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Comparison of the secondary metabolites in two scales of cephalosporin C (CPC) fermentation and two different post-treatment processes

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Abstract Cephalosporin C (CPC) is the precursor of a class of antibiotics that were more effective than traditional penicillins. CPC production is performed mainly through fermentation by *Acremonium chrysogenum*, whose secondary metabolism was sensitive to the environmental changes. In the present work, secondary metabolites were measured by ion-pair reversed-phase liquid chromatography tandemed with hybrid quadrupole time-of-flight mass spectrometry, and the disparity of them from two scales of CPC fermentations (pilot and industrial) and also two different post-treatment processes (oxalic acid and formaldehyde added and control) were investigated. When fermentation size was enlarged from pilot scale (50 l) to industrial scale (156,000 l), the remarkable disparities of concentrations and changing trends of the secondary metabolites in *A. chrysogenum* were observed, which indicated that the productivity of CPC biosynthesis was higher in the large scale of fermentation. Three environmental factors were measured, and the potential reasons that might cause the differences were analyzed. In the post-treatment process after industrial fermentation, the changes of these secondary metabolites in the tank where oxalic acid and formaldehyde were added were much less than the control tank where none was added. This indicated that the quality of the final product was more stable after the oxalic acid and formaldehyde were added in the post-treatment process. These findings provided new insight into industrial CPC production.

Keywords *Acremonium chrysogenum* · Secondary metabolites · Scale-up · Post-treatment process · Q-TOF-MS

Introduction

Cephalosporin C (CPC) is the precursor of more potent antibiotics against human infection caused by penicillinase-producing bacteria [16]. CPC was produced by a filamentous fungus, *Acremonium chrysogenum*, through fermentation. The biosynthesis pathway of CPC begins with the condensation of three amino acids: L-α-aminoadipic acid (α-AAA), L-cysteine, and L-valine, to form the tripeptide, L-α-aminoadipyl-L-cysteinyl-D-valine (ACV) by ACV synthetase (ACVS) [45]. The second step of the pathway is the cyclization of the ACV to form isopenicillin N (IPN) [24] catalyzed by a cyclase (IPNS) [25] with free oxygen as electron acceptor. Afterwards, the IPN is isomerized to penicillin N (Pen N) by a labile racemase. The forth and fifth reaction is the oxidative ring expansion of Pen N to deacetoxycephalosporin C (DAOC) [39] and then to deacetylcephalosporin C (DAC) by a bifunctional enzyme (expandase/hydroxylase, DAOCS/DACS) [8, 29], which requires α-ketoglutarate, Fe^{2+}, and O_2. Finally, DAC is transformed into CPC by an acetyl-CoA:DAC acetyltransferase as shown in Fig. 1.

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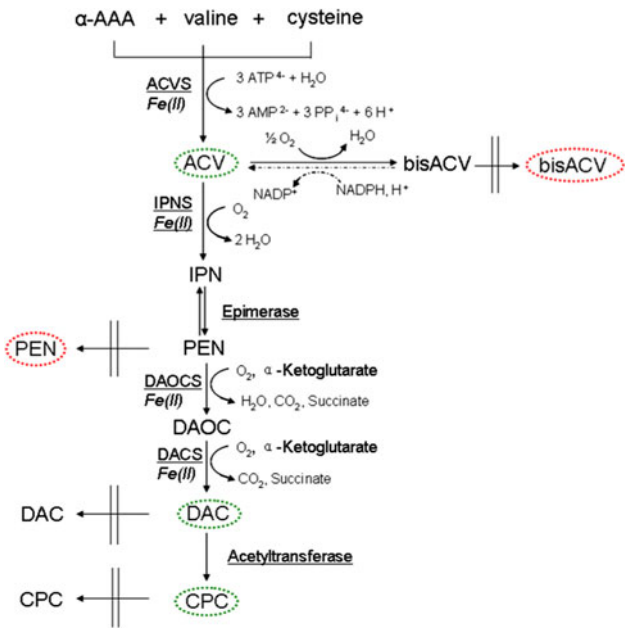

Fig. 1 Cephalosporin C biosynthesis pathway in *A. chrysogenum*. The detected intracellular and extracellular compounds are marked by *green* and *red dashed circles*, respectively

The secondary metabolism pathway of *A. chrysogenum* and its regulations have been studied since the 1980s. The rate-limiting step was identified on the formation of ACV from three amino acid precursors by constructing a kinetic model using in vitro kinetic data of the biosynthetic enzymes [21]. The DAOCS/DACS was also proven to play a key role in the biosynthesis pathway, since the level of the bifunctional enzyme in a high-CPC-producing strain was higher than that in a low-producing strain [9, 20, 37]. This was bolstered by increasing the copy number of DAOCS/DACS gene, which enhanced the CPC titer [33]. On the other hand, CPC biosynthesis and its secondary metabolism are greatly influenced by fermentation conditions. High concentrations of carbon [40, 43, 44], nitrogen [5, 31, 46], and phosphate sources [42] repressed β-lactam synthetases severely, among which ACVS and DAOCS were more easily repressed compared with IPNS [3, 10]. On the contrary, CPC production could be stimulated by methionine or norleucine because they could induce the formation of ACV [41], IPN, and DAOC [28]. The CPC production was also sensitive to the dissolved oxygen concentration (DOC), with 20 % of saturation as a critical level, below which the CPC production would be severely inhibited and even ceased [12]. The intensity of agitation also influenced secondary metabolism and CPC output greatly as it had a strong effect on the morphology of *A. chrysogenum* [17], which is closely related to CPC production [36]. These results indicated that the enzymes and intermediates involved in CPC biosynthesis pathway are very sensitive to the environment; and the changes of intermediates could present the productive capacity of *A. chrysogenum*.

In order to bring the CPC production process to its economic fruition, the fermentation was optimized through bench to pilot scale and finally to the industrial scale. Many environmental conditions in the industrial fermentation would changed compared with pilot and bench fermentations, because of the different mixing, shear stress, heat and mass transfer coefficient, and so on [38], which would subsequently bring effect on the secondary metabolism in *A. chrysogenum* as well as CPC output. On the other hand, before the separation of CPC product, the fermentation firstly should be left to stand for a couple of hours in order to make the mycelium being settled, which is called the post-treatment process. During the post-treatment process, oxalic acid and formaldehyde were added, since these two chemicals can facilitate the precipitation of impurity protein by changing the value of pH, and the formaldehyde can kill the fungus as well as avoid new bacterial contaminations. Adding oxalic acid and formaldehyde is an empirical operation in factory, however, the effect of these chemicals on the amount and quality of the CPC product was obscure.

In this work, the metabolites involved in the CPC biosynthesis pathway and their changes over time were detected by IP-RPLC coupled to Q-TOF-MS, which has the advantage of high resolution and sensitivity. By comparing and analyzing the disparity of the secondary metabolites between pilot and industrial fermentations, the potential reasons behind the differentiae and the interplay of these metabolites were illustrated. By analyzing the disparity of the secondary metabolites in two different post-treatment processes, the merits of adding oxalic acid and formaldehyde were revealed. This work might provide new fundamental data for modeling, improving and predicting industrial CPC production.

Materials and methods

Strains and fermentation conditions

The industrial strain of *A. chrysogenum* used for CPC production in this study was provided by Hebei Zhongrun Pharmaceutical Co., Ltd (Shijiazhuang, China). The seed medium consisted of 20 g/l sucrose, 80 g/l corn steep liquor, 2 g/l soybean meal, 20 g/l dextrin, 10 g/l soybean oil, and 10 g/l $CaCO_3$. Pilot and industrial fermentations were carried out in 50- and 156,000-l steel fermentors with a working volume of 35 and 147,000 l, respectively. Both fermentations lasted for 128 h. The medium for both pilot and industrial batch fermentations were the same, containing 30 g/l cornmeal, 40 g/l dextrin, 100 g/l corn steep

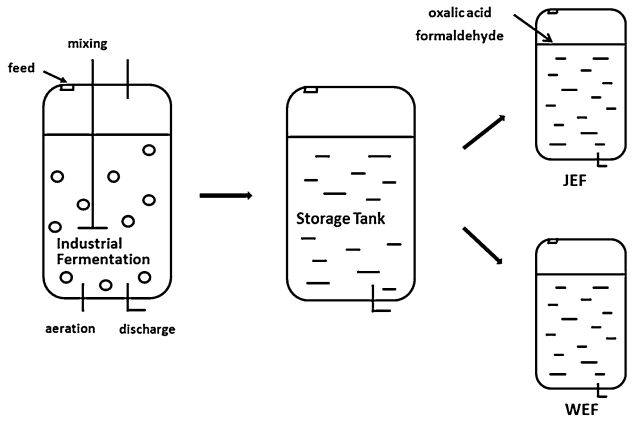

Fig. 2 Schematic drawing of the post-treatment processes. In the JEF process, oxalic acid and formaldehyde were added, and WEF was the control process

liquor, 50 g/l wheat gluten, 100 g/l soybean oil, 5 g/l $CaCO_3$, 2 g/l methionine, and 0.22 g/l $ZnSO_4$. $NH_3{\cdot}H_2O$ and $(NH_4)_2SO_4$ were added to the medium in order to maintain the value of pH at 5.55–5.65. Soybean oil and glucose were fed as carbon source to achieve fed-batch cultivation, and total sugars was measured by DNS method. The temperature was maintained at 28 ± 1 °C in the first 25 h; afterwards, it was kept at 25 ± 1 °C until the end of the fermentation. The beginning 28 °C was suitable for the hyphal growth, while the reduction of the temperature to 25 °C was beneficial for biosynthesis and accumulation of CPC. The aeration rate was kept above 1:1 (minute ventilation: working volume) and DOC maintained above 20 % of saturation. The value of pH and DOC was detected by Mettler-Toledo electrode 104654501 and 52200965, respectively.

Post-treatment processes

When the fermentation of industrial CPC production finished, the fermented mixture was settled for 8 h before separation. In our work, the fermented broth was separated into two post-treatment tanks as shown in Fig. 2. One of them was added with oxalic acid and formaldehyde, with concentration of 0.02 and 0.1 %, respectively. This process was named JEF. Another tank named WEF was also settled for 8 h without adding any chemicals as control process. The mixtures were sampled at 2, 5, and 8 h during the post-treatment process and extracellular secondary metabolites were measured after the mixtures were filtered.

Sampling, quenching, and extraction of intracellular secondary metabolites

There were two industrial and two pilot fermentations for parallel samplings, and both industrial and pilot fermentations were repeated once several months later. Seven samples were taken in each fermentor (three biological replicates were performed for each sample), and the interval of sampling in both fermentations was 16 h between the first four samples and 24 h between the following samples. The samples of broth were first filtered; the liquids were applied for extracellular detection and the cells were extracted according to the method described by Lu et al. [19] with slight modifications. The extraction process was as follows: first, the cells were washed with phosphate buffer solution (PBS) and the supernatant was descanted after centrifugation. The cells were then ground to a fine powder in liquid nitrogen with mortar and pestle and 200 mg of ground cells were firstly suspended with 300 µl of extraction buffer of methanol/water (80:20, v/v, −20 °C) and mixed thoroughly. The mixture was then frozen in liquid nitrogen for 15 min. At the end of 15 min, the sample was centrifuged at 10,000 rpm for 5 min at 4 °C and the soluble extract was removed. The pellet was then resuspended in 300 µl of extraction buffer and was placed in liquid nitrogen for 15 min. After centrifugation, a second clear extract yielded, which was combined with the first extract. The pellet was then again resuspended in 200 µl extraction buffer and the resulting suspension was frozen in liquid nitrogen for another 15 min. The sample was then spun down and the resulting soluble phase combined with the initial two extracts to give a total of 800 µl extract. Before analysis the extracts were mixed with 2 µg/ml penicillin V (HPLC grade, Tianjin Yi-Fang Technological Company, Tianjin, China) as internal standard (IS). The biomass dry weight (DW) was measured by 200 mg of dry grounded cells in an oven for 24 h at 105 °C. The intracellular metabolites were determined with the cell dry mass as a reference. The relative abundance (RA) of extracellular metabolites was calculated by normalization of peak area of each metabolite to IS.

Secondary metabolite analysis by IP-RPLC coupled to Q-TOF-MS

The IP-RPLC was performed as Seifar reported in 2008 [30]. All chromatographic separations were realized on a reversed-phase Symmetry C18 column 3.5 µm, 100 mm × 2.1 mm i.d. (Waters, Dublin, Ireland). The column was kept at ambient temperature during analysis, while samples in the auto sampler were kept at 6 °C. The mobile phase A consisted of 2 mM dibutylammonium acetate (DBAA, 0.5 mol/l served as concentrated solution, TIC, Tokyo, Japan) and mobile phase B was the mixture of 16 % (v/v) A with 84 % (v/v) acetonitrile. The linear gradient realized by a 1200 series pump system (Agilent Technologies, Palo Alto, CA, USA) was as follows: 5–50 % B from 0 to 15 min, 50–95 % for the following 5 min, and then

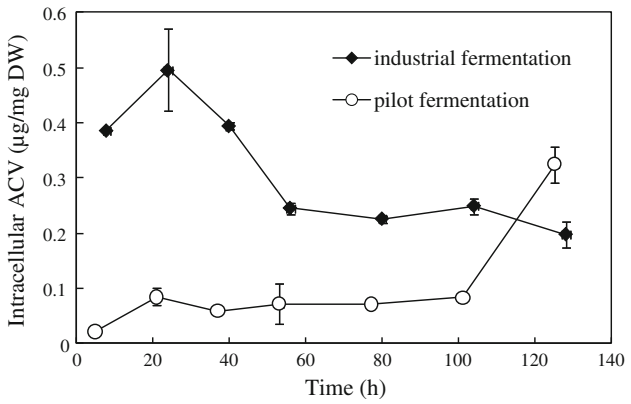

Fig. 3 Intracellular concentrations of ACV during industrial and pilot fermentations. *DW* dry weight (of *A. chrysogenum* cell)

maintain 95 % B from 20 to 25 min, returning to the initial condition (5 % B) at 27 min and maintained until 40 min, with 0.2 ml min^{-1} of flow rate. MS analysis of the samples was performed using micrOTOF-Q II mass spectrometer of Bruker Daltonics (Bremen, Germany). The ESI parameters were operated in negative ion mode and mass spectra was recorded in the range *m/z* 100–1,000. The capillary voltage was maintained at 2,600 V with the end plate offset at −500 V. Nitrogen was used as nebulizer and dry gas at flow rate of 6.0 and 0.8 min/l, respectively; source temperature was maintained at 180 °C.

Results

Intracellular ACV

As shown in Fig. 3, the pools of intracellular ACV were totally different between two scales of CPC fermentations. Before 56 h, ACV content presented downtrend in the industrial process and then kept constant until the end of fermentation; while in the pilot process, the concentration of intracellular ACV changed slightly except for the last 25 h. Despite their different changes, the contents of ACV also showed a large disparity between two fermentations: its concentration in industrial cells was always higher than that in the pilot ones except for the last 20 h, and the average concentrations of intracellular ACV in large fermentor was about three-fold higher than that in the small one.

Extracellular bis-ACV and PEN

ACV possesses a free cysteine thiol and can be oxidized into the dimer form with a disulphide bond: bis-δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (bisACV) [32]. The high

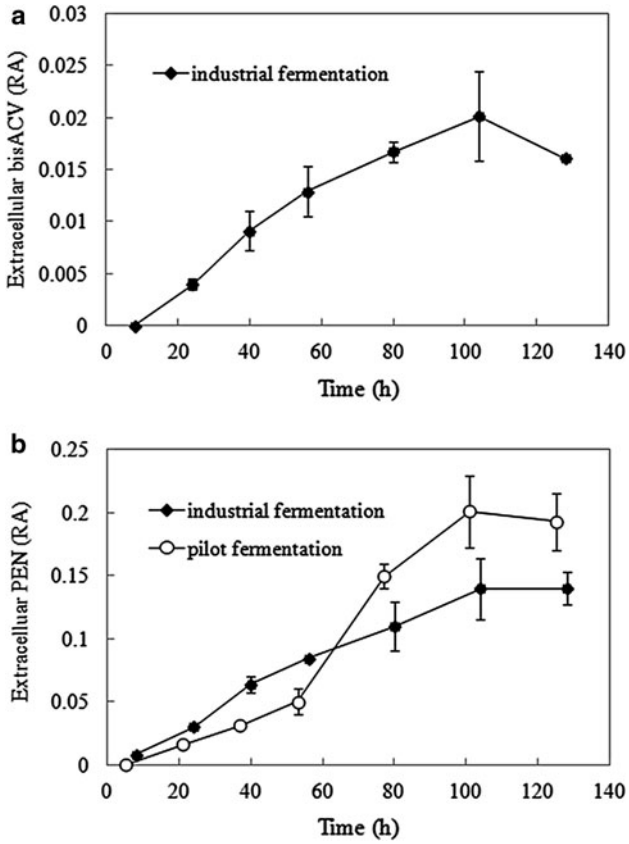

Fig. 4 Extracellular levels of PEN and bisACV during industrial and pilot fermentations. *RA* relative abundance

concentration of ACV in industrial cells led to a detectable concentration of bisACV in the fermentation broth as shown in Fig. 4a. Except for the last 25 h, its concentration presented an uptrend. In pilot fermentation, however, no bisACV was detected either in cells or in broth.

IPN and Pen N are the major products secreted into medium besides CPC [21, 33, 46], thus only extracellular contents were detected. Since the structures of penicillin N and isopenicillin N are identical except for the configuration of the α-aminoadipyl side chain, the compounds are indistinguishable by chromatography, electrophoresis, ninhydrin staining or by the rate of acid or penicillinase destruction [7]. Moreover, the molar ratio of IPN to Pen N was postulated to be unity; thus, their contents were added together and named PEN, whose contents and changes in the two scales of fermentations are presented in Fig. 4b. PEN pools showed an upward trend during fermentations. In the industrial process, the accumulation rate during the first 56 h was higher than that in pilot one. Then, PEN concentration in pilot fermentation surged higher than that in the industrial one, and kept this disparity until the end of the fermentation. The average concentration of PEN in pilot fermentation was about 1.1-fold higher than that in the industrial one.

Intracellular DAC and CPC

It has been observed that there are two phases during CPC fermentation [22]. The first phase is called the trophophase (the balanced growth phase) characterized by the intense accumulation of biomass, and CPC is not normally formed during this phase though occasionally small amounts are formed. The second phase is named as idiophase (unbalanced growth phase), during which the metabolic products begin to be synthesized at a very high speed. The two phase of CPC production was very obvious as presented in Fig. 5, which shows the intracellular DAC and CPC curves during the pilot and industrial fermentations and the noticeable disparities. It can be seen from Fig. 5a that DAC began its synthesis at 20 h in industrial cells, and increased for the following 60 h; thereafter it decreased linearly until the end of fermentation. In the pilot process, DAC started its synthesis at 40 h, which was 20 h later than that in industrial one. The DAC level also increased for the following 60 h but at a lower rate, and then it decreased slightly.

On the other hand, CPC levels in both fermentations were rather minor before 56 h, as shown in Fig. 5b. Afterwards, CPC began to be synthesized largely in both fermentations, but the rate of CPC accumulation in industrial cells was much higher.

The average concentration of DAC in the large process was 2.6-fold more than that in the small one, while the disparity of CPC concentrations was 2.7-fold. The similar disparity corresponded with other conclusions that besides being synthesized from DAOC, a substantial portion of DAC was derived by degradation of CPC [13, 14] and the concentration of DAC was proportional to CPC.

The disparity of secondary metabolites between two post-treatment processes

The changes of secondary metabolites during two different post-treatment processes are presented in Fig. 6 and obvious disparities can be observed from this figure. As shown in Fig. 6a, b, and c, the content of bisACV, PEN, and DAC stayed constant in the JEF process, but in the WEF process their contents changed dramatically during the last 3 h. As shown in Fig. 6d, the concentration of CPC in both tanks decreased, while in the WEF process the CPC pool decreased more noticeably.

Discussion

Cephalosporins have become more significant antibiotic medicines, since more and more pathogenic bacteria turned to have penicillins resistance. The secondary metabolism in *A. chrysogenum* is susceptible to fermentation conditions,

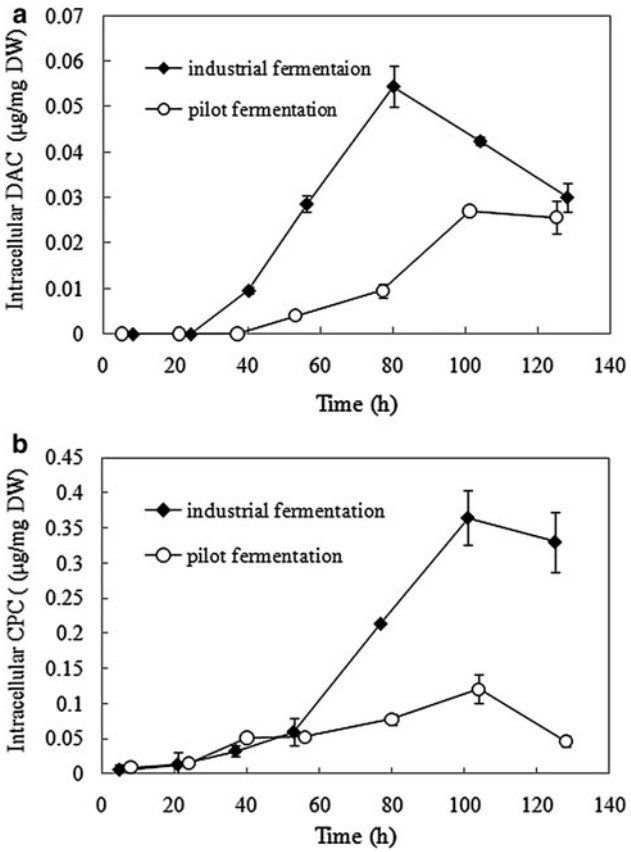

Fig. 5 Intracellular concentrations of DAC and CPC during industrial and pilot fermentations. *DW* dry weight (of *A. chrysogenum* cell)

such as the concentration of carbon or nitrogen source, the value of pH, and the force of shear stress. The different environmental stresses could put remarkable effect on the secondary metabolites, which would further have interaction with each other and also with the environmental conditions. In the present study, we detected the secondary metabolites involved in the CPC biosynthesis pathway in *A. chrysogenum* and analyzed their differentiae between pilot and industrial fermentations. Furthermore, the disparities of the secondary metabolites caused by two different post-treatment processes were also detected and analyzed.

Firstly, a large disparity of contents and changes of the tripeptide, ACV, was observed between pilot and industrial fermentations as shown in Fig. 3. ACV formation was the first and rate-limiting step in the CPC biosynthesis pathway [21]. It has been proven that there was a positive correlation between the capacity of CPC production and the concentration of ACV: in some adverse conditions, such as a too high amount of nitrogen [5], glucose [18], and very low oxygen content [11] less than the limiting value, the ACV content was several times less than the ordinary groups, which corresponded with very low CPC production flux. Considering the conclusions of the references, it is

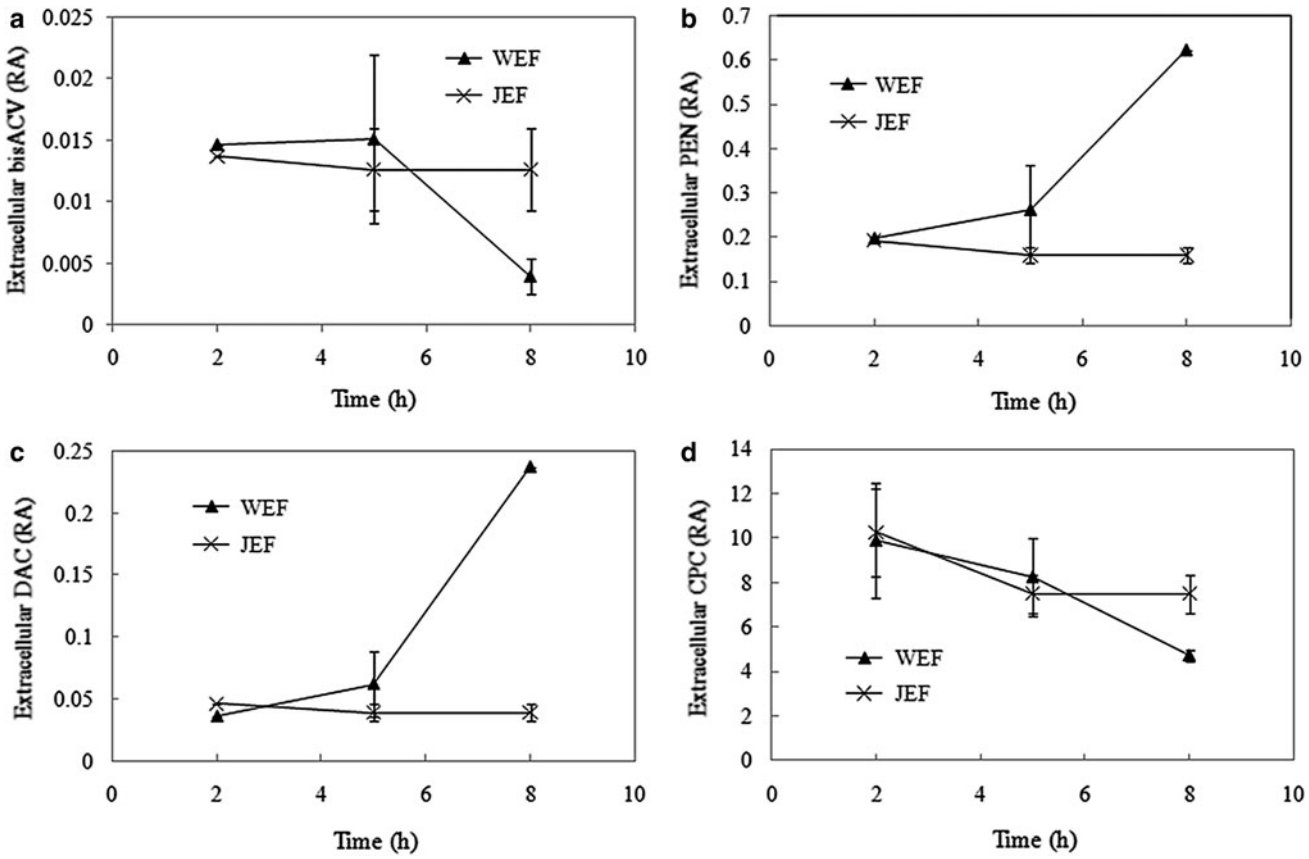

Fig. 6 Extracellular levels of secondary metabolites during JEF and WEF post-treatment processes. *RA* relative abundance

reasonable to deduce that in the present work CPC production in industrial fermentation was higher than that in pilot one because the concentration of ACV in industrial cells was three-fold higher than that in the pilot cells.

The relatively high concentration of ACV in the industrial process led to a detectable amount of bisACV content, which was secreted into the broth, as shown in Fig. 4a. bisACV was considered as an unwanted side-product in penicillin production because of its inhibitory effect on ACVS [34, 35]. However, the value of its inhibitory constant on ACVS (K_{bisACV}) was several times higher than that of K_{ACV} [34, 35]. Moreover, the bisACV was not even detected in industrial cells, thus it hardly could have any inhibitory effect on ACVS. Furthermore, it has been proven that bisACV can be converted to Pen N by crude lytic enzyme extracts [1] and can also be reduced back to ACV by means of the thioredoxin–thioredoxin reductase (TR) system [6]. Therefore, the formation of a small amount of bisACV in the industrial process should not be considered as waste or an inhibitory substance.

On the other hand, it can be seen from Fig. 3 that ACV content declined noticeably at the early stage of fermentation in the industrial process, while this change did not occur in the pilot one. It has been proven that when the concentration of ammonium was greater than 100 mM, the level of CPC production began to be repressed [31, 46]; and greater than 250 mM ammonium would severely inhibit the ACVS activity (50 % inhibition) [46]. Under adverse conditions, the concentration of ACV stayed almost constant. However, under normal conditions, which meant a higher flux of CPC biosynthesis, the ACV content dropped dramatically [5], which might be converted to the next metabolite. A similarly remarkable decrease of ACV pools in the high speed of penicillin productions [4, 15], which has the same beginning step with CPC biosynthesis pathway, also indicated that the intermediate metabolites were consumed largely when synthesis flux was high. Thus, the difference of ACV changing trends between two scales of fermentations also suggested that the capacity of CPC fermentation in industrial process was higher than that in the pilot one. The initially quick consumption of ACV might be due to its conversion into IPN and Pen N, as cyclase also showed a high peak of activity [10] in the first phase of fermentation. In fact, it can be seen from Fig. 4b that in the initial 56 h, the rate of PEN production in the industrial process was higher than that in the pilot one. This corresponded with the initial differentia of ACV changing trends.

The rate of PEN accumulation in the industrial process, however, declined since 56 h when CPC began to be synthesized, while that in pilot process increased and its average concentration was 1.1-fold more than that in the industrial one. Perez-Martinez and Peberdy [27] found an opposite correlation between PEN content and the capacity of CPC production by comparing the secondary metabolites between low- and high-producing strains. The reason behind this phenomenon was due to the different ability of converting this antibiotic to the later intermediates in the pathway. The lower content of PEN in industrial broth in idiophase indicated that the conversion of PEN into the later metabolites in CPC production phase was faster in the large scale of fermentation. This could be confirmed by the large disparity of DAC and CPC pools between two scales of fermentations as shown in Fig. 5: it could be seen that the speed of DAC and CPC accumulation in industrial cells was much higher than that in pilot cells. On the other hand, as shown in Fig. 5a, the beginning of DAC formation in industrial cells was 20 h earlier than that in pilot cells, which meant that the conversion of DAC into CPC started earlier in the large scale of fermentation. In sum, the disparities of the secondary metabolites between two scales of fermentations suggested that the productive ability of industrial cells was higher than that of pilot ones.

There were several examples that CPC production in the large scale of fermentors was better than that in the small ones. Firstly, the productivity of CPC fermentation at bioreactor levels was higher than that performed in shake flasks [33]. One reason for the disparity was the better aeration in bioreactors [12]. CPC production is very sensitive to the DOC, with the critical DOC value of 20 % saturation [12, 47]. In our work, DOC in both fermentations, however, was always kept above the limiting value, as shown in the supplementary Fig. 1. In fact, the average value of DOC in the pilot fermentation was sometimes even higher than that in the industrial one. Thus, the oxygen limitation could be lined out from the factors that might lead to different productivity between industrial and pilot fermentations. On the other hand, the nitrogen source was exactly the same between the two scales of fermentations, as shown in supplementary Fig. 2 and was a hundred-fold less than the inhibitory value (100 mM) [31]. Except for the amount of nitrogen source and dissolved oxygen, CPC production was also sensitive to the amount of carbon source. The concentration of total sugars presented some difference in pilot and industrial fermentations in our work, as shown in supplementary Fig. 3. It should be noticed that the beginning concentration of total sugars was the same between the two scales of fermentations; besides, the amount of glucose feeding per unit working volume during the fed-batch fermentations was also identical and was added at the same time in both productions. Nevertheless, the concentration of total sugars in pilot fermentation was still higher than that in the industrial one, as shown in supplementary Fig. 3, which suggested that the up-taking and consumption rate of sugars was lower in the pilot fermentation, especially during the last 30 h of fermentation. Moreover, the content of total sugars in the pilot fermentation was closer to the repressing level (5.5 %) [40], and the partial concentration of sugars even might higher than the repressing level because of incomplete mixing. The relatively more adverse environmental condition in the pilot fermentation would further damage cell viability and productivity as a vicious circle, since the consumption of sugars in the late phage of fermentation was even less.

Secondly, the CPC titer in the 30,000-l fermentor was higher than that in the 14-l one under the same fermentation conditions [2]. In this case, DOC was not the limiting factor because the aerations in both fermentors were sufficient. The disparity might result from different shear stress between the two scales of CPC fermentations. CPC fermentation is a strong aerobic fermentation, and the criteria for its scale-up maintaining geometric similarity are based on empirical or semi-empirical equations, which correlate the volumetric oxygen transfer coefficient (k_La) and the volumetric airflow rate per unit working volume (Q/V). Consequently, the stronger shear and mixing is needed in the larger scale of fermentation in order to maintain a good oxygen and mass transfer. As a result, the shear force, especially that around the tip zone, increased markedly after scale-up. It has been reported that the intensity of agitation influenced CPC production greatly as it had a strong effect on the morphology of *A. chrysogenum* [17]. During CPC fermentation, the morphology of *A. chrysogenum* was mainly classified into three types: hyphae, swollen hyphal fragments, and arthrospores. The morphological differentiation of *A. chrysogenum* in submerged culture was closely related to the production of CPC. Before the beginning of CPC synthesis, the long hyphae have to differentiate into highly swollen hyphal fragments, which then gradually differentiate into arthrospores during the CPC production phase [26]. Pazouki and Panda found that as agitation became more severe, the number of tips and arthrospores increased and the length of the hyphal became shorter [17]. It is generally known that a larger number of arthrospores and shorter hyphae were indicators of more effective CPC production [36]. As shown in Fig. 5, the DAC production started earlier in the industrial process and the CPC production rate was also higher than that in the pilot one. This corresponded with the above discussion that the beginning of CPC production was earlier and the CPC titer was higher in industrial fermentation because of the higher shear force. This was also another reason causing the productivity gap between the

fermentations performed in flasks and bioreactors in the first case, because the shear force was much less in flasks than that in the bioreactor, which stirred by impellers.

In the post-treatment process, the secondary metabolites in the JEF process, where oxalic acid and formaldehyde were added, changed much less than that in the control process (WEF). It can be seen from Fig. 6a and b that in the WEF tank, the change of bisACV and Pen N contents showed opposite trends. This was consistent with the above discussion that bisACV could be converted into PEN in vitro [1]. The increase of PEN, however, was not equal to the decrease of bisACV, which suggested that there might be other metabolites, such as ACV, being converted to IPN and Pen N. On the other hand, the changing trends of DAC and CPC concentrations in WEF process were also opposite. This corresponded with the previous conclusions that DAC was not only the precursor of CPC but also degraded from CPC [13, 14]. In the following chemical conversion process, CPC would be hydrolyzed into a more effective derivative: 7-aminocephalosporanic acid (7-ACA) [23]. The decrease of CPC would lead to a lower output of 7-ACA, while the increase of PEN and DAC content in the WEF process, however, would lower the quality of the final product because they could be transformed into undesirable impurities. The original objective of using oxalic acid and formaldehyde was to kill the fungus as well as to avoid new bacterial contaminations, while this technology could also enhance the quality of CPC product, as shown in our results.

Conclusions

Since its discovery in the 1950s, the production of CPC has increased dramatically by process and strain improvement. In the following 60 years, chemical derivatives of cephalosporin C (semisynthetic cephalosporins) have become the most widely used antibiotics in the world. The studies on optimization of fermentation conditions and scale-up strategies have been carried out by many scholars. However, the micro responses of cells on secondary metabolism level during scale-up and post-treatment processes were still obscure. The present study illustrated huge disparities between pilot and industrial fermentation, and between additives-adding and control post-treatment processes by analyzing the secondary metabolites involved in CPC biosynthesis. The disparities between two scales of fermentations indicated higher productivity in industrial production, while the disparity between two post-treatment processes suggested that additives-adding process has several merits over the control one. These findings provided new sights into the changes of *A. chrysogenum* cells in scale-up and post-treatment processes.

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