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Review

Bioreactors in solid state fermentation technology: Design, applications and engineering aspects

Sidharth Arora, Richa Rani, Sanjoy Ghosh*

Biochemical Engineering Laboratory, Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee, 247667, India

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ABSTRACT

In recent years, substantial credibility in employing Solid-State Fermentation (SSF) technique has been witnessed owing to its numerous advantages over submerged fermentation (SmF). In spite of enormous advantages, true potential of SSF technology has not been fully realized at industrial scale. The lack of rational and scalable bioreactor designs backed by mathematical models and automated control system that could successfully address heterogeneity with respect to heat and mass, and also operate aseptically, remains the prime reason for it. As a result, there still exists vast scope in SSF bioreactor research and development to facilitate broad spectrum of biotechnological applications. The present article reviews state-of-the-art in SSF technology with focus on bioreactors that have been employed for bioprocess applications, in particular, enzyme production. Based on the mode of operation, bioreactors are divided into four categories with emphasis on design features, effect of operating conditions on productivity, applications and limitations. Selected modeling studies developed over the years, have been revised and presented in problem specific manner in order to address the limitations. Some interesting designs including few recent ones that have been proposed and/or employed at pilot and industrial levels are discussed in more detail.

1. Introduction

The recent surge in demand for large quantity of biologically active secondary metabolites (antibiotics, bacterial toxins, immune drugs, and alkaloids), single cell proteins, enzymes, industrial chemicals, biofuel, food, phenolics, feed, and pharmaceutical products (Thomas et al., 2013; Pandey, 2001) has made SSF technology as an alternate production method to submerged fermentation (SmF), the need of the hour. In addition to the production of bio-active products of commercial interest, there is also a growing popularity of SSF to be used as waste management technology, applications of which may include bioremediation, detoxification, bioleaching and biopulping (Thomas et al., 2013; Singhania et al., 2009; Krishna, 2005). The technique with its broad application and operational advantage over SmF (Subramaniyam and Vimala, 2012; Cunha et al., 2012; Sun and Xu, 2009; Holker and Lenz, 2005) (Table 1) has led to significant research inputs eventually assisting in better reactor design, operation and scale-up strategies (Mitchell et al., 2006). In spite of advances, major hindrance in industrialization of SSF process remains the lack of simple, efficient and easily scalable bioreactors that could successfully address heat build-up, heterogeneity (heat and mass), and at the same time operate with utmost sterility (Papagianni, 2014). This is probably due to combination of three factors i.e. lack of efficient bioreactor design, lack of mathematical models describing the transport and kinetic phenomena at micro- and macro-scopic levels and the lack of effective online process monitoring and control strategies. However, in recent years there have been reports of few bioreactor systems that have at least partially overcome these challenges for a specific application, there still exists a vast scope for improvement to address a broad spectrum of biotechnological applications.

In this review, based on the mode of operation, SSF bioreactors have been classified into four categories. Description under each category

Abbreviations: AFR, Air flow rate; APP, air pressure pulsation; APP-SSF, air pressure pulsation solid-state fermentation; ASFB, air-solid fluidized bed bioreactor; a_W, Water activity; Bt, *Bacillus thuringiensis*; CER, carbon dioxide evolution rate; CFU, colony forming unit; CMC, Carboxy methyl cellulose; *D*, Axial dispersion coefficient; DDF, dimensionless design factor; DPS, discrete particle simulation; GDD-SSF, gas double dynamic solid-state fermentation; g-ds, gram-dry-solid/substrate; g-fs, gram-fermented-solid/substrate; HLD, honeycomb loading device; H_C, critical bed height; IM, intermittent mixing; IMC, initial moisture content; IU, international unit; K_La, mass transfer coefficient; NO, nitric oxide; OUR, oxygen uptake rate; P_e, peclet number; PA, pressure amplitude; PG, pectinase; PBR, packed bed bioreactor; RDB, rotating drum bioreactor; SB, sugarcane bagasse; SmF, submerged fermentation; SL, substrate loading; SSF, solid-state fermentation; TB, tray bioreactor; TPH, total petroleum hydrocarbons; VAC, volatile aroma compounds; vvm, volume of air under standard conditions per volume of medium per minute; WB, wheat bran; ~, Nearly to

* Corresponding author.
E-mail address: ghoshfbs@iitr.ac.in (S. Ghosh).

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Table 1
Advantages of SSF over SmF.

Parameters	Solid State Fermentation (SSF)	Submerged Fermentation (SmF)
Absence of free water	Lower reactor volume required. Negligible chances of contamination. No foam formation. Lower cost of treatment of liquid effluents.	Relatively large reactor volume is required. High water activities make the process highly susceptible to bacterial contamination. Extensive foam formation and high cost for treatment of liquid effluents.
Fermentation medium	Low cost and natural Minimal mineral supplement.	Highly purified analytical grade chemicals are used which usually cost multiple times higher than SSF media.
Natural Environment	Solid nature of the substrate mimics the natural environment of fungi.	The dissolved nature of substrate does not provide the natural habitat for fungi.
Volumetric productivity	Has been reported comparatively higher in many studies	Lower volumetric productivity is often associated with SmF for fungal based products.
Downstream processing	Simpler and easier since the product is highly concentrated.	Product concentration and purification costs are higher. Generally defines the process economics.
Environment aspects	Use of natural wastes as substrate helps in biomass energy conservation, waste management and pollution control.	Significant environmental sustainability is not possible with the use of synthetic and processed media constituents of high analytical grade
Product quality	Heat and pH resistant products reported in few cases.	Compared to SSF, superior product quality has not been observed with SmF.

begins with an introduction of the reactor type and highlights of recent case studies, with emphasis on enzyme production. The case studies present a holistic view of reactor configuration, effect of operating conditions on process productivity, advantages and limitations. Mathematical models are powerful tools which can aid in bioprocess optimization, provide scale-up guidelines and facilitate bioreactor control and automation. For instance, models could be embedded in control schemes (e.g., Model predictive control, PID control) and the resulting control algorithms shall control and provide automation, thereby, significantly increasing bioreactor performance. Selected modeling studies have been revisited and cited at the end of each category with an objective to address limitations, discuss scale-up strategies and promote greater interaction between biologists and engineers. However, model assumptions and solution techniques are not discussed in detail, and can be gleaned from the references cited herein. Table 2 shows recent examples of SSF bioreactor used for enzyme production, whereas, Table 3 cites recent examples of SSF bioreactors employed for the production of spores, antibiotics, pigments, chemicals etc.

2. Bioreactor classification

The bioreactors have been classified into following four categories, based on their mode of operation.

I Tray bioreactor
II Packed bed bioreactor
III Air pressure pulsation bioreactor
IV Intermittent or continuously mixed SSF bioreactors

2.1. Tray bioreactor

SSF in trays has traditionally been used for the production of fermented foods such as tempeh, miso, koji, and soy sauce (Zhu and Tramper, 2013; Nout and Aidoo, 2011) in some Asian countries (Chen and Zhu, 2013). Trays are generally made of wood, metal or plastic, with or without perforations, packed with substrate-support and stacked one above the other in temperature and humidity controlled rooms (Fig. 1). Scale-up is generally achieved by increasing the surface area and/or increasing the number of trays. The current section starts with reports describing the utility of different substrate-supports for enzyme production in tray bioreactor (TB). Emphasis has been on the operating bed heights, chemical nature and initial moisture content (IMC) of substrate-support and their effect on productivity. Operational difficulties, issues related to poor O_2 and moisture level, heat accumulation in bed and control strategies are discussed herein.

Laccase production by *Trametes hirsute* was studied using grape seeds as substrate-support in TB (Rodríguez Couto et al., 2006). Use of grape seeds as inert support over nylon cube sponge resulted in a threefold increase in laccase production. Using the same organism, increased laccase activity (12260 U L^{-1}) was achieved when orange peel was used as substrate-support in a TB of 1 cm bed height (Rosales et al., 2007). High activity was attributed to high pectin and cellulose content in orange peels and to the absence of mechanical stress. Application of laccase production was extended on to the removal and de-colorization of synthetic dyes in TB (0.5 cm thickness) under semi-solid-state condition using *Trametes pubescens* (Rodríguez-Couto et al., 2009). Initially, dyes were adsorbed on to dry sunflower shell seeds which were subsequently used as solid support for fermentation. Remarkably high laccase activity (40172 U L^{-1}) was obtained when 0.5 mM Cu^{2+} and 50 μM tannic acid were added as supplements to the growth media on the 3rd day of cultivation. TB was used for cellulolytic enzyme production using a co-fermentation technique involving *Trichoderma reesei* and *Aspergillus oryzae* (Brijwani et al., 2010). Soybean meal and wheat bran (4:1) were used as substrate (1 cm height) and optimum operating conditions of temperature (30 °C), moisture (70%) and pH (5.0) were also obtained. These workers stressed on the importance of an appropriate C:N in substrate and concluded that the process not only facilitated high cellulase titres but also resulted in balanced production of glucanase (endo- and exo-) and β-glucosidase, which is recommended in biomass processing for biofuel production. Higher β-glucosidase levels were reported (Dhillon et al., 2011b) during fermentation of apple pomace using *Aspergillus niger* and *Trichoderma reesei* in TB. Still higher β-glucosidase levels were obtained (91.8 IU g-fs^{-1}) when *Aspergillus niger* was cultivated in plastic trays (40 × 25 × 12 cm) (Dhillon et al., 2011a) and the operating conditions were optimized using response surface methodology technique. High IMC (> 70% w/w) resulted in lower enzyme activities possibly due to low O_2 levels, decrease in bed porosity and substrate aggregation. This was an important observation which asserts IMC, a critical design parameter for TB.

A modification of the traditional TB was proposed for spore production by *Clonostachys rosea* mutant strain CRM-16 (Zhang et al., 2014). Sporulation area in the bioreactor was two times more than a traditional TB. Wheat bran and maize meal (3:1, w/w) were covered by a porous polyethylene membrane both on top and the bottom. With just two mixing events, sporulation was reported to be ten times greater than the TB. The porous polyethylene membrane was also claimed to lower the risk of bacterial contamination. Das et al. (2015) worked on the production of fumaric acid on plastic trays (35 × 22 × 11 cm) using *Rhizopus oryzae* 1526. Apple pomace ultrafilteration sludge and apple