Electronic Supplementary Data

A novel strategy to alleviate medium acidosis for simultaneously yielding more bacterial cellulose and electricity

Qing Wang[a,b,c], Dong Tian[a,b], Jinguang Hu[c], Yongmei Zeng[a,b], Fei Shen*[a,b]

[a] *Institute of Ecological and Environmental Sciences, Sichuan Agricultural University, Chengdu, Sichuan 611130, P. R. China.*

[b] *Rural Environment Protection Engineering & Technology Center of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan 611130, P. R. China.*

[c] *Chemical and Petroleum Engineering, Schulich School of Engineering, the University of Calgary, Calgary, T2N 4H9, Canada.*

** Corresponding Author:* fishen@sicau.edu.cn

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1. Fermentation apparatus and the data acquisition system

The schematic diagram of the fermentation apparatus and the data acquisition system as shown in **Fig. 1**. This fermentation apparatus consists of polyethylene terephthalate (PET) vessel (8.5 cm × 3.0 cm × 13.5 cm in length × width × height) with a gas-permeable film, culture medium, adjustable resistor (switch to 2 Ω). Copper (Cu) and magnesium (Mg) were selected as anode and cathode (20 mm × 15 mm × 0.5 mm in length × width × thickness), respectively. The current was determined by a computer-controlled multi-meter (UT803, UNI-T, China) and the data is recorded by its control software (1 point/2 s). Voltages of the galvanic cell were monitored by a multi-meter (Keithley 2000, Tektronix, USA) and the generated data of voltage were collected using the Test Point software during the BC fermentation.

2. BC production

In this study, the strain *acetobacter xylinum* (ATCC 23767) was purchased from the American Type Culture Collection (Rockville, MD, USA). Hestrin-Schramm (HS) medium, consisting glucose 2.0 (%, w/v), peptone 0.5 (%, w/v), yeast extract 0.5 (%, w/v), $Na_2HPO_4 \cdot 12H_2O$ 0.7 (%, w/v), and citric acid 0.1 (%, w/v), was employed to prepare the culture medium for BC production.[1] The prepared culture medium was autoclaved under 105°C for 30 min, then using the sulfuric acid and sodium hydroxide to adjust the pH to approximately 5.0. Initially, 100 µL strain liquid was poured into 100 mL HS medium for 48 h dynamic incubation at 30°C to propagate the strain. After that, 10 mL inoculum was added into 90 mL medium and incubated in the designed fermentation apparatus at 30°C for 6-days static incubation.

After 6 days of fermentation, the obtained BC hydrogel was washed by 0.1 mol/L NaOH under 80°C for 30 min to remove the residual sugar and bacteria, then the BC was further washed by distilled water till the neutral pH. Finally, the washed BC film was dried in an oven under 105°C for 6 h and weighted to record the yield.

3. Analytic methods

The optical density (OD) of culture medium after fermentation was determined by a spectrophotometer (U-2910, HITACHI, Japan) at 610 nm; the dissolved oxygen (DO) was detected using a portable meter (JPB-607A, Lei-ci, China) with a probe (DO957, Lei-ci, China); the pH of the medium was determined by a pH meter (PHS-3C, Lei-ci, China); the concentration of residual glucose and glucuronic acid in the culture medium was determined by an HPLC system (1260 Infinity II, Agilent, Germany) with a refractive index detector (RID) (G7162A, Agilent, Germany) and a sugar column (I.D. × L= 8.0 × 300 mm, SH1011, Shodex, Japan) for separation; the eluent is 5.0 mmol/L H_2SO_4 under 0.6 mL/min flow rate, the column temperature is controlled at 60°C and the injection volume is controlled at 10 μL. 500 μL liquid was taken from the medium, heating 5 min under 100°C to inactivate the bacteria, and centrifuged at 12000 rpm for 10 min, then the collected liquid was diluted and filtered with 0.45 μm filter for HPLC analysis.

OriginPro (version 2020, OriginLab Corporation, USA) was employed for statistical analysis.

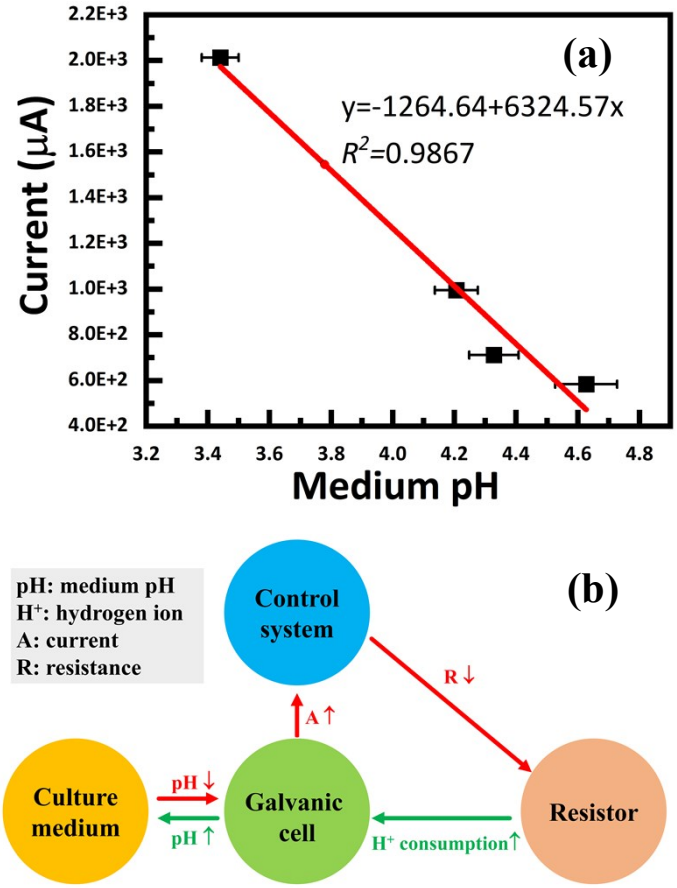

Fig. S1 Assumption of the automatic pH control system: (a) the correlativity between medium pH and electric current of built-in galvanic cell; (b) the regulation process of medium pH by the automatic pH control system.

Generally, the mechanism of a pH sensor is a well linear correlation between the behavior of a pH-sensitive chemical with the pH changes, and this correlation could be determined by the electrochemical system or spectrophotometric systems. [2-4] As shown in **Fig. S1a**, there is a well linear correlation between the formed current of the built-in galvanic cell and the medium pH ($\boldsymbol{R^2}$=0.9867). We believed this correlation could be employed to design an electrochemical pH sensor, and when the medium pH became larger, the current in the circuit became smaller, vice versa.

This pH-sensitive electrochemical reaction of the galvanic cell can be used to design a fully automatic pH control system as shown in **Fig. S1b**: when the culture medium begins to acidify, the H^+ concentration in the medium increases and the medium pH decreases accordingly; for the built-in galvanic cell, the increased H^+ can lead a higher power output capacity (appears as an increase in current or voltage in the circuit); the control system will detect these changes of current and gives a judgment of the value of medium pH according to the known correlation between current and medium pH; the control system will decrease the load resistance of the built-in galvanic cell when the medium pH is lower than a suitable range; then, the built-in galvanic cell will consume more H^+ to generate more electricity power; finally, the medium pH will increase till a suitable range and the control process will stop. This assumption is easy to implement under the existing technique, and we will also study this further.

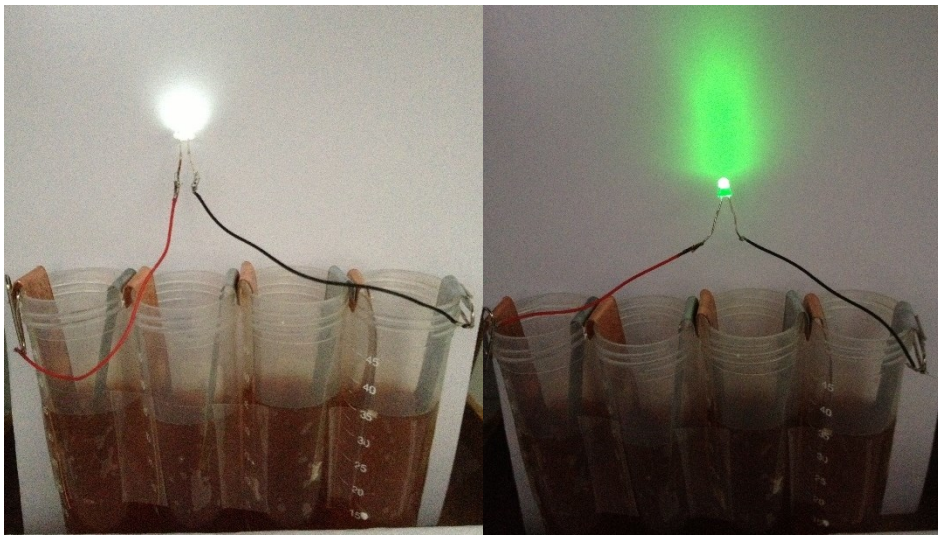

Fig. S2 Using the built-in galvanic cell in series to light a LED bulb:

(a) white LED bulb; (b) green LED bulb. The working voltage of these two bulbs is around 3.6 V. To facilitate this demonstration, 4 simple fermentation apparatus were connected.

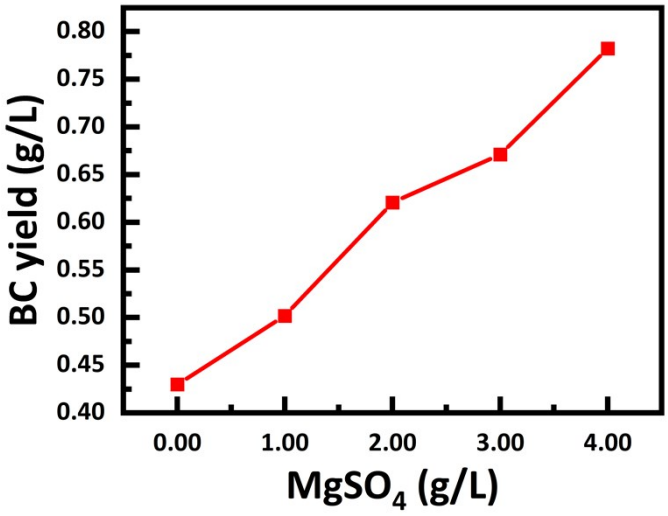

Fig. S3 Effect of Mg^{2+} on BC production.

The Mg^{2+} would release to culture medium when the built-in galvanic cell working. To investigate the effect of Mg^{2+} on BC production, $MgSO_4$ was added to the medium according to a concentration of 0.00 g/L to 4.00 g/L as shown in **Fig. S3**. As the increasing concentration of Mg^{2+} in the culture medium, the BC yield was distinctly increased from 0.43 g/L to 0.78 g/L. In this study, we tried to measure the weight of the Mg electrode after 6-days incubation, the quality of its weight loss is almost negligible. Therefore, it believed that the released Mg^{2+} will not adversely affect the bacterial metabolic activity, but instead, it will promote the BC yield.

References

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