Supporting Information

Sequential generation of hydrogen and lipids from starch by combination of dark fermentation and microalgal cultivation

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Experimental Section

Microorganisms and culture conditions

Seed sludge was obtained from a wastewater treatment plant in Heilongjiang province, China. Before use, the sludge was pretreated as the method in the literature to enrich the dark fermentative bacteria.[1] Previous reports showed that the green microalga *Scenedesmus* sp. had high growth rate and lipid content,[2] so it was used for lipid production in all the experiments.[3]

Experimental procedures

Dark fermentative hydrogen production was performed in 300 mL reactors (serum bottles) with a working volume of 200 mL by batch experiments. Four starch powers (i.e., cassava starch, corn starch, sweet potato starch and potato starch) were used. According to the manufacturer's instructions, the specific heat values of cassava

starch, corn starch, sweet potato starch and potato starch were 15.08, 14.4, 15.08 and 14.13 kJ g^{-1}, respectively. The initial starch concentration was set at 5 g L^{-1}. Other compositions of the medium were described in previous studies.[4, 5] The initial pH of the medium was adjusted to 7.0 ±0.2 and nitrogen gas was used to ensure an anaerobic environment. The medium was sterilizated at 121 ℃ for 15 min. Inoculum of 10% (v/v) was added to each reactor. Temperature of fermentation was maintained at 35 ±1 ℃ in a constant temperature incubation shaker (130 rpm).

The residual liquid from dark fermentation was centrifuged at 12,000 rpm for 10 min and filtered through a 0.22 μm membrane to remove the hydrogen produces. The supernatant was collected and the pH was adjusted to 7.0 ±0.2. Microalgal cultivation was carried out in batch mode using a series of 250 mL Erlenmeyer flasks containing 150 mL supernatant. The flasks were shaken at 130 rpm in darkness at constant temperature of 25 ±1 ℃. All the experiments were run in triplicates.

Analytical methods

Biogas was sampled from the head space of the reactors using a gas-tight glass syringe. The hydrogen content was determined by a gas chromatograph equipped with a thermal conductivity detector (Agilent 4890D, USA). Argon was adopted as the carrier gas at a flow rate of 30 mL min^{-1}. Temperatures of injection, oven and detector were 120, 35, and 120 ℃, respectively. Soluble metabolites in dark fermentative effluent were measured by a gas chromatograph equipped with a flame ionization detector (Agilent 7890A, USA). The carrier gas was nitrogen and the flow rate was 50 mL min^{-1}. Prior to analysis, the samples were centrifuged at 12,000 rpm for 5 min and

filtered through a 0.22 μm membrane to remove solids from the liquid medium. Temperatures of injection port, column and detector were 250, 300, and 300 ℃, respectively. The biochemical composition were measured as the methods described previously.[6] Biomass concentration was determined by filtering samples through a 0.22 μm Millipore filter, drying at 105 °C and measuring the constant dry weight. The pH value was adjusted with 1 M HCl or 1 M NaOH solution and was monitored using a digital pH meter (Mettler FE20K, Switzerland).

References

1. P. Mohammadi, S. Ibrahim, M. S. Mohamad Annuar and S. Law, *J. Cleaner Production*, 2011, **19**, 1654-1658.
2. Y.-H. Wu, Y. Yu and H.-Y. Hu, *Bioresour. Technol.*, 2015, **192**, 374-381.
3. H.-Y. Ren, B.-F. Liu, C. Ma, L. Zhao and N.-Q. Ren, *Biotechnol. Biofuels*, 2013, **6**, 143.
4. B.-F. Liu, N.-Q. Ren, D.-F. Xing, J. Ding, G.-X. Zheng, W.-Q. Guo, J.-F. Xu and G.-J. Xie, *Bioresour. Technol.*, 2009, **100**, 2719-2723.
5. X.-J. Wang, N.-Q. Ren, W.-S. Xiang and W.-Q. Guo, *Int. J. Hydrogen Energy*, 2007, **32**, 748-754.
6. H.-Y. Ren, B.-F. Liu, F. Kong, L. Zhao, G.-J. Xie and N.-Q. Ren, *Bioresour. Technol.*, 2014, **166**, 625-629.