

Short Communication

Development of Various Processing Strategies for New Generation Antibiotics Using Different Modes of Bioreactors

Ipsita Chakravarty¹, Kanika Kundu², Sumedha Ojha¹ and Subir Kundu^{1*}

¹School of Biochemical Engineering, Indian Institute of Technology (Banaras Hindu University), India

²Chemistry Section, Banaras Hindu University, India

***Corresponding author**

Subir Kundu, Chemistry Section, Banaras Hindu University, India, Tel: 91- 7080185543; 91-9415228077; Email: skundu.bce@itbhu.ac.in; subirbhu@gmail.com

Submitted: 14 December 2016

Accepted: 21 March 2017

Published: 22 March 2017

ISSN: 2333-7117

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Keywords

- Antibiotic
- Bioreactor design
- CSTBR
- Airlift and packed-bed bioreactor
- Immobilization techniques

Abstract

Bioreactor design is an integral part of bioprocess intensification. The sole aim of process design is to identify the thrust areas and improve the production of the desired metabolite. Complex secondary metabolites like antibiotics are of great importance. Several researchers have highlighted the significance of media formulation, feed strategies, and control of physico-chemical parameters, metabolic engineering and genetic improvement for enhanced antibiotic fermentation. But, there is a lacuna regarding various aspects of bioreactor design and analysis for antibiotic fermentation. In this short communication, we have summarized our research work/ findings on improved antibiotic production through strategic design and development of bioreactors as a case study. The physical design and operational conditions can significantly tackle problems of substrate inhibition, poor cell growth and improper oxygen mass transfer. Thus, helping in meliorated production of several life-saving drugs.

INTRODUCTION

The advent of bulk production techniques of antibiotics marks the rise of biochemical/ bioprocess engineering. The industrially important microbial products could now serve the mankind through its bulk availability. Penicillin was discovered in 1929 by Alexander Fleming but the methods of amplified production of this valuable drug were unknown until [1] other renowned scientists such as Howard Florey, Ernest Chain and Norman Heatley in close association with pharmaceutical companies managed to scale-up the microbial production in bioreactors to globally cure infections [2]. This biotechnological achievement revealed the path for the large scale bio production of several other useful antibiotics. There is a constant urge to improve the process control, better aseptic design and operation; and innovative ways to enhance mass transfer through bioreactor design [3]. Biochemical engineering involves the production of several useful metabolites through chemical reactions in biological systems such as microorganisms, plants and animals. Process optimization and its intensification is an important aspect of biochemical engineering to obtain higher product yields [4]. Especially on large scales, there are diversified technical issues that most of the bioreactors have in common transfer of mass

and energy [5]. The purpose of bioreactor design is to accomplish conditions where different cell biomass can grow efficiently and produce a variety of products. The technical design must adapt to compensate for cell magnification dynamics and supply of nutrients in a profound manner [6]. The bioreactor design and its analysis are an integral part of any bioprocess development. The choice of bioreactor influences the cultivation of microorganisms in a controlled manner, reaction kinetics, mass transfer, heat transfer and often the metabolic pathways inside a microbial system [7]. Microorganisms are quite sensitive and fragile. Their morphological state and physiological behavior are very much affected by their habitat. Bioreactor must therefore be designed to mimic the preferable habitat of the microorganism which is also suitable for the production of desired metabolite. High-shear tolerant microorganisms exhibit robustness towards mixing phenomenon [8]. Aeration with or without mechanical agitation play a key role in changing the morphology of microorganisms and rheology of the fermentation medium. Stirred tank bioreactors are still widely used as they have high oxygen transfer characteristics. They are easy to design but develop higher shear stress compared with airlift bioreactors. Several empirical scale-up relationships have been used to correlate variables

such as oxygen mass transfer and shear rates, with agitation speed and reactor dimensions [9,10] but the applicability of bioreactor design to synthesize day-to-day useful products must be understood. Considering the significant role of bioreactors in various industries, it is not surprising that they are emerging in various forms and built up of different materials. However, the objectives in bioreactor design are common. The purpose of a bioreactor is to economize the process and bulk production of useful biochemicals to serve the mankind [11,12]. In this short communication, the significance of bioreactor design in process intensification has been focused through our research work on Cephalosporin-C production (as a case study).

Strategy 1: Different operational modes of bioreactors

The biosynthesis of Cephalosporin-C takes place in few microorganisms, viz. molds, actinomycetes (*Streptomyces* sp.) and few bacteria. Both free and immobilized microbial cells [13] can be utilized under batch, fed-batch and continuous cultivation modes [14]. Cephalosporin-C is produced in batch (stirred tank bioreactor) [15] as well as airlift bioreactor (batch and continuous mode) [16]. It was seen that the increase in production of Cephalosporin was achieved as the flow rate of supplementary medium is reduced in the fed-batch fermentation process. (Figure 1A,1B) showed the independence of Cephalosporin-C production rate from the growth rate of the organism. This indicated that the antibiotic is non-growth associated type where the conditions required for cell growth are different from those of antibiotic production. The specific product formation rate of Cephalosporin-C was highest when the specific growth rate of *C. acremonium* was at its least. The initial phase of rapid growth followed by a second phase of very little or no growth is necessary for higher concentrations of Cephalosporin-C.

Lower growth rates are observed during Cephalosporin production in fed-batch mode. In general, slow addition of supplementary feed containing hydrolyzed sucrose resulted in reduction in catabolite repression and thus the batch process in airlift bioreactor. The fed batch fermentation favored maintenance of higher antibiotic production rates during the process, resulting in a higher specific antibiotic production. This strategy is a boon to control substrate inhibition. In continuous mode of fermentation, it can be produced both by packed bed bioreactor using different types of immobilization processes and in continuous stirred tank bioreactor.

Secondary metabolites like antibiotic production occurs in late stationary phase (Idio-phase) of growth. The culture is grown to saturation level and then, the flow of nutrient is controlled to maintain the stationary phase [17]. Oxygenation of the media controls the metabolic pathway to produce the target drug in case of aerobic processes.

Strategy 2: Whole-cell immobilization

Whole cell immobilization and its applicability for antibiotic production was explored using packed bed bioreactor, operated in batch or continuous mode. The residence time and microbial reusability are high in case of packed bed reactors. Silk sachets were used as immobilization matrices for significant increase in production [13]. Cephalosporin C fermentation is a highly aerobic process. The mass transfer limitation of oxygen is a critical issue due to immobilized cells [18]. Lack of oxygen penetration into the cells lead to formation of Penicillin N instead of cephalosporin C production. Synergistic mixed culture technique for improved oxygen supply to the immobilized cells was also investigated in a packed bed column [19-21]. The second microorganism was benefitted by the first microorganism's product of metabolite

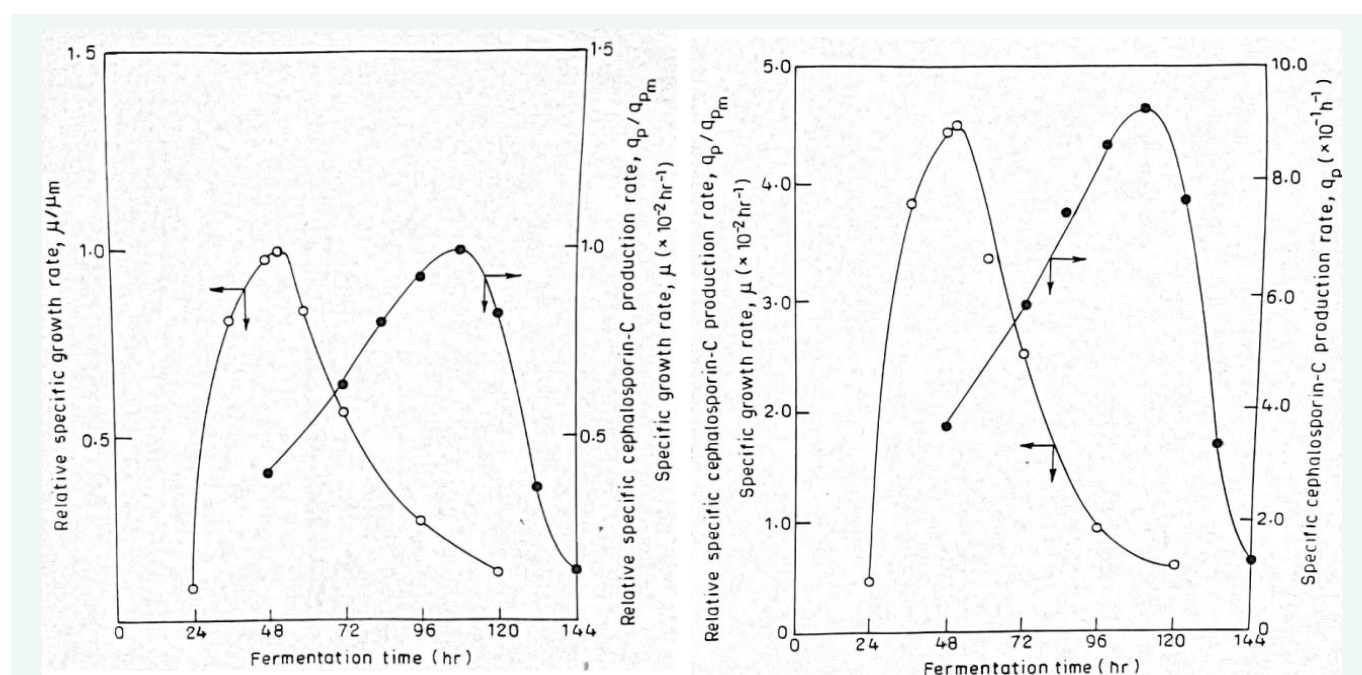


Figure 1 A) Plot of μ/μ_m and q_p/q_{pm} vs Fermentation Time of Batch Culture studies B) Variation of specific growth rate and specific product formation rate at different time periods of batch fermentation.

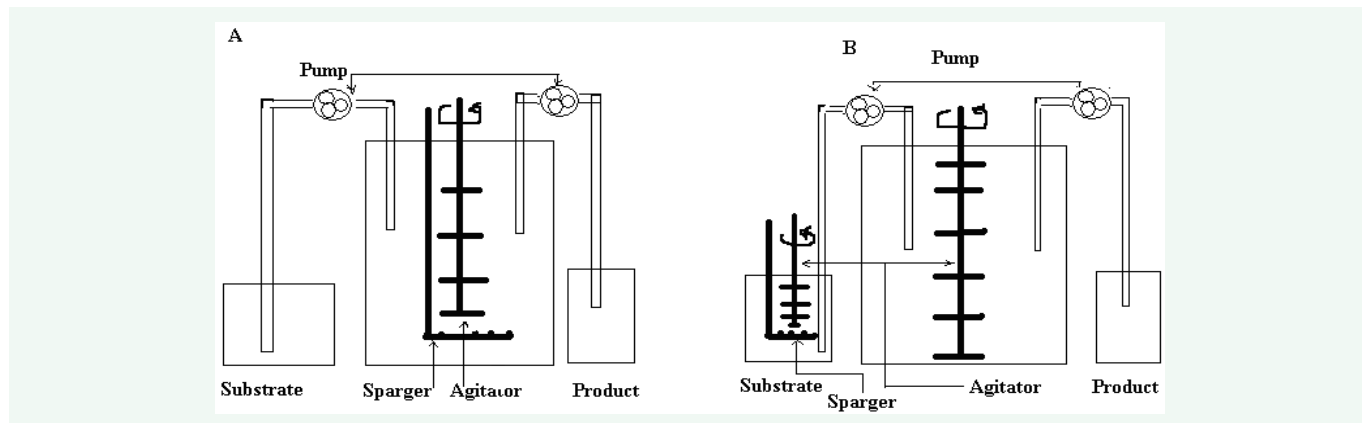


Figure 2 A) Continuous Bioreactor with oxygen Sparger B) Continuous Bioreactor with oxygen enriched fresh substrate.

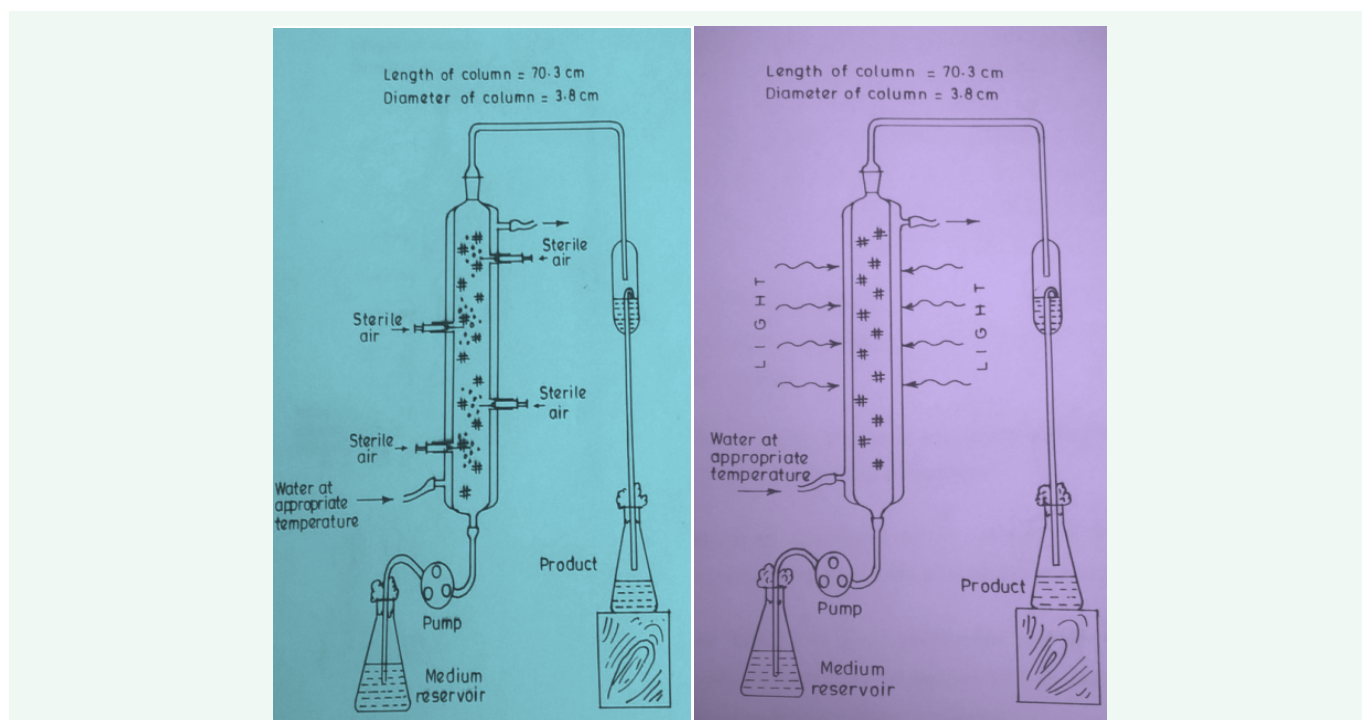


Figure 3 Packed Bed Reactor with a) monoculture b) Co-immobilized microbial cells (Algae and Fungi) for enhanced oxygenation [33,34].

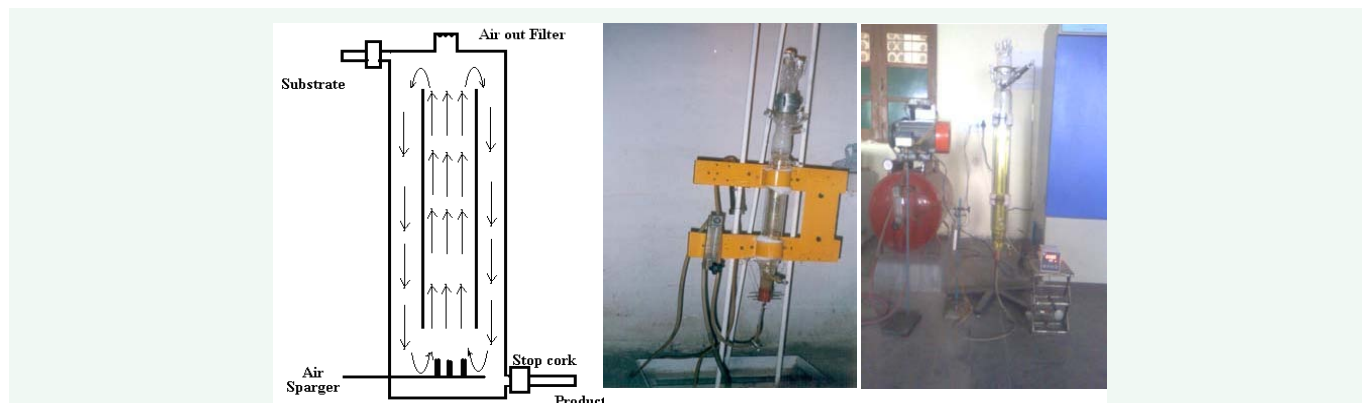


Figure 4 Internal air-loop reactor for Cephalosporin C production.

and the vice-versa. Photoautotrophic algae (*Chlorella* sp.) which produces oxygen *in situ* are coupled with fungi (*Cephalosporium acremonium*) which in turn produce the Cephalosporin C. The algae absorb carbon-di-oxide from air and media producing free oxygen which adds up oxygen to the media. Cephalosporin-C was also produced by immobilized microbial cells utilizing symbiotic mode (*in-situ* oxygen production) in a packed bed bioreactor using Bagasse, Silk sachets, calcium/Barium/strontium alginate and the same coated with poly-acrylamide resin [14,23]. Cephalosporin C acylase enzyme is involved in the conversion of Cephalosporin C to 7-ACA in single step mode of conversion. The microorganisms used for the synthesis of this enzyme are *Pseudomonas diminuta* [20], *Bacillus megaterium* and *E. coli* [22]. There is also study on continuous production of 7-ACA by loading immobilized microbial cell in a packed bed bioreactor at optimum cells to carrier ratio and at an optimum flow rate [15,25].

Strategy 3: Conventional and non-conventional bioreactors

The production of Cephalosporin-C was done using both conventional and non-conventional mode of bioreactors. Stirred tank bioreactor was used in batch or continuous mode as conventional bioreactor [19]. The morphological characteristics of the mold change under high agitation which in turn affects the yield of the Cephalosporin C [19]. Non-conventional mode involved packed bed bioreactors and airlift bioreactors [15,17,25,26]. Submerged culture was favored for improved oxygenation as antibiotic production is mostly aerobic. Actinomycetes and molds are filamentous in nature and take long time to reach stationary phase. Therefore, microorganisms are first allowed to grow under batch condition and then continuous mode of operation is started [17]. The non-conventional mode involves in either packed bed bioreactor or Airlift bioreactor. Airlift Bioreactors solve shear sensitivity issue of filamentous microorganisms and the oxygenation problem.

Internal loop Air Lift Bioreactor was found to be more suitable as compared to external loop air lift bioreactor for biosynthesis of Cephalosporin C. Due to its superiority for Cephalosporin C production. Cultivation of mold in external loop air lift bioreactor leads to cell deficient broth, which results in decreased Cephalosporin C concentration. Laboratory handling of external loop bioreactor is also more difficult as compared to internal loop air lift bioreactor. The internal loop airlift reactors have better volumetric oxygen mass transfer rate than external loop bioreactor [15,17,25,26]. The air lift reactor ensures low shear than conventional process agitator, improving production. The process is costlier and tough. But, it ensures improved production of this low volume and high value product. Amongst few novel antibiotics, lipopeptide antibiotics have emerged as stars of the recent times. Different modes of bioreactors have influenced the growth of the microorganism and the production of the antibiotic. Fed-batch strategy or the semi-continuous mode is beneficial in case of several antibiotic production as they often suffer precursor or carbon source inhibition [27-29]. Design and development of bioreactor and their analysis holds a great importance for both conventional and non-conventional antibiotics [30-32]. Daptomycin is a promising member of the lipopeptide antibiotic family, which has displayed a broad

spectrum of activity *in vitro* against a wide range of gram-positive bacteria, including Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococci. Daptomycin is not affected by mechanisms that confer specific resistance to beta-lactam agents (including methicillin), glycopeptides (such as vancomycin), quinupristin/dalfopristin, linezolid or other agents potentially useful against Gram-positive bacteria species. The unique mechanism of action and low resistance profile, together with rapid bactericidal action make Daptomycin a promising alternative in future to act against resistant organisms. Improved production of Daptomycin was achieved through improved broth rheology and controlled hydrodynamics. Also, we have tried to study the pattern of oxygen mass transfer using air-lift bioreactor with different modes of cell growth. The change in morphological features of the microbe inside the bioreactor was useful for process intensification [33,34].

CONCLUSIONS

Different operational conditions have been considered for various process development. The control over process variables using different bioreactors for Cephalosporin-C production was highlighted in these studies, based on design and functionality. The scale-up of a process is definitely an important aspect. Several problems concerned with antibiotic fermentation were dealt using various types of conventional and non-conventional modes of bioreactors. Non-conventional type of bioreactors (absence of mechanical agitators) dealt with shear sensitivity issues of *Cephalosporium acremonium* and other microbes which are primarily responsible for antibiotic fermentation. Drugs like Cephalosporins and Daptomycin effectively deal with life-threatening infections, especially drug-resistant taphylococcal infections. Several literary reports suggest antibiotic improvement through genetic engineering and metabolic engineering. Antibiotics are low volume and high value products. Therefore, efforts to improve their bulk production using suitable type of bioreactor would help to bring down the cost and improve the productivity. Latest techniques involve micro and mini-multi bioreactor systems, statistical methodologies for optimization, scaled-down process unit systems and other bio analytical instruments. Conventional strategies discussed here can be combined with latest tools and techniques to have substantial impact on the product and process development.

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Cite this article

Chakravarty I, Kundu K, Ojha S, Kund S (2017) Development of Various Processing Strategies for New Generation Antibiotics Using Different Modes of Bioreactors. *JSM Biotechnol Bioeng* 4(1): 1073.