Weifeng Cao¹Yujue Wang^{1,2}Jianquan Luo^{1,2}Junxiang Yin³Yinhua Wan^{1,2}

Research Article

Role of oxygen supply in α , ω -dodecanedioic acid biosynthesis from n-dodecane by *Candida viswanathii* ipe-1: Effect of stirring speed and aeration

¹State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, P. R. China

²University of the Chinese Academy of Sciences, Chinese Academy of Sciences, Beijing, P. R. China

³China National Center for Biotechnology Development, Beijing, P. R. China

α , ω -Dodecanedioic acid (DC₁₂) usually serves as a monomer of polyamides or some special nylons. During the biosynthesis, oxygenation cascaded in conversion of hydrophobic n-dodecane to DC₁₂, while the oxidation of n-dodecane took place in the intracellular space. Therefore, it was important to investigate the role of oxygen supply on the cell growth and DC₁₂ biosynthesis. It was found that stirring speed and aeration influenced the dissolved oxygen (DO) concentration which in turn affected cell growth as well as DC₁₂ biosynthesis. However, the effect of culture redox potential (Orp) level on DC₁₂ biosynthesis was more significant than that of DO level. For DC₁₂ biosynthesis, the first step was to form the emulsion droplets through the interaction of n-dodecane and the cell. When the stirring speed was enhanced, slits in the surface layer of the emulsion droplets would be increased. Thus, the substances transportation by water through the slits would be intensified, leading to an enhanced DC₁₂ production. Compared with the batch culture at a lower stirring speed (400 rpm) without culture redox potential (Orp) control, the DC₁₂ concentration was increased by 5 times up to 201.3 g/L with Orp controlled above 0 mV at a higher stirring speed (800 rpm).

Keywords: *Candida viswanathii* / α , ω -Dicarboxylic acids / α , ω -Dodecanedioic acid / n-Dodecane / Redox potential

Received: August 15, 2017; revised: October 12, 2017; accepted: November 20, 2017

DOI: 10.1002/elsc.201700142

1 Introduction

α , ω -Dicarboxylic acids (DC) are versatile chemical intermediates with different chain length, generally represented as HOOC-(R)_n-COOH. These chemical compounds are used as raw materials for the preparation of perfumes, polymers, adhesives, and macrolide antibiotics for many years and are well known polymer building block [1]. Among the DC, α , ω -dodecanedioic acid (DC₁₂) usually serves as a monomer of polyamides or some special nylons such as nylon 612 and nylon 1212 [2,3]. DC₁₂ is also of interest as potential alternate fuel substrates in parenteral nutrition [4]. Nowadays, most long chain DCs are produced by microbial biotransformation from the corresponding alkane [5,6]. The first step in the alkane degradation pathway is the terminal oxidation (α -oxidation) of alkane to α -monocarboxylic acid

(MCA) by cytochrome P450; MCA can be further oxidized to the corresponding DC by ω -oxidation. The reactions cannot be completed in one organelle, so transportation among the organelles is needed.

For an industrial production of DC, bioprocess engineering was carried out to enhance the production of DC by optimizing parameters, such as medium composition [7,8], pH [9,10], oxygen availability [11,12]. Scheller et al. [11] reported that oxygenation cascaded in conversion of n-alkanes to α , ω -dioic acids catalyzed by cytochrome P450, and Ji et al. [13] also indicated that the oxygen supply was of crucial importance for the oxygenation. In addition, since alkanes are insoluble in water and the oxidation of alkanes took place in the intracellular space, the cells must first associate with alkane droplets and then form emulsion droplets for alkane transporting into the cell [14]. Both the biosynthesized MCA and DC will be degraded to acetyl-CoA in microbodies by β -oxidation, and the acetyl-CoA can be transported into the mitochondrial TCA cycle for energy generation [5]. Therefore, it was important to investigate how to intensify the oxygen transport into the cell and the release of DC into the medium.

Correspondence: Prof. Yinhua Wan (yhwan@ipe.ac.cn), State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, P. R. China

Abbreviations: DC₁₂, α , ω -Dodecanedioic acid; DC/DCA, α , ω -Dicarboxylic acid; DO, Dissolved oxygen; MCA, α -Monocarboxylic acid

Table 1. Effect of control strategies for oxygen supply on DC₁₂ production

Operation strategy		DC ₁₂ (g/L)	Biomass (g/L)	Productivity (g·L ⁻¹ ·h ⁻¹)	Y _{p/x} (g/g)
Strategy I	800 rpm+4 L/min	201.3 ± 16.1	18.6 ± 1.5	1.76 ± 0.14	10.8 ± 0.86
Strategy II	800 rpm+1 L/min	158.7 ± 8.1	18.9 ± 1.9	1.39 ± 0.07	8.40 ± 0.43
Strategy III	400 rpm+4 L/min	39.7 ± 2.9	22.4 ± 2.0	0.35 ± 0.03	1.77 ± 0.13
Strategy IV	400 rpm-800 rpm+4 L/min	138.6 ± 7.2	19.7 ± 1.8	1.22 ± 0.06	7.04 ± 0.37
Strategy V	800 rpm-400 rpm+4 L/min	78.2 ± 4.3	21.2 ± 1.9	0.67 ± 0.04	3.69 ± 0.20
Strategy VI	400 rpm+O ₂ +4 L/min	112.7 ± 6.1	18.9 ± 1.6	0.99 ± 0.05	5.96 ± 0.32
Strategy VII	800 rpm+Na ₂ S+4 L/min	69.4 ± 4.1	14.4 ± 1.2	0.61 ± 0.04	4.82 ± 0.28
Strategy VIII	800 rpm+H ₂ O ₂ +4 L/min	177.7 ± 12.3	17.4 ± 1.7	1.56 ± 0.11	10.18 ± 0.70
Strategy IX	800 rpm+4 L/min with pH gradient	196.2 ± 11.3	17.6 ± 1.5	1.72 ± 0.10	11.15 ± 0.64

“800 rpm + 4 L/min” and “800 rpm+1 L/min” mean that the aeration rate was respectively controlled at 4 L/min and 1 L/min with the same stirring speed of 800 rpm. “400 rpm+4 L/min” means that the aeration rate was controlled at 4 L/min with a stirring speed of 400 rpm. “400 rpm-800 rpm+4 L/min” means that the stirring speed was controlled at 400 rpm in the first 42 h and then was adjusted to 800 rpm, while the aeration rate was kept at the same value of 4 L/min. “800 rpm-400 rpm+4 L/min” means that the stirring speed was controlled at 800 rpm in the first 42 h and then was adjusted to 400 rpm, while the aeration rate was kept at the same value of 4 L/min. “400 rpm+O₂” means that pure oxygen was added in the broth under the stirring speed of 400 rpm to keep a DO of around 40%. “800 rpm+Na₂S” means that the Orp of the broth was adjusted to below 0 mV by exogenous addition of Na₂S between 8 h and 42 h, and then the control was stopped. “800 rpm+H₂O₂” means that the Orp of the broth was adjusted to above 50 mV between 18 h and 26 h by exogenous addition of H₂O₂ whenever required. “800 rpm+pH gradient” means that the pH was gradually increased to 8.0 from 5.0 between 18 h and 26 h to avoid rapid dropping of Orp. The control strategies have the same meaning as those in Figs. 3 and 5.

It was well known that the stirring speed and aeration rates affected the gas-liquid oxygen transport and productivity in fermentation process [15, 16]. The effect of stirring speed on morphology, cell growth and product yield has been investigated for several fermentation processes. Stirring speed played an important role in the improvement of mass and heat transfer in the fermentor [17]. An increase in the stirring speed improved mixing, mass and heat transfer, but might also have negative effects such as disrupting of the cells, vacuolation and autolysis, which in turn decreased the overall productivity [18]. Similarly, aeration rate was also an important parameter for supply oxygen in large-scale fermentation [19, 20]. However, there is no literature focusing on the effects of stirring speed and aeration rate on the biosynthesis of DC.

Considering its high price and growing demand, DC₁₂ was chosen as the object in this study. The effect of stirring speed and aeration on DC₁₂ biosynthesis from n-dodecane by *Candida viswanathii* ipe-1 was investigated in order to reveal the role of oxygen supply in a stirred bioreactor.

2 Materials and methods

2.1 Microorganism, media, and cultivation conditions

Candida viswanathii ipe-1 (CGMCC No. 8824), cultivation conditions, and the medium for DC₁₂ production were the same as described by Cao et al. [21]. A 7.5 L bioreactor (BioFlo[®] 110, New Brunswick Scientific, USA) containing 3.0 L of the fermentation culture medium was used in this study. After the fermentation medium was sterilized, an initial 200 mL (i.e. 149.8 g) sterile n-dodecane per liter fermentation medium was added in the broth, and another 200 mL (i.e. 149.8 g) sterile n-dodecane per liter fermentation medium was added in the broth after culturing for 42 h. 200 mL seed culture per liter fermentation medium was transferred into the reactor at the initial stage of fermentation. Dissolved oxygen (DO) was detected by a DO electrode (Mettler

Toledo, Switzerland). Briefly, the procedure for DO electrode calibration involved reducing the pO₂ in the saturated sodium sulfite solution to zero and then following the increase in pO₂ over time after the resumption of aeration and stirring speed, until maximum O₂ saturation was reached in the sterilized broth before inoculation under pH 5.0, 30°C and air saturation at the gage pressure of 0.03 MPa. The maximum O₂ saturation value was calibrated as 100% of the DO probe. Then DO value detected by the DO probe in the fermentation process under 1.0 atm was the relative value compared with the calibration. To identify the dominant parameter on DC₁₂ production (DO or stirring speed), experiments were conducted by using a gas mixing station with a mass flow controller (BioFlo[®] 110, New Brunswick Scientific, USA), which allowed precise DO control (±1% saturation) independently of the stirring speed, through the addition of pulses of nitrogen or oxygen as required. The main operation strategies used in this paper were listed in Table 1. All fermentation experiments were performed at least twice to ensure the data reproducibility. The data presented in the figures are the average values.

2.2 Analytical methods

Biomass and DC₁₂ concentrations were measured as described by Cao et al. [21]. Redox potential (Orp) in the culture was measured by a platinum electrode (Mettler Toledo, Switzerland) and controlled by exogenous addition of Na₂S or H₂O₂ whenever required.

3 Results and discussion

3.1 Effect of stirring speed on DC₁₂ production

The stirring speed (i.e. shearing stress) produced by impellers has a significant effect on cell growth and product biosynthesis

Figure 1. Effect of stirring speed on DC₁₂ production by *C. viswanathii* ipe-1. The time courses refer to the effect of stirring speed on DC₁₂ (A), biomass (B) and DO (C).

in a fermentation process. To study the effect of shearing rate on cell growth and DC₁₂ production by *C. viswanathii*, batch fermentations were carried out at different stirring speeds (400, 600, 700, 800, and 1000 rpm) at a controlled pH with an aeration rate of 4.0 L/min (30°C). It is evident from Fig. 1A that there is a considerable increase in DC₁₂ production with increasing stirring speed from 400 to 800 rpm. A high stirring speed might increase the mass transfer and gas dispersion in the medium, thus leading to a higher DC₁₂ production. However, with further enhancing the stirring speed to 1000 rpm, DC₁₂ concentration kept almost the same as that at 800 rpm. The maximum DC₁₂

concentration achieved 201.3 g/L at 800 rpm. Differently, Fig. 1B shows that the final cell concentration decreased with increasing stirring speed, and the highest biomass was 22.4 g/L at 400 rpm which was decreased by 16.9% at 800 rpm. This might be due to the higher shear stress caused by the blade tips of the impeller, which disrupted the cells. Ho et al. [22] and Large et al. [23] also reported that higher shear stress could disrupt cells. In addition, the biosynthesis of DC₁₂ was related to the cells which could successfully transform from growth phase to conversion phase [24], and this was different from those growth-related product [16, 17]. Therefore, shearing effect induced by the higher stirring speed on the cells might have a negative effect on the cell growth and DC₁₂ production. In addition, with the same aeration rate of 4 L/min (Fig. 1C), DO levels in the first 36 h were close at stirring speeds of 600, 700, and 800 rpm. While their DO levels became different at 42 h, and then kept almost constant until the end of the fermentation. Meanwhile, DC₁₂ concentration in the broth obviously increased from 42 h. However, with further enhancing stirring speed to 1000 rpm, DC₁₂ concentration did not increase, though DO level went up by approximately 26% at 1000 rpm compared with that at 800 rpm (Fig. 1C). At stirring speeds from 400 to 800 rpm, it was hard to distinguish a clear correlation between DC₁₂ concentration and DO level or stirring speed in the end of the fermentation (DO level was cascaded with stirring speed). And there was a trade-off effect of stirring speed on DC₁₂ production and cell growth, because DC₁₂ production was increased by higher stirring while the cell growth showed oppositely.

3.2 Effect of aeration on DC₁₂ production

Aeration was another important factor which affected the gas-liquid oxygen transport. Though aeration was less effective than agitation for regulating oxygen supply [15], oxygen concentration could be adjusted to a certain value using gas blending at different agitation speeds [18]. Therefore, changing the oxygen supply with aeration at the same selected speed of 800 rpm was carried out (Fig. 2). The DC₁₂ concentration associated with DO level could be observed, and the DO profiles significantly varied with different aeration rates. However, when the aeration rate was larger than 4 L/min, DO level stopped increasing and kept around 50% in the conversion phase. Rywińska et al. [25] also reported that when aeration rate was higher than a critical value, it had no impact on DO and citric acid production. Oxygen limitation was probably the reason for the lower DC₁₂ production at the aeration rates lower than 4 L/min. These results indicated that aeration could influence DO significantly, which would in turn affect DC₁₂ production. However, the final cell concentration was not obviously affected by the aeration rate (Fig. 2B), which was different from the effect of DO level induced by stirring speed. Therefore, the enhancing oxygen transfer with increasing aeration rate from 3 to 4 L/min may mainly contribute into the DC₁₂ biosynthesis, since the DO level was similar at aeration rates from 3 to 4 L/min. Furthermore, it could be concluded that the detected DO levels with the DO detector in the broth could not really reflect the oxygen supply for DC₁₂ biosynthesis, since the detected DO level was the same though DC₁₂ production was

Figure 2. Effect of aeration rate on DC₁₂ production by *C. viswanathii* ipe-1. The time courses refer to the effect of aeration rates on DC₁₂ (A), biomass (B) and DO (C).

different due to oxygen transfer variation at aeration rates from 3 to 5 L/min (Fig. 2).

3.3 Effect of oxygen supply on DC₁₂ production

3.3.1 Effect of oxygen supply by varying stirring speed and aeration

During DC₁₂ biosynthesis, oxygenation cascaded in conversion of n-alkanes to α , ω -dioic acids [11, 12], and because the oxi-

Figure 3. Variation of Orp during oxygen supply with stirring speed and aeration and its effect on DC₁₂ production by *C. viswanathii* ipe-1. “800 rpm + 4 L/min” and “800 rpm + 1 L/min” mean that the aeration rate was respectively controlled at 4 L/min and 1 L/min with the same stirring speed of 800 rpm. “400 rpm + 4 L/min” means that the aeration rate was controlled at 4 L/min with the stirring speed of 400 rpm. “800 rpm-400 rpm + 4 L/min” means that the stirring speed was controlled at 800 rpm in the first 42 h and then was adjusted to 400 rpm, while the the aeration rate was kept at the same value of 4 L/min. “400 rpm-800 rpm + 4 L/min” means that the stirring speed was controlled at 400 rpm in the first 42 h and then was adjusted to 800 rpm, while the the aeration rate was kept at the same value of 4 L/min.

dation of alkanes took place in the intracellular space, the cells must first be surrounded with alkane droplets and then form emulsion droplets for alkane transporting into the cell [14]. The formed alkane droplets may be destroyed by shearing or aeration. Therefore, operation strategies with varied stirring speed and aeration were carried out to investigate their effect on oxygen supply. As shown in Fig. 3A, at a stirring speed of 800 rpm and an aeration rate of 4 L/min (Strategy I), the culture redox potential (Orp) was above 0 mV in the conversion phase, while at a stirring speed of 400 rpm and an aeration rate of 4 L/min (Strategy III), the Orp was below -50 mV in most of the time of conversion phase. As a result, the DC₁₂ concentration in Strategy I was 5.1 times of that in Strategy III (Table 1). The difference of DC₁₂ production in Strategy I and Strategy III might be mainly caused by the different Orp level in the conversion phase induced by

stirring speeds, which would be further explored at the same aeration of 4 L/min by changing stirring speed at 42 h because the DC₁₂ began to be largely biosynthesized at that point. When the stirring speed was controlled at 800 rpm in the first 42 h and then was adjusted to 400 rpm (Strategy V), the Orp was below 0 mV in most of the time of conversion phase. Meanwhile, the DC₁₂ concentration in Strategy V decreased by 61.2% compared with that in Strategy I, while it was increased by 97.0% compared with that in Strategy III. Moreover, when stirring speed was controlled at 400 rpm in the first 42 h and then was adjusted to 800 rpm (Strategy IV), the Orp was above 0 mV in most of the time of conversion phase. Meanwhile, the DC₁₂ concentration in Strategy IV was decreased by 31.1% compared with that in Strategy I, while it was increased by 2.4 times compared with that in Strategy III (Table 1). Therefore, a higher Orp level in the conversion phase could be achieved by a higher stirring speed, leading to an enhancement of DC₁₂ production. However, the DC₁₂ concentration in Strategy IV or Strategy V was still lower than that in Strategy I. Thus, besides the stirring speed, there should have other factors affecting Orp.

3.3.2 Effect of oxygen supply by varying Orp

Orp could represent the sum of all oxido-reduction processes in fermentation, especially the metabolic processes in cells [26]. Orp was determined by reversible oxide-reduction couples as well as irreversible reducing agents coupled with the action of free oxygen and hydrogen. For example, by addition of potassium ferrocyanide, Orp was maintained between 280 and 80 mV to obtain a high citric acid productivity in *Aspergillus niger* fermentation [27]. In addition, Orp also played an important role in the cellular physiology of microorganisms such as growth capacity [28] and thermal resistance [29,30]. Since DO level could not perfectly reflect the biosynthesis of DC₁₂ (Fig. 2), the variation of Orp during oxygen supply might better reflect the DC₁₂ biosynthesis by *C. viswanathii* ipe-1, which should be further studied. In this section, we are going to figure out whether the Orp level could be adjusted by DO level. First, pure oxygen was introduced into the broth at a lower stirring speed of 400 rpm (Strategy VI) to control the DO level around 40% through a gas mixing station with a mass flow controller. As a result, the Orp level was enhanced by raising DO level (Fig. 4), and DC₁₂ concentration in Strategy VI was increased by 1.84 times compared with that in Strategy III. However, the Orp level was still lower in Strategy VI than that in Strategy I although the DO level was about the same between them, and the DC₁₂ production was decreased by 44.0% in Strategy VI compared with that in Strategy I. Second, DO level was decreased with decreasing aeration rate at higher stirring speed of 800 rpm. When the aeration rate was decreased to 1 L/min (Strategy II), the DO level decreased, leading to a decrease in Orp (Fig. 3) and a 21.2% decrease in DC₁₂ concentration (Table 1) compared with Strategy I. Therefore, the Orp also could be regulated by the DO level in the broth.

In addition, compared Strategy I with Strategy IV, besides the stirring speed, the main difference between them was that the Orp level was lower between 6 and 42 h in Strategy IV. It is required to know whether such lower Orp could decrease DC₁₂ production. In order to achieve this goal, a solution of Na₂S was added into the broth to create a lower Orp level between 6 h and

Figure 4. Regulation of Orp during the production of DC₁₂ by *C. viswanathii* ipe-1 with the same aeration rate of 4 L/min. “800 rpm+H₂O₂” means that the Orp of the broth was adjusted to above 50 mV between 18 h and 26 h by exogenous addition of H₂O₂ whenever required. “800 rpm+Na₂S” means that the Orp of the broth was adjusted to below 0 mV by exogenous addition of Na₂S between 8 h and 42 h, and then the control was stopped. “800 rpm+pH gradient” means that the pH was gradually increased to 8.0 from 5.0 between 18 h and 26 h to avoid hastily dropping of Orp. “400 rpm+O₂” means that pure oxygen was added in the broth under the stirring speed of 400 rpm to keep a DO of around 40%.

42 h (Strategy VII) (Fig. 4). As a result, the DC₁₂ concentration in Strategy VII was 65.5% lower than that in Strategy I (Table 1). Therefore, the decreasing DC₁₂ production in Strategy III was caused by the lower Orp level. Moreover, there was another interesting phenomenon that the Orp decreased rapidly when the pH rose to 8.0 from 5.0 (adjusting the fermentation process into the conversion phase from cell growth phase in Strategy I). Meanwhile, the Orp only jumped between 18 and 26 h, and then kept around 30 mV. It needed further to verify whether such rapid Orp decrease had a negative effect on DC₁₂ production by changing culture pH to 8.0 (Fig. 4) (Strategy IX). It could be seen that there was no difference of DC₁₂ concentration (< 5%) between Strategy I and Strategy IX (Table 1). Therefore, the rapid decrease in Orp caused by the increasing pH had no significant effect on DC₁₂ production. Previously, Jiao et al. [12] reported that the H₂O₂ not only could enhance the oxygen supply but also change the metabolism by inducing cytochrome P450, the

Figure 5. Schematic mechanism of oxygen supply with stirring speed and aeration in the DC_{12} production by *C. viswanathii* ipe-1. The flow charts refer to the effect of aeration rates and stirring speed (A), and Orp (B).

key enzyme of α, ω -oxidation. Thus, the H_2O_2 was used here for enhancing the oxygen supply and keeping Orp constant between 18 and 26 h (Strategy VIII) during the pH increase period (Fig. 4). At the end of the fermentation, the broth volume was 11.2% larger than that in Strategy I. As shown in Table 1, there was no difference of DC_{12} concentration ($< 5\%$) between Strategy I and Strategy VIII (after correcting the volume). Therefore, further enhancing Orp between 18 h and 26 h could not improve DC_{12} production, while lower Orp between 6 and 42 h could decrease DC_{12} production although the stirring speed and DO level were about the same (Fig. 4).

Above results confirmed that Orp level was important for DC_{12} biosynthesis, and Orp level could be partly regulated by the DO level or stirring speed. However, As shown in Fig. 2, different oxygen transfers could be achieved at the same detected DO level in the broth at 800 rpm by varying the aeration rate from 3 to 5 L/min. Although the DO level was regulated as the same in Strategy VI and Strategy I, the higher DC_{12} production or Orp was obtained at higher stirring speed. Therefore, under a constant aeration rate, it could speculate that slits in the surface layer of the emulsion droplets would be increased at higher stirring speed, and an increasing oxygen transfer caused by a higher stirring speed would improve the oxygenation. As a result, DC_{12} production was increased. When oxygen transfer was enhanced, the oxygenation rose, and the DC_{12} production was accordingly increased. However, oxygen was offered by aeration, and stirring speed could not produce oxygen. Therefore, oxido-reduction processes were highly affected by stirring speed under a proper aeration rate, and DC_{12} concentration could be increased by keeping Orp above 0 mV in the conversion phase.

3.4 Mechanism discussion

For further understanding the role of oxygen supply in DC_{12} biosynthesis from n-dodecane by *Candida viswanathii* ipe-1, the effect mechanism of oxygen supply on DC_{12} production

would be expounded in this section (Fig. 5). DC_{12} was biosynthesized by microbial biotransformation from the corresponding n-dodecane, and n-dodecane is mostly very hydrophobic substances. Previously, it was reported that for the uptake of alkanes in the yeast cells, a passive, diffusion-like process was proposed, and the conversion rate of alkanes greatly depended on the emulsification of the substrate in the medium [1, 31]. Therefore, for DC_{12} biosynthesis, the first step was to form the emulsion droplets through the interaction of n-dodecane and the cells. However, the substance transportation in the DC_{12} biosynthesis was achieved through water. Because the hydrophobic n-dodecane was around the cells, the transportation of DO or other nutrient substances through water into the cells became difficult, meanwhile there was also a trouble for the transportation of intracellular DC_{12} to the outside of the cells. Since oxygen availability was important for DC_{12} production [11, 12], the formed emulsion droplets would also be detrimental to DC_{12} biosynthesis. Moreover, it was pointed out that both the biosynthesized MCA and DC can be degraded to acetyl-CoA in microbodies by β -oxidation, and the acetyl-CoA can be transported into the mitochondrial TCA cycle to generate energy [5]. As a result, the cells would grow faster under lower stirring (400 rpm). When higher stirring speed was introduced into the fermentation process, slits in the surface layer of the emulsion droplets would be formed by the higher shear stress caused by the blade tips of the impeller, which would enhance the contact chance between cells and water through the slits. Thus, the substances transportation by water through the slits would be intensified. However, if the stirring speed was too high, the emulsion droplets would be destroyed, or the cell would be killed. That was why the cell concentration was higher while DC_{12} concentration was lower at 400 rpm, and cell concentration decreased with the stirring speed, while higher DC_{12} production could be obtained at a stirring speed higher than 800 rpm.

When alkanes were used as sole carbon and energy sources for microbial growth, oxygen serves as electron acceptor under aerobic conditions [13]. If oxygen is available, the degradation of

alkanes is initiated by cytochrome P450 monooxygenases in most cases especially in eukaryotic microorganisms. These enzymes are used for the oxidation of the n-alkanes to alcohols [11]. For DC biosynthesis, diterminal degradation primary alcohols from the monoterminial pathway are hydroxylated at their ω -position resulting in the formation of diols. These are subsequently oxidized to ω -hydroxy fatty acids and then to dicarboxylic acids which are degraded by β -oxidation (Fig. 5B) [14, 32]. The second step (Fig. 5B) is performed either by membrane-bound or soluble NAD⁺-or NADP⁺-dependent fatty-alcohol dehydrogenases (FADH, ADH genes) or by hydrogen peroxide-producing fatty-alcohol oxidases (FAOD; FAO genes), which convert the terminal hydroxy groups of 1-alkanols, 1, ω -diols, or ω -hydroxy fatty acids into corresponding fatty aldehydes [11, 14]. During the oxidation process of alkanes, the Orp level would be varied. Moreover, Jiao et al. [12] reported that the activities of P450 could be induced at an appropriate level of H₂O₂. Therefore, it was necessary to control the Orp level at proper values for DC biosynthesis. Moreover, in order to avoid degradation of DC through β -oxidation, it was also important to intensify the exchange of the intracellular and extracellular substances through the slits created by shear force.

4 Concluding remarks

DC₁₂ biosynthesis could be largely affected by oxygen supply, while the effect of Orp level on DC₁₂ biosynthesis was more significant than that of DO level. During DC₁₂ biosynthesis, the emulsion droplets first formed through the interaction of n-dodecane and the cells, impeding the transportation of DO and other nutrient substances into the cells with water. When the stirring speed was enhanced with a proper aeration, slits formed in the surface layer of the emulsion droplets, and thus DC₁₂ production was enhanced by the better exchange of the intracellular and extracellular substances through these slits.

Practical application

α , ω -Dodecanedioic acid (DC₁₂) usually serves as a monomer of polyamides or some special nylons. During the biosynthesis, oxygenation cascaded in conversion of n-dodecane to DC₁₂, while n-dodecane is insoluble in water and the oxidation of alkanes took place in the intracellular space. In this study, the effect of oxygen supply on the cell growth and DC₁₂ biosynthesis from n-dodecane by *Candida viswanathii* ipe-1 was investigated in a bioreactor. Compared with the batch culture at a lower stirring speed (400 rpm) without culture redox potential (Orp) control, the DC₁₂ concentration was increased by 5 times with Orp controlled above 0 mV at a higher stirring speed (800 rpm). Therefore, the control strategies of DC₁₂ production in this study would not only provide a good reference for process development and optimization for DC₁₂ production but also provide a good reference for process development and optimization of other petroliferous fermentation systems.

The authors thank the National High Technology Research and Development Program of China (No.2015AA021002 and No.2014AA021005), and the National Natural Science Foundation of China (No.21406240) for the financial supports.

The authors have declared no conflict of interest.

5 References

- [1] Huf, S., Krügener, S., Hirth, T., Rupp, S. et al., Biotechnological synthesis of long-chain dicarboxylic acids as building blocks for polymers. *Eur. J. Lipid Sci. Tech.* 2011, 113, 548–561.
- [2] Zheng, W., Mcauley, K. B., Marchildon, E. K., Yao, K. Z., Effects of end-group balance on melt-phase nylon 612 polycondensation: experimental study and mathematical model. *Ind. Eng. Chem. Res.* 2004, 44, 2675–2686.
- [3] Yu, J., Yuan, X., Zeng, A., A novel purification process for dodecanedioic acid by molecular distillation. *Chinese J. Chem. Eng.* 2015, 23, 499–504.
- [4] Salinari, S., Bertuzzi, A., Gandolfi, A., Greco, A. V. et al., Dodecanedioic acid overcomes metabolic inflexibility in type 2 diabetic subjects. *Am. J. Physiol. Endocrinol. Metab.* 2006, 291, E1051–E1058.
- [5] Jiao, P., Ma, S., Hua, Y., Huang, Y. et al., Isolation and enzyme determination of *Candida tropicalis* mutants for DCA production. *J. Gen. Appl. Microbiol.* 2000, 46, 245–249.
- [6] Schaffer, S., Haas, T., Biocatalytic and fermentative production of α , ω -bifunctional polymer precursors. *Org. Process Res. Dev.* 2014, 18, 752–766.
- [7] Picataggio, S., Deanda, K., Mielenz, J., Determination of *Candida tropicalis* acyl coenzyme A oxidase isozyme function by sequential gene disruption. *Mol. Cell Biol.* 1991, 11, 4333–4339.
- [8] Yi, Z. H., Rehm, H. J., Formation of α , ω -dodecanedioic acid and α , ω -tridecanedioic acid from different substrates by immobilized cells of a mutant of *Candida tropicalis*. *European J. Appl. Microbiol. Biotechnol.* 1982, 16, 1–4.
- [9] Liu, S., Li, C., Fang, X., Cao, Z. A., Optimal pH control strategy for high-level production of long-chain α , ω -dicarboxylic acid by *Candida tropicalis*. *Enzyme Microb. Tech.* 2004, 34, 73–77.
- [10] Liu, S., Li, C., Xie, L., Cao, Z. A., Intracellular pH and metabolic activity of long-chain dicarboxylic acid-producing yeast *Candida tropicalis*. *J. Biosci. Bioeng.* 2003, 96, 349–353.
- [11] Scheller, U., Zimmer, T., Becher, D., Schauer, F. et al., Oxygenation cascade in conversion of n-alkanes to α , ω -dioic acids catalyzed by cytochrome P450 52A3. *J. Biol. Chem.* 1998, 273, 32528–32534.
- [12] Jiao, P., Huang, Y., Li, S., Hua, Y. et al., Effects and mechanisms of H₂O₂ on production of dicarboxylic acid. *Biotechnol. Bioeng.* 2001, 75, 456–462.
- [13] Ji, Y., Mao, G., Wang, Y., Bartlam, M., Structural insights into diversity and n-alkane biodegradation mechanisms of alkane hydroxylases. *Front Microbiol.* 2013, 4, 1–13.
- [14] Fickers, P., Benetti, P. H., Wache, Y., Marty, A. et al., Hydrophobic substrate utilisation by the yeast *Yarrowia lipolytica*, and its potential applications. *FEMS Yeast Res.* 2005, 5, 527–543.
- [15] Fenice, M., Barghini, P., Selbmann, L., Federici, F., Combined effects of agitation and aeration on the chitinolytic enzymes

- production by the Antarctic fungus *Lecanicillium muscarium* CCFEE 5003. *Microb. Cell. Fact.* 2012, 11, 12.
- [16] Tinoco-Valencia, R., Gómez-Cruz, C., Galindo, E., Serrano-Carreón, L., Toward an understanding of the effects of agitation and aeration on growth and laccases production by *Pleurotus ostreatus*. *J. Biotechnol.* 2014, 177, 67–73.
- [17] Silva, D. P., Pessoa, A., Jr., Roberto, I. C., Vitolo, M., Effect of agitation and aeration on production of hexokinase by *Saccharomyces cerevisiae*. *Appl. Biochem. Biotechnol.* 2001, 91–93, 605–613.
- [18] Amanullah, A., Tuttiett, B., Nienow, A., Agitator speed and dissolved oxygen effects in xanthan fermentations. *Biotechnol. Bioeng.* 1998, 57, 198–210.
- [19] Dick, O., Onken, U., Sattler, I., Zeeck, A., Influence of increased dissolved oxygen concentration on productivity and selectivity in cultures of a colabomycin-producing strain of *Streptomyces griseoflavus*. *Appl. Microbiol. Biot.* 1994, 41, 373–377.
- [20] Kempf, M., Theobald, U., Fiedler, H. P., Influence of dissolved O₂ on the fermentative production of gallidermin by *Staphylococcus gallinarum*. *Biotechnol. Lett.* 1997, 19, 1063–1065.
- [21] Cao, W., Liu, B., Luo, J., Yin, J. et al., α , ω -Dodecanedioic acid production by *Candida viswanathii* ipe-1 with co-utilization of wheat straw hydrolysates and n-dodecane. *Bioresour. Technol.* 2017, 243, 179–187.
- [22] Ho, C. H., Henderson, K. A., Rorrer, G. L., Cell damage and oxygen mass transfer during cultivation of *Nicotiana tabacum* in a stirred-tank bioreactor. *Biotechnol. Progr.* 1995, 11, 140–145.
- [23] Large, K. P., Ison, A. P., Williams, D. J., The effect of agitation rate on lipid utilisation and clavulanic acid production in *Streptomyces clavuligerus*. *J. Biotechnol.* 1998, 63, 111–119.
- [24] Cao, W., Li, H., Luo, J., Yin, J. et al., High-level productivity of α , ω -dodecanedioic acid with a newly isolated *Candida viswanathii* strain. *J. Ind. Microbiol. Biot.* 2017, 44, 1191–1202.
- [25] Rywińska, A., Musiał, I., Rymowicz, W., Zarowska, B. et al., Effect of agitation and aeration on the citric acid production by *Yarrowia lipolytica* grown on glycerol. *Prep. Biochem. Biotech.* 2012, 42, 279–291.
- [26] Unden, G., Trageser, M., Duchene, A., Effect of positive redox potentials (>+ 400mV) on the expression of anaerobic respiratory enzymes in *Escherichia coli*. *Mol. Microbiol.* 1990, 4, 315–319.
- [27] Berovi, M., Scale-up of citric acid fermentation by redox potential control. *Biotechnol. Bioeng.* 1999, 64, 552–557.
- [28] Zhuge, X., Li, J., Shin, H.-d., Liu, L. et al., Improved propionic acid production with metabolically engineered *Propionibacterium jensenii* by an oxidoreduction potential-shift control strategy. *Bioresour. Technol.* 2015, 175, 606–612.
- [29] Riondet, C., Cachon, R., Wache, Y., Alcaraz, G. et al., Extracellular oxidoreduction potential modifies carbon and electron flow in *Escherichia coli*. *J. Bacteriol.* 2000, 182, 620–626.
- [30] George, S., Richardso, L., Pol, I., Peck, M., Effect of oxygen concentration and redox potential on recovery of sublethally heat-damaged cells of *Escherichia coli* O157: H7, *Salmonella enteritidis* and *Listeria monocytogenes*. *J. Appl. Microbiol.* 1998, 84, 903–909.
- [31] Käppeli, O., Müller, M., Fiechter, A., Chemical and structural alterations at the cell surface of *Candida tropicalis*, induced by hydrocarbon substrate. *J. Bacteriol.* 1978, 133, 952–958.
- [32] Coon, M. J., Omega Oxygenases: Nonheme-iron enzymes and P450 cytochromes. *Biochem. Biophys. Res. Commun.* 2005, 338, 378–385.