Advances in Bioscience and Biotechnology, 2014, 5, 966-977
Published Online October 2014 in SciRes. http://www.scirp.org/journal/abb
http://dx.doi.org/10.4236/abb.2014.512110

Influence of Substrate Feeding and Process Parameters on Production of Coenzyme Q_{10} Using *Paracoccus denitrificans* ATCC 19367 Mutant Strain P-87

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Received 18 August 2014; revised 19 September 2014; accepted 8 October 2014

Abstract

Coenzyme Q_{10} (CoQ_{10}), an important antioxidant molecule playing a major role in electron transport chain, has been commercially produced by fermentation process for the use in oral nutraceutical formulations. Constructing the high-yielding CoQ_{10} producing strains is a pre-requisite for cost-effective production. A superior mutant strain P-87 generated from *Paracoccus denitrificans* ATCC 19367, which showed 1.25-fold improvement in specific CoQ_{10} content higher than the wild type strain at shake flask level, was selected to carry out the studies on CoQ_{10} yield improvement through fermenter process optimization. In the course of study, initially the cane-molasses-based medium and fed-batch fermentation strategy using pHBA in combination with sucrose were standardized in shake flask using wild type strain. This strategy was subsequently translated at 2 L laboratory fermenter while optimizing the fermentation process parameters using improved mutant strain P-87. Under optimized fermentation condition, mutant strain P-87 produced 49.85 mg/L of CoQ_{10} having specific content of 1.63 mg/g of DCW, which was 1.36 folds higher than the specific CoQ_{10} content of wild-type strain under similar optimized condition. The temperature and DO were found to be critical parameters for CoQ_{10} production by mutant strain P-87. The optimum temperature was found to be 32˚C and the optimum DO concentration to be maintained throughout the fermentation cycle was found to be 30% of air saturation. Overall, a new cost-effective process has been established for the production of CoQ_{10} using the cheaper substrate "cane molasses" and higher CoQ_{10} producing mutant strain P-87.

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How to cite this paper: Tokdar, P., Ranadive, P., Kshirsagar, R., Khora, S.S. and Deshmukh, S.K. (2014) Influence of Substrate Feeding and Process Parameters on Production of Coenzyme Q_{10} Using *Paracoccus denitrificans* ATCC 19367 Mutant Strain P-87. *Advances in Bioscience and Biotechnology*, **5**, 966-977. http://dx.doi.org/10.4236/abb.2014.512110

Keywords

CoQ_{10}, *Paracoccus denitrificans*, Cane Molasses, pHBA, Sucrose, DO, Process Optimization

1. Introduction

CoQ_{10} is 2,3-dimethoxy-5-methylbenzoquinone with 10 units of isoprenoid side chain at the 6-position of the quinine ring [1]. Recently it has received great attention for its application as therapeutic agent as well as in related fields such as a potential antioxidant [2]. CoQ_{10} can be produced by chemical synthesis [3], semi-chemical synthesis [4], extraction from animal tissues [5] and microbial fermentation [6] including bacteria (e.g. *Agrobacterium*, *Paracoccus*, *Cryptococcusi*, *Rhodobacter*, *Tricosporon*), molds (e.g. *Neurospora*, *Aspergillus*), yeasts (e.g. *Candida*, *Sporidobolus*, *Rhodotorula*), etc. In the wake of environmental awareness, the first three options became least desirable due to inherent uses of solvents and chemicals in the process. Microbial fermentation, on the contrary, offers an environmentally benign option based on the enzymatic catalysis at the cellular level for CoQ_{10} assembly. Moreover, this approach is attractive to the industry because the process is easy to control at a relatively low production cost. But the potency and the capability of the producing strain are the key factors for an economically viable biological process.

A genetically engineered microorganism synthesizing CoQ_{10} has also been constructed [7]-[11]. Despite of recent accomplishments in metabolic engineering in *Escherichia coli* cells for CoQ_{10} production, the production levels are not yet competitive with the levels presently produced by fermentation or random mutagenesis and screening [11]. The successful approaches for the commercial production of CoQ_{10} have relied predominantly on bacterial and yeast mutants selected for their high CoQ_{10} content. So far, the isolation of strains by mutagenesis and selection on inhibitors has proved to be the most successful strategy to increase yields of CoQ_{10} [12]. The coupled fermentation-extraction process and two-phase conversion system has been reported with enhanced production of CoQ_{10} by *Sphingomonas* sp. [13] [14]. The fermentation optimization process for *A. tumefaciens* has been reported with respect to restricted electron flux, viscosity of the broth, controlling sucrose concentration and NADH/NAD + ratio [15]-[19]. The influences of aerobic-dark and anaerobic-light cultivation on CoQ_{10} production by *Rhodobacter sphaeroides* in the submerged fermenter and in the stirred tank, airlift reactor were reported [20] [21].

As reported previously, one strain of *Rhizobium* sp. was elected as a potent CoQ_{10} producer. Along with the progress of strain improvement, many fermentation condition experiments had been carried out and it has been observed that oxygen supply is one of the important environmental factors in CoQ_{10} production or other secondary metabolite production by microorganisms [22]-[24]. It was reported that a green mutant (carotenoid-deffi-cient mutant, Co-22-11) derived from *Rhodopseudomonas sphaeroides* KY-4113 produced 350 mg/L of CoQ_{10} under culturing conditions with a limited supply of air, the CoQ_{10} content being 8.7 mg/g of DCW. In this case, the amount and content were 2.8 and 3.6 times larger than those given by the wild type strain, respectively [6]. Optimization of the media and culture conditions is also one of the most effective strategies to maximize the production of CoQ_{10} by fermentation [25] [26]. For example, the optimal oxidation-reduction potential was −150 mV for cell growth and −200 mV for CoQ_{10} accumulation in cells [23]. The cultivation of *R. sphaeroides* under the situation of aerobic-dark at 0% dissolved oxygen (DO) was suggested to be applied in the scale-up of CoQ_{10} production [20].

In our previous study mutant strain P-87 generated through mutagenesis and selection process, showed 1.25-fold improvement in specific CoQ_{10} content higher than *Paracoccus denitrificans* ATCC 19367 under shake flask cultivation. In the present study, efforts were made to optimize the CoQ_{10} fermentation process for mutant strain P-87 in 2 L laboratory fermenter. Initially the media modification and dosing strategy were employed at shake-flask level using *Paracoccus denitrificans* ATCC 19367 and subsequently the process was transferred to the laboratory fermenter for optimizing fermentation parameters for improved mutant strain P-87. The research work is focused on improving the CoQ_{10} fermentation process in order to make it cost-effective.

2. Materials and Methods

2.1. Strain, Media and Materials

The bacterial strain *Paracoccus denitrificans* ATCC 19367 and its induced mutant strain P-87 were maintained

at 4˚C - 8˚C on Tryptic Soy Agar (TSA) slants. All dehydrated media and media components were procured from Hi-Media, India. All solvents (AR grade) were procured from Merck [27].

2.2. Optimization of Shake Flask Fermentation

The seed medium contained 60 g of sucrose, 15 g of yeast extract, 15 g of peptone, 5 g of NaCl in 1 L demineralized water and pH 7.2. The 50 ml seed medium in 500 ml conical flask was inoculated with loopful of wild type strain culture on slant and incubated at 30˚C with shaking at 220 rpm. The 10% of grown seed was transferred to 50 ml of different production media in 500 ml conical flask. The production medium PM-A contained 25 g of sucrose, 10 g of $(NH_4)_2 \cdot SO_4$, 0.5 g of K_2HPO_4, 0.5 g of KH_2PO_4, 0.25 g of $MgSO_4 \cdot 7H_2O$, 20 g of corn steep liquor (CSL), 20 g of $CaCO_3$, trace element solution 1 ml/L in 1 L demineralized water and pH 7.0. PM-A medium was further modified by changing the concentration of other ingredients to suit CoQ_{10} fermentation. These media are as followed: PM-B consisting of 50 g of cane molasses, 10 g of $(NH_4)_2 \cdot SO_4$, 0.5 g of K_2HPO_4, 0.5 g of KH_2PO_4, 0.25 g of $MgSO_4 \cdot 7H_2O$, 20 g of CSL, 20 g of $CaCO_3$ in 1 L demineralized water and pH 7.0, PM-C consisting of 50 g of sucrose, 10 g of $(NH_4)_2 \cdot SO_4$, 0.5 g of K_2HPO_4, 0.5 g of KH_2PO_4, 0.25 g of $MgSO_4 \cdot 7H_2O$, 40 g of CSL, 20 g of $CaCO_3$ in 1 L demineralized water and pH 7.0 and PM-D consisting of 80 g of cane molasses, 13 g of $(NH_4)_2 \cdot SO_4$, 0.5 g of K_2HPO_4, 0.5 g of KH_2PO_4, 0.25 g of $MgSO_4 \cdot 7H_2O$, 40 g of CSL, 20 g of $CaCO_3$ in 1 L demineralized water and pH 7.2. The production flasks were incubated at 30˚C with shaking at 220 rpm for 120 h. The best production medium was dosed intermittently with different concentrations of para-hydroxy benzoic acid (pHBA) (5, 10, 20, 25, 40 and 50 mg/L) at 24 h [28] and with different concentration of sucrose (5%, 10%, 20%, 30% and 50%) at 48 h, 72 h solely [17].

2.3. CoQ_{10} Extraction Method

The 20 ml of broth was centrifuged at 12,000 rpm for 20 min to get biomass pellet, which was extracted with 20 ml ethanol by heating in shaking water bath at 60˚C for 3 h. The cells were removed by centrifugation and ethanol layer was re-extracted with 20 ml of hexane. The hexane layer was separated, concentrated till dryness and finally reconstituted with 1 ml of hexane. The titer was estimated by comparing the area of sample and standard of known concentration and expressed as mg of CoQ_{10}/L of broth (mg/L). The titer value was divided with DCW to get specific CoQ_{10} content (mg/g of DCW) [29].

2.4. Quantification of CoQ_{10}

The CoQ_{10} extracted from cell biomass was quantified on HPLC (Agilent 1100) using normal phase Kromasil silica column (250 mm × 4.6 mm, 5 µ particle size) and hexane:isopropyl alcohol (95:5) as mobile phase with a flow rate of 1 ml/min. Detection was carried out at 273 nm [30].

2.5. Dry Cell Weight (DCW) Measurement

The 10 ml of broth was centrifuged at 12,000 rpm for 20 min in a pre-weighed centrifuge tube. The cell mass was quantified by drying at 60˚C until a constant mass was obtained.

2.6. Estimation of Total Sugar

The total sugar was estimated by Anthrone method [31].

2.7. Optimization of Process Parameters in 2 L Fermenter

The fermentation process was optimized for mutant strain P-87 using PM-D medium in 2 L Applicon fermenter. A 10% (v/v) seed culture was inoculated into a 2 L fermenter with a working volume of 1 L. The fermentation was carried out by altering the parameters *i.e.* temperature (25˚C, 28˚C, 30˚C, 32˚C, 35˚C), agitation (300 rpm, 500 rpm, 700 rpm and 900 rpm), aeration (0.3 vvm, 0.5 vvm, 0.7 vvm and 1 vvm) and DO (10%, 20%, 30%, 40% and 50%) of appropriate air saturation [32] [33]. The intermittent feeding was started after 24 h of growth with 25 mg/L pHBA followed by 100 ml of 30% sucrose dosing at 48 h and 72 h respectively [17] [28]. The batch was harvested at 120 h. The broth samples were analyzed for CoQ_{10} production, packed cell volume (PCV), DCW, total sugar consumption and specific CoQ_{10} content. To study the effect of optimized parameters with

Paracoccus denitrificans ATCC 19367, batches were carried out and harvested at the same time as mentioned above. The broth samples were also analyzed in the same way as mentioned above.

2.8. Statistical Analysis

For analyzing differences between two groups, student's t-test was used based on PRISM-5 software. P values below 0.05 were considered statistically significant. The values in all graphs are an average of 3 trials. All error bars represent standard error of mean.

3. Results and Discussions

The wild type strain of *Paracoccus denitrificans* ATCC 19367 was found to produce considerably less amount of CoQ_{10} than the other few bacterial strains [6]. There are very few published reports on the improvements of *Paracoccus denitrificans* strain as well as process conditions for over production of CoQ_{10} [27] [32]-[34]. Therefore this strain has been selected in the present study. The previously generated mutant strain P-87 was used as a starting organism for fermenter optimization studies.

3.1. Optimization of Production Media

Being a primary metabolite, a longer cell growing stage would tend to accumulate more biomass and lead to a higher CoQ_{10} concentration being produced. Hence for CoQ_{10} fermentation, most researchers have made an effort to increase the biomass by substrate feeding or maintaining high substrate concentration in medium [17] [35]. The effect of type and concentration of carbon and nitrogen source on CoQ_{10} fermentation kinetics parameters using *Paracoccus denitrificans* was studied in order to optimize the nutritional requirement [32]. The improvement in cultivation medium for enhanced production of CoQ_{10} by photosynthetic *R. rubrum* using response surface methodology was reported [36]. Ca^{2+} increases the specific CoQ_{10} content in *Agrobacterium tumefaciens* was also reported [37]. Considering the above aspects, modification of the sucrose based PM-A medium was carried out by increasing the concentration of carbon and nitrogen sources (PM-B, PM-C, PM-D) and also by changing the carbon source (PM-B, PM-D) from sucrose to cane molasses. The cheaper carbon source cane molasses was introduced to reduce the cost of medium in order to make the process economically viable. The organism was able to tolerate higher amount of cane molasses in PM-D medium very efficiently than pure sucrose based PM-A medium as mentioned earlier with *Agrobacterium tumefaciens* [30].

Figure 1 describes the specific CoQ_{10} content and titer obtained with different production media. The highest CoQ_{10} production of 19.55 mg/L with 0.6821 mg/g of DCW specific content was obtained on PM-D medium whereas in PM-A medium CoQ_{10} production was 16.3 mg/L with 0.5689 mg/g of DCW specific content. The

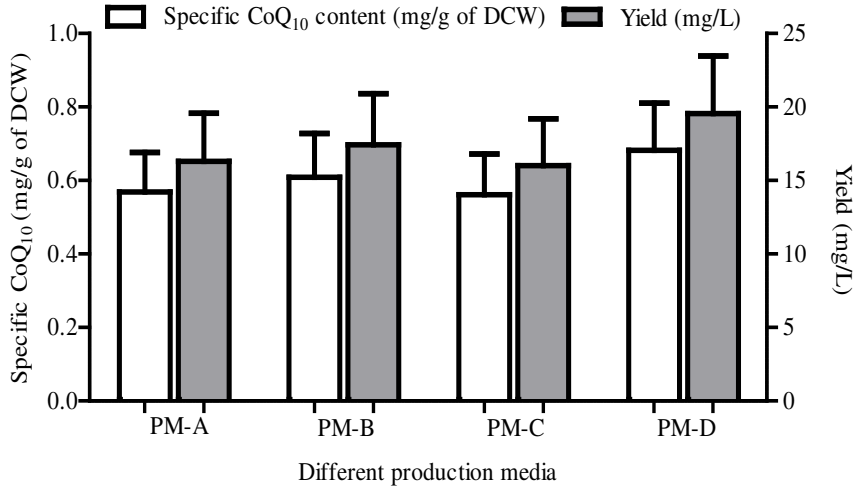

Figure 1. Comparison of specific CoQ_{10} content and titer between different production media using *Paracoccus denitrificans* ATCC 19367 at shake flask level. Medium PM-D showing improvement in specific CoQ_{10} content and titer.

other two media namely PM-B and PM-C showed relatively less titer as well as specific CoQ_{10} content. Based on the media studies, medium PM-D containing 80 g/L of cane molasses and 40 g/L of CSL was found to be best suited medium for screening of mutants in shake flasks.

3.2. Optimization of Dosing Strategy in Shake Flask

pHBA is a precursor of aromatic ring of CoQ_{10} biosynthesis and hence has been used in fed batch fermentation for CoQ_{10} production. It was observed that significant improvement in CoQ_{10} content of *Sporidiobolus johnsonii* was achieved by feeding pHBA precursors, resulted in achieving the maximum content of 10.5 mg/g DCW [28]. Different concentrations of pHBA like 5, 10, 25, 40 and 50 mg/L were independently dosed at 24 h in shake flask and the results are expressed in **Figure 2**. It was observed that dosing of pHBA at 25 mg/L concentration helped in improvement of CoQ_{10} titer and specific CoQ_{10} content. As compared to batch fermentation, the fed batch fermentation using pHBA (25 mg/L) showed 1.24 folds improvement in specific CoQ_{10} content and 1.11 folds improvement in titer. The pHBA concentration above 25 mg/L showed reduction in the specific content and titer due to toxicity.

The concentration of sucrose in the fermentation medium is one of the major factors for CoQ_{10} production using microorganisms [17]. Different concentrations of sucrose were used for dosing in independent experiments in order to maintain the total sugar concentration throughout the process and results are expressed in **Figure 3**. Out of different concentration tried, 30% sucrose dosing at 48 h and 72 h was found to be beneficial as it showed highest CoQ_{10} production having titer of 20.92 mg/L with specific CoQ_{10} content of 0.8307 mg/g of DCW. This dosing strategy helped in maintaining the total sugar concentration between 22.5 - 24.5 g/L till the end of fermentation cycle. Based on the above results, 25 mg/L of pHBA concentration and 30% of sucrose concentration were selected as feeding parameters in subsequent fed batch optimization studies.

The best pHBA and sucrose in combination were used for feeding purpose in the next trials of shake flask. **Figure 4** describes the different dosing strategies and their respective CoQ_{10} production values. The combination of pHBA (25 mg/L at 24 h) and sucrose (30% at 48 h and 72 h) was useful in improving the CoQ_{10} titer of 25 mg/L with 0.8720 mg/g of DCW of specific CoQ_{10} content. The above mentioned shake flask feeding strategy was implemented at 2 L laboratory fermenter level for process optimization using improved mutant strain P-87. The mutant strain P-87 was previously generated by iterative rounds of mutagenesis and selection showed improvement in CoQ_{10} production and specific content [27]. The mutant strain P-87 was deposited in Microbial Culture Collection (MCC) of National Center for Cell Sciences (NCCS), Pune, India under accession number MCC2520.

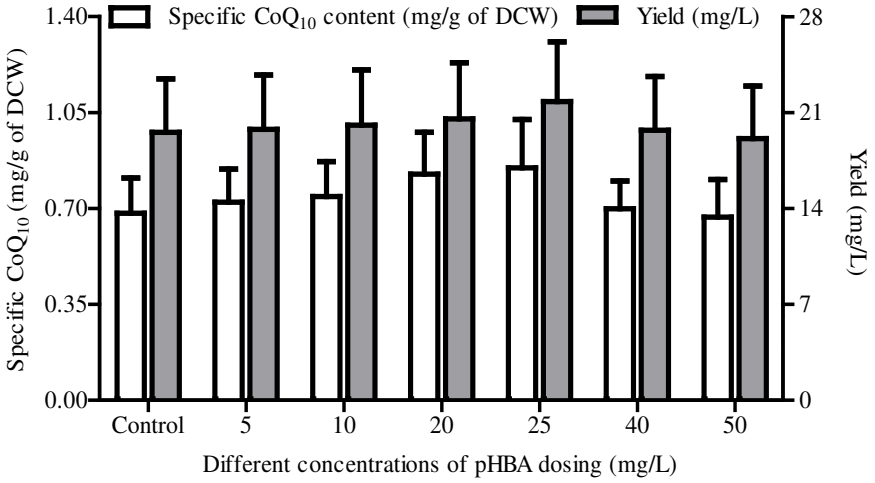

Figure 2. Comparison of specific CoQ_{10} content and titer between different pHBA concentrations dosed intermittently at 24 h of fermentation cycle with PM-D medium using *Paracoccus denitrificans* ATCC 19367 at shake flask level. The pHBA concentration of 25 mg/L dosed at 24 h showing improvement in specific CoQ_{10} content and titer.

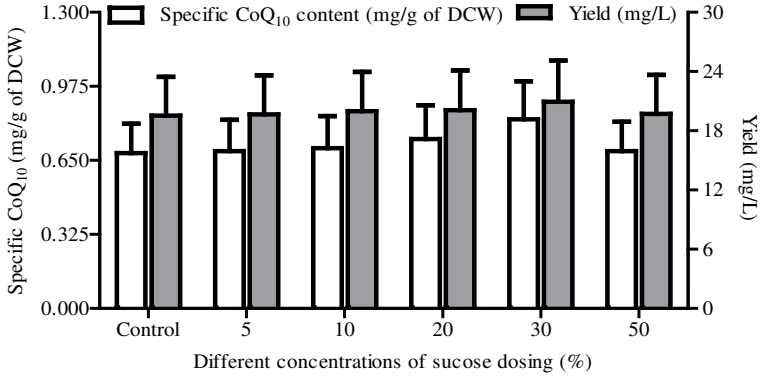

Figure 3. Comparison of specific CoQ_{10} content and titer between different sucrose concentrations dosed intermittently at 48 h and 72 h of fermentation cycle with PM-D medium using *Paracoccus denitrificans* ATCC 19367 at shake flask level. Sucrose concentration of 30% dosed at 48 h and 72 h showing slight improvement in specific CoQ_{10} content and titer.

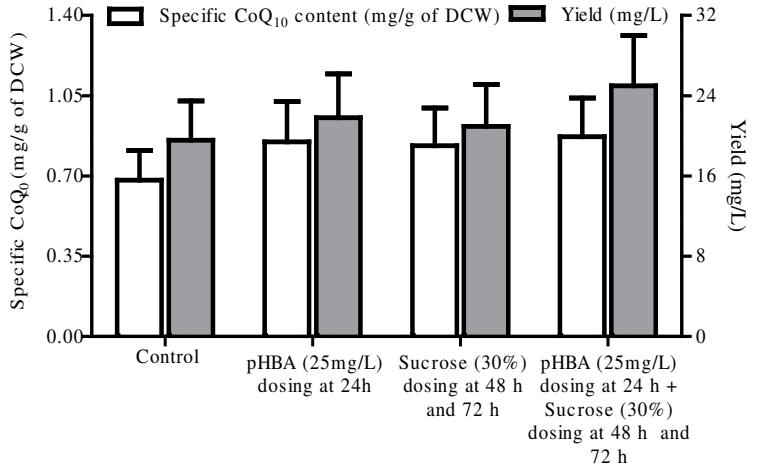

Figure 4. Comparison of specific CoQ_{10} content and titer between different dosing strategies performed with PM-D medium using *Paracoccus denitrificans* ATCC 19367 at shake flask level. Combination of pHBA and sucrose dosing showing improvement in specific CoQ_{10} content and titer.

3.3. Optimization of Process Parameters in 2 L Fermentor

The optimized fed batch process from the shake flask was transferred to the fermenter level with additional optimization studies with respect to fermentation parameters. For this purpose an improved mutant strain P-87 previously generated from *Paracoccus denitrificans* ATCC 19367 showing improvement in CoQ_{10} production in shake flask level was used [27]. In order to make the fermentation process cost effective, the mutant strain P-87 and cheaper raw materials were utilized during process optimization.

During fermentation of *Paracoccus denitrificans* ATCC 19367 on cane molasses based PM-D medium at shake flask level, dosing was carried out using 25 mg/L of pHBA at 24 h followed by 30% of sucrose solution at 48 h and 72 h respectively. The total sugar concentration dropped from 40 g/L to 17.5 - 18.5 g/L at 46 h and then it was maintained at around 22.5 - 24.5 g/L till end with the help of intermittent dosing at 48 h and 72 h. The same fed batch strategy was adopted for further optimization in fermenter by altering the parameters using mutant strain P-87. During optimization of temperature condition in fermenter, we have tried different temperatures like 25˚C, 28˚C, 30˚C, 32˚C, 35˚C and the results are expressed in **Figure 5**. It was observed that rise in temperature showed gradual increase in titer and specific CoQ_{10} content till 32˚C where we found the maximum CoQ_{10} production was 35.87 mg/L with 1.2286 mg/g of DCW specific content keeping identical aeration 0.5 vvm and agitation 500 rpm, but beyond that it was showed reduction in titer as well as specific CoQ_{10} content.

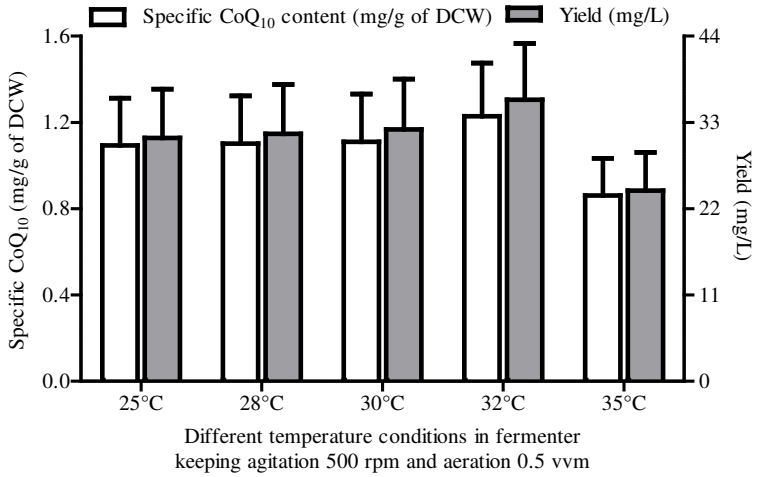

Figure 5. Comparison of specific CoQ_{10} content and titer between different temperature conditions with standardized dosing strategies in PM-D medium using mutant strain P-87 at fermenter level. Temperature 32˚C showing improvement in specific CoQ_{10} content and titer.

Both agitation and aeration are involved to a different extent in overall mass and oxygen transfer in the process fluid. Agitation controls the nutrient transfer and distribution of air and oxygen. Aeration not only determines the oxygenation of the culture, but also contributes to bulk mixing of the fermentation broth. High agitation promotes good mass transfer but is energy-intensive which increases the production cost. Agitation creates shear forces which cause morphological changes, variation in their growth and product formation, and also damages the cell structure. The combined effect of aeration/agitation and fed batch strategy on CoQ_{10} production by *Pseudomonas diminuta* was reported [38]. The fermentation process conditions were optimized for CoQ_{10} production in 150-L reactor using a mutant strain of *R. sphaeroides* with respect to temperature, aeration and fed batch strategy [39]. They observed that, aeration shift from the adequate aeration at early growth phase to the limited aeration in active cellular metabolism, was a key factor in CoQ_{10} production.

The effect of limited supply of air on CoQ_{10} production by *R. sphaeroides* was studied. The high aeration decreased the CoQ_{10} content [6]. Earlier it was described that high aeration suppresses the CoQ_{10} production in *Paracoccus denitrificans* [33]. During optimization of aeration condition in fermenter, we have tried different aerations like 0.3 vvm, 0.5 vvm, 0.7 vvm, 1 vvm and the results are expressed in **Figure 6**. It was observed that rise in aeration showed gradual decrease in titer and specific CoQ_{10} content. Aeration of 0.3 vvm showed maximum CoQ_{10} production of 34.55 mg/L with 1.1935 mg/g of DCW specific content, keeping standardized temperature 32˚C and agitation 500 rpm.

During optimization of agitation condition in fermenter, we have tried different agitations like 300 rpm, 500 rpm, 700 rpm, 900 rpm and the results are expressed in **Figure 7**. It was observed that rise in agitation from 300 rpm to 500 rpm showed improvements in titer and specific CoQ_{10} content but beyond 500 rpm it was gradually decreased due to high shear stress. As per our present observation, 500 rpm was found to be the optimal agitation and showed maximum CoQ_{10} production of 38.3 mg/L with 1.2480 mg/g DCW specific content.

It was proposed that cell growth and CoQ_{10} production were affected by various DO concentrations with *Agrobacterium* sp. and it was also been showed earlier that DO concentration had a great effect on the specific cell growth rate and DCW of *Rhizobium radiobacter* WSH2601 [22]. Effect of oxygen on CoQ_{10} production by *Paracoccus denitrificans* was also reported [34]. The pH and dissolved oxygen levels were found to be the key factors affecting CoQ_{10} productions in *A. tumefaciens* [40]. In the current study, during optimization of DO condition in fermenter, we have tried different DO concentrations like 10%, 20%, 30%, 40%, 50% and a typical fermenter kinetics at different DO concentrations with respect to specific content at different time was shown in **Figure 8(a)**. It was observed that the optimum DO concentration for CoQ_{10} biosynthesis was 30% (of air saturation), and higher or lower DO concentration was less favorable for CoQ_{10} production. The appropriate DO concentration of 30% was found to be the optimal and showed maximum CoQ_{10} production of 49.85 mg/L with 1.63 mg/g DCW specific content of as shown in **Figure 8(b)**.

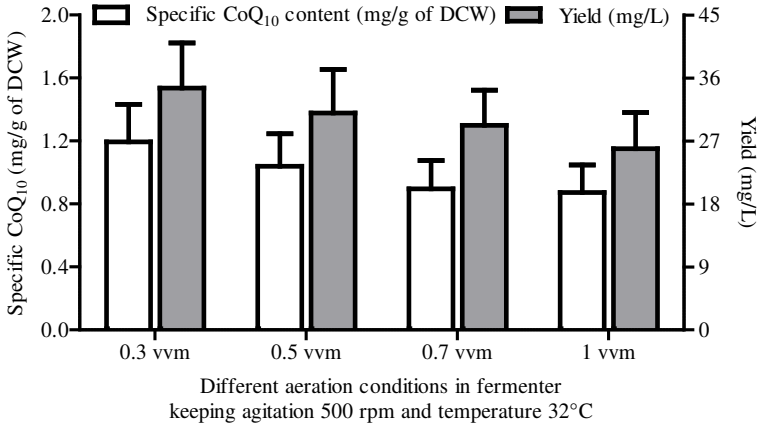

Figure 6. Comparison of specific CoQ_{10} content and titer between different aeration conditions with standardized dosing strategies in PM-D medium using mutant strain P-87 strain at fermenter level. Aeration of 0.3 vvm showing improvement in specific CoQ_{10} content and titer.

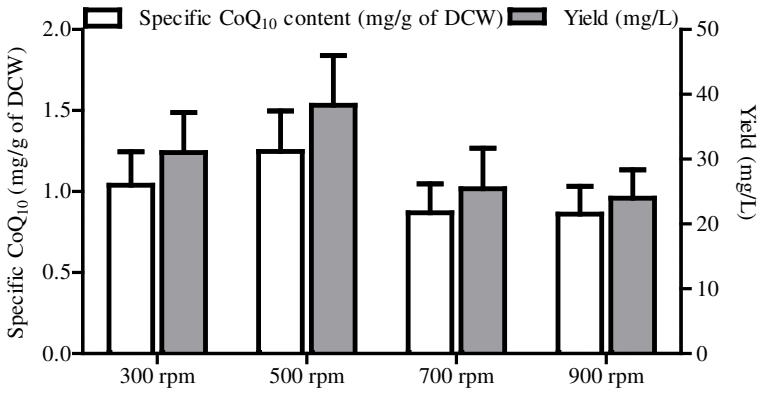

Figure 7. Comparison of specific CoQ_{10} content and titer between different agitation conditions with standardized dosing strategies in PM-D medium using mutant strain P-87 at fermenter level. Agitation of 500 rpm showing improvement in specific CoQ_{10} content and titer.

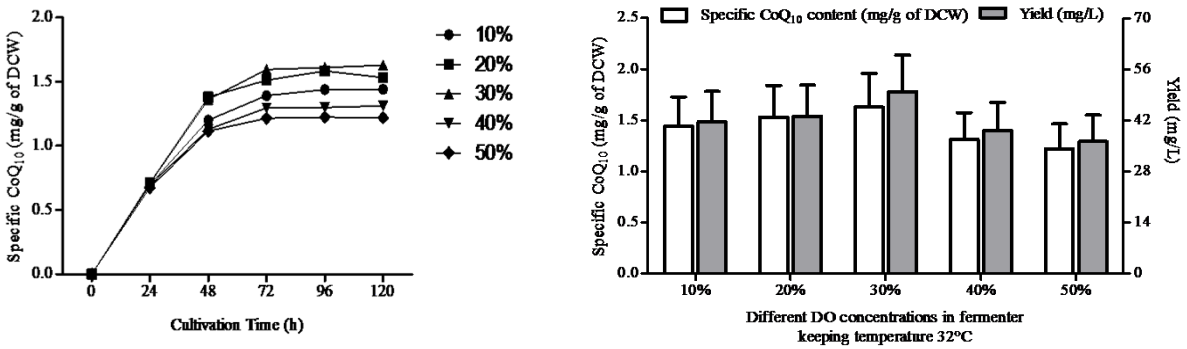

Figure 8. Comparison of specific CoQ_{10} content and titer between different DO concentrations with standardized dosing strategies in PM-D medium using mutant strain P-87 at fermenter level. (a) Fermenter kinetics with different DO concentrations and 30% DO showing improvement in specific CoQ_{10} content; (b) The appropriate DO concentration of 30% showing improvement in specific CoQ_{10} content and titer.

Fermenter operated at temperature 32˚C with appropriate DO concentration of 30% (of air saturation) showed 1.3 folds improvement in titer and specific CoQ_{10} content than above standardized parameters *i.e.* agitation 500 rpm, aeration 0.3 vvm and temperature 32˚C as shown in **Figure 9**. There was not much fluctuation observed in pH throughout the cycle and it was automatically maintained in the range of 7.2 to 7.5 till the end of fermentation cycle due to presence of high amount of buffering agent like $CaCO_3$ and intermittent dosing of 30% sucrose at 48 h and 72 h.

At identical fermenter condition *i.e.* 30˚C, 500 rpm, 0.5 vvm, *Paracoccus denitrificans* ATCC 19367 found to produce 29.23 mg/L with 0.8960 mg/g of DCW specific content whereas at optimized fermenter condition, it produced 34.55 mg/L with 1.1935 mg/g of DCW specific content as shown in **Figure 10(a)**. Similarly in case of mutant strain P-87, at identical fermenter condition it produced 32.10 mg/L with 1.1096 mg/g of DCW specific content whereas at optimized fermenter condition, it produced 49.85 mg/L with 1.63 mg/g of DCW specific content as shown in **Figure 10(b)**. So it was observed that, mutant strain P-87 showed 1.23 folds improvement

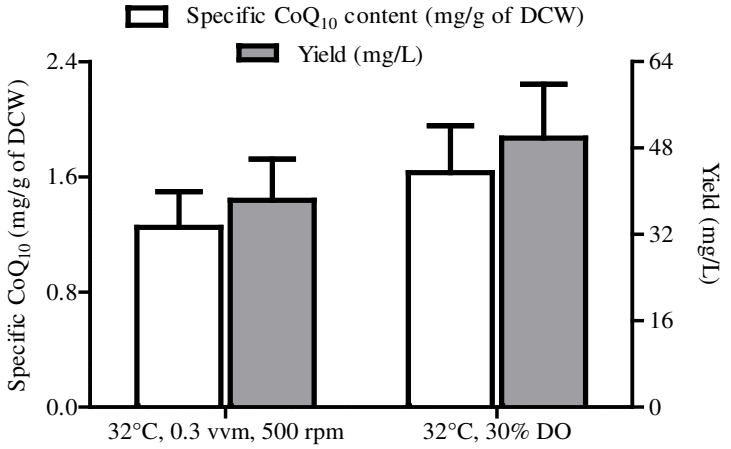

Figure 9. Comparison of specific CoQ_{10} content and titer between standardized fermenter parameters and appropriate DO concentrations of 30% at 32˚C with standardized dosing strategies in PM-D medium using mutant strain P-87 at fermenter level. The appropriate DO concentration of 30% and temperature 32˚C showing improvement in specific CoQ_{10} content and titer.

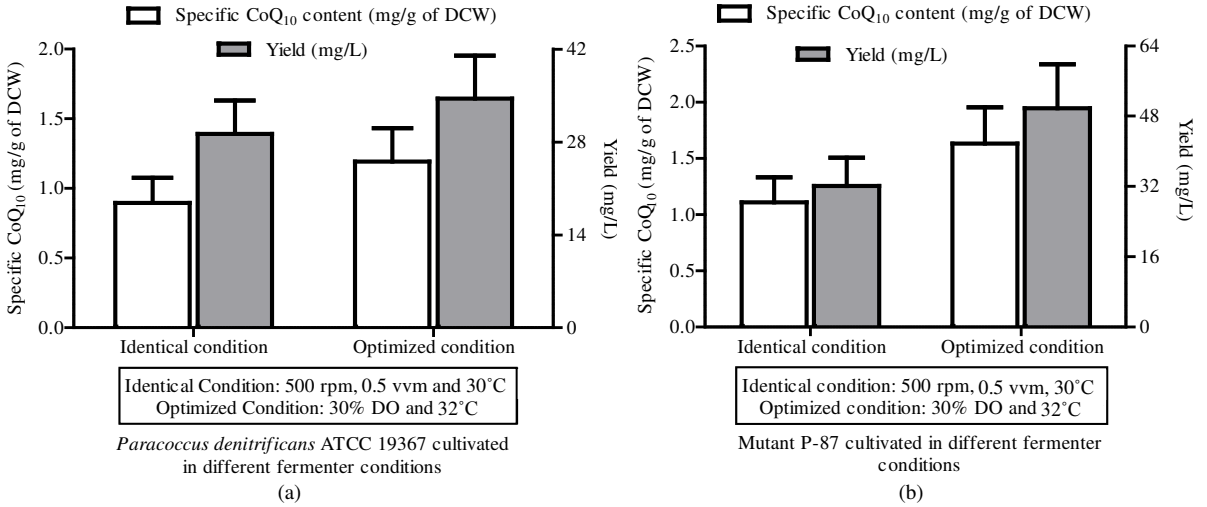

Figure 10. Comparison of specific CoQ_{10} content and titer between identical and optimized parameters using mutant strain P-87 and *Paracoccus denitrificans* ATCC 19367 with standardized dosing strategies in PM-D medium at fermenter level. (a) *Paracoccus denitrificans* ATCC 19367 cultivated in different conditions; (b) Mutant strain P-87 cultivated in different conditions. Mutant strain P-87 showing improvement in specific CoQ_{10} content and titer in both conditions.

Table 1. Comparison of CoQ_{10} production, PCV and specific CoQ_{10} content under different fermentation conditions with wild type and mutant P-87.

Strain	Condition	Aeration (vvm)	Agitation (rpm)	Temperature (˚C)	Cycle (h)	PCV (%)	Yield (mg/L)	Specific CoQ_{10} content (mg/g of DCW)
Wild type strain	Identical	0.5	500	30	120	8.9	29.23	0.8960
	Optimized	Appropriate DO concentration of 30% (of air saturation)		32	120	13.3	34.55	1.1935
Mutant strain P-87	Identical	0.5	500	30	120	11.4	32.1	1.1096
	Optimized	Appropriate DO concentration of 30% (of air saturation)		32	120	17.6	49.85	1.63

in specific CoQ_{10} content at identical fermentation condition and 1.36 folds improvements in specific CoQ_{10} content at optimized fermentation condition than *Paracoccus denitrificans* ATCC 19367 as shown in **Figure 10(a)**, **Figure 10(b)**.

At optimized fermenter condition mutant strain P-87 found to produce 49.85 mg/L at 120 h of fermentation cycle with a PCV of 17.6% whereas wild type strain produced 34.55 mg/L with a PCV of 13.3%. It was observed that mutant strain P-87 produced more biomass and titer than wild type strain under two different fermentation conditions as mentioned in **Table 1**. The highest biomass (1.3 folds improvement) and titer (1.44 folds improvement) was produced by mutant strain P-87 under optimized fermentation condition. On the whole, it is observed that the optimized production medium, intermittent dosing and process parameters has resulted in higher CoQ_{10} titer and specific CoQ_{10} content.

4. Conclusion

A mutant strain P-87 derived from *Paracoccus denitrificans* ATCC 19367 was utilized for CoQ_{10} fermentation optimization studies in 2 L laboratory fermenter. The fed-batch fermentation strategy developed in the shake flask level was transferred to the fermenter with optimization of the fermentation process parameters. The optimized fed-batch condition includes dosing of pHBA at a concentration of 25 mg/L at 24 h followed by 30% of sucrose solution at 48 h and 72 h respectively. The optimized fermentation parameters are at the temperature of 32˚C and at the appropriate DO concentration of 30% (of air saturation). Under optimized condition mutant strain P-87 produced 49.85 mg/L of CoQ_{10} having specific content of 1.63 mg/g of DCW which is 1.36 folds higher than that produced by wild type strain under optimized fermentation condition. The use of cane molasses in the medium-improved mutant strain and optimized fermentation parameters helped in reducing the cost of production with improved yield. Overall the laboratory scale process for production of CoQ_{10} using mutant strain P-87 was established.

Acknowledgements

We acknowledge Dr. Arun Balakrishnan, Senior Vice President, Piramal Enterprises Ltd., Mumbai, India for his support and encouragement towards this project.

References

[1] Wolf, D.E., Hoffman, C.H., Trenner, N.R., Arison, B.H., Shunk, C.H., Linn, B.D., McPherson, J.F. and Folkers, K. (1958) Structure Studies on the Coenzyme Q Group. *Journal of the American Chemical Society*, **80**, 47-52. http://dx.doi.org/10.1021/ja01550a096

[2] Wu, Z.F., Weng, P.F., Du, G.C. and Chen, J. (2001) Advances of Coenzyme Q_{10} Function Studies. *Journal of Ningbo University*, **2**, 85-88.

[3] Keinan, E. and Eren, D. (1998) Total Synthesis of Polyprenoid Natural Products via Pd (0)-Catalyzed Oligomerization. *Pure and Applied Chemistry*, **60**, 89-98.

[4] Lipshutz, B.H., Mollard, P., Pfeiffer, S.S. and Chrisman, W. (2002) A Short, Highly Efficient Synthesis of Coenzyme Q_{10}. *Journal of the American Chemical Society*, **124**, 14282-14283. http://dx.doi.org/10.1021/ja021015v

[5] Laplante, S., Souchet, N. and Bry, P. (2009) Comparison of Low-Temperature Processes for Oil and Coenzyme Q_{10} Extraction from Mackerel and Herring. *European Journal of Lipid Science and Technology*, **111**, 135-141.

http://dx.doi.org/10.1002/ejlt.200800133

[6] Yoshida, H., Kotani, Y., Ochiai, K. and Araki, K. (1998) Production of Ubiquinone-10 Using Bacteria. *The Journal of General and Applied Microbiology*, **44**, 19-26. http://dx.doi.org/10.2323/jgam.44.19

[7] Zhu, X.F., Yuasa, M., Okada, K. Suzuki, K.K., Nakagawa, T., Kawamukai, M. and Matsuda, H. (1995) Production of Ubiquinone in *Escherichia coli* by Expression of Various Genes Responsible for Ubiquinone Biosynthesis. *Journal of Fermentation and Bioengineering*, **79**, 493-495. http://dx.doi.org/10.1016/0922-338X(95)91268-A

[8] Okada, K., Kainou, T., Tanaka, K., Nakagawa, T., Matsuda, H. and Kawamukai, M. (1998) Molecular Cloning and Mutational Analysis of the ddsA Gene Encoding Decaprenyl Diphosphate Synthase from *Gluconobacter suboxydans*. *European Journal of Biochemistry*, **255**, 52-59. http://dx.doi.org/10.1046/j.1432-1327.1998.2550052.x

[9] Lee, J.K., Her, G., Kim, S.Y. and Seo, J.H. (2004) Cloning and Functional Expression of the dps Gene Encoding Decaprenyl Diphosphate Synthase from *Agrobacterium tumefaciens*. *Agricultural and Biological Chemistry*, **48**, 1347-1348.

[10] Saiki, R., Nagata, A., Kainou, T. Matsuda, H. and Kawamukai, M. (2005) Characterization of Solanesyl and Decaprenyl Diphosphate Synthases in Mice and Humans. *FEBS Journal*, **272**, 5606-5622. http://dx.doi.org/10.1111/j.1742-4658.2005.04956.x

[11] Park, Y.C., Kim, S.J., Choi, J.H., Lee, W.H., Park, K.M., Kawamukai, M., Ryu, Y.W. and Seo, J.H. (2005) Batch and Fed-Batch Production of Coemzyme Q_{10} in Recombinant *Escherichia coli* Containing Decaprenyl Diphosphate Synthase Gene for *Gluconobacter suboxydans*. *Applied Microbiology and Biotechnology*, **67**, 192-196. http://dx.doi.org/10.1007/s00253-004-1743-y

[12] Kawamukai, M. (2009) Biosynthesis and Bioproduction of Coenzyme Q_{10} by Yeasts and Other Organisms. *Biotechnology and Applied Biochemistry*, **53**, 217-226. http://dx.doi.org/10.1042/BA20090035

[13] Zhong, W., Fang, J., Liu, H. and Wang, X. (2009) Enhanced Production of CoQ_{10} by Newly Isolated *Sphingomonas* sp. $ZUTEO_3$ with a Coupled Fermentation-Extraction Process. *Journal of Industrial Microbiology Biotechnology*, **36**, 687-693. http://dx.doi.org/10.1007/s10295-009-0538-7

[14] Zhong, W., Wang, W., Kong, Z., Wu, B., Zhong, L., Li, X., Yu, J. and Zhang, F. (2011) Coenzyme Q_{10} Production Directly from Precursors by Free and Gel-Entrapped *Sphingomonas* sp. $ZUTEO_3$ in a Water-Organic Solvent, Two-Phase Conversion System. *Applied Microbiology and Biotechnology*, **89**, 293-302. http://dx.doi.org/10.1007/s00253-010-2876-9

[15] Gu, S.B., Yao, J.M., Yuan, Q.P., Xue, P.J., Zheng, Z.M., Wang, L. and Yu, Z.L. (2006) Kinetics of *Agrobacterium tumefaciens* Ubiquinone-10 Batch Production. *Process Biochemistry*, **41**, 1908-1912. http://dx.doi.org/10.1016/j.procbio.2006.04.002

[16] Koo, B.S., Gong, Y.J., Kim, S.Y., Kim, C.W. and Lee, H.C. (2010) Improvement of Coenzyme Q_{10} Production by Increasing the NADH/NAD^+ Ratio in *Agrebacterium tumefaciens*. *Bioscience*, *Biotechnology and Biochemistry*, **74**, 895-898. http://dx.doi.org/10.1271/bbb.100034

[17] Ha, S.J., Kim, S.Y., Seo, J.H., Lee, K.M. and Lee, J.K. (2007) Controlling the Sucrose Concentration Increases Coenzyme Q_{10} Production in Fed-Batch Culture of *Agrebacterium tumefaciens*. *Applied Microbiology and Biotechnology*, **76**, 109-116. http://dx.doi.org/10.1007/s00253-007-0995-8

[18] Kuratsu, Y. and Inuzuka, K. (1985) Factors Affecting Broth Viscosity and Coenzyme Q_{10} Production by *Agrobacterium* Species. *Applied Microbiology and Biotechnology*, **21**, 55-59.

[19] Choi, G.S., Kim, Y.S., Seo, J.H. and Ryu, Y.W. (2005) Restricted Electron Flux Increases Coenzyme Q_{10} Production in *Agrobacterium tumefaciens* ATCC4452. *Process Biochemistry*, **40**, 3225-3229. http://dx.doi.org/10.1016/j.procbio.2005.03.038

[20] Yen, H. and Chiu, C. (2007) The Influences of Aerobic Dark and Anaerobic-Light Cultivation on CoQ_{10} Production by *Rhodobacter sphaeroides* in the Submerged Fermenter. *Enzyme and Microbial Technology*, **41**, 600-604. http://dx.doi.org/10.1016/j.enzmictec.2007.05.005

[21] Yen, H.W. and Shih, T.Y. (2009) Coenzyme Q_{10} Production by *Rhodobacter sphaeroides* in Stirred Tank and in Airlift Bioreactor. *Bioprocess and Biosystems Engineering*, **32**, 711-716. http://dx.doi.org/10.1007/s00449-008-0294-5

[22] Kuratsu, Y., Hagino, H. and Inuzuka, K. (1984) Effect of Ammonium Ion on Coenzyme Q_{10} Fermentation by *Agrobacterium* Species. *Agricultural and Biological Chemistry*, **48**, 1347-1348. http://dx.doi.org/10.1271/bbb1961.48.1347

[23] Sakato, K., Tanaka, H., Shibata, S. and Kuratsu, Y. (1992) Agitation-Aeration Studies on Coenzyme Q_{10} Production Using *Rhodopseudomonas spheroids*. *Biotechnology and Applied Biochemistry*, **16**, 19-28.

[24] Pfefferle, C., Theobald, U., Gurtler, H. and Fiedler, H.P. (2000) Improved Secondary Metabolite Production in the Genus *Streptosporangium* by Optimization of Fermentation Conditions. *Journal of Biotechnology*, **80**, 135-142. http://dx.doi.org/10.1016/S0168-1656(00)00249-2

[25] Wu, Z.F., Du, G.C. and Chen, J. (2003) Effects of Dissolved Oxygen Concentration and DO-Stat Feeding Strategy on

CoQ_{10} Production with *Rhizobium radiobacter*. *World Journal of Microbiology and Biotechnology*, **19**, 925-928. http://dx.doi.org/10.1023/B:WIBI.0000007322.19802.57

[26] Zhang, D., Shrestha, B., Li, Z. and Tan, T. (2007) Ubiquinone-10 Production Using *Agrobacterium tumefaciens* dps Gene in *Escherichia coli* by Expression of Various Genes Responsible for Ubiquinone Biosynthesis. *Journal of Bioscience and Bioengineering*, **79**, 493-495.

[27] Tokdar, P., Vanka, R., Ranadive, P., George, S., Khora, S.S. and Deshmukh, S.K. (2014) Protoplast Fusion Technology for Improved Production of Coenzyme Q_{10} Using *Paracoccus denitrificans* ATCC 19367 Mutant Strains. *Journal of Biochemical Technology*, **5**, 685-692.

[28] Dixson, D.D., Boddy, C.N. and Doyle, R.P. (2011) Reinvestigation of Coenzyme Q10 Isolation from *Sporidiobolus johnsonii*. *Chemistry Biodiversity*, **8**, 1033-1051. http://dx.doi.org/10.1002/cbdv.201000278

[29] Ranadive, P., Mehta, A. and George, S. (2011) Strain Improvement of *Sporidiobolus johnsonii* ATCC 20490 for Biotechnological Production of Coenzyme Q_{10}. *International Journal of Chemical Engineering and Applications*, **2**, 216-220. http://dx.doi.org/10.7763/IJCEA.2011.V2.106

[30] Tokdar, P., Wani, A., Kumar, P., Ranadive, P. and George, S. (2013) Process and Strain Development for Reduction of Broth Viscosity with Improved Yield in Coenzyme Q_{10} Fermentation by *Agrobacterium tumefaciens* ATCC4452. *Fermentation Technology*, **2**, 110. http://dx.doi.org/10.4172/2167-7972.1000110

[31] Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, **26**, 350-356. http://dx.doi.org/10.1021/ac60111a017

[32] Bule, M.V. and Singhal, R.S. (2013) Fermentation Kinetics of Production of Ubiquinone-10 by *Paracoccus denitrificans* NRRL B-3785: Effect of Type and Concentration of Carbon and Nitrogen Sources. *Food Science and Biotechnology*, **23**, 607-613.

[33] Matsumura, M., Kobayashi, T. and Aiba, S. (1983) Anaerobic Production of Ubiquinone-10 by *Paracoccus denitrificans*. *European Journal of Applied Microbiology and Biotechnology*, **17**, 85-89. http://dx.doi.org/10.1007/BF00499856

[34] Kaplan, P., Kucera, I. and Dadak, V. (1993) Effect of Oxygen on Ubiquinone-10 Production by *Paracoccus denitrificans*. *Biotechnology Letters*, **15**, 1001-1002. http://dx.doi.org/10.1007/BF00129925

[35] Yen, H.W., Feng, C.Y. and Kang, J.L. (2009) Cultivation of *Rhodobacter sphaeroides* in the Stirred Bioreactor with Different Feeding Strategies for CoQ_{10} Production. *Applied Biochemistry and Biotechnology*, **160**, 1441-1449. http://dx.doi.org/10.1007/s12010-009-8576-1

[36] Tian, Y., Yue, T., Yuan, Y., Soma, P.K. and Lo, Y.M. (2010) Improvement of Cultivation Medium for Enhanced Production of Coenzyme Q_{10} by Photosynthetic *Rhodospirillum rubrum*. *Journal of Biochemical Engineering*, **51**, 160-166. http://dx.doi.org/10.1016/j.bej.2010.06.011

[37] Ha, S.J., Kim, S.Y., Seo, J.H., Jeya, M., Zhang, Y.W., Ramu, T., Kim, I.W. and Lee, J.K. (2009) Ca^{2+} Increases the Specific Coenzyme Q_{10} Content in *Agrebacterium tumefaciens*. *Bioprocess and Biosystems Engineering*, **32**, 697-700. http://dx.doi.org/10.1007/s00449-009-0318-9

[38] Bule, M.V. and Singhal, R.S. (2010) Combined Effect of Agitation/Aeration and Fed-Batch Strategy on Ubiquinone-10 Production by *Pseudomonas diminuta*. *Chemical Engineering Technology*, **33**, 885-894. http://dx.doi.org/10.1002/ceat.201000072

[39] Kien, N.B., Kong, I., Lee, M. and Kim, J.K. (2010) Coenzyme Q_{10} Production in a 150-l Reactor by a Mutant Strain of *Rhodobacter sphaeroides*. *Journal of Industrial Microbiology Biotechnology*, **37**, 521-529. http://dx.doi.org/10.1007/s10295-010-0699-4

[40] Ha, S.J., Kim, S.Y., Seo, J.H., Oh, D.K. and Lee, J.K. (2007) Optimization of Culture Conditions and Scale-Up to Pilot and Plant Scales for Coenzyme Q_{10} Production by *Agrobacterium tumefaciens*. *Applied Microbiology and Biotechnology*, **74**, 974-980. http://dx.doi.org/10.1007/s00253-006-0744-4

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