J Ind Microbiol Biotechnol (2010) 37:521–529
DOI 10.1007/s10295-010-0699-4

ORIGINAL PAPER

Coenzyme Q_{10} production in a 150-l reactor by a mutant strain of *Rhodobacter sphaeroides*

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Received: 20 November 2009 / Accepted: 10 February 2010 / Published online: 27 February 2010

Abstract For the commercial production of CoQ_{10}, batch-type fermentations were attempted in a 150-l fermenter using a mutant strain of *R. sphaeroides*. Optimum temperature and initial aeration rate were found to be 30°C and 2 vvm, respectively. Under optimum fermentation conditions, the maximum value of specific CoQ_{10} content was achieved reproducibly as 6.34 mg/g DCW after 24 h, with 3.02 g/l of DCW. During the fermentation, aeration shift (from the adequate aeration at the early growth phase to the limited aeration in active cellular metabolism) was a key factor in CoQ_{10} production for scale-up. A higher value of the specific CoQ_{10} content (8.12 mg/g DCW) was achieved in fed-batch fermentation and comparable to those produced by the pilot-scale fed-batch fermentations of *A. tumefaciens*, which indicated that the mutant strain of *R. sphaeroides* used in this study was a potential high CoQ_{10} producer. This is the first detailed study to demonstrate a pilot-scale production of CoQ_{10} using a mutant strain of *R. sphaeroides*.

Keywords Coenzyme Q_{10} · Photosynthetic bacteria · *Rhodobacter sphaeroides* · Mutant · Pilot scale · Aeration shift

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Introduction

Ubiquinones, which are also referred to as coenzyme Q, are membrane-bound lipid components. They are naturally occurring oil-soluble materials found abundantly in animals, plants, and microorganisms as a coenzyme involved in biological reactions. They play an important role not only as an electron carrier in the respiratory chain, but also as an antioxidant and prooxidant [1–4]. The number of isoprene units in the prenyl side chain of ubiquinones varies depending on the living organism. Coenzyme Q_{10} (CoQ_{10}), 2,3-dimethoxy-5-methyl-benzoquinone with a side chain of ten monosaturated isoprenoid units, is the only ubiquinone homolog found in human organs [5]. In human beings, CoQ_{10} boosts energy, enhances the immune system, and acts as an antioxidant [1]. Recently, CoQ_{10} has been widely used for pharmaceuticals, cosmetics, food supplements, etc., because of its various physiological activities [6–8].

CoQ_{10} is able to be produced by chemical [9], semi-chemical [10], or biological synthetic methods. The biological synthesis of CoQ_{10} is more diversely used than the chemical and semi-chemical syntheses. This is because the starting materials used during chemical synthesis of CoQ_{10} are different from those used in microorganisms and human beings [11]. Therefore, the commercial production of CoQ_{10} biologically synthesized from microorganisms has attracted increasing attention [12], and construction of genetically engineered microorganism and metabolic modification have been attempted to improve yields of CoQ_{10} [6, 13–16]. Despite the recent accomplishments in metabolic engineering of *Escherichia coli* for CoQ_{10} production, production levels are not yet competitive with the levels produced by isolation or fermentation [14], illustrating the need for a

careful assessment of the physiological and metabolic bottlenecks limiting CoQ_{10} biosynthesis [17]. In addition, low yields from microbiological production of CoQ_{10} on an industrial scale have resulted in a high cost for CoQ_{10} [11]. Accordingly, intensified efforts in the development of bioprocess have been made for the commercial production of CoQ_{10} [17], and recently a coupled fermentation-extraction process has been suggested with enhanced production of CoQ_{10} by *Sphingomonas* sp. [18].

Up to now, the isolation of natural producers has been the most successful strategy in the development of microbial strains for the commercial CoQ_{10} production [17], and further improvements in CoQ_{10} production were achieved by chemical mutagenesis [19]. Among the natural producers, *Agrobacterium tumefacients* and *Rhodobacter sphaeroides* have been identified as good candidates for CoQ_{10} production [11, 12]. In our previous study [20], *R. sphaeroides* was found to be able to grow photosynthetically under strictly anaerobic conditions and aerobically in either light or dark conditions, which may lead to make scale-up of the CoQ_{10} fermentation difficult. This is because the main bottleneck in scale-up of phototrophic fermentation has been found to be the low efficiency of light energy conversion to the desired product, which is caused by an excessive dissipation of light energy to heat [21]. Even though photosynthetic bacteria have been diversely applied [6, 22–24], especially to the hydrogen production to meet the increasing demand for energy in recent years [25, 26], a few of their pilot-scale studies have been reported to date [27].

Although an interest in CoQ_{10} has been recently renewed due to the growing demands of the pharmaceutical industry [17], a few scale-up studies of the CoQ_{10} fermentation have been conducted using microorganisms other than photosynthetic bacteria: optimization of culture conditions and scale-up to pilot and plant scales using *A. tumefaciens* [11] and statistical optimization of culture conditions and operation parameters in a 150-l fermenter using *Paracoccus denitrificans* [28]. As a result, a pilot-scale fermentation of *R. sphaeroides* is necessary to verify its potential for commercial CoQ_{10} production. Recently, we identified a high-coenzyme-Q_{10}-producing *R. sphaeroides* mutant of an isolated strain [29] and deposited it into the Korean Agricultural Culture Collection (KACC) as *R. sphaeroides* KACC 91339P. Therefore, in this study, a scale-up fermentation was attempted from a 1-l fermenter to a 150-l fermenter, upon which the optimum fermentation conditions and operation parameters were investigated for high CoQ_{10} production using a mutant strain of *R. sphaeroides*.

Materials and methods

Microorganism and culture medium

R. sphaeroides KACC 91339P, a mutant strain, was used in this study, the wild-type cell of which was isolated from the silt of the Nakdong River (Busan, Korea) by our laboratory [29]. The strain was maintained on a solid agar plate that contained (per l): 1 g of malic acid, 2 g of casamino acid, 3 g of yeast extract, 1 ml of vitamin solution, 1 ml of mineral solution, and 15 g of agar. The vitamin solution contained (per l): 0.2 g of nicotinic acid, 0.4 g of thiamine-HCl, 0.2 g of nicotinamide, and 0.008 g of biotin. The mineral solution contained (per l): 3 g of $FeSO_4 \cdot 7H_2O$, 0.01 g of H_3BO_3, 0.01 g of $Na_2MoO_4 \cdot 2H_2O$, 0.02 g of $MnSO_4 \cdot H_2O$, 0.01 g of $CuSO_4 \cdot 5H_2O$, 0.01 g of $ZnSO_4$, and 0.5 g of ethylenediamine tetraacetic acid. The pH of the culture medium was adjusted to 7.2 before autoclaving at 121°C for 15 min. The above liquid culture medium excluding agar was used to cultivate cells for seed culture and laboratory- and pilot-scale fermentations. The photosynthetic bacterium was regularly checked under a microscope to ensure that contamination had not occurred and was transferred to a fresh agar plate every 2 weeks.

Seed culture and chemical mutation

To prepare pure seed-culture for pilot-scale fermentation, cells were first transferred to a 10-ml tube after being taken from the colony using tip, and incubated at 30°C, 180 rpm, and 50 lux. When cells were grown to the end of the exponential growth phase, a 5-ml culture broth was then inoculated into a 250-ml flask containing 60 ml of the fresh culture medium and incubated under the same culture conditions. Likewise, 60 ml of the culture broth was finally transferred to a 1-l reactor (Marubishi, Japan) in a 600-ml working volume. Cells in the reactor were cultivated at 30°C, 200 rpm, 2 vvm, and 50 lux. 'Antifoam 204' (diluted 10-fold) was occasionally used when severe foaming occurred. The seed culturing for pilot-scale fermentation was continued by repeated-batch culture until 90 g of cells (wet weight) were collected. After each batch culture, the cell pellet was prepared by centrifuging the culture broth at 6,000 rpm for 10 min and then decanting the supernatant after two washes with distilled water.

To produce mutant cells from wild-type *R. sphaeroides* cells, the following steps were carried out. Ninety grams of wet cells obtained from the seed culturing were suspended in a 900-ml 0.5 M Tris-maleate buffer (pH 6.2) containing 30 mg/ml of menadinone, and mixed severely at a room temperature for 20 min. Then, cells were washed twice with 0.85% saline. After being centrifuged, the cells were

resuspended in a 2-l flask containing 1.2-l fresh culture medium and incubated for 2 h at 30°C, 180 rpm, and 50 lux in order to allow mutant cells to adapt to the culture medium. The mutant cells prepared in this way were used as a seed culture for the pilot-scale fermentation. To ensure the chemical mutation had been properly achieved, the mutant cells were spread on a bouillon medium containing 0.5% meat extract, 0.5% peptone, 0.5% NaCl and 2% agar (pH 7.2) [30]. It was confirmed that mutant cells formed green colonies on the agar plates.

Laboratory-scale fermentation for CoQ_{10} production

To investigate the effect of temperature on CoQ_{10} production, batch-type fermentations were carried out in a 1-l reactor at various temperatures (27, 30, 34, 37, and 40°C). After 60 ml of the seed culture was inoculated into the reactor filled with 540 ml of the culture medium, the fermentation was started at 200 rpm and 50 lux under each temperature. Air was initially supplied at 2 vvm until the DO level dropped to 0.9 mg/l, after which the aeration rate was controlled by on-off air supply to maintain the DO level at less than 1.0 mg/l, based on our previous study [31]. 'Antifoam 204' (diluted 10-fold) was occasionally used when severe foaming occurred. Samples were taken periodically from the reactor to measure the concentrations of dry cells and CoQ_{10}. The pH and DO were measured in real time using Labo Controller (Marubishi, Japan). The dry-cell weight (DCW) of the bacteria was determined by weighing the cell pellet after it was dried in an oven at 105°C for 12 h.

Pilot-scale fermentation for CoQ_{10} production

To scale up the data achieved from the laboratory scale for the commercial CoQ_{10} production, pilot-scale fermentations were carried out in a 150-l conventional continuous-stirred-tank reactor filled with 90 l of the culture medium. The floor of the whole working room was thoroughly cleaned using the detergent Terg-A-Zyme (Alconox, USA). The reactor was filled with a 3 mg/l chloroform solution for 1 day for its sterilization. After the chloroform solution was evacuated, the reactor was filled with hot (higher than 80°C) autoclaved dissolved water (DW) and placed for 7 h in order to wash the remaining chloroform. When this procedure for the sterilization of the reactor was completed, 1.2-l resuspended mutant cells (90 g wet cells) was inoculated into the reactor containing 90 l of the fresh culture medium, and fermentation was started at 30°C and 200 rpm. The air from the air compressor (set at 2 kg_f/cm^2) was supplied into the reactor initially at various aeration rates (1, 2, and 3 vvm) to investigate the effect of the initial aeration rate on CoQ_{10} production. The air was supplied into the reactor through the air filter packed with sterile glass wool, and air bubbles were distributed by three ceramic disk-typed diffusers (12-cm diameter) installed at the bottom of the reactor. A sterile air filter was also installed at the air exit in order to prevent contamination from the outside. When severe foaming occurred, the DO level was controlled at less than 1.0 mg/l by on-off air supply. By the use of two incandescent bulbs (100 W), 50 lux of light was supplied through the two-sided glass (8 cm × 35 cm) installed on the body of reactor. Ten-fold diluted 'Antifoam 204' was used when severe foaming occurred during fermentation. Samples were collected periodically from the reactor to measure the concentrations of dry cells and CoQ_{10}.

To obtain more CoQ_{10} accumulation in the fermenter, a fed-batch operation was adopted using the four-stage feeding strategy proposed by Gu et al. [32]. The working volume in the fed-batch fermentation increased from 50 to 90 l by intermittent feeding of concentrated culture medium. The concentration of each component in the concentrated culture medium was decided by considering the change of working volume, and thus, the initial concentration in every fed-batch operation was the same as that of culture medium used in batch fermentation. When the DO level was increased over 1 mg/l without air supply, the feeding solution of 10 l was added intermittently four times. Just after new fed-batch operation was started, pH of the culture medium was adjusted to 7 with sterile 3-N HCl in order to maintain high cell activity. The other culture conditions of the fed-batch fermentation were the same as those of the batch fermentation.

Extraction and measurement of CoQ_{10}

CoQ_{10} was extracted from the *R. sphaeroides* mutant strain and analyzed by the methods of Matsumura et al. [33] and Takahashi et al. [7] with modifications. Ten grams of cells (wet weight) was suspended in 70 ml of methanol, and the slurry was heated at 55°C for 5 min. Chloroform (140 ml) was added, and the suspension was stirred at 30°C for 20 min and filtered through filter paper (Whatman no. 1). NaCl solution (0.58%, w/v) was added by one-fifth of the filtrate volume. The filtrate and the NaCl solution were gently mixed and then allowed to separate into two phases. The lower phase was evaporated, and the residue was resuspended in ethanol. CoQ_{10} contained in this solution was analyzed by HPLC (Agilent 1200, USA) on a Zorbax Eclipse Plus C18 column (100 mm × 4.6 mm, 5 μm) with ethanol as the mobile phase at a flow rate of 1 ml/min. The CoQ_{10} was quantified by an external standard method, based on the peak area, and detected at 275 nm. The intracellular content of CoQ_{10} was estimated by the relationship between dry cell weight and the amount of CoQ_{10}

Table 1 Comparative results of CoQ_{10} production in a 1-l reactor at various temperatures

Temperature (°C)	DCW (g/l)	Specific CoQ_{10} content (mg/g DCW)	CoQ_{10} productivity (mg/l/h)
27	2.65 ± 0.05^{b}	3.80 ± 0.03^{b}	0.438 ± 0.03^{b}
30	2.93 ± 0.03^{a}	4.66 ± 0.03^{a}	0.569 ± 0.03^{a}
34	2.68 ± 0.07^{b}	3.78 ± 0.04^{b}	0.442 ± 0.04^{b}
37	2.03 ± 0.05^{c}	3.02 ± 0.04^{c}	0.255 ± 0.03^{c}
40	0.96 ± 0.03^{d}	1.98 ± 0.03^{d}	0.079 ± 0.03^{d}

Means with different superscript are significantly different ($P < 0.05$). Values represent mean ± SD of three replicates

in the broth. The CoQ_{10} measurement was carried out in triplicate.

Statistical analyses

Statistical analyses were done with measurements obtained from this study. Since the sample observations were not arranged in a frequency distribution, the standard deviations were calculated by the following procedures: each deviation was squared, the sum of the squares was divided by $(n - 1)$, one less than the sample size (n) (this resulted in the sample variance), and finally extraction of the square root recovered the original scale of measurement. Comparisons of means were performed by the Tukey method [34] using the SAS program, since all sample sizes were equal. Differences were considered significant at $P < 0.05$.

Results and discussion

Effect of temperature on CoQ_{10} production

The effect of temperature on CoQ_{10} production by *R. sphaeroides* mutant cells was investigated in a 1-l reactor. The mutant strain of *R. sphaeroides* used in this study formed green colonies on the bouillon medium, which was found to be superior to its wild-type strain for CoQ_{10} production in our previous study [29]. This was likely because the mutant strain of *R. sphaeroides* could overcome growth inhibition by menadinone during ubiquinone biosynthesis or its related metabolisms, and thus this might result in the overproduction of CoQ_{10} [30]. Up to now, successful approaches for the commercial production of CoQ_{10} have relied predominantly on bacterial mutants selected for their high CoQ_{10} content [17].

In all fermentations carried out at various temperatures, all profiles of cell growth and DO level were almost similar: Cells grew steadily until approximately 24 h, and then cell growth decreased slowly. The DO level in the reactor was rapidly dropped to 0.9 mg/l within 3–4 h with the occurrence of foam; after that it was maintained at less than 1.0 mg/l until approximately 24-h fermentation, and it was slowly increased without aeration in the end. The maximum values of specific CoQ_{10} content in all fermentations were achieved similarly at the point where the maximum DCW was obtained. As seen in Table 1, the maximum specific CoQ_{10} content (4.66 mg/g DCW) was achieved at 30°C in three replicate experiments. Sasaki and Nagai [35] reported that the maximum CoQ_{10} production by *Rhodopseudomonas gelatinosa* was obtained at 35°C. In this study, however, the increase of temperature over 30°C resulted in the decrease CoQ_{10} production with the decrease of DCW. This difference may exist because different species of photosynthetic bacteria are able to ferment under many different environmental conditions [36]. At the maximum CoQ_{10} production (4.66 mg/g DCW), the value of CoQ_{10} productivity was 0.569 mg/l/h. A similar result could be found in the recent study of Yen and Shih [37], in which 4.6 mg CoQ_{10}/g DCW of the maximum CoQ_{10} content (with 0.48 mg/l/h of the maximum CoQ_{10} productivity) was achieved in a 5-l airlift bioreactor using *R. sphaeroides*. Moreover, Yoshida et al. [30] reported that high production of CoQ_{10} was not always reproduced in subsequently repeated experiments using a mutant strain. This is due to reversion of a mutant strain to a wild-type strain, which is a well-known cause of disappointment in the industrial application [38]. However, the values of CoQ_{10} content were not significantly different in our three replicate experiments, indicating that the mutant strain used in this study was reproduced stably by the chemical mutation.

Effect of initial aeration-rate on CoQ_{10} production

In our previous study [20], it was found that the control of the DO level during the fermentation of *R. sphaeroides* was crucial to obtain higher CoQ_{10} content. According to this finding, the effect of initial aeration-rate on the CoQ_{10} production was investigated in a 150-l reactor at various aeration rates (Table 2). During fermentations at all aeration rates, all profiles of cell growth, DO level, and CoQ_{10} production exhibited almost the same tendency. The maximum value of the specific CoQ_{10} content, 6.34 mg/g DCW, was achieved at 2 vvm, from which 0.798 mg/l/h of

Table 2 Comparative results of CoQ_{10} production in a 150-l reactor at various aeration rates

Aeration rates (vvm)	DCW (g/l)	Specific CoQ_{10} content (mg/g DCW)	CoQ_{10} productivity (mg/l/h)
1	2.85 ± 0.03^{b}	4.68 ± 0.04^{b}	0.556 ± 0.04^{b}
2	3.02 ± 0.03^{a}	6.34 ± 0.03^{a}	0.798 ± 0.04^{a}
3	2.65 ± 0.04^{c}	4.05 ± 0.03^{c}	0.444 ± 0.04^{c}

Means with different superscript are significantly different ($P < 0.05$). Values represent mean ± SD of three replicates

CoQ_{10} productivity was obtained. These values were higher than those obtained from a 1-l reactor. This result indicated that the increase of air flow rate would be necessary in scale-up for higher CoQ_{10} production. This may happen because the maximum permissible air-flow rate is more limited in a smaller reactor than that in a larger reactor. However, a further increase of the initial aeration rate (at 3 vvm) in a 150-l reactor resulted in lower CoQ_{10} production with significantly lower DCW. It has been reported that a high aeration rate would lead to the increase of cell growth rate, but a slight decrease of CoQ_{10} content [37]. In this study, however, not only CoQ_{10} production, but also DCW was lower at 3 vvm, indicating some exertion of shear stress on the cells.

Optimum CoQ_{10} production in a batch fermentation

The best result of fermentation operated in a 150-l reactor is seen in Fig. 1. The fermentation was carried out initially under the optimum conditions (30°C, 200 rpm, 2 vvm and 50 lux) achieved from this study and our previous study as well [20, 31]. Under the above conditions, the DO level in the reactor was rapidly decreased to 1.5 mg/l within 3 h, and then further decreased to 0.6 mg/l around 7 h with the occurrence of foam. The DO level dropped a little more severely than in a 1-l reactor, which indicated that microbial oxygen-consumption in the larger reactor appeared to be higher during the early growth phase. After cells entered active cellular metabolism (after 7 h), the DO level was maintained at a low rate (less than 1.0 mg/l) by DO control. The DO level was increased slowly after 24 h of fermentation without aeration. This result may be attributed to the oxygen requirements of *R. sphaeroides*, a facultative microorganism, at the early growth phase, but its metabolism may have to switch to fermentation metabolism afterwards for higher CoQ_{10} production [36, 39]. From the beginning, the pH was steadily increased up to 9.35 until 24 h, and then decreased a little. Likewise, both the cell concentration and the specific CoQ_{10} content were increased until 24 h, and then decreased slowly. The maximum CoQ_{10} production was 6.34 mg/g DCW, with 3.02 g/l of DCW. The profile of CoQ_{10} production exhibited that it was growth-associated, possibly clarified as a primary metabolite. The same results were found in other studies of *Rhodobacter* sp. by Yamada et al. [40] and *R. sphaeroides* by Yen and Chiu [41] as well. In those studies, CoQ_{10} biosynthesis occurred predominantly during the exponential growth phase. Therefore, *R. sphaeroides* mutant cells must be harvested at the late-exponential growth phase for commercial use.

It is clear that problems of scale-up in a bioreactor are associated with the behavior of liquid in the bioreactor and the metabolic reactions of the microorganisms [42, 43]. Accordingly, transport limitation is considered to be one of the major factors responsible for phenomena observed at large scale. For this reason, scale-up is still regarded more as an art than a science [44]. In our previous study [31], it was found that poorer conditions of aeration-agitation during active fermentation resulted in higher production of CoQ_{10}, and thus the level of DO in the reactor was detrimental to the cell growth and CoQ_{10} production, which was in agreement with the results reported in the studies of *Agrobacterium* species by Kuratu et al. [45], *A. tumefaciens* by Ha et al. [11], *Rhizobium radiobacter* by Wu et al. [46], and *R. sphaeroides* by Urakami and Yoshida [47]. Specifically, Yen and Chiu [41] suggested that the cultivation of *R. sphaeroides* under the conditions of aerobic-dark at 0% DO could be applied to the CoQ_{10} production for scale-up. Accordingly, the DO control during fermentation, which enhances the CoQ_{10} biosynthesis, plays an important role in commercial CoQ_{10} production. In this study, fermentation started with sufficient aeration at 2 vvm, which could increase the cell growth rate at the early growth phase [39], and then aeration was controlled after rapid decrease of the DO level (after 7 h) to maintain the DO level at less than 1.0 mg/l. This aeration shift in active cellular metabolism could lead to a higher CoQ_{10} content successfully in a pilot-scale fermentation. Consequently, this aeration shift was a key factor in CoQ_{10} production by *R. sphaeroides* for scale-up.

Increased CoQ_{10} production in a fed-batch fermentation

Time course analysis of pH, DO, DCW, and CoQ_{10} was performed in a fed-batch fermentation (Fig. 2). After 24, 36, 47, and 58 h of fermentation, 10 l of fresh culture medium was fed to the 150-l fermenter. Cell activity was maintained well enough during the fed-batch fermentation.

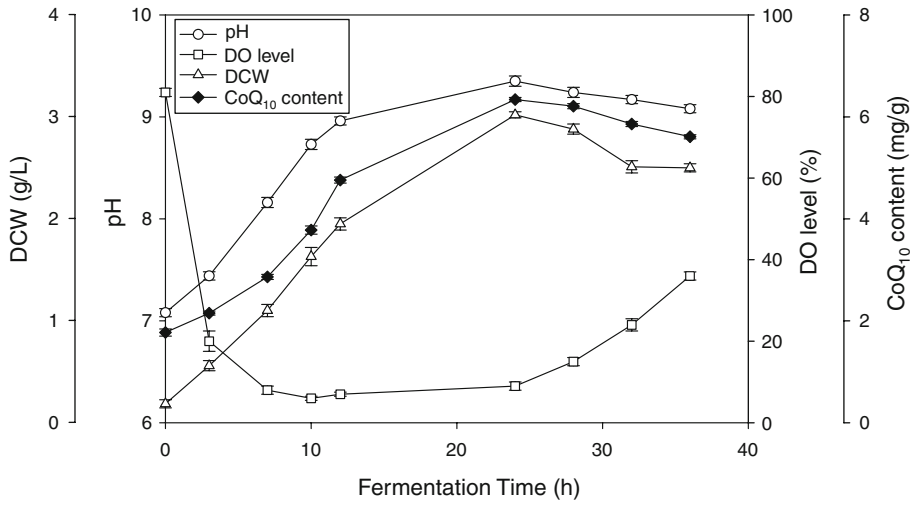

Fig. 1 Profiles of pH, DO level, DCW, and CoQ_{10} content in batch fermentation under 30°C, 200 rpm, and 50 lux. *Error bars*: mean ± SD of three replicates

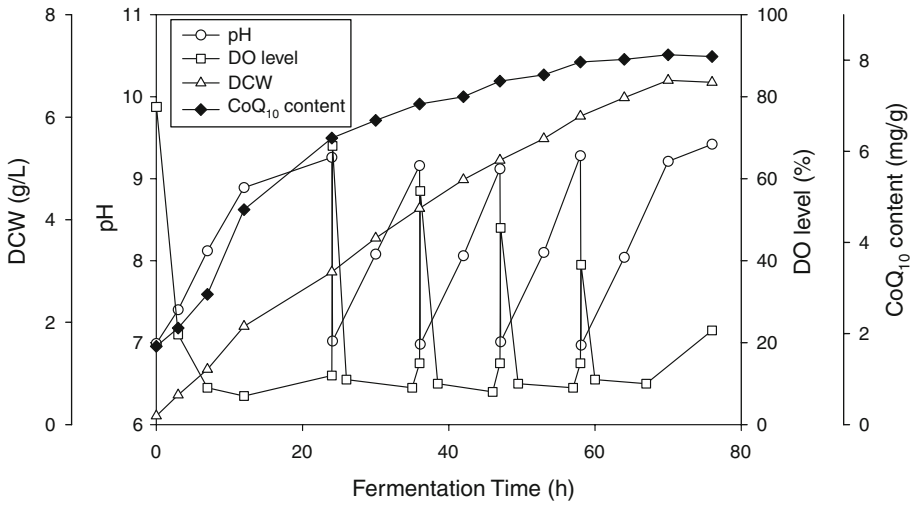

Fig. 2 Profiles of pH, DO level, DCW, and CoQ_{10} content in fed-batch fermentation using four stage feeding strategy under 30°C, 200 rpm, and 50 lux

The value of specific CoQ_{10} content reached 8.12 mg/g DCW in 70 h of fermentation, and the biomass (DCW) increased up to 6.72 g/l. This value of the specific CoQ_{10} content corresponded to 1.28 times more than that achieved from batch operation. However, the increase of CoQ_{10} production in the fed-batch fermentation was not high, compared with the result of fed-batch culture by Ha et al. [11] (1.3 times higher) or Gu et al. [32] (3.5 times higher), which was carried out in laboratory-scale fermenters. This was caused probably by difficulty of scale-up and/or feeding strategy in fed-batch operation [21].

Comparison of pilot-scale CoQ_{10} production

The result of this study was compared to representative CoQ_{10} productions by photosynthetic bacteria, which have been reported in literature to date. As seen in Table 3, the values of specific CoQ_{10} content varied. Although intensified efforts in the development of bioprocess have been made recently for the commercial CoQ_{10} production [17], the CoQ_{10} productions achieved in the 1990s were somewhat higher than those achieved in recent years. The highest value of specific CoQ_{10} content (12.5 mg/g DCW) was achieved from fed-batch fermentation by the study of Sakato et al. [19], which was higher than that (8.12 mg/g DCW) achieved from fed-batch fermentation by this study. Among these strains of photosynthetic bacteria seen in Table 3, however, direct comparison for commercial CoQ_{10} production was difficult because all results were achieved in laboratory-scale experiments except for this study. Their potential for commercial CoQ_{10} production must be verified in a pilot-scale fermenter, since their scale-up has been known to be difficult [21].

There have been few reports of pilot-scale or plant-scale CoQ_{10} fermentations to date (Table 4). In addition to these reports, Sakato et al. [19] reported that successful fermentation in a 80-kl fermenter was accomplished by the control of ORP during the last phase of fermentation. However,

Table 3 Comparative results of CoQ_{10} productions obtained from various fermentations using photosynthetic bacteria

Strain	Fermentation scale (l)	Mode of fermentation	Specific CoQ_{10} content (mg/g DCW)	Reference
Rhodopseudomonas spheroides	30	Fed-batch	12.5	Sakato et al. [19]
Rhodobacter capsulatus	1	Batch	4.61	Urakami and Yoshida [47]
Rhodobacter sphaeroides	0.3	Batch	8.70	Yoshida et al. [30]
Rhodobacter sphaeroides	5	Batch	4.61	Yen and Chiu [41]
Rhodobacter sphaeroides	5	Airlift (batch)	4.6	Yen and Shih [37]
Rhodobacter sphaeroides	150	Batch	6.34	This study
		Fed-batch	8.12	

Table 4 Comparative results of CoQ_{10} productions by various microbial fermentations in pilot scales or plant scales

Strain	Fermentation scale (l)	Mode of fermentation	Specific CoQ_{10} content (mg/g DCW)	Reference
Agrobacterium tumefaciens	300	Fed-batch	8.24	Ha et al. [11]
Agrobacterium tumefaciens	5,000	Fed-batch	8.05	Ha et al. [11]
Agrobacterium tumefaciens	300	Fed-batch	9.25	Ha et al. [48]
Rhodobacter sphaeroides	150	Batch	6.34	This study
		Fed-batch	8.12	

detailed results for CoQ_{10} production could not be acquired from that study because it was not explicitly reported. As seen in Table 4, Ha et al. [11] produced 8.24 mg CoQ_{10} per a gram of DCW in a 300-l fermenter by a fed-batch fermentation using *A. tumefaciens*, and a not significantly lower level of CoQ_{10} production was obtained satisfactorily even in a 5,000-l fermenter. Moreover, Ha et al. [48] could achieve improved CoQ_{10} production in fed-batch fermentation by optimized control of the substrate concentration. In this study, on the other hand, we achieved 6.34 mg CoQ_{10} per a gram of DCW by batch fermentation using *R. sphaeroides*, and we achieved a higher specific CoQ_{10} content (8.14 mg/g DCW) by fed-batch fermentation, which was comparable to those of *A. tumefaciens*. Accordingly, *R. sphaeroides* used in this study was verified as a potential producer for commercial CoQ_{10} production.

Recently, economical production of CoQ_{10} by microbes has become more important because of the growing demands of the pharmaceutical industry [18]. Among the natural producers, *A. tumefacients* and *R. sphaeroides* have been identified as good candidates for CoQ_{10} production [11, 12]. Up to now, there have been some scale-up studies of the CoQ_{10} fermentation using *A. tumefacients*, but no studies of CoQ_{10} fermentation using *R. sphaeroides*. Accordingly, CoQ_{10} production using *R. sphaeroides* in a pilot-scale fermenter in this study was valuable to verify its potential for commercial CoQ_{10} production. Now, we are preparing a clinical test of CoQ_{10} as an ointment for the removal of wrinkles.

Acknowledgments This research was supported by a grant (M2007-05) from the Marine Bioprocess Research Center of the Marine Bio 21 Center funded by the Ministry of Land, Transport and Maritime, Republic of Korea.

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