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Surfactant Supplementation to Enhance the Production of Vitamin K_2 Metabolites in Shake Flask Cultures Using *Escherichia* sp. Mutant FM3-1709

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Summary

The effects of the addition of various surfactants on the cell growth and production of vitamin K_2 metabolites, such as intracellular menaquinone-4 (MK-4), extracellular MK-4, intracellular MK-6 and extracellular MK-6, were studied in the submerged culture of *Escherichia* sp. All of the added surfactants caused the extension of the exponential phase. Betaine, polyoxyethylene oleyl ether and Tween-80 were favourable to the cell growth of *Escherichia* sp. mutant strain FM3-1709. The highest cell growth (X_{max}), biomass production rate (Q_x) and biomass yield ($Y_{x/s}$) were (12.6±0.2) g/L, (0.21±0.01) g/(L·h) and (2.42±0.02) g/g, respectively. The results show that the addition of all surfactants led to a lower production of intracellular MK-4, whereas the production of extracellular MK-4 increased remarkably. Among the five investigated surfactants, the addition of the nonionic surfactant polyoxyethylene oleyl ether (1.0 g/L) led to the highest production of extracellular MK-4 ((33.6±0.4) mg/L), MK-6 ((2.56±0.07) mg/L) and the highest yield of total MK-4 ((47.6±0.4) mg/L) and MK-6 ((6.0±0.1) mg/L). The addition of polyoxyethylene oleyl ether proved to be more beneficial for the secretion of MK-4 than MK-6.

Key words: surfactant, vitamin K_2, shake flask cultures

Introduction

Vitamin K consists of a series of compounds (phylloquinone and menaquinones) that have a common 2-methyl-1,4-naphthoquinone nucleus but differ in the structure of a side chain (*1–3*). Phylloquinone (vitamin K_1) is involved in electron transport during photosynthesis and can be recovered by extraction from green plants. Menaquinones (vitamin K_2, MK-*N*) have variable side chains (*N*=4–13) where *N* represents the number of isoprene units on the isoprenoid tail. Menaquinones are constituents of plasma membranes in some bacteria, and they play important roles in electron transport, oxidative phosphorylation and active transport (*4,5*). In recent years, vitamin K has been proven to have important roles not only in blood coagulation but also in bone metabolism (*6,7*). In addition, it has been reported that MK-4 can stimulate testosterone production in testes and testicular tumour cells *via* activation of protein kinase A (*8*), and prevent hepatocarcinogenesis (*9*). Moreover, as an electron carrier, vitamin K_2 may possibly be used to treat mitochondrial pathologies such as Parkinson's disease and amyotrophic lateral sclerosis (*10–12*). Because of its phys-

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iological functions, further detailed study is warranted to determine how to increase the production of vitamin K_2.

Even though *Escherichia coli* as a microbial platform for vitamin K_2 production has been reported (*13*), the focal point of this research is genetic modification in metabolic engineering. The control of fermentation process has not been studied in *Escherichia* sp. From the viewpoint of achieving an economical process, the higher the intracellular MK content, the higher the product yield, assuming the biomass concentration remains constant. Therefore, most industrial microbiologists have primarily investigated how to increase, as much as possible, the content of bacteria. However, it has been found that the content always remains at a stable level in bacteria, since 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase, the first regulatory enzyme in the shikimate pathway, is feedback-regulated by chorismate and MK-4 (*14*). If the cells could be induced to excrete excessive vitamin K_2 extracellularly, and the concentration kept below the level of feedback inhibition of DAHP synthase, then an improvement in the total vitamin K_2 concentration would likely be realized.

Surfactants, which are amphiphilic compounds being both water and oil soluble, have been used successfully to enhance the production of biologics, such as enzymes (*15–17*), polysaccharides (*18*,*19*) and unsaturated fatty acids (*20*). The mechanism of this effect is that low concentrations of surfactants increase the membrane permeability and thus the extracellular productivity by removing the restriction of intracellular diffusion of target metabolites. However, the extracellular accumulation of vitamin K_2 by *Escherichia* sp. has not been documented. The overall effects of different surfactants on cell growth, intracellular vitamin K_2 biosynthesis and extracellular vitamin K_2 accumulation by *Escherichia* sp. are still not clear. This study attempts to expand our understanding of the effects of surfactant addition in submerged culture on the cell growth of and vitamin K_2 production by *Escherichia* sp.

Materials and Methods

Microorganism and inoculum

Strain FM3-1709, which was derived from *Escherichia* sp. 1-1, was used in this study. The strain was obtained as a 1-hydroxy-2-naphthoate-resistant mutant and further mutated by nitrogen ion beam irradiation. It was maintained on beef extract-peptone agar slants, which were made up of the following components (in g/L): beef extract 3, peptone 10, NaCl 5 and agar 20. The pH value was initially adjusted to 7.0. The slants were incubated at 37 °C for 96 h and then stored at 4 °C. The components of the plate medium were the same as those of the slant medium.

Inoculum preparation

The medium for seed culture consisted of the following components (in g/L): glycerol 10, peptone 10, yeast extract 1.5, K_2HPO_4 4.5, NaCl 3 and $MgSO_4·7H_2O$ 0.3. The pH value was initially adjusted to 7.0, and the medium was then autoclaved at 103.4 kPa and 121 °C for 20 min. *Escherichia* sp. was transferred to the medium by punching out 0.7-mm diameter agar discs from the culture grown on the beaf-extract-peptone plates. Five discs were used to inoculate 100 mL of liquid media. The seed culture was grown in a 500-mL Erlenmeyer flask at 37 °C and 200 rpm (4.44×*g*) on a shaking incubator with a rotational radius of 10 cm for 24 h.

Shake flask cultures

The flask culture experiments were performed in 500-mL flasks containing 100 mL of the basal medium inoculated with 10 % (by volume) of the seed culture. The basal medium contained (in g/L): glycerol 10, peptone 10, yeast extract 1.5, K_2HPO_4 4.5, NaCl 3, $MgSO_4·7H_2O$ 0.3 and cedar oil 1. The initial pH value was adjusted to 7.0 with the addition of either 1 M NaOH or 1 M HCl. Apart from the basal medium, various kinds and concentrations of surfactants, such as the anionic surfactant sodium dodecyl benzene sulphonate (SDBS), cationic surfactant alkyl dimethyl benzyl ammonium chloride (ADBAC), amphoteric surfactant betaine, and nonionic surfactants Tween-80 and polyoxyethylene oleyl ether (POE), were added to the medium to investigate their influence on the formation of vitamin K_2 metabolites. All media were sterilized at 121 °C for 20 min. Each culture was incubated at 37 °C on a rotary shaker incubator at 200 rpm (4.44×*g*) for 120 h, and samples were collected at various intervals from the shake flasks to measure the dry biomass, glycerol concentration, pH value and intra- and extracellular MK-4 and MK-6 production. Three sets of shake flasks were prepared at the same time for each test. The values are the means of triplicate determinations.

Extraction and measurements of menaquinones

Cells and culture fluid were separated by centrifugation of the culture broth at 7104×*g* for 5 min. The collected cells were dissolved with 15 mL of deionized water and destroyed by ultrasonic waves at a frequency of 20 kHz. Menaquinones were extracted from the cells after homogenizing them in 10 mL of *n*-butanol by shaking at room temperature for 10 min. In each run, the mixture was stirred well and then centrifuged at 999×*g* for 10 min to separate the organic and aqueous layers. The amounts of intracellular and extracellular MK in the upper organic phase were determined by high performance liquid chromatography (HPLC) (*21*). Extracellular MK was extracted by homogenizing 15 mL of culture fluid and 10 mL of *n*-hexane by shaking at room temperature for 10 min.

Determination of biomass and glycerol concentration

For biomass determination, the dry cell mass (DCM) was calculated from the absorbance of the culture broth measured at 660 nm after 10-fold dilution using a calibration curve.

A colourimetric method was used to determine the residual glycerol in the fermentation broth (*20*). The method is based on acidic periodate oxidation of alditols resulting in the formation of formaldehyde. A volume of 1 mL of the appropriately diluted fermentation broth and 1 mL of 0.015 M sodium metaperiodate in 0.12 M HCl were mixed in test tubes. A volume of 2 mL of 0.1 %

rhamnose solution was then added to each tube in order to remove excess periodate ions. After mixing, 4 mL of Nash reagent (20 mM of ammonium acetate buffer and 20 mg of acetyl acetone) were then added to the mixture. Colour was allowed to develop for 15 min in a water bath at 53 °C. Upon cooling, the yellow reaction product had an absorption maximum at 412 nm. The linear calibration curve for glycerol was obtained in the range of 0–25 μg/mL.

Results and Discussion

Batch flask culture

Fig. 1 shows the kinetics of cell growth and metabolite accumulation by *Escherichia* sp. mutant FM3-1709 in shake flask cultures. These data were obtained with 100 mL of basal medium at an initial pH=7.0, 37 °C and 200 rpm (4.44×*g*). A rapid increase in the biomass was observed within the first 48 h, during which time the cells were in the exponential growth phase. The highest concentration of dry cell biomass was (9.3±0.2) g/L. However, the production of intracellular MK-4 increased rapidly during the first 96 h, and intracellular MK-6 increased rapidly during the first 108 h. According to the results shown in Fig. 1, the formation of intracellular MK-4 and MK-6 followed a mixed-growth-associated product formation pattern and their concentration increased to the highest level of (22.0±0.2) mg/L at 96 h and (4.7±0.1) mg/L at 108 h, respectively. Extracellular MK-4 and MK-6 were hardly detected. The following fermentations were mainly performed for 96 or 108 h to investigate the influence of the addition of surfactant on the formation of vitamin K_2 metabolites.

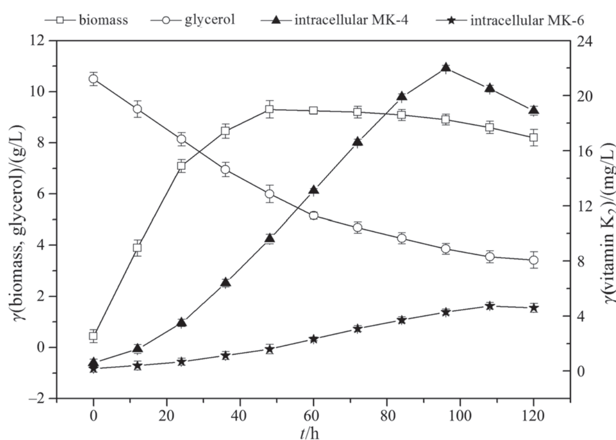

Fig. 1. Time course of cell growth and metabolite formation during submerged fermentation of *Escherichia* sp. in shake flask culture. Cultivation on the basal medium and menaquinone (MK) determination were carried out as described in Materials and Methods. All experiments were carried out in triplicate and data are expressed as mean values±standard deviations (S.D.)

Effect of the addition of surfactant

Fig. 2 shows the time profile of biomass and metabolite concentrations produced in the culture with the addition of 1.0 g/L of POE. The results indicate that dry cell biomass rose to (12.6±0.2) g/L in 60 h. The amount

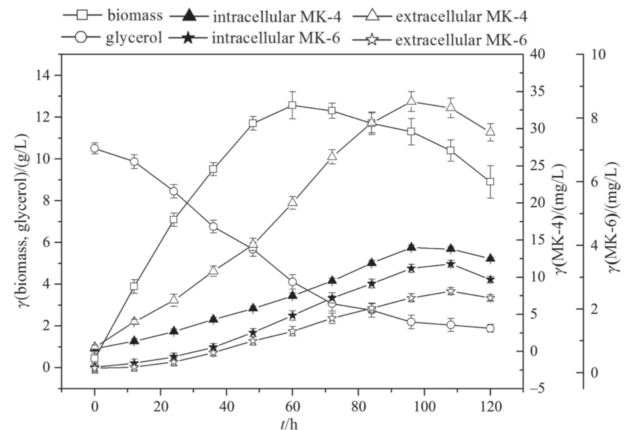

Fig. 2. Time course of cell growth and metabolite formation of *Escherichia* sp. in a shake flask culture with the addition of 1.0 g/L of polyoxyethylene oleyl ether (POE). Cultivation and menaquinone (MK) determination were carried out as described in Materials and Methods. All experiments were carried out in triplicate and data are expressed as mean values±S.D.

of intracellular MK-6 reached a maximum of (3.42±0.09) mg/L of culture broth in 108 h of cultivation. Extracellular MK-6 could hardly be detected up to the stationary phase, and then its amount increased gradually and reached a maximum of (2.56±0.07) mg/L in 108 h of cultivation. In contrast to MK-6, extracellular MK-4 was detectable from the beginning of cultivation and continued to increase after the stationary phase. The amount of extracellular MK-4 reached a maximum of (33.6±0.4) mg/L of culture broth in 96 h of cultivation. On the other hand, the amount of intracellular MK-4 increased more slowly compared to that of extracellular MK-4, and reached a maximum of (14.0±0.5) mg/L of culture broth in 96 h of cultivation. The total amount of MK-*N* was (62.8±0.5) mg/L of culture broth at 96 h of cultivation. Compared with the control described above, the total amount of MK-*N* was 2 times higher and the amount of extracellular MK-4 was markedly increased. The data demonstrate that POE can effectively enhance the total production of vitamin K_2 metabolites, especially of the extracellular MK-4.

Effect of different kinds of surfactants

To further investigate the effect of surfactant on vitamin K_2 production, SDBS, ADBAC, betaine, Tween-80 and POE were individually added to submerged cultures of strain FM3-1709. The surfactants were added at the beginning of cultivation, all at suitable concentrations. Fig. 3 shows the influence of various kinds of surfactants on cell growth, intracellular and extracellular MK-*N* production.

The cell growth of strain FM3-1709 in 48 h was found to increase after the addition of POE or Tween-80, whereas it decreased after the addition of SDBS or ADBAC in 96 h of cultivation. It increased slightly when betaine was added, compared to the control. The inhibition of cell growth by SDBS and ADBAC may have been caused by the higher concentration of these ionic surfactants, which dissolved the cell membrane components (*23*), leading to breakage of the membrane. Under these conditions,

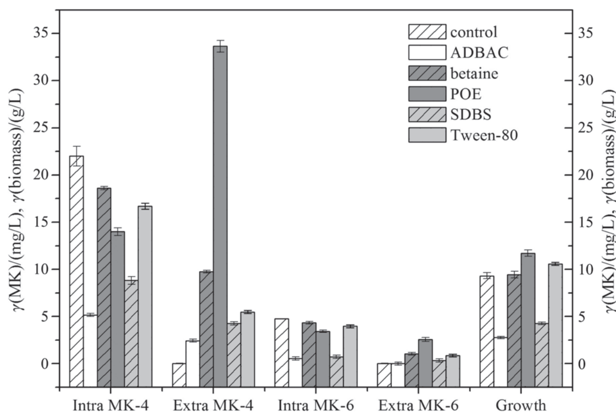

Fig. 3. Effects of different surfactants on the production of intracellular menaquinone-4 (MK-4), extracellular MK-4, intracellular MK-6, extracellular MK-6 and cell growth. The biomass was measured at 48 h, MK-4 at 96 h, MK-6 at 108 h. All experiments were carried out in triplicate and data are expressed as mean values±S.D. Control: without the addition of surfactant. Concentrations of surfactants (in g/L): alkyl dimethyl benzyl ammonium chloride (ADBAC) 0.1, betaine 0.5, polyoxyethylene oleyl ether (POE) 1.0, sodium dodecyl benzene sulphonate (SDBS) 0.1 and Tween-80 1.0

cells cannot reproduce. In contrast, the biomass was higher than the control, and increased when the concentration of POE or Tween-80 was increased. The highest cell concentration of (12.6±0.1) g/L was obtained after 48 h of cultivation in a medium to which 0.5 g/L of POE was added. The result was also consistent with a previous report that compared the effects of anionic, cationic and nonionic surfactants on the growth of *Saccharomyces cerevisiae*, which indicated that only the nonionic surfactant was biocompatible with *S. cerevisiae* (*24*).

The addition of all surfactants led to a lower intracellular but higher extracellular MK-4 production by strain FM3-1709. The extracellular MK-4 concentration obtained from the cultivation with the addition of POE was much higher than with the addition of other surfactants. This result showed that the surfactants had the ability to secrete intracellular MK-4 into the extracellular broth, so that POE enhanced the total MK-4 production. However, different surfactants gave different values of MK-4 production. The highest extracellular MK-4 production ((33.6±0.4) mg/L) was obtained when 1.0 g/L of POE was supplemented. On the other hand, the lowest extracellular MK-4 production ((2.4±0.4) mg/L) was obtained when 0.1 g/L of ADBAC was added. The reason may be a conflict between the biocompatibility of the surfactant with the microorganism and the ability of the surfactant to enhance secretion of an intracellular product into the extracellular broth. High extracellular MK-4 concentration was only observed when the nonionic surfactant POE was added, which maintained good biocompatibility with *Escherichia* sp. Maintaining good biocompatibility with the cells and/or intensification of the secretion of intracellular MK-4 into the extracellular broth in the presence of POE may be the factors that cause its high extracellular concentration. The low extracellular concentration obtained from the broth to which SDBS, ADBAC or betaine was added may have been caused by poor biocompatibility with *Escherichia* sp. and less efficient secretion. These results were similar to a previous study that showed that the activity of lysozyme in a nonionic surfactant (Triton-X100) solution was stable within the concentration range between 1.0 and 5.6 mg/mL, but decreased dramatically in the presence of cationic surfactant (cetyl trimethyl ammonium bromide) within the concentration range between 0.0 and 0.2 mg/mL (*25*).

The addition of all surfactants enhanced the production of extracellular MK-6 in the fermentation broth, and POE was the most effective, enhancing the production of extracellular MK-6 from (0.00±0.05) mg/L in the control to (2.56±0.07) mg/L in 108 h of cultivation. The tests demonstrated that ADBAC and SDBS tended to prolong the exponential phase and also slow the formation of MK-6 in 108 h. However, up to 120 h, the MK-6 concentration rose to a higher level than that of the control with the addition of surfactants (Table 1). POE, betaine and Tween-80 were the surfactants that could accelerate the cell growth and rapidly stimulate the accumulation of both extracellular MK-4 and MK-6 (Table 1).

Effect of concentrations of POE

Various amounts of POE were added to the media to investigate their influence on cell growth and production of MK-*N*, and to determine an appropriate amount.

Table 1. Comparison of fermentation kinetic parameters in the cultures with the addition of different surfactants

Surfactant	Biomass					Total MK-4				Total MK-6			
	μ_{max}/h^{-1}	X_{max}/(g/L)	Q_x/(g/(L·h))	$Y_{x/s}$/(g/g)	t/h	P1$_{max}$/(mg/L)	Q_{P1}/(mg/(L·h))	$Y_{P1/x}$/(mg/g)	t/h	P2$_{max}$/(mg/L)	Q_{P2}/(mg/(L·h))	$Y_{P2/x}$/(mg/g)	t/h
Control	0.15±0.01	9.3±0.2	0.19±0.01	2.19±0.02	48	22.0±0.2	0.23±0.02	0.86±0.02	96	4.7±0.1	0.04±0.01	0.09±0.01	108
ADBAC	0.03±0.01	2.8±0.2	0.05±0.01	0.63±0.01	60	7.6±0.1	0.08±0.01	0.39±0.02	96	0.54±0.07	0.01±0.01	0.03±0.01	120
Betaine	0.14±0.01	9.4±0.2	0.16±0.01	1.71±0.01	60	28.4±0.2	0.17±0.02	1.17±0.02	96	5.4±0.2	0.05±0.01	0.12±0.01	108
POE	0.18±0.01	12.6±0.2	0.21±0.01	2.42±0.02	60	47.6±0.4	0.50±0.02	2.54±0.02	96	6.0±0.1	0.06±0.01	0.13±0.01	108
SDBS	0.06±0.01	4.3±0.1	0.07±0.01	0.74±0.02	60	13.1±0.1	0.14±0.01	0.45±0.02	96	1.05±0.08	0.01±0.01	0.04±0.01	120
Tween-80	0.17±0.01	10.6±0.2	0.18±0.01	2.25±0.01	60	22.26±0.1	0.23±0.02	0.96±0.02	96	4.8±0.1	0.04±0.01	0.10±0.01	108

μ_{max}=maximum specific growth rate, X_{max}=highest cell growth, Q_x=biomass production rate, $Y_{x/s}$=biomass yield, P1=menaquinone-4 production, Q_{P1}=menaquinone-4 production rate, $Y_{P1/x}$=menaquinone-4 yield, P2=menaquinone-6 production, Q_{P2}=menaquinone-6 production rate, $Y_{P2/x}$=menaquinone-6 yield, MK=menaquinone, ADBAC=alkyl dimethyl benzyl ammonium chloride, POE=polyoxyethylene oleyl ether, SDBS=sodium dodecyl benzene sulphonate

Based on the results shown in Fig. 4, the cell growth reached a maximum value of (12.6±0.1) g/L after 48 h of cultivation when 0.5 g/L of POE was added. However, the cell growth of strain FM3-1709 was found to decrease when the concentration of POE was increased. Supplementation of POE did not seem to favour the production of intracellular MK-4 but it stimulated the production of extracellular MK-4. Indeed, the addition of POE resulted in a lower intracellular concentration of MK-4. On the other hand, the production of extracellular MK-4 was increased significantly; in particular, when the concentration of POE was 1.0 g/L, the yield reached (33.6±0.4) mg/L. However, instead of increasing, the extracellular production of MK-4 decreased with further increase in POE concentration. When the concentration of POE was 1.5 and 2.0 g/L, the yields were (32.7±0.7) and (31.6±0.8) mg/L, respectively. The reason might be that at higher concentrations of surfactant, the permeability of the cell membrane increases. However, the higher concentration of surfactant might also cause interaction with other biocompounds in the cell and then result in low cell growth and production (Fig. 4).

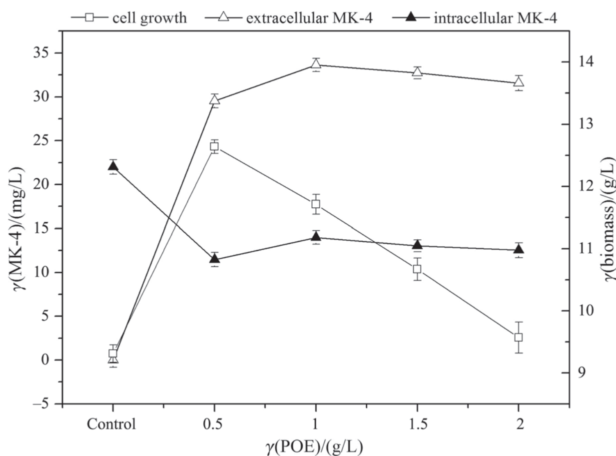

Fig. 4. Effects of different concentrations of polyoxyethylene oleyl ether (POE) on the production of menaquinone-4 (MK-4). The biomass was tested at 48 h, and MK-4 at 96 h. All experiments were carried out in triplicate and data are expressed as mean values±S.D.

The effect of the concentration of POE on the formation of MK-6 is shown in Fig. 5. When the data obtained at 96 h were compared with those at 108 h, the total amounts of MK-6 with different concentrations of POE remained almost constant and exhibited a slight increase at 108 h. The addition of POE resulted in a lower intracellular concentration of MK-6. Moreover, the production of extracellular MK-4 was slightly increased. When the concentrations of POE were 0.5, 1.0, 1.5, 2.0 g/L, the total yields of MK-6 were (4.19±0.06), (5.6±0.1), (5.49±0.09), (4.90±0.08) mg/L at 96 h and (4.5±0.1), (6.0±0.1), (5.7±0.1), (5.10±0.09) mg/L at 108 h, respectively.

From the above results, it is worth mentioning that the addition of POE can stimulate the secretion of MK-4 and MK-6, and is especially beneficial for the secretion of MK-4. This result is consistent with previous reports (*26*). This might be because the molecular mass and diameter of MK-4 are lower than those of MK-6 and secretion therefore occurs more easily through the cell membrane.

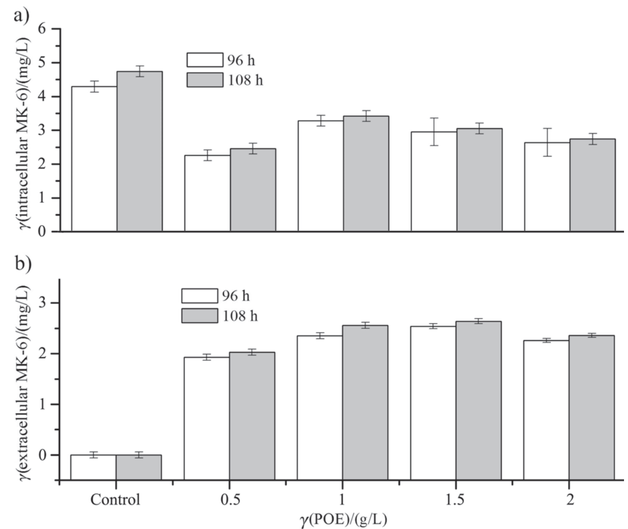

Fig. 5. Effects of different concentrations of polyoxyethylene oleyl ether (POE) on the production of: a) intracellular menaquinone-6 (MK-6) and b) extracellular MK-6 in 108 h of cultivation. All experiments were carried out in triplicate and data are expressed as mean values±S.D.

Comparison of fermentation kinetic parameters

Fermentation kinetics parameters of all shake flask cultures were calculated and are listed in Tables 1 and 2 for comparison. Surfactants added to the media obviously had a great influence on the cell growth and metabolism. According to the results in Table 1, the addition of all surfactants caused the extension of the exponential phase. Nonionic surfactants (POE, Tween-80) and the amphoteric surfactant (betaine) were beneficial for the cell growth. On the contrary, the anionic surfactant (SDBS) and cationic surfactant (ADBAC) inhibited the cell growth. Compared with the highest cell growth (X_{max}) of (9.3±0.2) g/L, biomass production rate (Q_x) of (0.19±0.01) g/(L·h) and biomass yield ($Y_{x/s}$) of (2.19±0.02) g/g in the control, the addition of POE (1.0 g/L) was able to increase these parameters to X_{max}=(12.6±0.2) g/L, Q_x=(0.21±0.01) g/(L·h) and $Y_{x/s}$=(2.42±0.02) g/g (Table 1). When the added amounts ranged from 0.5 to 1.5 g/L, interestingly, the biomass production rate (Q_x) and biomass yield ($Y_{x/s}$) remained constant at about 0.20 g/(L·h) and 2.40 g/g, respectively (Table 2).

Betaine, POE and Tween-80 showed the ability to induce MK-4 excretion (Table 1). In particular, 1.0 g/L of POE effectively increased the MK-4 production rate from (0.23±0.02) (control) to (0.50±0.02) mg/(L·h), more than 2-fold increase. Moreover, 1.0 g/L of POE was also optimal concentration for MK-4 production, as shown in Table 2, as the addition of 0.5 g/L reduced the production rate to (0.41±0.01) mg/(L·h).

Based on the results presented in Table 1, compared with the MK-6 production rate of (0.04±0.01) mg/(L·h) in the control, 1.0 g/L of POE increased the value to (0.06±0.01) mg/(L·h). On the other hand, with the addi-

Table 2. Comparison of fermentation kinetic parameters in the cultures with the addition of different concentrations of POE

γ(POE)	Biomass				Total MK-4				Total MK-6			
	X_{max}	Q_x	$Y_{x/s}$	t	$P1_{max}$	Q_{P1}	$Y_{P1/x}$	t	$P2_{max}$	Q_{P2}	$Y_{P2/x}$	t
g/L	g/L	g/(L·h)	g/g	h	mg/L	mg/(L·h)	mg/g	h	mg/L	mg/(L·h)	mg/g	h
Control	9.3±0.2	0.19±0.01	2.19±0.02	48	22.0±0.2	0.23±0.02	0.86±0.02	96	4.7±0.1	0.04±0.01	0.09±0.01	108
0.5	13.6±0.3	0.23±0.01	2.43±0.01	60	41.0±0.3	0.41±0.01	2.42±0.02	96	4.5±0.1	0.05±0.01	0.10±0.01	108
1.0	12.6±0.2	0.21±0.01	2.42±0.02	60	47.6±0.4	0.50±0.02	2.54±0.02	96	6.0±0.1	0.06±0.01	0.13±0.01	108
1.5	11.3±0.3	0.19±0.01	2.39±0.01	60	46.2±0.3	0.48±0.02	2.53±0.02	96	5.7±0.1	0.05±0.01	0.12±0.01	108
2.0	10.6±0.3	0.18±0.01	2.23±0.01	60	45.1±0.3	0.47±0.01	2.53±0.02	96	5.10±0.09	0.05±0.01	0.12±0.01	108

POE=polyoxyethylene oleyl ether, X_{max}=highest cell growth, Q_x=biomass production rate, $Y_{x/s}$=biomass yield, P1=menaquinone-4 production, Q_{P1}=menaquinone-4 production rate, $Y_{P1/x}$=menaquinone-4 yield, P2=menaquinone-6 production, Q_{P2}=menaquinone-6 production rate, $Y_{P2/x}$=menaquinone-6 yield

tion of ADBAC and SDBS, the production of MK-6 extended to 120 h, which thus led to a decrease in the production rate to (0.01±0.01) mg/(L·h). A lower cell growth rate slowed the formation of MK-6 down. Higher concentrations of 1.5 and 2.0 g/L of POE might retard cell growth and therefore slow the formation of MK-6 down. They also decreased the production rate to (0.05±0.01) mg/(L·h). The obtained results were useful in the regulation and optimization of *Escherichia* sp. culture for the efficient production of cell mass and vitamin K_2 metabolites in submerged cultures.

Conclusions

The feasibility of using surfactants to enhance the cell growth and production of intracellular and extracellular MK-4 and MK-6 was investigated in this study. It can be concluded that surfactants such as Tween-80, betaine and especially POE could be favourably used as additives to improve the cell growth of *Escherichia* sp. strain FM3-1709. The addition of all surfactants led to a lower intracellular MK-*N* but higher extracellular MK-*N* concentration, and 1.0 g/L of POE led to the highest extracellular MK-4 concentration of (33.6±0.4) mg/L and total MK-*N* concentration of (62.8±0.5) mg/L. Moreover, the addition of POE improved the secretion of MK-4 compared to MK-6.

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