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A review on production of echinocandins by *Aspergillus sp.*

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Abstract

For the last two decades Echinocandins have successfully emerged out & gained considerable importance when introduced in the World Pharma Market. Echinocandins are new novel class of drugs for fungal infections. Echinocandins inhibit an enzyme necessary for the formation of fungal cell wall components, thus disrupts the integrity of the cell wall and eventually leads to cell death. They are fungicidal and less toxic to the host by virtue of their novel mechanism of action. *Aspergillus sp* is the most investigated species among many fungal species producing secondary metabolites of this kind. Bioprocess parameters help us to understand the relationship between the fungal growth and its secondary metabolite (Echinocandins) in large scale The objective of the present review is to provide an updated & thoughtful overview for the appropriate fermentative production methodology.

Key words: Fermentation; Echinocandins, antibiotics, Bioprocess and Growth

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Introduction

Microorganisms had long been used for the production of innumerable daily consumable fermented products. With the involvement of improved techniques like bioconversion using fermentation and enzymatic application ,the scale of microbial production had been increased by many folds.

The majority of these microbial products technically are secondary metabolites in nature. Secondary metabolites are natural products which are different from primary metabolites, as they are not directly involved in growth, development, and reproduction of a living organism and are often used in defense against predation and habitat encroachment, or even used as means of communication. So far, 1500 compouds (Secondary metabolites) in fungi have already been isolated. The most heard & important among them are antibiotics and pigments. Besides being to be the antibacterial/ antifungal ,their newly bio-transformed versions are used in other applications like anticancerous, immunosuppressor, bio-insecticidal/ herbicidal functions.

The Echinocandins are non competitive β-1, 3-glucan synthase inhibitors (Chen *et al.* 2011) manufactured synthetically through considerable modification in lipopeptides, after obtaining from fermentation broths of enormous fungi. Echinocandins are a novel class of antifungals that inhabits minimal toxicity to the host by virtue of their unique mode of action.. It is most often used against Candidiasis and Aspergillosis. They are fungicidal against some yeasts (most species of Candida, but not against Cryptococcus, trichosporon and Rhodotorula), fungistic against some molds (Aspergillus, but not Fusarium and Rhizopus), and modestly or minimally active against dimorphic fungi (Mukherjee *et al.* 2011).
The identification of the earliest lead compound (for anidulafungin) was first highlighted in 1974. For the treatment of invasive fungal infections (IFIs) in adults caspofungin was approved in January 2001 by the US FDA (July 2008 for use in children >3 months of age). Approval for clinical application was licensed to two more Echinocandins, micafungin (approved March 2005) and anidulafungin (approved February 2006) have been licensed. Caspofungin and anidulafungin are available worldwide, whilst micafungin is marketed in the EU, US, Japan and parts of Asia. A large clinical efficacy trials have clearly given the evidence of the promise of the Echinocandins as effective against the anti-*Candida*

and anti-*Aspergillus* agents has been reflected by; these agents have had significant impact on the treatment of invasive Candidiasis (IC) and invasive Aspergillosis (IA) (Chen et al. 2011; Mukherjee et al. 2011).

Liver serve as the prominent site for the metabolic degradation of Echinocandins (also in the adrenals and spleen) through hydrolysis and *N*-acetylation. After the initial distribution phase, hepatic uptake—and final degradation—is slow (for caspofungin and micafungin), leading to a long terminal half-life.The extensive uptake of micafungin by red-blood cells was reported(Sucher *et al.* 2009). Two unfamiliar metabolites from micafungin possess the antifungal characterstic. These degradation products are excreted slowly over many days, mainly in the bile. Studies through radio labeling techniques has suggested most residual drug or metabolite are contained in the liver, renal cortex, and skin (Cappelletty & Eiselstein-McKitrick 2007).

Through combination therapy Echinocandins provide an exciting option (as it provides no cross resistance) with other antifungals especially against the fulminant fungal infections. More antifungal agents have reached clinical use in the past two decades than at any other time. The Echinocandins have been a welcome addition to this group, with the latest being anidulafungin (Morris & Villmann 2006). There are several lines of evidence to support anidulafungin's role as primary therapy for the treatment of invasive candidiasis in non-neutropenic patients, and as alternative therapy to fluconazole in patients with esophageal candidiasis with azoles intolerance or triazole-resistant *Candida* (Kim *et al.* 2007).
Numerous worldwide industries where researches and manufacturing of Echinocandin are ongoing are Concord biotech, Biocon, Richcore laboratories, Pfizer and Eli Lilly, Aventis Pharma, Astellas Pharma etc.

Normally,the production of Echinocandins from microorganisms is greatly influenced by media components, e.g. variation in C/N ratio, presence of some easily metabolizable sugars, such as glucose (Beg *et al.* 2002), and metal ions (Varela *et al.* 1996). Antibiotic synthesis is also strongly affected by rapid metabolizable nitrogen sources, such as amino acids present within the medium. Alongwith that, there exist so many other physical parameters, like aeration, inoculum density, pH, temperature and incubation, also influence over the amount of antibiotic produced (Hameed *et al.* 1999; Puri *et al.* 2002). In order to upscale the antibiotic production from microorganisms at the industrial & commercial level, biochemical and bioprocess engineers employ many advance technological methodologies & strategies to achieve tremendous productivity of antibiotic in a bioreactor. Recent years has witnessed that there has been an extensive amount of research and development efforts are focused especially on the use of statistical approach methods, applying many different mathematical & statistical software packages during process optimization studies, with the target of attaining an increment in the yield of biosynthetic products in the fermentation medium (De Coninck *et al.* 2000; Puri et al. 2002; Varela et al. 1996). Great number of complicated mechanisms and operations control the production during the transition state between log and the stationary phase (Priest 1977; Strauch & Hoch 1993).
As far as our knowledge is concerned, so far not much report was communicated for Echinocandins production in large scale. Hence, in the present review, we mainly emphasized in the discussion over the effect of nutritional and physicochemical parameters in fermentative production of Echinocandins. For analysis of the fermentation process with respect to the biological significance of each parameter and their levels with statistical consistency to design and operate a bioreactor for enhanced yield and productivity this productive piece of information is highly essential to be under consideration.

Origin of Review

It was the studies on Papulacandins that was isolated from the strain of *Papularia sphaerosperma* (Pers.).But these papulacandins were narrow spectrum antifungals & had therapeutic application only on Candida spp. In 1970,after fungal fermentation the screening of products led to the discovery of Echinocandins that shows broad range of activity.The first Echinocandin was pneumocandin though the isolated product from the fermentation was Echinocandin B but it could not find much application due to its side effect of hemolysis. In 1980, through the semi synthetic modification of the Echinocandins, Cilofungin production was reported. It was the first Echinofungin analog to enter the clinical trials. But it was withdrawn due to its enhanced toxicity level. The first approved Echinocandin was Caspofungin, followed by micafungin and anidulafungin to get the approval. Fungal infections are common problem in high risk patients such as immunocompromised patients, resulting from AIDS infections, cancer treatment, the growing use of organ transplant and other nosocomial situations (Chen et al. 2011; Fujie 2007). Serious deep mycosis such as invasive candidiasis can often be fatal disease. The risk of developing deep mycosis has increased due to an increase in the number of patients with defects in the immune system (Mukherjee et al. 2011). Candida is currently predominant fungal pathogen in such patients. However, invasive Aspergillosis has been increasing in incidence and Aspergillus is the most causative pathogen during bone marrow transplantation (Chen et al. 2011).

Amphotericin B, flucytosine, the imidazole miconazole, trizoles fluconazole, itraconazole and voriconazole are some of the synthetically available fungicides. But these drugs exhibits certain adverse side effects regarding antifungal spectra of activity, toxicity or resistance. Amphotericin B was the only systemic antifungal agent for the treatment of invasive fungal infections (IFIs) for the last so many years. In the 1990s, the advent of the triazoles and lipid amphotericin B formulations provided the alternative therapeutic options. However, renal toxicity remains a major drawback of amphotericin B formulations, whilst drug interactions, hepatotoxicity and limitations to use in renal failure are primary concerns with newer-generation azoles (Chen et al. 2011; Ostroumova *et al.* 2012; Young *et al.* 2012). Fluocytosine has problem regarding tolerance and is rarely used alone today. As for azole antifungal agents such as miconazole and flucanazole their wide application led to the development of azole resistance in *Candida* species (Ji *et al.* 2011; Schmalreck *et al.* 2012).

Taking account of these drawbacks, the requirement of much safer, more effective and greater clinical utility antifungal agent. Researchers focused on 1, 3-β-D-glucan, a key component of fungal cell wall, which is not present in human cells and thus developing an antifungal agent that inhibits the synthesis of 1, 3-β-D-glucan (Thompson *et al.* 2009; Zonios & Bennett 2008). Mammalian cells do not contain 1, 3-β-D-glucan polymers,thus causes the inability of the presence of mechanism that is based on toxicity .Hence account for good tolerability of the Echinocandins.

An advance & novel class of drug, the Echinocandins, has been successfully used against Candidiasis and Aspergillosis and other human mycosis. They demonstrate a wide range of antifungal activities that constitute the fungicidal action as against some yeasts (most species of *Candida*, but not against *Cryptococcus*, *Trichosporon* and *Rhodotorula*), fungistatic against some molds

(*Aspergillus*, but not *Fusarium* and *Rhizopus)*, and modestly or minimally active against dimorphic fungi (*Blastomyces* and *Histoplasma)*. All the Echinocandins have a similar spectrum of antifungal activity but have significant illustrative structural differences. Caspofungin, Anidulafungin and Micafungin are the three so far clinically proven Echinocandins, i.e ,the antifungal agents that acts as potent inhibitors of the 1, 3-β-D-glucan lipopeptides present in the fungal cell wall. (Chen et al. 2011; Mukherjee et al. 2011).

Different Forms of Echinocandins

Caspofungin Acetate (MK-991, formerly L-743, 872)

The first noncompetitive glucan synthase inhibitor that is semi-synthetic, water-soluble cyclic lipopeptide antifungals is Caspofungin Acetate.The patients suffering from invasive Aspergillosis who are sensitive or failed to respond against the synthetic forms of the antifungals like azoles ..The chemical structure of CAS is shown in Figure 1.

Figure 1: Chemical structure of Caspofungin Acetate.(CAS).

Mechanism of Action

The synthesis of β-(1,3)-d-glucan,(Chitin and glucan fibrils are very necessary of cell division and cell growth (Krause *et al.* 2004a; Vazquez 2005).) a homopolysaccharide component of the cell wall get blocked. (Phai Pang *et al.* 2011; Yao *et al.* 2011).by CAS. As Mammalian cells do not contain 1, 3-β-D-glucan polymers, indicating good tolerability of the Echinocandins (Vazquez 2005). The MIC (minimal inhibitory concentrations) and MFC (minimal fungicidal concentrations) for Echinocandins are low for a broad spectrum of fungi (Phai Pang et al. 2011; Yao et al. 2011).

Spectrum of activity

The *in vitro* and *in vivo* antifungal activity of CAS has shown its efficacy against *Aspergillus spp*. like *A. fumigatus, A. flavus, A. niger, A. terreus, and A. Nidulans* and *Candida spp.* (the most common cause of nosocomial fungal infections) like *C. albicans, C. glabrata, C. krusei and C. tropicalis*, including non-albicans species (Ernst *et al.* 2000) and isolates resistant to other drugs. In animals, it also has activity against the cyst form of *Pneumocystis carinii* (Morrison 2002; Pacetti & Gelone 2003) the drug has little or no activity against *Cryptococcus neoformans* (Krishnarao & Galgiani 1997). Hence, it is evident that CAS belongs to broad spectrum of antifungals.

Adverse effects

Histamine sensitive reactions like fever and rash, infusion-related reactions including phlebitis, transient elevations in liver transaminase levels, headache, nausea and anemia (Keating & Figgitt 2003; Sable *et al.* 2002), facial flushing has occurred during infusion, embryotoxicity in animals are some of the undesirable effects of CAS reported so far.Along with that few incidents of pulmonary infiltrates and hypercalcemia , two drug-related unfavourable events has been also reported (Keating & Jarvis 2001). Further study conducted on 623 patients, it was found that no serious clinical or laboratory drug-related event was happened.(Sable et al. 2002). But when compared to AMB , CAS has been related with fewer adverse effects , and hence more preferable. (Kartsonis *et al.* 2002).

Anidulafungin (LY303366; [ANIDU])

Anidulafungin is a semi-synthetic lipopeptide of the echinocandin B class, synthesized from afermentation product of *Aspergillus nidulans*. The chemical name for anidulafungin is 1-[(4R, 5R)-4,5-dihydroxy-N(2)-[[4"-(pentyloxy)[1,1':4',1"-terphenyl]-4-l]carbonyl]-L-ornithine] echinocandin B. It also possess the similar mechanism of action & a promising broad-spectrum, antifungal activity in vitro and in vivo as CAS . FDA has given its approval against invasive candidiasis, candidemia and esophageal candidiasis (Mukherjee et al. 2011; Spreghini *et al.* 2011). The chemical structure of Anidulafungin is shown in Figure 2.

Figure 2: Chemical structure of Anidulafungin.

Spectrum of Activity

Anidulafungin demonstrated the most immediate noticeable activity against Candida species including C. glabrata and C. Krusei but showed negligible activity against *Cryptococcus neoformans* (Krishnarao & Galgiani 1997). The clinically tested assured activity of ANIDU against *Aspergillus spp*. and other species of filamentous fungi has also been seen (Pfaller *et al.* 1998). Against *C. albicans, C. tropicalis, C. Glabrata and C. krusei,* but less against *C. famata and C. parapsilosis*, the in vitro activity of ANIDU was found superior compared to ITRA and FLUC.

Adverse Effects

The relevant combinational adverse event rate was 46%, but only 5% of these were directly related to the anidulafungin (Krause et al. 2004a) has been reported. The most familiar unfavourable events included hypotension (13%), vomiting (13%), constipation (11%),

nausea (11%) and pyrexia (11%), but none of them needs dosification. No systemic infusion-related undesirable reactions or anaphylactic reactions occurred when 1700 doses of anidulafungin were given. In another study, treatment related hilarious events were reported in only 9.3% of patients (Krause *et al.* 2004b).

Micafungin (FK-463; [MICA])

A new lipopeptide echinocandin with a broad-spectrum *in vitro* and *in vivo* antifungal activity, against both *Aspergillus* and *Candida species*. comes to successfully meet in micafungin. After its justified screening as fermentation product,the final synthesis take place by chemical modification. The mode of action is similar to CAS (Farowski *et al.* 2012). The structure of micafungin is shown in Figure 3.

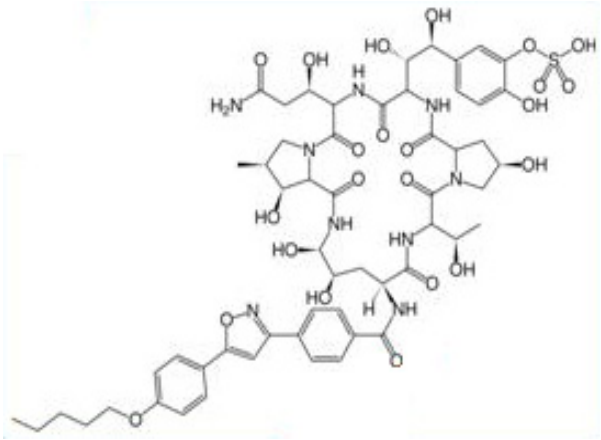

Figure 3. Chemical structure of Micafungin.

Spectrum of activity

Aspergillus species are the most diverse group of prominent species against which the MICA are found to be most effective in its action .Moreover (except for certain clinical isolates) it is active against the dematiaceous fungi *Cladosporium trichoides, Exophiala spinifera, Fonsecaea pedrosoi*, and *Exophiala dermatitidis*. However, no activity against *Fusarium solani, Pseudallescheria boydii, and the zygomycetes Absidia corymbifera, Cunninghamella elegans, Rhizopus oryzae* and *Rhizopus microsporus var. rhizopodiformis* had been found. The infections caused by the *Candida* and *Aspergillus species* and dematiaceous fungi (Nakai *et al.* 2002)are particularly treated by MICA.

Safety and efficacy

At a dose of 2-10 mg/ kg body weight, MICA was tested enormously effective than AMB or FLUC against an AMB and FLUC-resistant *C. tropicalis* specimen (Warn *et al.* 2002). Although MICA appears to be a validated promising agent for invasive fungal infections it requires further clinical evaluation & confirmatory testings.

Fermentative production of echinocandins

The technology of fermentation process (bioprocess) which is a sophisticated,multi-phase,multi-component system is basically employed having microbial cells with their favourable raw materials, biomass, and with proper aeration and agitation. Appropriate & convenient technique is applied for sterilization of medium. And aseptically transferred to fermenter, equipped with agitators, baffles, air sparger as per the design of the process. Various sensing devices control the various physical parameters of the fermentation. Lab Inoculum is a pure strain of microorganism. Favourable conditions such as optimum temperatures, pH, sufficient substrate, nutritional salts, vitamins and oxygen (for aerobic organisms) should be properly maintained inside the fermenter , enabling the organism to grow and produce finished metabolites. The exponential multiplication of cells will start after a certain period of lag phase and eventually reach to a maximum cell concentration as the medium goes on depletion. In a batch culture, the fermentation process will be terminated at the end of the cycle and the broth will be transferred to the Down Stream Processing Department. Hence, the entire process can be distinguished into three stages;

Stage I: Upstream processing that primarily involves preparation & sterlisation of submerged medium, air purification & separation of particulate and inhibitory chemicals from the medium.
Stage II: Fermentation process where the fundamental conversion of substrates to the desired finished product with the aid of biological agents & biocatalyst such as microorganisms and enzymes are performed.
Stage III: Downstream processing mainly involves separation of biodebris and biomass from the fermentation broth, purification and concentration of the desired product .After that waste disposal or recycling of waste depending on the type & nature of waste is ultimately done.

On the basis of many factors like type of product, the concentration levels production and the desired purity,the fermentation stage can constitute between 5-50% of the total fixed and overall operating costs of the process. Hence, operation of a bioreactor with its optimal design frequently dominates the overall technological and economic performance of the process. This process can be operated either in a batch mode or continuously.

On a large scale basis, to carry out the process it is necessary to examine the three distinguished principle areas of implication :

1. to screen out the best possible acquirable biocatalyst (microorganisms, animal cell, plant cell, or enzyme) and media optimization for a desired product formation.
2. To make possible the adequate provision of the best environment for the biocatalyst to perform the designing of the bioreactor and operating it in an efficient manner.
3. to churn out the desired products from the reaction mixture in the most economical & beneficial way

Microorganism

Selection of a good & improved microorganism is imperative to obtain the desired product. The selected microorganism should be able to produce sufficient productivity ,to secrete large amounts of proteases and production of toxins or any other undesired products should be minimum. Potential hosts should be suitable for industrial fermentations and produce large cell mass per volume quickly on cheap media (Kirk & Othmer 1994).

Aspergillus nidulans is filamentous fungi species that belong to the phylum of Ascomycota. The genus of *Aspergillus* consist of numerous mold species in many climatic conditions across the world. They possess commercially important medical significance also an important research organism for the study of eukaryotic cell biology since the last 50 years. *Aspergillus* species including *Aspergillus nidulans* proved to be a great source of secondary metabolites producton. The genomic sequencing of *Aspergillus nidulans* exposed that there are more than 40 secondary metabolite

pathways present in this species, but at the time of compilation of the genome only less than ten had been discovered (Galagan *et al.* 2005). Echinocandin, lectin, polyketides and non ribosomal factors are contained by *Aspergillus nidulans* that produces the secondary metabolites .

Medium Design

Among the presence of several secondary metabolite producers, fungi are exclusively employed for the metabolite production though the levels of production do not reported to go beyond few tens of milligrams per litre (Gouka *et al.* 1997). Filamentous fungi have the feature to utilize a great variety of carbon and nitrogen sources by secreting a range of different metabolite into their existing surrounding . Some key intermediates of primary metabolism serve as branching points of biosynthetic pathways leading to end products of primary and secondary metabolism. Many essential factors like precursors, carbon sources, nitrogen sources, phosphate, trace elements, induction of enzymes of secondary metabolism, catabolic repression and inhibition, feedback repression and inhibition mainly regulates the production of secondary metabolites whereas the control is done by autoregulators (Betina 1994).

The level of presence of the cell precursor may regulate secondary metabolite biosynthesis most commonly antibiotic, especially when the particular synthase is already active in the cells (Betina 1994). There are differences between the carbon sources for growth and secondary metabolism. For example, glucose is normally the best source for the growth but can hindered the production of the secondary metabolism. As it has been revealed to influence the production of actinomycin, benzodiazepine alkaloids, cephalosporin, chlorotetracycline, enniantin, ergot alkaloids, erythromycin, kanamycin, oleandomycin, penicillin, puromycin, tetracycline and tylosin (Chadwick & Whelan 1992).

The impact of nitrogen sources on secondary metabolism are restricted by numerous factors that includes the type of metabolic pathway, the producing organism, the type and concentration of the nitrogen sources and the stage of cultures whether in stationary or submerged phase. Nitrogen sources are favourable for growth but exhibits negative effects over secondary metabolic pathways (Betina 1994). For example, negative impact of ammonium salts have been demonstrated in the production of cephalosporine, penicillin, erythromycin, tylosin, leucomycin, chloramphenicol, macbecin, rifamycin, streptomycin, streptothricin and tetracycline (Chadwick & Whelan 1992). It is known that many trace elements areimportant for microbial growth because of their involvement as metalloenzymes or as enzyme activators. Zinc, iron and manganese are the most important trace elements in promoting the secondary metabolism,. Numerous reports have been published on the importance of these three elements in secondary metabolite biosynthesis (Betina 1994).

While improving a biotechnological industrial process, designing of the fermentation medium is of utmost importance as it influence the product concentration and volumetric productivity. It is also important to reduce the cost of the medium as much as possible, as this may affect the overall process economics. Medium screening studies are very time consuming and expensive. This is because the number of possible media combinations that can be tested and the number of fermentation substrates that are available are also very large. Rapid identification of the variables essentially should be controlled for optimizing production of useful metabolites thus economizing the efforts.

Effect of medium sterilization on metabolite production

Shake flask cultures are mainly applicable when submerged aerobic cultures are employed & the improvement of media and selection of microorganisms, are conducted in industrial microbiology This is because the scale is convenient and replication can be easily achieved. Production variables such as autoclaving procedures, inoculum size, temperature, medium composition, pH and agitation can be identified and their effect on fermentation productivity are determined by the involvement of sequential cultures where only one variable at a time is varied. These investigations provide a basis for the manipulation of production variables in order to optimize the product concentrations.

In benchscale studies it is imperative to examine the effect of autoclaving of the fermentation medium on bio productivity. Sterilization of fermentation media causes the denaturation of the vital nutrients (e.g. vitamins, amino acids, and sugars). The formation of toxic and noncompatible media may interfere the growth and product formation.(Anderson *et al.* 1986). Analysis in the compromise between the substrate quality and the risk of contamination of the raw materials requires to be done. Sterilization conditions have been shown to affect the overall performance of the fermentation p. For example, Efrotomycin production by *Nocardia lactamdurans* was greatly improved by sterilizing glucose together with the rest of the medium components (Jain & Buckland 1988),Zaragozic acid production by *Leptodontidium elatius* was also improved under different autoclaving conditions (Connors *et al.* 1995).

Optimization of physicochemical parameters

Inoculum size, oxygen transfer rate, pH and temperature shows diverse effects on product formation during any bioreactor operation while performing the aerobic fermentation processes by altering the metabolic pathways and influencing the metabolic fluxes (Çalık *et al.* 2001). The morphology of the colony is mainly affaected by the inoculum size. A close relationship between a particular morphology and increased process productivity is characteristic of a number of industrially important fermentations (Calam 1987; Kristiansen *et al.* 1999). The role of fungal morphology in relation to formation and secretion of metabolite has been evaluated in *Aspergillus niger* (Papagianni & Moo-Young 2002). Manipulation in the morphology is mainly done by means of inoculum levels. Thus, morphological development in filamentous fungal fermentations can be manipulated by inoculum level (Angelova *et al.* 1998; Chen *et al.* 1999; Domingues *et al.* 2000; Papagianni & Moo-Young 2002). The pattern of expression of metabolite in *Aspergillus niger* can be modified by altered in the inoculum size (Papagianni & Moo-Young 2002). Quality inoculum,as reflected in terms of size, type or age, is of utmost importance in evaluating the outcome of filamentous fungal fermentations (Dobson *et al.* 2008; Gancheva & Dimova 1984; van Suijdam *et al.* 1980; Vecht-Lifshitz *et al.* 1990).

Bioprocess parameters such as incubation temperature and pH of the growth medium are the most important that are mainly tried to keep both these variables constant at their optimal values throughout the fermentation process. The influence of temperature and pH on a bioprocess can be very different, and since the growth process is the result of many enzymatic processes the influence of both culture parameters on the overall bioreaction is quite complex (Çalık et al. 2001). It has also been demonstrated that the production of secondary metabolites is influenced by pH (Murphy & Power 2001; Song *et al.* 2008). This parameter normally affects the morphology and the metabolite production pattern of the genus *Aspergillus*

(Michelin *et al.* 2011; Schugerl *et al.* 1998) and can improve its stability (Wiebe 2003).

The influence of temperature as for the activity of an enzyme is found to be same as on the maximum specific growth rate of a microorganism .Specific growth rate is gradually increased up to optimum temperature. But beyond the optimum value a rapid decrease in the specific growth rate was observed (Nielsen & Villadsen 1994). The mechanism of temperature control of metabolite production is not well understood (Chaloupka 1985). However, studies performed by Frankena *et al*. (Frankena *et al.* 1986) showed that a link existed between metabolite synthesis and energy metabolism in microorganism, that was controlled by temperature and oxygen uptake.

The pH of culture strongly influence the tremendous enzymatic processes and transport of several species across the cell membrane. Variation in pH changes the acid-base equilibria and fluxes of various nutrients, inducers and growth factors between the abiotic and biotic phase (Moon & Parulekar 1991). The influence of pH on cellular activity is determined by the sensitivity of the individual enzymes to changes in pH. Enzymes are normally active only within a optimum pH interval and the total enzyme activity of the cell is therefore a complex function of the environmental pH. Microbial cells possess a remarkable power to maintain the intracellular pH at a constant level even when the remarkable variations in the pH of the extracellular medium are done (Nielsen & Villadsen 1994).

The medium turned acidic when ammonium ions were used,, while it turned alkaline when organic nitrogen, such as consumption of the amino acids or peptides (Moon & Parulekar 1993). The decline in the pH may also be due to production of acidic products (Moon & Parulekar 1991). Taking account of the close relationship between protease synthesis and the utilization of nitrogenous compounds, variations in pH during fermentation may provide the kinetic information about the metabolite production, such as the start and end of the metabolite production period. Temperature also serve as another very important physiological factor that needs to be controlled and altered from one type of microorganism to another.
The aeration rate indirectly indicates the dissolved oxygen level in the fermentation broth. Different dissolved oxygen calculations & profiles can be obtained by: (i) variations in the aeration rate; (ii) variations in the agitation speed of the bioreactor; or (iii) use of oxygen rich or oxygen deficient gas phase (appropriate air-oxygen or air-nitrogen mixtures) as the oxygen source (Michalik *et al.* 1995; Moon & Parulekar 1991). The variation in the agitation speed affects the extent of mixing in the shake flasks or the bioreactor and will also affect the availability of the nutrient. However, lowering the aeration rate caused a sharp drastic reduction in the metabolite yields (Moon & Parulekar 1991). This indicates that a reduction in oxygen supply is a critical limiting factor for growth as well as biosynthesis of antibiotic.

Diverse influence of oxygen transfer take place on the product formation in aerobic fermentation processes by affecting the metabolic pathways and variations in metabolic fluxes. As per the analysis of cell growth conditions and metabolic pathway some biosynthetic processes need high oxygen transfer rate conditions while others require controlled oxygen transfer rate conditions (Calik *et al.* 1999). It has been extensively investigated in defined (Calik *et al.* 2000; Çalık *et al.* 1998; Calik et al. 1999) and molasses based complex medium (Çalık *et al.* 2003) medium oxygen transfer conditions were found to be favorable for metabolite production.
Bioreactor

The harvesting & involvement of bacteria, yeasts and fungi, is an ancient fermentation technology whereas the application of bioreactors for the production of fermentation products is a modern approach. This particular process involving sterile, aseptic and controlled conditions for the production of antibiotics that was started in the early 1940's.During the scale up process it is necessary to investigate the type of bioreactor is the most efficiently suitable according to the physical and physiological requirements of the microorganisms so used before starting further optimizations. It has been found that the stirred tank reactors are the most widely used type in the production of pharmaceutical compounds. These types of reactors offer a few advantages, such as independent control of mixing conditions and aeration rate. But the high energy consumption and high shear stress affecting such fragile cells as fungal cells are few drawbacks.

Bubble column bioreactors (BC) proved to be a nice alternative to the stirred tank bioreactors (STR). Bubble column reactors possess a relatively simple construction with the absence of mechanically moving friction porionts. So,they have low maintainenance & operating costs. With regard to the internal flow and efficiency behaviour, they offer a large interfacial area and transport rates leading to excellent heat and mass transfer characteristics and more suitable shear conditions for fungal growth and production. From laboratory scale to production, scale up of recently developed fermentation bioprocesses is normally found to be a complicated and time consuming. Particularly, the scale up from shake flask to bioreactor is a tough job. It has been reported that the oxygen transfer rate and the specific power input are most often used for scale up .(Bapat & Wangikar 2004; Humphrey 1998; Jin *et al.* 2004)

Conclusion

Microbial metabolism is a complex biogenetic pathway, differing in terms of biomass and associated bioactive secondary metabolite production with only slight variation in physiological, fermentation and medium components. Hence it is a comprehensive quantitative and mechanistic preliminiary evaluation for complete metabolic information of processes, mass balances, inhibitions and production processes that is most often very difficult or impossible in the case of normal fermentation process results. So, in the present article, an attempt has been elucidated to explain the effect of nutritional and physicochemical parameters on fungal Echinocandins production. This is important for obtaining higher Echinocandins production as well as for the reduction of process operating cost.

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