhibition on biological nutrient removal from saline wastewater in a sequencing batch reactor. Enzyme and Microbial Technology, 34: 313-318.
[2] Pernetti, M., Di Palma, L. (2005) Experimental evaluation of inhibition effects of saline wastewater on activated sludge. Environmental Technology, 26: 695-703.
[3] Zeng, F.J., Wu, Y.T., Bo, L., Zhang, L.H., Liu, W.F., Zhu, Y.M. (2020) Coupling of electricity generation and denitrification in three-phase single-chamber MFCs in high-salt conditions. Bioelectrochemistry, 133.
[4] Lefebvre, O., Tan, Z., Kharkwal, S., Ng, H.Y. (2012) Effect of increasing anodic NaCl concentration on microbial fuel cell performance. Bioresource Technology, 112: 336-340.
[5] Wang, T., Li, J., Zhang, L.H., Yu, Y., Zhu, Y.M. (2017) Simultaneous heterotrophic nitrification and aerobic denitrification at high concentrations of NaCl and ammonia nitrogen by Halomonas bacteria. Water Science and Technology, 76: 386-395.