Research Article

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Simultaneously enhanced production of poly(β-malic acid) and pullulan using a dissolved oxygen shift (DO-shift) control strategy

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Abstract

BACKGROUND: Poly(β-malic acid) (PMA) and pullulan are two valuable biopolymers that can be produced simultaneously by certain strains of *pullulans*. In previous studies, PMA production was successfully improved with reducing pullulan production. In the present study, a DO-shift control strategy was developed to obtain simultaneous high yields of PMA and pullulan.

RESULTS: PMA and pullulan co-production at various constant DO levels ranging from 10% to 90% saturation were carried out in a 5 L stirred tank fermentor. Based on the analysis of batch fermentation kinetics at various constant DO levels, a DO-shift control strategy that shifted the DO level from 30% saturation to 70% saturation at 72 h was developed to effectively obtain high productions of both PMA and pullulan.

CONCLUSION: By applying the DO-shift control strategy, the maximum concentrations of PMA and pullulan reached 118.6 g L^{-1} and 27.2 g L^{-1}, respectively. PMA production was successfully improved without sacrificing pullulan production.

Keywords: poly(malic acid); pullulan; dissolved oxygen; co-production

INTRODUCTION

Biopolymers are the most abundant molecules in living matter. Microorganisms are capable of producing a wide variety of biopolymers, including polynucleotides, polyamides (protein), polyesters, polysaccharides, etc. Poly(β-malic acid) (PMA) is a water-soluble polyester consisting of L-malic acid monomer. Owing to its biocompatibility, degradability, and water solubility, PMA has been studied primarily for biomedical applications, which can be applied to make various compression molded pellets, films and microparticles for drug delivery.[1–3] Moreover, PMA can be easily hydrolyzed to L-malic acid, which is widely used as an acidulant and taste modifier in food industries.[4] L-malic acid is also considered one of the C4 platform chemicals in biorefinery engineering.[5] Therefore, development of efficient biological processes for PMA production is required for practical applications of PMA.

Pullulan is another kind of valuable biopolymer; this polysaccharide is made mainly of maltotriose repeating units interconnected by α-1,6 linkages.[6] Pullulan is of economic importance with increased applications in food, pharmaceutical, agricultural and chemical industries.[7–9] Pullulan and PMA can be produced simultaneously due to their tightly interrelated metabolic pathway. *Aureobasidium pullulans* is the most commonly used species for the co-production of PMA and pullulan.[10] Leathers *et al.* recently completed a multilocus molecular phylogeny of *A. pullulans*, they found that certain phylogenetically defined clades of *A. pullulans* could produce PMA and pullulan simultaneously.[11] Zhang *et al.* investigated the effect of $CaCO_3$ on PMA and pullulan co-production by *A. pullulans* ZD-3d, the addition of $CaCO_3$ strongly stimulated PMA production, while the biosynthesis of pullulan was simultaneously depressed.[12] In the work reported by Cao *et al.* the DO was maintained above 70% saturation during the fermentation process, which was aimed at obtaining an abundant PMA production and depressing pullulan production.[13] The above studies focused on the enhancement of only one of the products, PMA production was successfully improved by sacrificing pullulan production. From the biorefinery perspective, since both PMA and pullulan are valuable, it would be interesting and economical to obtain high production of both PMA and pullulan in a unified fermentation process.

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Oxygen is an indispensable substrate during PMA and pullulan fermentation, researchers have focused on optimizing dissolved oxygen concentration (DO) for the sake of a high yield of pullulan or PMA. Rho *et al.* observed that the rate of pullulan synthesis varied inversely with DO in a controlled oxygen environment.[14] Wecker *et al.* found that pullulan production was enhanced under the combined conditions of low DO and low shear rate.[15] Gibbs *et al.* performed pullulan production at several agitation rates and DO levels, they found that pullulan production can be improved by maintaining DO at a low level during the initial phase of fermentation.[16] On the other hand, Zhang reported that the DO level for PMA production was to be >10% during the whole fermentation process, while the reason for this DO control strategy was not presented.[12] Cao *et al.* examined the effects of DO and agitation rate on PMA production, and they proposed a DO and agitation rate combined control strategy to enhance PMA production.[13] Therefore, it appears feasible to regulate the co-production of PMA and pullulan by DO control.

In the present study, detailed research on the effects of DO and agitation rate on the co-production of PMA and pullulan with *A. pullulans* HA-4D was carried out. Based on the analysis of kinetic parameters during the fermentation process, a novel DO-shift control strategy was designed to obtain simultaneous high yields of PMA and pullulan. Attempts were also made to determine whether PMA and pullulan co-production was mainly affected by DO or by agitation rate. The strategy proposed here is the first attempt to improve co-production of PMA and pullulan based on DO control.

MATERIALS AND METHODS

Microorganism and media

Aureobasidium pullulans HA-4D (CGMCC No.7.208) used in this study was deposited in the China General Microbiological Culture Collection Center, Beijing, China. This strain was maintained on potato dextrose agar slants at 4 °C The composition of seed medium was (g L^{-1}): glucose 80, Yeast extract 1, $NaNO_3$ 2, KH_2PO_4 0.1, $MgSO_4$ 0.1, KCl 0.5 and $CaCO_3$ 20. The production medium consisted of (g L^{-1}): glucose 100, Yeast extract 1, $NaNO_3$ 2, KH_2PO_4 0.1, $MgSO_4$ 0.1, KCl 0.5, $ZnSO_4$ 0.1 and $CaCO_3$ 30.

Culture conditions

The seed culture of strain HA-4D was prepared by inoculating cells maintained on potato dextrose agar into a 500 mL flask containing 100 mL of the seed medium, the seed culture was aerobically incubated at 25 °C and 200 rpm for 48 h. For batch fermentation, 300 mL of the seed culture was inoculated into 2.7 L of the fermentation medium in a 5 L stirred tank fermentor (FS-02 series, Winpact, USA). Fermentation was performed at 25 °C under aeration at 1 vvm. The pH of the culture broth was maintained at approximately 6.5 with the addition of $CaCO_3$. To investigate the effect of DO on the co-production of PMA and pullulan, the agitation rate was automatically adjusted based on DO value (DO was cascaded to agitation rate through PID control), which was detected by an on-line DO probe (P52200967, S3490197, Mettler Toledo, Switzerland). To determine whether the co-production of PMA and pullulan was mainly affected by DO or agitation rate, experiments were carried out using a gas mixing system (FS-02 series, Winpact, USA), which allows precise DO control independent of the agitation rate, through the addition of oxygen or nitrogen as required. For fed-batch fermentation, the concentrated glucose solution (500 g L^{-1}) was continuously fed after 72 h to maintain the residual sugar at approximately 10 g L^{-1}. Approximately 0.7 L of the feeding solution was added to the fermentor, and the final volume of culture broth was approximately 3.2 L because of the losses from sampling and water evaporation.

Analytical methods

The DO value of the culture broth were measured by inductors of the bioreactor. Dissolved oxygen (DO) concentrations under different conditions were expressed in terms of DO saturation level (%), in which 100% corresponds to the actual DO concentration when the maximum agitation rate and aeration were both applied (supplied at 25 °C and 1 atm). The culture broth (5 mL) was centrifuged at 8000 × *g* for 10 min, and the resulting supernatant was used to determine the concentrations of PMA, pullulan and residual sugar. PMA and pullulan were purified by repeated ethanol precipitation.[11,12] The first addition of 0.5 volume of cold ethanol was to selectively remove pullulan as precipitates. The precipitates were centrifuged at 8000 × *g* for 10 min and dried at 80 °C to constant weight.[17] After removal of the pullulan, the supernatant was added to two volumes of ethanol, the mixture was kept at 4 °C overnight for precipitation of PMA. Precipitated PMA dissolved in deionized water and the concentration of PMA was estimated from the concentration of malic acid after hydrolysis. The dissolved PMA solution was mixed with an equal volume of 2 mol L^{-1} H_2SO_4 and incubated at 90 °C for 9 h. After neutralization of the solution with 2 mol L^{-1} NaOH solution, the concentration of malic acid was determined by an HPLC apparatus (Agilent 1260 series, USA) equipped with a Spursil C18-EP column (5 µm, 300 × 7.8 mm; Dikma, China) and detected with a UV detector at 210 nm. The mobile phase was 25 mmol L^{-1} KH_2PO_4 (adjusted to pH 2.5 by phosphoric acid)/methanol (9:1, v/v) with a flow rate of 0.5 mL min^{-1} at 30 °C. PMA concentration was then estimated with the following formula: [PMA] (g L^{-1}) = 0.87 × [malic acid] (g L^{-1}).[18] For the measurement of dry cell weight (DCW), excess $CaCO_3$ in the culture broth was eliminated by adding 1 mol L^{-1} HCl before measurement. The broth was then centrifuged and the resulting precipitate was collected, washed three times with distilled water and dried to constant weight.

Statistical analysis

The data from the batch and fed-batch fermentations were the average of three independent samples, and the error bars indicated the standard deviation (SD) from the mean of the triplicates.

RESULTS AND DISCUSSION

Co-production of PMA and pullulan without DO control

To obtain the mechanism of co-production of PMA and pullulan, batch and fed-batch fermentations with *A. pullulans* HA-4D were carried out in a 5 L stirred tank fermentor without DO control. As shown in Fig. 1(a) and (b), the agitation rate was constant 400 rpm, DO fell rapidly during the first 12 h, and increased subsequently at 18 h. At the end of the batch fermentation, 32.6 ± 1.3 g L^{-1} PMA and 18.2 ± 0.7 g L^{-1} pullulan were obtained, while the final concentration of PMA and pullulan in fed-batch fermentation reached 88.5 ± 2.1 g L^{-1} and 18.5 ± 1.2 g L^{-1}, respectively. It is noted that PMA production was obviously enhanced while there was no significant increase of pullulan concentration after feeding with glucose. The specific formation rates of

PMA and pullulan during the fed-batch process were also calculated. As shown in Fig. 1(c), the specific formation rate of pullulan (q_p-pullulan) achieved its highest level at about 20 h with 0.04 h^{-1}, then q_p-pullulan decreased to 0 at 96 h, and thereafter, the concentration of pullulan remained nearly unchanged. By contrast, the specific formation rate of PMA (q_p-PMA) reached its highest level of about 0.06 h^{-1} at 20 h, then q_p-PMA slowly decreased to 0.014 h^{-1} at 168 h, and the concentration of PMA continuously increased during the whole fermentation process. The above results showed that fed-batch culture was better than batch culture in term of obtaining high productions of total products, and the biosynthesis of PMA and pullulan by *A. pullulans* HA-4D were not synchronous, so that the co-production of PMA and pullulan in a fed-batch fermentation process should be separately regulated: cultivation conditions suitable for pullulan production were preferred in the early stage, and cultivation conditions favorable for PMA biosynthesis were then required in the later stage.

Effect of DO on PMA and pullulan co-production

Owing to the presence of $CaCO_3$ in the fermentation medium, the culture pH was maintained around 6.5 during the fermentation process, thus a pH-shift control strategy was not available for the co-production of PMA and pullulan. It is well known that the microbial fermentation of biopolymer, such as welan gum, poly(lysine) and (glutamic acid), is sensitive to the DO concentration in the culture broth.[19–21] Thus the effects of DO on cell growth and co-production of PMA and pullulan were examined. Batch fermentations of the strain HA-4D were performed at several DO levels ranging from 10% to 90% saturation. The initial agitation rate is 400 rpm, then DO was cascaded to the agitation rates through PID control (Fig. 2(e)). As shown in Fig. 2(b), when DO was maintained at 10% saturation, only 20.4 ± 1.1 g L^{-1} biomass was obtained, whereas DCW increased to 29.1 ± 1.5 g L^{-1} when DO was controlled at 70% saturation. PMA production was positively correlated with DO concentration within a certain range. When the DO level was maintained at 10% saturation, only 22.3 ± 0.9 g L^{-1} PMA was accumulated after 78 h of batch fermentation. However, PMA concentration was as high as 36.7 ± 1.3 g L^{-1} when the DO concentration was controlled at 70% saturation (Fig. 2(c)). By contrast, the optimal DO level for pullulan production was 30%, where pullulan concentration reached its highest value (26.8 ± 1.3 g L^{-1}, Fig. 2(d)). With the aforementioned information, it can be concluded that cell growth, PMA production and pullulan production were all sensitive to DO concentration in the culture broth. High DO level was favorable for cell growth and PMA production, whereas low DO level was more suitable for pullulan biosynthesis.

To analyze the kinetic characteristics of the effects of DO on cell growth, product formation and substrate consumption, specific cell growth rate (μ), q_p-PMA, q_p-pullulan and specific substrate consumption rate (q_s) were calculated based on the data in Fig. 2. As shown in Fig. 3, the maximum values of the above kinetic parameters were all obtained at 6–12 h, although these parameters varied greatly with DO levels. The increased DO concentration was favorable for cell growth and the maximum μ was obtained at 70% DO (Fig. 3(a)). As for PMA production, the maximum q_p-PMA of 0.08 h^{-1} was observed at 70% DO, and the highest PMA concentration (36.7 ± 1.3 g L^{-1}) was also obtained at this DO level (Fig. 3(b)). By contrast, q_p-pullulan obtained at low DO concentrations were much higher than those obtained at high DO levels during the batch fermentation process, the highest value of q_p-pullulan was obtained at 30% DO before 32 h, while the value of q_p-pullulan at 10% DO was higher than other DO levels after 32 h (Fig. 3(c)), the maximum pullulan concentration (26.8 ± 1.3 g L^{-1}) was observed when the DO concentration was controlled at 30% saturation. With the aforementioned results, it can be concluded that the optimal DO level for pullulan production was 30% saturation, while 70% DO was optimal for PMA production.

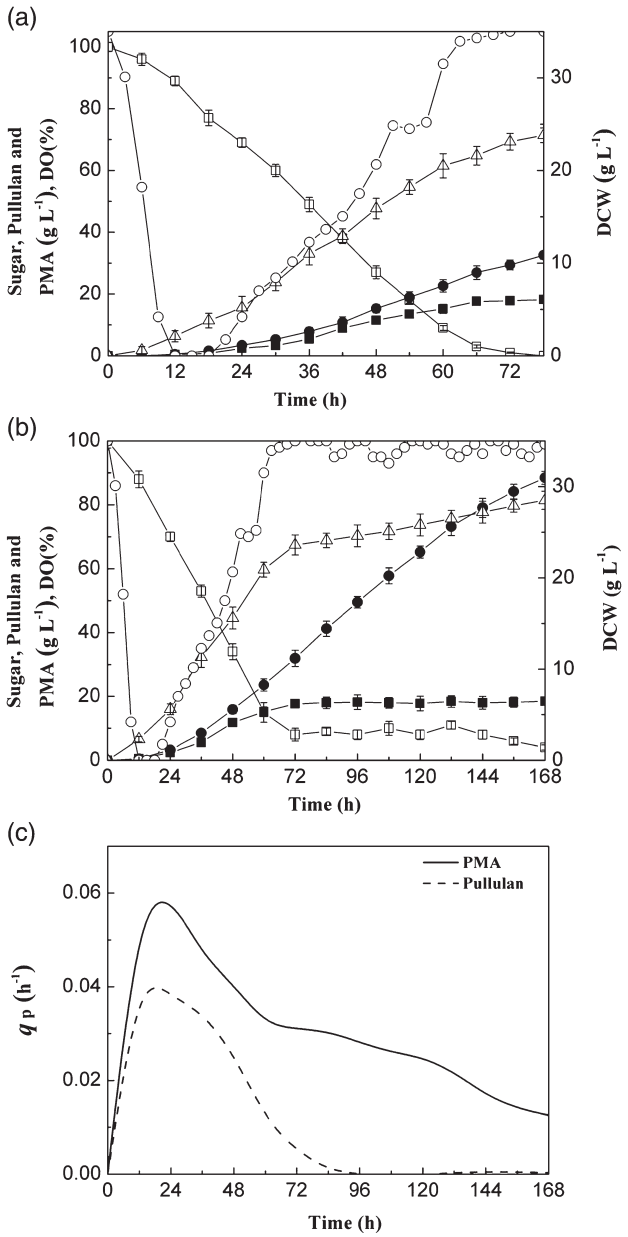

Figure 1. The co-production of PMA and pullulan in a 5 L fermentor. Cultivations were conducted at 25 °C, aeration rate of 1 vvm and agitation rate of 400 rpm, while DO was not controlled in these two cases. (a) batch fermentation; (b) fed-batch fermentation; (c) specific formation rates of PMA and pullulan in fed-batch fermentation. Symbols: PMA(●); Pullulan(■); DCW(△); DCW(□); DO(○).

Effects of agitation rate and DO on PMA and pullulan co-production

In the above experiments, DO was cascaded to the agitation rates through PID control, high agitation rates had to be maintained when high DO levels were required, thus it was hard to determine whether the co-production of PMA and pullulan was

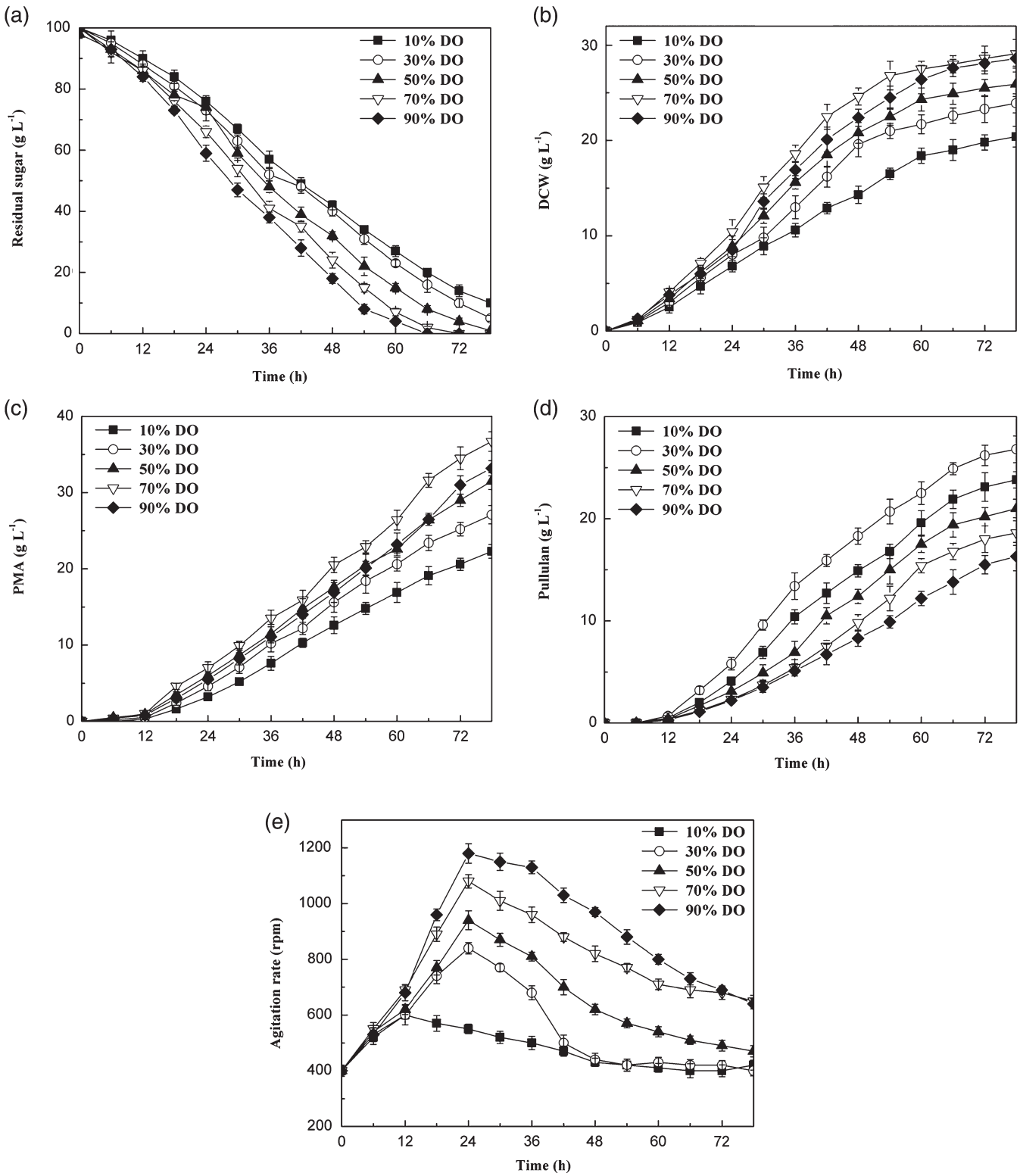

Figure 2. Effects of DO on PMA and pullulan co-production by *A. pullulans* HA-4D. (a) glucose; (b) cell growth; (c) PMA production; (d) pullulan production; and (e) agitation rate. When DO was dropped to the set values, DO was maintained constant at this value by automatically controlling agitation rate.

mainly affected by DO or by agitation rate. Previous studies on the individual production of PMA or pullulan have shown that pullulan production was depressed with increasing agitation rates, the increased DO levels instead of increased shear forces were responsible for this effect.[16] On the other hand, PMA production was mainly affected by DO.[13] To verify whether the above conclusions are valid for the co-production of PMA and pullulan by *A. pullulans* HA-4D, several fermentations were carried out using a gas mixing system at different constant agitation rates, which could investigate the effects of DO levels independent of agitation rates on

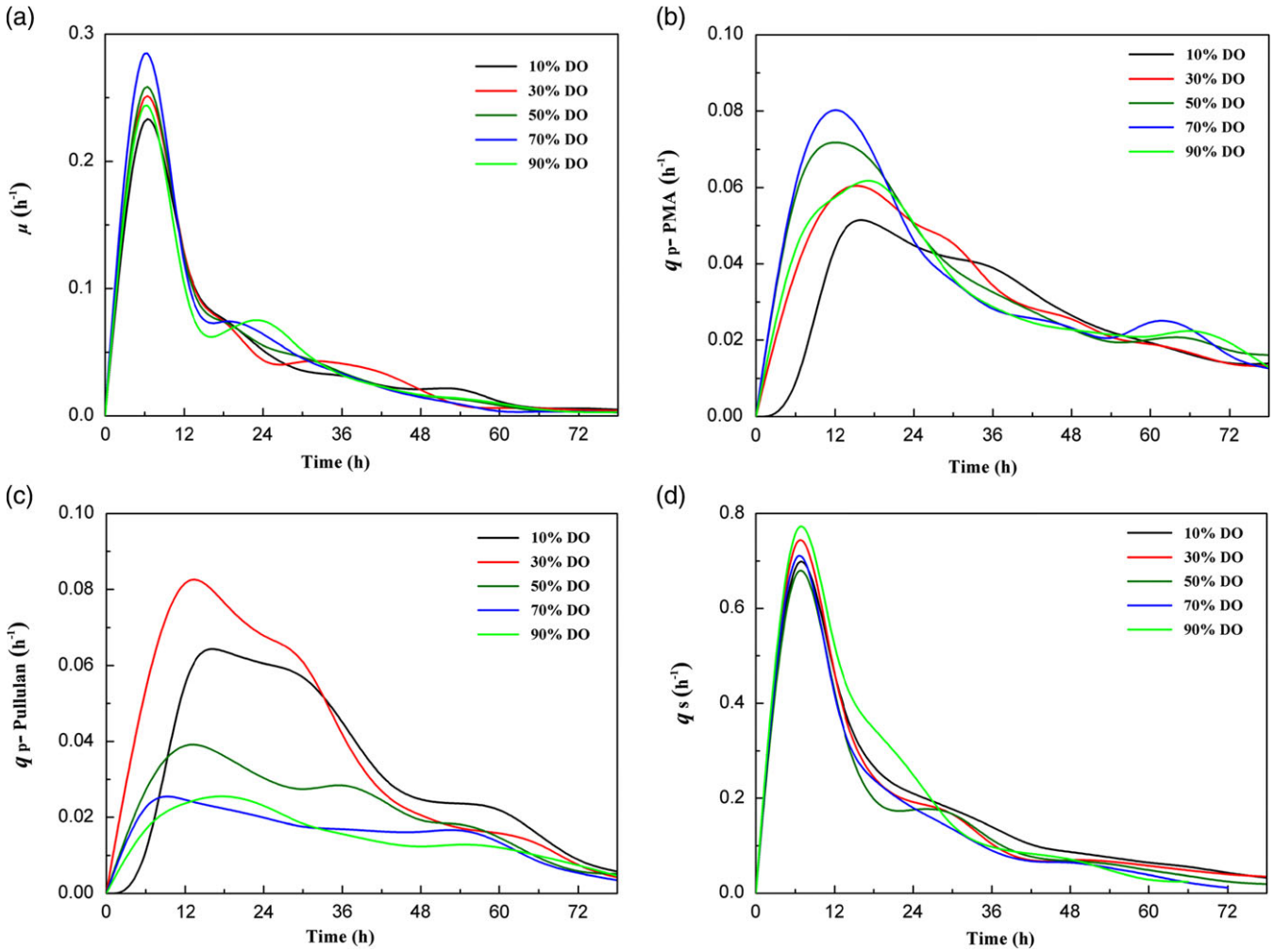

Figure 3. Time courses of kinetic parameters during batch fermentation in a 5 L fermentor at different constant DO levels. The kinetic parameters include (a) specific growth rate, (b) specific PMA formation rate, (c) specific pullulan formation rate, and (d) specific glucose consumption rate.

the co-production of PMA and pullulan. As shown in Fig. 4(a), the production of PMA was higher at 70% DO than at 30% DO no matter which agitation rate was used, the final PMA concentration was comparable for the same DO level despite different agitation rates being applied. These results indicated that the DO level in the culture, not the agitation rate, was the main factor affecting PMA production, which was in good agreement with Cao *et al.*'s research.[13] On the other hand, the pullulan production at 30% DO was higher than that of 70% DO at the two tested agitation rates (Fig. 4(b)). The final pullulan concentrations obtained at 400 rpm and 800 rpm were almost the same when DO was maintained at 70% saturation. However, when the DO level was maintained at 30% saturation, pullulan concentration at 800 rpm was lower than that at 400 rpm. It seems that DO might be the major controlling factor for pullulan production, because when the agitation rate was controlled at the same speed, the variation of DO levels obviously affected pullulan production. Moreover, it is noted that both DO and agitation rate affected pullulan production at a low DO level; combined conditions of low DO level and low agitation rate were required for a high yield of pullulan.

The agitation rate is an important factor controlling DO supply in the stirred tank fermentor, because agitation is important to ensure complete mixing of the medium components (including oxygen) within the fermentor. However, agitation also creates shearing forces that may influence the morphology of *A. pullulans* and even exert negative effects on cell growth.[16] As for PMA production, Cao *et al.* found that a high DO level was beneficial for PMA production[13] and our study was in good agreement with their research. On the other hand, the effects of agitation rates and DO on pullulan production by *A. pullulans* is disputed, there have been claims of enhanced pullulan production under conditions of both high and low oxygen transfer. For example, Ono *et al.* and McNeil *et al.* both reported pullulan production was enhanced at high agitation rates, and they attributed this phenomenon to the increased mass transfer of oxygen in the culture.[22,23] However, Wecker *et al.* found that the optimum production of pullulan occurred under combined conditions of low agitation rate and low DO concentration,[15] and the latter idea was supported by the observations of the present study.

Enhanced co-production of poly(β-malic acid) and pullulan by a DO-shift control strategy

In a previous study, Wang *et al.* improved S-adenosylmethionine and glutathione co-production simultaneously in a unified fermentation process by optimization of amino acids feeding.[24] This operation made the cultivation process more efficient and economical by achieving multiple objectives optimization. In the

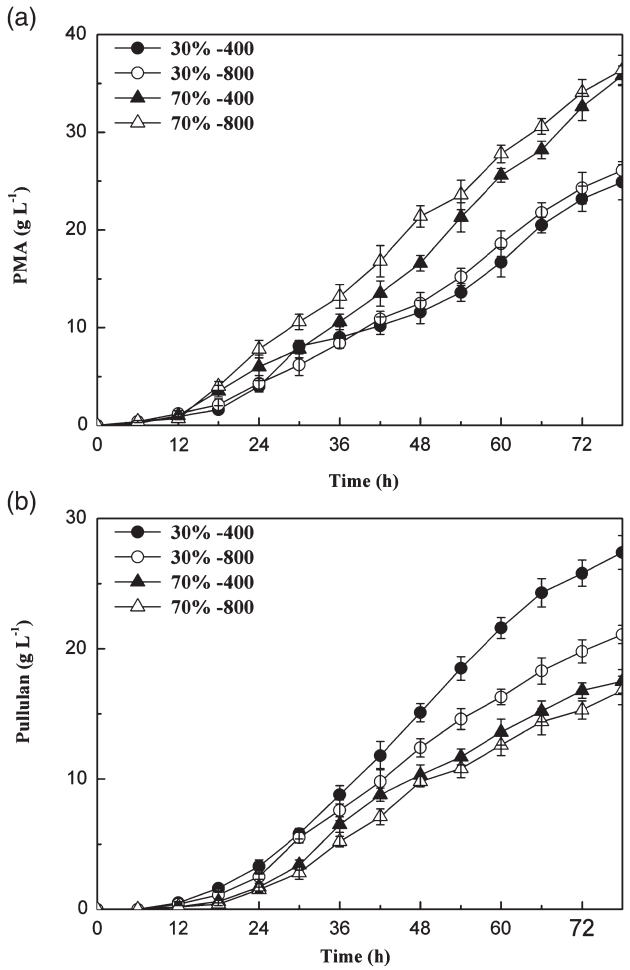

Figure 4. Effects of agitation rate and DO on the co-production of PMA and pullulan. Experiments were conducted using a gas mixing system, which allowed precise DO control independently of the agitation rate. Symbols: constant 800 rpm varied DO: 30% (○) and 70% (△); constant 400 rpm varied DO:30% (●) and 70% (▲).

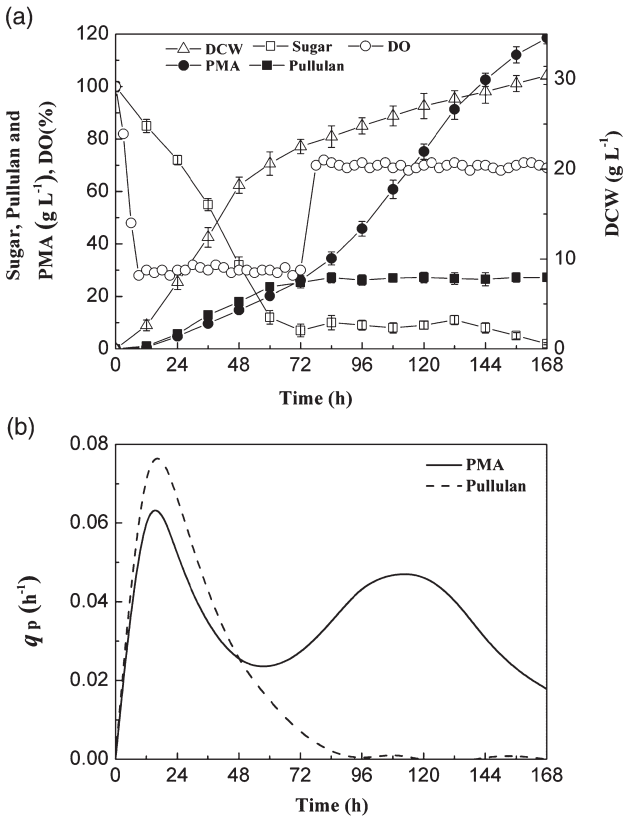

Figure 5. The co-production of PMA and pullulan in fed-batch fermentation with DO-shift control strategy (a), the specific formation rates of PMA and pullulan (b) during the fermentation process were also calculated.

present study, to obtain high productions of both PMA and pullulan in a unified fermentation process, a DO-shift control strategy was performed. Two control phases were designed in the fermentation process. In the first phase, DO was controlled at 30% saturation for pullulan production. In the second phase, DO was increased to 70% saturation for better PMA production. As depicted in Fig. 5(a), pullulan accumulated rapidly in the first phase because of the appropriate DO levels. Figure 3(c) indicated that pullulan biosynthesis rate was very slow after 72 h, thus DO level was increased to 70% saturation at this time and the process reached the second phase. q_p-pullulan and q_p-PMA under the DO-shift control strategy were also calculated (Fig. 5(b)). Compared with PMA and pullulan co-production without DO control (Fig. 1(c)), the maximum q_p-pullulan in the first phase was increased from 0.04 h^{-1} to 0.075 h^{-1}, as a result, the final pullulan concentration was obviously increased from 18.5 ± 1.2 g L^{-1} to 27.2 ± 1.5 g L^{-1}. On the other hand, the maximum q_p-PMA under these two culture conditions was nearly unchanged, but another peak of q_p-PMA profile occurred in the second phase (i.e. DO was maintained at 70% saturation), and PMA production was obviously enhanced during this phase. Therefore, the above results showed the feasibility and effectiveness of this two-stage DO control strategy.

With the aforementioned fermentation strategy, PMA and pullulan concentrations reached 118.6 ± 2.3 g L^{-1} and 27.2 ± 1.5 g L^{-1}, respectively. In order to find if the DO-shift strategy is optimal for the co-production of PMA and pullulan, fed-batch fermentations with constant 30% DO and constant 70% DO were also performed, the PMA and pullulan production levels are summarized in Table 1. When the DO was maintained at 30% saturation during the whole fermentation process, the final PMA and pullulan concentrations reached 91.4 ± 1.6 g L^{-1} and 27.8 ± 1.2 g L^{-1}, respectively. In contrast, 125.8 ± 2.0 g L^{-1} PMA and 10.7 ± 0.7 g L^{-1} pullulan were obtained when constant 70% DO was applied. These results indicated that the constant 30% or 70% DO level was favorable for the production of only one of the products, and simultaneous high productions of PMA and pullulan cannot be obtained at a constant DO level. In contrast, the DO-shift strategy can lead to the accumulation of both PMA and pullulan at high concentrations, as a result, the total product (PMA plus pullulan) concentration, productivity and production yield reached 145.8 ± 2.7 g L^{-1}, 0.87 ± 0.02 g L^{-1} h^{-1} and 0.75 ± 0.01 at the end of fermentation. All of these parameters were superior to those obtained at constant 400 rpm or constant DO levels, thus the co-production system for PMA and pullulan with DO-shift control was efficient and economical.

Aureobasidium pullulans is a biotechnologically important fungus that can be used in different fields, this fungus produces numerous valuable bioproducts, including industrial enzymes, PMA, pullulan and heavy oil.[8,10,25] Leathers *et al.* examined PMA production by 56 strains of *A. pullulans* representing genetically diverse phylogenetic clades, they found that eight strains from

Table 1. Comparison of PMA and pullulan co-production under different cultivation modes in a 5 L fermentor

	Without DO control	Constant 30% DO	Constant 70% DO	Shifting DO (30% to 70%)
PMA ($g\,L^{-1}$)	88.53 ± 2.13	91.36 ± 1.63	125.83 ± 1.96	118.60 ± 2.27
Pullulan ($g\,L^{-1}$)	18.26 ± 1.19	27.77 ± 1.22	10.67 ± 0.71	27.17 ± 1.50
Total product ($g\,L^{-1}$)	106.80 ± 3.16	119.13 ± 1.79	136.50 ± 2.67	145.77 ± 2.68
PMA yield on glucose ($g\,g^{-1}$)	0.46 ± 0.011	0.47 ± 0.008	0.65 ± 0.010	0.61 ± 0.012
Pullulan yield on glucose ($g\,g^{-1}$)	0.09 ± 0.006	0.14 ± 0.006	0.06 ± 0.004	0.14 ± 0.008
Total product yield on glucose ($g\,g^{-1}$)	0.55 ± 0.016	0.61 ± 0.009	0.70 ± 0.014	0.75 ± 0.014
PMA productivity ($g\,L^{-1}\,h^{-1}$)	0.53 ± 0.013	0.54 ± 0.010	0.75 ± 0.012	0.71 ± 0.014
Pullulan productivity ($g\,L^{-1}\,h^{-1}$)	0.11 ± 0.007	0.17 ± 0.007	0.06 ± 0.004	0.16 ± 0.009
Total product productivity ($g\,L^{-1}\,h^{-1}$)	0.64 ± 0.019	0.71 ± 0.011	0.81 ± 0.016	0.87 ± 0.016

Table 2. PMA and pullulan production by various microorganisms and processes

Strain	PMA ($g\,L^{-1}$)	Pullulan ($g\,L^{-1}$)	Key process	Reference
A.pullulans RSU 7	8.9	9.8	Wild strains isolation	11
A.pullulans CCTCC M2012223	46.5	28.8	Tween 80 addition	17
A.pullulans ipe-1	37.9	ND	Combined control of pH, DO and agitation	13
A.pullulans ZX-10	123.7	ND	FBB immobilization	18
A.pullulans var. pullulans MCW	152.5	ND	Wild strains isolation and medium optimization	27
A.pullulans Y68	ND	59	Medium optimization	28
A.pullulans CCTCC M2012259	ND	27.4	Agitation rate control	29
Aspergillus japonicus VIT-SB1	ND	39	Wild strains isolation	30
A.pullulans HA-4D	118.6	27.2	DO control	This study

Abbreviations: FBB, fibrous-bed bioreactor; ND, no data.

different phylogenetic clades of *A. pullulans* can produce PMA, pullulan and heavy oil simultaneously.[11] To date, the key enzymes for the biosynthesis of PMA and pullulan have not been identified, thus it is difficult to create the engineered microorganisms in which the metabolic pathways were redesigned. From energy saving and economic perspectives, if it is impossible to redesign the metabolic pathways, it is important to maximize the existing metabolic pathways.[26] However, most of the existing literature on PMA and/or pullulan production has focused exclusively on only one of the biopolymers (Table 2),[11,13,17,18,27–30] and some researchers tried to minimize the production of one to improve that of the other.[13] By contrast, simultaneously enhanced production of PMA and pullulan were achieved using a DO-shift control strategy in this study, the PMA and pullulan levels in this work were superior to other reports in the literature, but inferior to the highest level of the individual production of PMA or pullulan. Through this co-production system, the PMA production was successfully improved without reducing pullulan production. Similar work has also been reported in literature. For example, Tu *et al.* investigated the role of Tween 80 in the co-production of PMA and pullulan with *A. pullulans* CCTCC M2012223, Tween 80 acted as an effective stimulatory agent for both PMA and pullulan biosynthesis.[17] This type of fermentation, which produced multiple commercial products in a unified process is attractive, because the raw material utilization was maximized, and the diversity of the products was enriched.

CONCLUSIONS

The co-production of PMA and pullulan was sensitive to DO concentration in the culture broth, so that high productions of PMA and pullulan cannot be obtained simultaneously at a constant DO level. Based on the analysis of batch fermentation kinetics at various constant DO levels, a novel DO-shift control strategy that enables the co-production of these two biopolymers in one fermentation process was designed. Finally, the co-production of PMA and pullulan was simultaneously improved with this DO-shift control strategy. The present work provides a feasible approach for efficient and economical co-production of PMA and pullulan.

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