Process strategies for cephalosporin C fermentation

Pradeep Srivastava*, Punita Mishra and Subir Kundu
School Of Biochemical Engineering, Institute of Technology, Banaras Hindu University, Varanasi 221 005

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Cephalosporin C (CPC), a β-lactam antibiotic, is starting molecule for industrial production of semi synthetic cephalosporins. CPC fermentation has been carried out in Air Lift Reactor (indigenously made up of borosilicate glass) in different fermentation modes i.e., batch and fed batch modes using a mold Acremonium chrysogenum (synn. Cephalosporium acremonium). A definite correlation exists between the antibiotic productivity and flow rate of supplementary feed. Fed batch process was shown to be more efficient than the batch one, and the process in which the lowest supplementary feed flow rate was used resulted in significantly higher antibiotic production.

Keywords: Acremonium chrysogenum, Cephalosporin C, Batch fermentation, Fed batch fermentation, Flow rates

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Introduction

Cephalosporin-C (CPC), a β-lactam antibiotic, which like all secondary metabolites, is synthesized in bioprocesses involving microorganisms. Industrial production of this antibiotic is still carried out in conventional batch fermentation in aerated stirred tank bioreactors by fermentation of submerged cultures of wild and mutant strain of Acremonium chrysogenum. Regulatory mechanisms for secondary metabolism in this process include repression and inhibition of the β-lactam synthetases by glucose and other carbon and energy sources easily metabolized by the microorganism. These conditions are essential for cell growth, but drastically suppress the production of many antibiotics. The development of an improved strategy for CPC fermentation requires proper control of specific growth rates of cells using certain key nutrients. Various strategies have been evaluated which include batch, fed batch, semi continuous and continuous modes. Another strategy uses, repeated batch reactors in tower reactors for CPC production. Some earlier studies suggest the requirement of a certain level of specific growth rate of the organism for its maximum biosynthetic activity.

In batch process, CPC production by A. chrysogenum in a synthetic medium containing glucose and sucrose, diauxic phenomenon is observed. It is characterized by the rapid consumption of sucrrose to form biomass (trophophase) at the beginning of the process. After depletion of this carbohydrate by the slow consumption of sucrose, the more difficult carbohydrate to assimilate, during which the majority of the CPC is produced (idiophase) and cell growth is insignificant. Even when added slowly, sucrose tends to accumulate in the reaction medium in virtue of the low activity of the enzyme that hydrolyzes this carbohydrate, thereby limiting its consumption rate. The use of sucrose is, therefore, a good strategy to obtain high antibiotic concentration in the conventional batch process, but is unsuitable for the fed batch process. A viable alternative is to use hydrolyzed sucrose in the supplementary medium, which together with the low cost of this sugar in relation to other carbohydrates such as glucose and fructose may make the fed batch process even more advantageous.

Control on biomass and hence specific growth rate in fermentation with sufficient supplies of dissolved oxygen enables maximization of antibiotic production. A direct correlation has been developed between morphological variation and oxygen availability in the broth. Present study describes fermentation of CPC in batch and fed batch and an attempt is made to compare the various kinetic analyses of the processes. The feed strategy employed was effective in avoiding oxygen limitation during the fermentation and helped in process improvement.
Materials and Methods
Organism
A. chrysogenum, obtained from M/s J K Pharmaceuticals Ltd, Cuddalore, Pondichery, was maintained on Potato-Dextrose-Agar after incubation for 5-8 days at 28°C. The surface growth from a 7-day old slant was suspended in sterilized distilled water (10% inoculum) and used for inoculation of seed media.

Culture Medium and Conditions
Germination carried and inoculum prepared. The medium for main fermentation of the batch process contained sucrose, DL methionine and the remaining components in the same amounts as those in preculture. Composition of the initial batch medium of the fed batch process was the same as that used in the conventional batch process (Run # 1). The supplementary medium utilized in Run # 2, #3 and # 4 contained the same components as the initial batch and previously hydrolyzed sucrose with different flow rates.

The sucrose used in the supplementary medium was hydrolyzed at 40°C in a buffer solution (pH 4.5) of $10^{-2}M$ sodium acetate, employing invertase enzyme in soluble powder form. The fermentation Runs (Table 1) were carried out in borosilicate fabricated Air Lift reactor (ALR; Internal loop, 2l) equipped with instruments for temperature and dissolved oxygen monitor, and a pH meter. The optimum temperature is maintained by flowing water through the jacket and DO by adjustment of air supply. Basic design of the lab scale ALR was determined.

All Runs were carried out at 28°C and dissolved oxygen concentration was maintained around 40% or above with appropriate airflow. Silicone oil was added to control foaming whenever necessary.

The batch fermentation Run #1 contained 1.8 l of main fermentation medium. The feed profile was designed to control the growth at a constant rate through the addition of growth limiting carbon source. The high product formation rate, required at slow growth rate, depends on having a high viable cell concentration. Initially, sub optimal substrate concentration ($S_0= 20g/l$) of sucrose was used during the early growth and after trophophase, substrate feed was made at constant rate. Fed batch fermentations, Runs #2, #3 and #4, were started with 1.2 l of medium and after the completion of trophophase i.e., maximum growth attained (37 h), supplementary medium was fed at established flow rates until 1.8, 2.0 and 1.6 l, respectively were attained at the end of 100 h of feed supply. Purpose of the first fed batch experiment, Run #2, was to reproduce the sucrose consumption rate during the idiophase of the conventional 1.8 l batch Run (Run #1).

Fermentation Runs were performed in triplicate. During each Runs, samples were collected and analyzed for substrate, cell mass and CPC. Substrate consumption rate was determined by DNS method. The weight of growing cell mass was estimated by dry cell weight method. CPC was estimated by Hydroxylamine method and confirmed by HPLC. Dissolved oxygen measurements were carried using a pre calibrated DO probe (Bioengineering AG, Sweden).

Results and Discussion
Under batch mode of CPC fermentation Run #1 in ALR (internal mode), variation of growth and product profiles (Fig. 1) is in accordance with earlier studies. Maximum CPC titer (3.1 gl$^{-1}$) was obtained after 120 h of fermentation while cell growth was highest at 47 h of fermentation. In batch culture, some process leading to production of antibiotics are sequential i.e. they exhibit a distinct growth phase (trophophase) followed by a production phase (idiophase). Maximum antibiotic yield is obtained by achieving high cell mass concentration, which leads to high rate

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<th>Table 1: Operational condition of the fed batch Runs</th>
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Fig. 1—Biomass, substrate and cephalosporin C fermentation in Air Lift Reactor (batch fermentation), Run # 1
of CPC production and maintaining these values as long as possible. Cell growth (maximum, 13.5 g/l) took approx 47 h. Synthesis of CPC increased up to 3.1 g/l then decreasing together with cell concentration possibly due to substrate depletion in total processing time.

Cell concentration results show that maximum values are reproducible and are close to those for the biomass in conventional batch process (about 13.5 g cell/l). Due to addition of the medium, cell concentration of the all fed batch fermentation process remained practically constant and was near its maximum value during the entire phase; however, the substrate was used for its maintenance. Depletion of carbon source and the subsequent start of the idiophase in the fed batch Runs were anticipated in relation to the simple batch fermentation. Addition of supplementary medium was started after about 37 h fermentation at all flow rates.

In Run #2 (Fig. 2), with the operational conditions equivalent to the batch fermentation (Run #1), the maximum CPC concentration was on the order of that obtained in the batch fermentation (3.1 g CPC/l), the difference being that this concentration was attained after 75 h of fermentation and remained practically constant during the entire feeding stage. The growth rate observed was quite low compared to growth rate during batch fermentation. Run #3 (Fig. 3) was performed at a flow rate 33% higher than that in Run #2 and the maximum CPC concentration obtained (2.1 g CPC/l) was 33% less. The results suggest the occurrence of significant catabolic repression due to the higher mass of sugars supplied in relation to the former condition. Run #4 (Fig. 4) reproduced the production phase of the conventional batch process for 1.6 l, supplying feed at a flow rate 33% lower than that in Run #2. High production rates can be observed after 70 h of fermentation. After this, production can be seen to be increasing although at lower rates, attaining 3.9 g/l at 120 h and 3.6 g/l at the end of the process without any signs of apparent degradation of the antibiotic was approx 30% higher.

Results showed high rates of CPC production as
well as upkeep of maximum cell concentration during the entire idiophase under all feeding conditions. Several conditions studied resulted in a significantly higher productivity in the lowest flow rate at a lower specific growth rate (0.0441 h⁻¹), certainly due to lower catabolic repression. A lower flow rate may still be investigated, taking care to maintain the integrity of the cell population by supplying a minimum of the remaining nutrients.

Conclusions
The increase in production of CPC is achieved as the flow rate of supplementary medium is reduced in the fed batch fermentation process. Lower growth rates are observed during CPC production in fed batch mode. In general, slow addition of supplementary feed containing hydrolyzed sucrose resulted in reduction in catabolic repression and thus it can be more advantageous strategy than the batch process in ALR. The fed batch fermentation favored maintenance of higher antibiotic production rates during the process in relation to those obtained in the batch process, resulting in a higher specific antibiotic production.

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References