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## Efficient production of coenzyme Q<sub>10</sub> from acid hydrolysate of sweet sorghum juice by *Rhodobacter sphaeroides*

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In order to achieve efficient bioconversion of biomass-derived sugars, acid hydrolysate of sweet sorghum juice (SSJAH) containing abundant fermentable sugars was used for coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) fermentation by *Rhodobacter sphaeroides* CQ-09-1. The synthesis of CoQ<sub>10</sub> was facilitated when the initial concentration of total sugar was 80.00 g L<sup>-1</sup>. And the highest CoQ<sub>10</sub> titer was obtained when the pH and temperature were maintained at 7.00 and 30.00 °C, respectively. Moreover, corn steep powder (CSP) was proved to be an efficient nitrogen & salt supplement to SSJAH. Under the optimized conditions, the titer of CoQ<sub>10</sub> reached 141.95 mg L<sup>-1</sup> in a fed-batch fermentation. The CoQ<sub>10</sub> titer reported was about two times higher than that obtained in the previous study using wild strains. This process introduces a potential way to produce CoQ<sub>10</sub> using the concept of biorefinery, while making full use of sweet sorghum juice (SSJ).

## 1 Introduction

Coenzyme Q, which is composed of a benzoquinone group and a varying length side chain of isoprenoid groups, is an essential component of the electron transfer system in the plasma membrane of prokaryotes and the inner mitochondrial membrane of eukaryotes.<sup>1</sup> And CoQ<sub>10</sub> containing ten isoprenoid units in the side chain has been widely accepted as a potent antioxidative dietary supplement which is related to energy boosting, immune enhancement and ease of hypertension.<sup>2,3</sup>

CoQ<sub>10</sub> could be produced by extraction from animal tissues<sup>4</sup> or chemical synthesis,<sup>5</sup> however, in order to produce biologically potent CoQ<sub>10</sub> without optical isomers and reduce the production cost, microbial fermentation has been considered the most viable approach.<sup>6,7</sup> Various microorganisms including bacteria,<sup>8,9</sup> moulds,<sup>10</sup> yeasts<sup>11</sup> and genetically engineered *Escherichia coli*,<sup>12</sup> have been reported for CoQ<sub>10</sub> production. In particular, photosynthetic bacteria such as *Rhodobacter*<sup>1,13</sup> exhibit excellent ability in bio-synthesis of CoQ<sub>10</sub>. Recently, in order to achieve the improvement of CoQ<sub>10</sub> titer, extensive efforts have been made in designing alternative biosynthetic pathway by metabolic engineering and strain development by natural isolation & chemical mutagenesis.<sup>14</sup> However, cheap

and alternative substrates for CoQ<sub>10</sub> production have been barely studied.

Sweet sorghum (*Sorghum bicolor* L. Moench) has been considered as an attractive raw material in biorefinery process, and it offers great advantages: greater biomass yield per hectare, high photosynthetic efficiency and wide adaptability to harsh environmental conditions.<sup>15</sup> The content of fermentable sugars in sweet sorghum juice (SSJ) from the stalk could reach 160.00–180.00 g L<sup>-1</sup>.<sup>16,17</sup> Furthermore, corn steep powder (CSP) obtained from corn starch processing provides an inexpensive source of proteins, amino acids, minerals, vitamins and trace elements.<sup>18</sup> A previous study indicated that CSP could be used as a satisfactory source of nitrogen and metal ions to replace yeast extract (YE) & salts in L-lactic acid fermentation, while resulting in 83.75% reduction in the production cost.<sup>19</sup> Thus, this study focused on the cost-efficient production of CoQ<sub>10</sub> using cheap and alternative substrates using the concept of biorefinery, and the conditions and modes of the fermentation with *R. sphaeroides* CQ-09-1 were optimized.

## 2 Methods

### 2.1 Raw materials

Sweet sorghum, which was kindly provided by the Institute of Millet Crops of Hebei Academy of Agricultural and Forestry Sciences, was harvested in the national high-tech industrial development zone of Shijiazhuang (114° E, 38° N), October 2018. A three-roller mill (SY-20, Guangzhou Fukang Co., Ltd, China) was used to obtain SSJ, and the husks and leaves were stripped from the stem by hand before juice squeezing. The fresh juice obtained was boiled for 5.00 min to achieve denaturation and precipitation of heteropolymeric proteins, and the

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suspended solids was removed by the vacuum suction filtration coupled filter paper using diatomite as filter-aids. The clarified SSJ contained 156.60 g L<sup>-1</sup> sucrose, 19.60 g L<sup>-1</sup> glucose and 19.20 g L<sup>-1</sup> fructose. The acid hydrolysis of SSJ was conducted as previously reported,<sup>20</sup> and the acid hydrolysate of sweet sorghum juice (SSJAH) which consisted of 102.02 g L<sup>-1</sup> glucose and 101.62 g L<sup>-1</sup> fructose was stored at -20.00 °C before use.

YE and sodium L-glutamate were purchased from Aobox Biotechnology Co., Ltd. (Beijing, China). CSP was provided by Beijing Mannafeed International Group (Beijing, China). Biotin, niacin and thiamine hydrochloride were obtained from Sigma (Shanghai, China). All other chemicals used in this study were of analytical grade and commercially available.

## 2.2 Microorganism and culture media

The *R. sphaeroides* CQ-09-1, which was derived from CICC 10287 (China Center of Industrial Culture Collection, Beijing, China), was screened by evolutionary engineering and used for CoQ<sub>10</sub> production in this study.

The medium for agar slant contained 3.00 g L<sup>-1</sup> glucose, 8.00 g L<sup>-1</sup> YE, 2.00 g L<sup>-1</sup> NaCl, 1.30 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 0.13 g L<sup>-1</sup> MgSO<sub>4</sub>, 150.00 µg L<sup>-1</sup> biotin, 1.00 mg L<sup>-1</sup> niacin, 1.00 mg L<sup>-1</sup> thiamine hydrochloride and 15.00 g L<sup>-1</sup> agar. And the composition of inoculum preparation medium was the same as that of agar slant except the addition of agar.

To investigate the effect sugar concentrations on CoQ<sub>10</sub> fermentation, mixed sugar with total sugar concentrations ranging from 40.00–120.00 g L<sup>-1</sup> were adopted, and the weight ratio of individual sugars was the same as that of SSJAH. In addition, the media contained 2.00 g L<sup>-1</sup> NaCl, 3.00 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 6.30 g L<sup>-1</sup> MgSO<sub>4</sub>, 2.00 g L<sup>-1</sup> CaCO<sub>3</sub>, 8.00 g L<sup>-1</sup> YE, 3.00 g L<sup>-1</sup> sodium L-glutamate, 150.00 µg L<sup>-1</sup> biotin and 1.00 mg L<sup>-1</sup> niacin.

Also, different nitrogen sources (CSP, ammonium nitrate, ammonium sulfate and urea) were adopted to examine the feasibility of a cheap alternative to YE, and the nitrogen content was the same as that of 8.00 g L<sup>-1</sup> YE. Moreover, the concentration optimization of CSP which was selected as the suitable nitrogen source was conducted, and CSP concentrations ranging from 8.00 g L<sup>-1</sup> to 16.00 g L<sup>-1</sup> were adopted. Besides nitrogen source, the media used also contained 2.00 g L<sup>-1</sup> NaCl, 3.00 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 6.30 g L<sup>-1</sup> MgSO<sub>4</sub>, 2.00 g L<sup>-1</sup> CaCO<sub>3</sub>, 120.00 g L<sup>-1</sup> mixed sugar, 3.00 g L<sup>-1</sup> sodium L-glutamate, 150.00 µg L<sup>-1</sup> biotin and 1.00 mg L<sup>-1</sup> niacin.

The media used for the optimization of temperature & pH contained 2.00 g L<sup>-1</sup> NaCl, 3.00 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 6.30 g L<sup>-1</sup> MgSO<sub>4</sub>, 2.00 g L<sup>-1</sup> CaCO<sub>3</sub>, 120.00 g L<sup>-1</sup> mixed sugar, 12.00 g L<sup>-1</sup> CSP, 3.00 g L<sup>-1</sup> sodium L-glutamate, 150.00 µg L<sup>-1</sup> biotin and 1.00 mg L<sup>-1</sup> niacin.

In batch and fed-batch fermentation, the media consisted of SSJAH (containing 120.00 g L<sup>-1</sup> total sugar), 2.00 g L<sup>-1</sup> NaCl, 3.00 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 6.30 g L<sup>-1</sup> MgSO<sub>4</sub>, 2.00 g L<sup>-1</sup> CaCO<sub>3</sub>, 12.00 g L<sup>-1</sup> CSP, 3.00 g L<sup>-1</sup> sodium L-glutamate, 150.00 µg L<sup>-1</sup> biotin and 1.00 mg L<sup>-1</sup> niacin. Concentrated SSJAH containing a total sugar concentration of 420.00 g L<sup>-1</sup> was fed as supplement of carbon source in fed-batch fermentation.

In particular, the pH of all media was adjusted to 7.00 before use.

## 2.3 Culture conditions

The strain *R. sphaeroides* CQ-09-1 was maintained on agar slants, and the stock culture was transferred to fresh medium monthly. After incubation at 30.00 °C for 48.00 h, the stock culture was stored at 4.00 °C. Inoculum preparation was carried out in 250.00 mL conical flasks with a working volume of 100.00 mL, which were incubated at 30.00 °C and 220.00 rpm for 96.00 h on a rotary shaker, and the pH was maintained at 7.00. The seed culture was then inoculated into fermentation medium with an inoculum volume of 10.00% (v/v).

The optimization of total sugar concentration, nitrogen source, temperature and pH were conducted in 250.00 mL conical flasks with a working volume of 100.00 mL. In particular, different temperatures (from 28.00 °C to 34.00 °C) and pH levels (from 6.80 to 7.00) were adopted. And other conditions were the same as those of inoculum preparation.

In order to confirm the optimized conditions obtained and to further achieve improvement in CoQ<sub>10</sub> titer, batch and fed-batch fermentations were conducted in dark condition, and a 5 L fermentor (SGB-5L, Changzhou Sungod Bio-technology & Engineering Equipment Co., Ltd., Jiangsu, China) was used. The pH and agitation rate were maintained at 7.00 and 400.00 rpm, respectively, and an aeration rate of 0.50 vvm with a temperature of 30.00 °C was adopted.

## 2.4 Analytical methods

Three parallel replicates were conducted in all fermentations, and deviations from the mean are given in tables or provided as error bars in figures. The cell density was measured by a spectrophotometer (UV-1100, Beijing Eternal Cause Instrument Co., Ltd, China) at 600.00 nm,<sup>13</sup> and a calibration curve which indicated the relationship between OD<sub>600</sub> and dry cell weight (DCW) (1.00 OD<sub>600</sub> = 0.61 g DCW L<sup>-1</sup>) was adopted. Dry cells were obtained by centrifugation and lyophilization in this study. Monosaccharides in fermentation broth were determined by high-performance liquid chromatography (HPLC) equipped with a Sugar-Pak 1 Column (Waters Ltd., USA) at 80.00 °C. A refractive index (RI) detector was used and deionized water was adopted as mobile-phase (0.50 mL min<sup>-1</sup>).<sup>21</sup> To compare the amounts of amino acids in YE, CSP and SSJAH, an amino acid analyzer (Sykam S433D, German) equipped with a cation separation column (LCA K06/Na 1.6 mm × 150 mm; Sykam GmbH, Eresing, German) was used to determine the amounts of amino acids. The amounts of metal ions and phosphorus were measured by inductively coupled plasma-optical emission spectrometer (ICP-OES) (PerkinElmer Optima 8300, USA).

The extraction and determination of CoQ<sub>10</sub> were according to methods previously reported.<sup>22</sup> The cells of *R. sphaeroides* CQ-09-1 were ruptured at 75.00 °C for 15.00 min, and 200.00 µL HCl (pH 2.00) was added into 1.00 mL fermentation broth. A centrifugation (5000.00 rpm, 10.00 min) was further conducted to collect solid phase, and an solution (ethyl acetate/ethanol =



5/3, v/v) with an amount of 4.00 mL was used for CoQ<sub>10</sub> extraction. The mixture, which was vortexed vigorously for 15.00 min, was kept in dark and incubated for 1.00 h at room temperature. Then, the supernatant obtained by centrifugation (8000.00 rpm, 5.00 min) was filtered through a 0.22  $\mu$ m filter and further analyzed by HPLC equipped with a Synergi Max-RP 80A column (Phenomenex, USA) at 35.00 °C. A UV detector at 275.00 nm was used and a mixture of methanol/ethanol (3/7, v/v) was used as the mobile phase (0.80 mL min<sup>-1</sup>).

### 3 Results and discussion

#### 3.1 Optimization of the total sugar concentration in SSJAH

Various carbon sources including glucose, fructose, xylose, acetate and malate have been reported in fermentation with *R. sphaeroides*.<sup>23–25</sup> SSJAH used in this study mainly contained glucose and fructose, and the effects of total sugar concentrations on CoQ<sub>10</sub> fermentation were investigated to avoid the carbon catabolite repression (CCR). As shown in Fig. 1, when the initial concentration of total sugar increased from 20.00 g L<sup>-1</sup> to 80.00 g L<sup>-1</sup>, the CoQ<sub>10</sub> titer was correspondingly enhanced from 7.28 mg L<sup>-1</sup> to 30.70 mg L<sup>-1</sup>. However, when the initial concentration of total sugar reached 100.00 g L<sup>-1</sup>, a CoQ<sub>10</sub> titer of 24.60 mg L<sup>-1</sup>, which decreased 19.87% of that obtained at 80.00 g L<sup>-1</sup> total sugar, was achieved. The tendency of cell density was similar to that of CoQ<sub>10</sub> titer, and the a top value of 18.60 g L<sup>-1</sup> was obtained with 80.00 g L<sup>-1</sup> total sugar. Moreover, the residual sugar concentrations were kept at relative low levels when the total sugar concentration ranged from 20.00 g L<sup>-1</sup> to 80.00 g L<sup>-1</sup>. And a significant increase in residual sugar concentration was observed with a total sugar concentration of 100.00 g L<sup>-1</sup>. The results indicated that a total sugar concentration of 80.00 g L<sup>-1</sup> should be adopted with high CoQ<sub>10</sub> titer and cell density while controlling the residual sugar concentration at a relative low level.

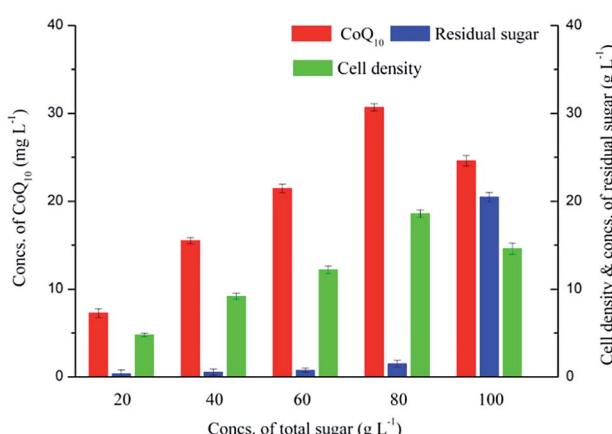


Fig. 1 Effects of different initial concentrations of total sugar on CoQ<sub>10</sub> titer, residual sugar and cell density. All experiments were carried out at 30.00 °C and 220.00 rpm for 96.00 h on a rotary shaker, the pH was maintained at 7.00 and 250.00 mL flasks with a working volume of 100.00 mL were used.

#### 3.2 The feasibility of a cheap alternative to YE in CoQ<sub>10</sub> fermentation

Nitrogen source significantly affects the growth of *R. sphaeroides*.<sup>26</sup> Two inorganic nitrogen sources and three organic nitrogen sources were used in this study as shown in Fig. 2. In particular, the nitrogen contents of various nitrogen sources were kept at the same level which was equal to 8.00 g L<sup>-1</sup> YE. For inorganic nitrogen sources, ammonium sulfate was more suitable than ammonium nitrate for CoQ<sub>10</sub> fermentation with *R. sphaeroides* CQ-09-1, and the CoQ<sub>10</sub> titer and cell density with ammonium sulfate reached 17.30 mg L<sup>-1</sup> and 10.48 g L<sup>-1</sup>, respectively, which were higher than those obtained with ammonium nitrate (13.10 mg L<sup>-1</sup> and 8.37 g L<sup>-1</sup>). Moreover, considerable amounts of residual sugar were observed (10.70 g L<sup>-1</sup> and 7.40 g L<sup>-1</sup>) when ammonium nitrate and ammonium sulfate were used. For organic nitrogen sources, the CoQ<sub>10</sub> titer and cell density with urea were 16.30 mg L<sup>-1</sup> and 9.96 g L<sup>-1</sup>, respectively. And CoQ<sub>10</sub> titer and cell density obtained with CSP reached 28.50 mg L<sup>-1</sup> and 18.56 g L<sup>-1</sup>, respectively, which were 95.00% and 96.02% of those provided by YE (30.00 mg L<sup>-1</sup> and 19.33 g L<sup>-1</sup>). Moreover, the residual sugar concentration was 1.60 g L<sup>-1</sup> with CSP, which was only 34.04% of that with urea (4.70 g L<sup>-1</sup>), and YE provided a residual sugar concentration of

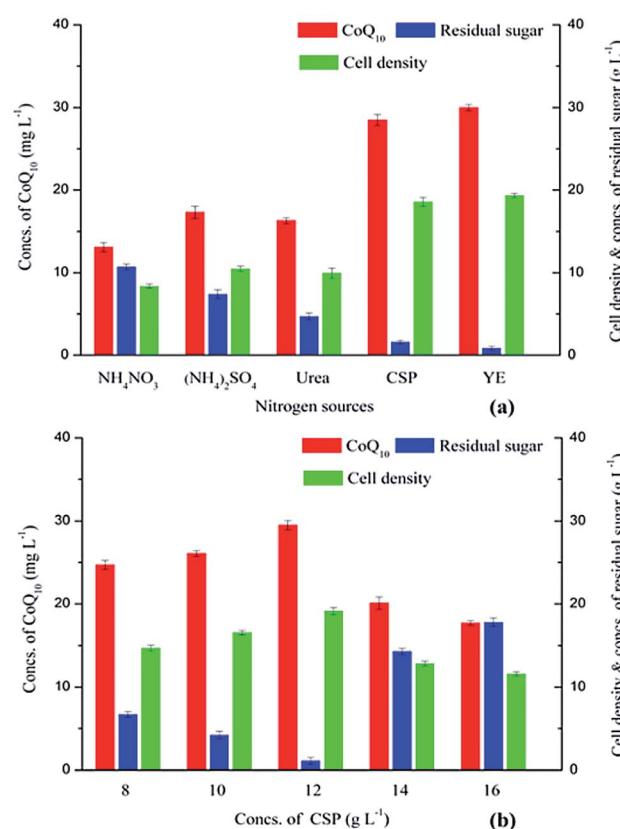


Fig. 2 Effects of different nitrogen sources on CoQ<sub>10</sub> titer, residual sugar and cell density (a) and the optimization of CSP concentration (b). All experiments were carried out at 30.00 °C and 220.00 rpm for 96.00 h on a rotary shaker, the pH was maintained at 7.00 and 250.00 mL flasks with a working volume of 100.00 mL were used.



Table 1 Constituents of metal ions and phosphorus of selected raw materials

Constituents (mg L <sup>-1</sup> )	SSJAH (containing a total sugar concentration of 80.00 g L <sup>-1</sup> )	YE solution (containing 8.00 g L <sup>-1</sup> YE)	CSP solution (containing 12.00 g L <sup>-1</sup> CSP)
K	103.52 ± 5.16	130.74 ± 3.36	317.19 ± 6.62
Na	7.72 ± 1.21	139.48 ± 2.82	27.70 ± 2.24
Ca	57.16 ± 2.26	14.28 ± 1.15	29.09 ± 1.95
Mg	106.44 ± 2.52	1.28 ± 0.36	73.25 ± 0.62
Mn	0.20 ± 0.03	—	0.29 ± 0.04
P	12.68 ± 0.61	109.80 ± 4.46	239.34 ± 7.46

0.85 g L<sup>-1</sup>. The results indicated CSP could replace YE without a significant drop in product titer and cell density, and the residual sugar concentration was kept at a relative low level.

In fact, CSP has been used as an effective nutrition supplement in CoQ<sub>10</sub> production by *Agrobacterium tumefaciens*.<sup>27</sup> As shown in Fig. 2b, the effect of CSP concentration on CoQ<sub>10</sub> production was further studied. The CoQ<sub>10</sub> titer increased from 24.70 mg L<sup>-1</sup> to 29.50 mg L<sup>-1</sup> when the CSP concentration was enhanced from 8.00 g L<sup>-1</sup> to 12.00 g L<sup>-1</sup>. However, the CoQ<sub>10</sub> titers obtained with 14.00 g L<sup>-1</sup> CSP and 16.00 g L<sup>-1</sup> CSP were only 68.14% and 60.00% of that provided by 12.00 g L<sup>-1</sup> CSP. The cell density trend was similar to that of product titer, and a top value of 19.13 g L<sup>-1</sup> was obtained with 12.00 g L<sup>-1</sup> CSP. As expected, when the CSP concentration was enhanced from 8.00 g L<sup>-1</sup> to 16.00 g L<sup>-1</sup>, the residual sugar first decreased from 6.70 g L<sup>-1</sup> to 1.10 g L<sup>-1</sup>, then increased to 17.80 g L<sup>-1</sup>, and the bottom value was achieved with 12.00 g L<sup>-1</sup> CSP. It indicated that the suitable CSP concentration for CoQ<sub>10</sub> fermentation was 12.00 g L<sup>-1</sup>. The negative effect provided by high concentration of CSP may be the excess organic salts, which needs further research.

Constituents of metal ions and phosphorus of SSJAH (containing a total sugar concentration of 80.00 g L<sup>-1</sup>), CSP and YE were determined in this study. As shown in Table 1, except for Na<sup>+</sup>, the concentrations of K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup> and P in CSP solution were all higher than those in YE solution. The lack of Na<sup>+</sup> could be supplemented by the addition of NaCl and sodium L-glutamate in the fermentation medium. Moreover, the contents of K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Mn<sup>2+</sup> in SSJAH solution approached or exceed the levels of those in YE solution. As shown in Table 2, amino acids composition of 8.00 g L<sup>-1</sup> YE, 12.00 g L<sup>-1</sup> CSP and SSJAH solution (containing a total sugar concentration of 80.00 g L<sup>-1</sup>) were analyzed. Only the contents of Glu, Gly, Tyr, Trp, Lys, Arg and Gln in CSP solution were significantly lower than those in YE solution, and all other amino acids contents in CSP solution were at or above the levels of those in YE solution. Fortunately, the contents of Glu (204.16 μmol L<sup>-1</sup>), Gly (78.88 μmol L<sup>-1</sup>), Tyr (97.92 μmol L<sup>-1</sup>), Trp (50.24 μmol L<sup>-1</sup>), Lys (80.16 μmol L<sup>-1</sup>), Arg (178.88 μmol L<sup>-1</sup>) and Gln (1400.32 μmol L<sup>-1</sup>) in CSP solution could be an efficient supplement when CSP and SSJAH were used together for the CoQ<sub>10</sub> fermentation. It is worth to mention that the total amino

Table 2 Amino acids composition of selected raw materials

Amino acid (μmol L <sup>-1</sup> )	YE <sup>a</sup>	CSP <sup>b</sup>	SSJAH <sup>c</sup>
Asp	236.32 ± 0.52	161.53 ± 0.76	693.42 ± 0.36
Thr	345.13 ± 0.38	271.95 ± 0.91	260.11 ± 0.55
Ser	267.36 ± 0.82	200.18 ± 1.11	440.32 ± 1.21
Glu	568.00 ± 1.55	190.82 ± 0.61	204.16 ± 0.96
Gly	1769.70 ± 2.26	529.29 ± 0.96	78.88 ± 0.82
Ala	980.98 ± 1.82	1991.54 ± 2.11	190.88 ± 1.51
Val	466.89 ± 0.72	833.54 ± 1.85	324.16 ± 1.26
Met	88.50 ± 2.24	189.85 ± 1.36	44.96 ± 0.78
Ile	358.72 ± 0.61	430.57 ± 1.45	112.64 ± 0.61
Leu	737.12 ± 1.54	1326.96 ± 1.16	124.48 ± 0.83
Tyr	328.65 ± 2.06	121.92 ± 0.91	97.92 ± 0.65
Phe	384.82 ± 3.16	446.19 ± 0.74	67.20 ± 1.11
His	164.65 ± 0.92	115.46 ± 0.51	127.04 ± 0.92
Trp	84.90 ± 0.81	13.69 ± 1.17	50.24 ± 0.53
Lys	608.17 ± 1.46	271.47 ± 2.01	80.16 ± 0.97
Arg	524.06 ± 1.81	226.56 ± 1.65	178.88 ± 1.24
Pro	175.33 ± 0.96	834.72 ± 0.62	98.08 ± 0.77
Gln	24.90 ± 1.51	—	1400.32 ± 2.22
Total amino acid	8114.19 ± 25.15	8156.24 ± 19.89	4573.84 ± 17.30

<sup>a</sup> 8.00 g L<sup>-1</sup> YE solution. <sup>b</sup> 12.00 g L<sup>-1</sup> CSP solution. <sup>c</sup> SSJAH containing a total sugar concentration of 80.00 g L<sup>-1</sup>.



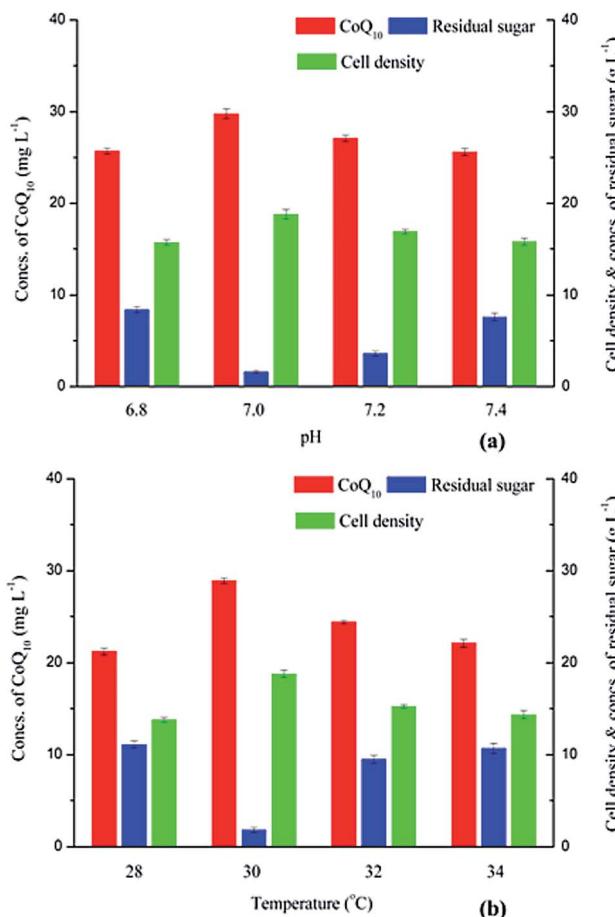


Fig. 3 Effects of pH (a) and temperature (b) on CoQ<sub>10</sub> titer, residual sugar and cell density. All experiments were carried out at 220.00 rpm for 96.00 h on a rotary shaker, and 250.00 mL flasks with a working volume of 100.00 mL were used.

acid content in the combination of CSP and SSJAH solution reached 12 730.08  $\mu\text{mol L}^{-1}$  which was 1.57 times higher than that in YE solution. Thus, it indicated that CSP can serve as an efficient nitrogen & salts supplement to SSJAH in the replacement of YE.

### 3.3 The effects of temperature and pH on CoQ<sub>10</sub> fermentation

As a facultative microorganism, *R. sphaeroides* can be cultivated by photoheterotrophy, photoautotrophy, chemoheterotrophy and fermentation.<sup>28</sup> And the effects of pH and temperature on CoQ<sub>10</sub> production using *R. sphaeroides* were investigated under fermentation conditions in this study.

CoQ<sub>10</sub> fermentations using *R. sphaeroides* CQ-09-1 were conducted at different pHs, and the effects of pH on CoQ<sub>10</sub> titer, residual sugar and cell density were shown in Fig. 3a. The CoQ<sub>10</sub> titer first increased from 25.70 mg L<sup>-1</sup> to 29.80 mg L<sup>-1</sup>, then decreased to 25.60 mg L<sup>-1</sup>, when the pH level was enhanced from 6.80 to 7.40, and the top value of CoQ<sub>10</sub> titer (29.80 mg L<sup>-1</sup>) was obtained at pH 7.00. The change of cell density was similar to that of product titer, and the highest cell density (18.80 g L<sup>-1</sup>)

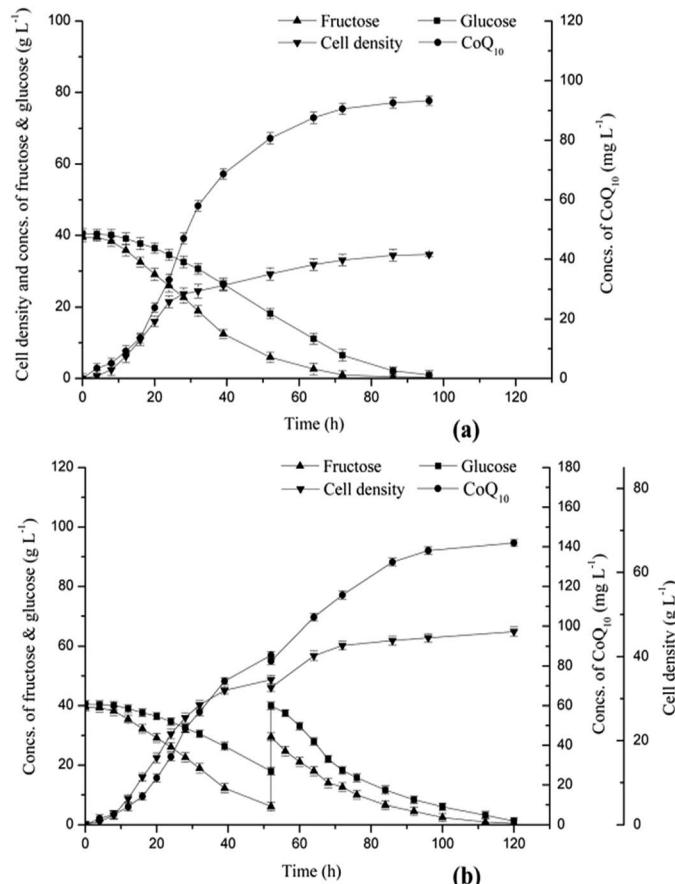


Fig. 4 Time-course of CoQ<sub>10</sub> production, cell density accumulation and carbon source consumption in batch (a) and fed-batch (b) fermentations. A 5 L fermentor at 400.00 rpm was used to perform the fermentation, and 40.00% NaOH (w/w) was automatically added as a neutralizer to keep the pH at 7.00. The temperature was maintained at 30.00 °C, and an aeration rate of 0.50 vvm was adopted.

was also achieved when the pH was maintained at 7.00. Jeong *et al.*<sup>29</sup> has reported that the CoQ<sub>10</sub> production was growth-associated, possibly as a primary metabolite, and the product titer increased as the cells accumulated. The concentration of residual sugar decreased from 8.40 g L<sup>-1</sup> to 1.60 g L<sup>-1</sup> when pH was increased from 6.80 to 7.00. However, the residual sugar concentration reached 3.60 g L<sup>-1</sup> and 7.60 g L<sup>-1</sup>, when the pHs were maintained at 7.20 and 7.40, respectively. The results indicated that the pH of 7.00 was the optimized value for CoQ<sub>10</sub> production under fermentation conditions.

In order to investigate the effects of temperature on CoQ<sub>10</sub> production, different temperatures ranging from 28.00 °C to 34.00 °C were adopted for *R. sphaeroides* CQ-09-1 cultivation. As shown in Fig. 3b, the CoQ<sub>10</sub> titer increased from 21.20 mg L<sup>-1</sup> to 28.90 mg L<sup>-1</sup> when the temperature was enhanced from 28.00 °C to 30.00 °C. However, the CoQ<sub>10</sub> titers decreased to 24.40 mg L<sup>-1</sup> and 22.10 mg L<sup>-1</sup>, when the temperatures reached 32.00 °C and 34.00 °C, respectively. As expected, the change of cell density was similar to that of product titer, and the top value (18.81 g L<sup>-1</sup>) was obtained at 30.00 °C. For the concentration of residual sugar, a bottom value of 1.80 g L<sup>-1</sup> was obtained at

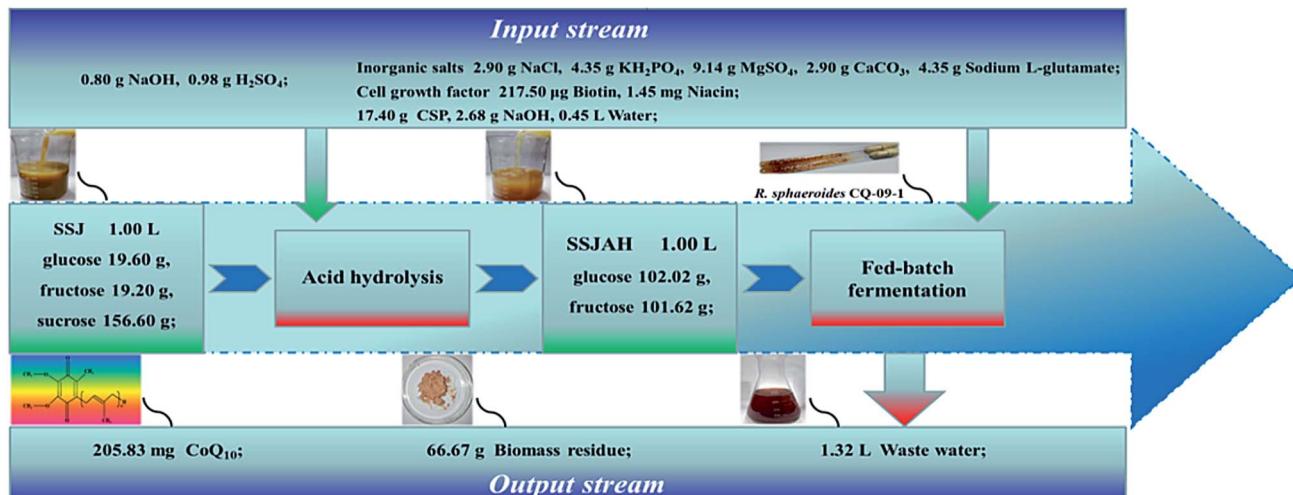


Fig. 5 The mass balance of the experimental process based on 1.00 L of dry SSJ.

30.00 °C. And the residual sugar concentrations were 11.10 g L<sup>-1</sup>, 9.50 g L<sup>-1</sup> and 10.70 g L<sup>-1</sup>, when the temperatures were maintained at 28.00 °C, 32.00 °C and 34.00 °C, respectively. Thus, the temperature was maintained at 30.00 °C in the following fermentations conducted in fermentor to achieve further improvement of CoQ<sub>10</sub> titer.

#### 3.4 Batch and fed-batch fermentation with *R. sphaeroides* CQ-09-1

A 5 L fermentor with an initial broth volume of 2.00 L were adopted for batch and fed-batch fermentations. As shown in Fig. 4a, the CoQ<sub>10</sub> titer increased as the cells of *R. sphaeroides* CQ-09-1 accumulated. The CoQ<sub>10</sub> titer was rapidly enhanced to 58.00 mg L<sup>-1</sup> within 32.00 h, and the cell density reached 24.50 g L<sup>-1</sup> at 32.00 h. The rising tendency of product titer slowed down with the slow growth of cell density after 32.00 h. A final product titer of 93.30 mg L<sup>-1</sup> was obtained at 96.00 h, and the final cell density was 34.67 g L<sup>-1</sup>. It is worth to mention that, although the concentrations of sugars were at the same level (40.00 g L<sup>-1</sup>), fructose and glucose were sequentially metabolized by *R. sphaeroides* CQ-09-1, and a lag phase as long as 12.00 h in glucose metabolism was observed. The fructose was exhausted at 72.00 h, and the residual glucose concentration declined to 1.01 g L<sup>-1</sup> at 96.00 h.

A logistic growth model combined with the Luedeking–Piret equation has been reported to model the batch production of CoQ<sub>10</sub> in the cultivation of *R. sphaeroides*, and the results indicated CoQ<sub>10</sub> production was a primary metabolite.<sup>30</sup> A longer cell growing stage would facilitate the accumulation of biomass, which would lead to a higher product titer. Thus, a fed-batch fermentation was conducted to further improve the titer of CoQ<sub>10</sub>. As shown in Fig. 4b, from 0.00 h to 52.00 h, the CoQ<sub>10</sub> titer reached 85.48 mg L<sup>-1</sup>, and the cell density accumulated to 34.52 g L<sup>-1</sup>. Because of the difference of utilization efficiency in glucose and fructose, the residual sugar concentrations of glucose and fructose were 17.96 g L<sup>-1</sup> and 6.15 g L<sup>-1</sup>, respectively, at 52.00 h. The supplement medium containing 420.00 g L<sup>-1</sup> total sugar was added to the fermentation broth, and the concentrations of glucose and fructose reached 39.98 g L<sup>-1</sup> and 29.50 g L<sup>-1</sup>, respectively. From 50.00 h to 120.00 h, a CoQ<sub>10</sub> titer of 141.95 mg L<sup>-1</sup> was finally obtained, and the cell density reached 45.98 g L<sup>-1</sup>. Interestingly, a simultaneous utilization of glucose and fructose was observed after 50.00 h. It was speculated that the cells preferring to utilize glucose survived and reproduced in the fermentation medium, and an evolution of strain CQ-09-1 could be achieved, which caused an enhanced capability of using glucose.

Table 3 Comparison of CoQ<sub>10</sub> production based on different carbon sources using wild strains

Microorganism	Carbon source	Fermentation type	CoQ <sub>10</sub> concentration (mg L <sup>-1</sup> )	Maximum cell density (g L <sup>-1</sup> )	Overall productivity (mg L <sup>-1</sup> h <sup>-1</sup> )	Reference
<i>R. sphaeroides</i> BCRC 13100	Molasses	Fed-batch	45.65	10.14	0.420	Yen and Shih <sup>1</sup>
<i>R. rubrum</i> ATCC 25852	Malic acid	Batch	10.81	1.71	0.113	Tian <i>et al.</i> <sup>33</sup>
<i>P. dinitrificans</i> NRRL B-3785	Glycerol	Batch	14.12	9.65	0.147	Bule and Singhal <sup>34</sup>
<i>Rhodotorula glutinis</i>	Glucose	Fed-batch	60.10	53	0.501	Balakumaran and Meenakshisundaram <sup>35</sup>
<i>R. sphaeroides</i> CQ-09-1	SSJAH	Fed-batch	141.95	45.98	1.183	This work



Comparing with the industrial price of yeast extract (YE) (\$9.2 kg<sup>-1</sup>), the cost of industrial corn steep liquor is only \$ 0.18 kg<sup>-1</sup>.<sup>31</sup> In addition, the yield of fermentable sugars obtained from sweet sorghum could reach 7.6 Mg ha<sup>-1</sup> which was higher than that of corn (5.7 Mg ha<sup>-1</sup>).<sup>32</sup> It indicated the raw material cost could be reduced by 54.06% using SSJ and CSP for CoQ<sub>10</sub> production. Fig. 5 briefly showed a mass balance of the experimental process, and a CoQ<sub>10</sub> yield of 205.83 mg L<sup>-1</sup> SSJ was obtained by fed-batch fermentation, which indicated the feasibility of this process for cost-efficient CoQ<sub>10</sub> production from abundant biomass-derived sugars. The comparison of CoQ<sub>10</sub> production based on different carbon sources using wild strains was shown in Table 3, and the CoQ<sub>10</sub> concentration, maximum cell density and overall productivity in this work were all higher than the results obtained in previously literatures. However, to achieve the commercially production of CoQ<sub>10</sub>,<sup>14</sup> further improvement of fermentation technology or metabolic regulation of *R. sphaeroides* CQ-09-1 will be necessary.

## 4 Conclusions

A cost-efficient way to produce CoQ<sub>10</sub> from abundant SSJ was introduced in this study. An optimization of total sugar concentration, nitrogen source, cultivation temperature and pH was conducted. In particular, CSP, which was used as a nitrogen & salt supplement to SSJAH, was proved to be an efficient alternative to YE. Based on SSJ with the optimized conditions, the CoQ<sub>10</sub> titer reached 93.30 mg L<sup>-1</sup> within 96.00 h in a batch fermentation. To further improve the product titer, a fed-batch fermentation was carried out, and a CoQ<sub>10</sub> titer of 141.95 mg L<sup>-1</sup> was obtained. Totally, the product yield reached 205.83 mg L<sup>-1</sup> SSJ, and the simultaneous utilization of glucose and fructose was achieved in the fed-batch fermentation.

## Conflicts of interest

There are no conflicts to declare.

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## Notes and references

- 1 H. W. Yen and T. Y. Shih, *Bioprocess Biosyst. Eng.*, 2009, **394**, 711.
- 2 L. Ernster and G. Dallner, *BBA, Mol. Basis Dis.*, 1995, **1271**, 195.
- 3 F. M. Sohet, A. M. Neyrinck, B. D. Pachikian, F. C. de Backer, L. B. Bindels, P. Niklowitz, T. Menke, P. D. Cani and N. M. Delzenne, *Biochem. Pharmacol.*, 2009, **78**, 1391.
- 4 L. Serge, S. Nathalie and B. Piotr, *Eur. J. Lipid Sci. Technol.*, 2009, **111**, 135.
- 5 I. Ehud and E. Doron, *Pure Appl. Chem.*, 1998, **60**, 89.
- 6 J. H. Choi, Y. W. Ryu and J. H. Seo, *Appl. Microbiol. Biotechnol.*, 2005, **68**, 9.
- 7 P. C. Corinne, M. B. Adam and J. J. M. Vincent, *Trends Biotechnol.*, 2007, **25**, 514.
- 8 Y. Natori and T. Nagasaki, *Agric. Biol. Chem.*, 1981, **45**, 2175.
- 9 H. Yoshida, Y. Kotani and K. Ochiai, *J. Gen. Appl. Microbiol.*, 1998, **44**, 19.
- 10 K. Kroll, E. Shekhova, D. J. Mattern, A. Thywissen, I. D. Jacobsen, M. Strassburger, T. Heinekamp, E. Shelest, A. A. Brakhage and O. Kniemeyer, *Mol. Microbiol.*, 2016, **101**, 92.
- 11 M. Kawamukai, *Biotechnol. Appl. Biochem.*, 2009, **53**, 217.
- 12 H. S. Zahiri, S. H. Yoon, J. D. Keasling, S. H. Lee, S. W. Kim, S. C. Yoon and Y. C. Shina, *Metab. Eng.*, 2008, **8**, 406.
- 13 J. Zhang, D. Gao, J. Cai, H. Liu and Z. Qi, *Biochem. Eng. J.*, 2018, **135**, 98.
- 14 S. Shukla and K. Dubey, *3 Biotech*, 2018, **8**, 249.
- 15 E. M. Rubin, *Nature*, 2008, **454**, 841.
- 16 L. Laopaiboon, S. Nuanpeng, P. Srinophakun, P. Klanrit and P. Laopaiboon, *Bioresour. Technol.*, 2009, **100**, 4176.
- 17 C. V. Ratnavathi, S. K. Chakravarhy, V. V. Komala, U. D. Chavan and J. V. Patil, *Sugar Tech*, 2011, **13**, 399.
- 18 A. C. Silva, P. M. Guimaraes, J. A. Teixeira and L. Domingues, *J. Ind. Microbiol. Biotechnol.*, 2010, **37**, 973.
- 19 Y. Wang, C. Chen, D. Cai, Z. Wang, P. Qin and T. Tan, *Bioresour. Technol.*, 2016, **218**, 1098.
- 20 Y. Wang, J. Chang, D. Cai, Z. Wang, P. Qin and T. Tan, *J. Chem. Technol. Biotechnol.*, 2017, **92**, 1848.
- 21 H. Ye, Y. Jin, S. Lin, M. Liu, Y. Yang, M. Zhang, P. Zhao and G. Jones, *Int. J. Biol. Macromol.*, 2012, **50**, 1315.
- 22 W. Lu, L. Ye, H. Xu, W. Xie, J. Gu and H. Yu, *Biotechnol. Bioeng.*, 2014, **111**, 761.
- 23 G. Kars, U. Gündüz, M. Yücel, G. Rakely, K. L. Kovacs and İ. Eroğlu, *Int. J. Hydrogen Energy*, 2009, **34**, 2184.
- 24 T. Liu, L. Zhu, W. Wei and Z. Zhou, *Int. J. Hydrogen Energy*, 2014, **39**, 4215.
- 25 W. Pattanamanee, W. Choorit, D. Kantachote and Y. Chisti, *Int. J. Hydrogen Energy*, 2012, **37**, 15855.
- 26 L. Hakobyan, L. Gabrielyan and A. Trchounian, *Int. J. Hydrogen Energy*, 2012, **37**, 6519.
- 27 S. J. Ha, S. Y. Kim, J. H. Seo, D. K. Oh and J. K. Lee, *Appl. Microbiol. Biotechnol.*, 2007, **74**, 974.
- 28 H. Koklu, I. Eroğlu, U. Gündüz, M. Yücel and L. Türker, *Int. J. Hydrogen Energy*, 2002, **27**, 1315.
- 29 S. K. Jeong, V. T. Dao, N. Kien and J. K. Kim, *J. Fish. Sci. Technol.*, 2008, **11**, 219.
- 30 H. W. Yen, C. Y. Feng and J. L. Kang, *Appl. Biochem. Biotechnol.*, 2010, **160**, 1441.
- 31 P. Maddipati, H. K. Atiyeh, D. D. Bellmer and R. L. Huhnke, *Bioresour. Technol.*, 2011, **102**, 6494.
- 32 T. H. Regassa and C. S. Wortmann, *Biomass Bioenergy*, 2014, **64**, 348.
- 33 Y. Tian, T. Yue, Y. Yuan, P. K. Soma and Y. M. Lo, *Biochem. Eng. J.*, 2010, **51**, 160.
- 34 M. V. Bule and R. S. Singhal, *Food Sci. Biotechnol.*, 2011, **20**, 607.
- 35 P. A. Balakumaran and S. Meenakshisundaram, *Prep. Biochem. Biotechnol.*, 2015, **45**, 398.

