Design Aspects of Bioreactors for Solid-state Fermentation: A Review

H. Kh. Q. Ali* **and M. M. D. Zulkali**
School of Bioprocess Engineering, University Malaysia Perlis,
Kompleks Pusat Pengajian, Jejawi 3, Arau 02600, Perlis, Malaysia

Review
Received: August 14, 2010
Accepted: April 26, 2011

Solid-state fermentation has gained renewed attention, not only from researchers but also from industries, due to several advantages over submerged fermentations. This is partly because solid-state fermentation has lower energy requirements, higher yields, produces less wastewater with less risk of bacterial contamination, and partly because of environmental concerns regarding the disposal of solid wastes. This paper reviews different types of bioreactors that have been used for various purposes and the recent process developments in solid-state fermentation.

Key words:
Solid-state fermentation, classification of bioreactors, general features

Introduction

Solid-state fermentations (SSF) are fermentations of solid substrates at low moisture levels or water activities; however, the substrate must possess enough moisture to support growth and metabolism of the microorganism. The water content of a typical submerged fermentation (SmF) is more than 95 %. The water content of a solid mash in solid-state fermentation often varies between 40 % and 80 %.[1]

SSF was used for the production of enzymes in the early 1900s and for the production of penicillin in the 1940s. The interest in SSF began in the mid-1970s. However, the theoretical base for SSF bioreactor technology only began to be established around 1990.[2] Over the last twenty years there has been a significant improvement in understanding how to design, operate and scale-up SSF bioreactors. Now, it has built up credibility in recent years in biotechnical industries due to its potential applications in the production of biologically active secondary metabolites, a part of feed, fuel, food, industrial chemicals, and pharmaceutical products.[3,4]

One of the unique characteristics of SSF is its operation at low moisture levels, which provides a selective environment for the growth of mycelial organisms, such as molds. In fact, most solid-state fermentations are mold fermentation producing extracellular enzymes on moist agricultural substrates. Since bacteria cannot tolerate low moisture levels, they might not be suitable for SSF. Through this characteristic the chances of contamination of the fermentation media by bacteria is greatly reduced in SSF.[5]

*Corresponding author: e-mail: dr.hayder_ali74@yahoo.com

Comparison between SSF and SmF

Several authors have discussed the advantages of SSF over SmF, while others have compared some of the features of the products that are produced by SSF and SmF. The major advantages of solid-state fermentation over submerged fermentation systems are:[6,7]

1. Small volume of fermentation mash or reactor volume, resulting in lower capital operating costs
2. Lower chance of contamination due to low moisture levels
3. Easy product separation
4. Energy efficiency
5. Simple technology
6. Product yields are usually higher
7. Oxygen is typically freely available at the surface of the particles.

Holker *et al.*,[8] Mitchell *et al.,*[9] Sun,[10] Long *et al.*,[11] and Neto *et al.*[12] described several other features of SSF that have an edge over submersed fermentation:

1. No waste production in the case of enzyme fermentation
2. Resembles the natural environment for several microorganisms
3. Longer production phase in amyloglucosidase production
4. Absence of co-produced carbohydrates
5. Use of waste or spent low value raw materials to produce high value products
6. No foam generation
7. Elimination of the need for rigorous control of many parameters during fermentation.

The major disadvantage is the heterogeneous nature of the media due to poor mixing characteristics, which results in control problems (pH and temperature) within the fermentation mash.[13] Therefore, it can be concluded that the SSF processes are economically advantageous in some cases when compared with SmF processes. This justifies the recent interest in SSF.[6,14]

Types of bioreactors

Many different kinds of bioreactors have been used in SSF processes, either on a laboratory scale, which uses quantities of dry solid mediums from a few grams up to a few kilograms; this category comprises many designs, with more or less sophistication. When several kilograms up to several tons are used, this is on an industrial scale.[15] The design of SSF bioreactors are based on similarities and operation so it can be divided into groups on the basis of how they are mixed and aerated:

Group 1: unforced aeration, without mixing

Group 2: forcefully-aerated, without mixing

Group 3: unforced aeration, continuous or intermittent mixing

Group 4: forcefully-aerated, continuous or intermittent mixing.

Bioreactors are categorized on the basis of operation rather than the physical design features of the bioreactor. Within each category, some of the bioreactors can operate in aseptic conditions.[16] Table 1 lists some of the bioreactors that have been used to produce different kinds of products in solid-state fermentation.

Group 1 (Tray bioreactor)

Tray bioreactors represent the simplest type of bioreactors that are used for SSF. They have been used for centuries in the production of traditional fermented foods such as tempeh, miso, koji[40] (a fermented steamed rice dish), and soy sauce.

The basic design features of the tray bioreactors are shown in Fig. 1:

– The chamber consists of a large number of individual trays one above the other with a gap in between for aeration. The tray chamber may be smaller for laboratory research or larger for industrial production.

– The tray may be constructed of various materials, such as wood, bamboo, metal (the metal should be painted to prevent corrosion),[41,42] or plastic.[43,44]

– The top of the trays is typically opened in every category, the bottom and sides of the trays may be perforated for allowing aeration of the undersurface.

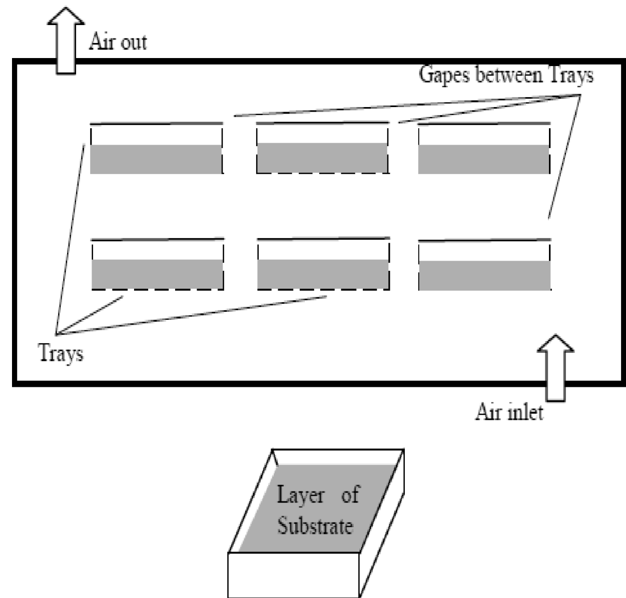


Fig. 1 – *Schematic diagram of the tray bioreactor and for individual tray*

– The temperature of the fermentation substrate is controlled by circulating warm or cool air as necessary; also, the relative humidity can be controlled by passing saturated or dry air through the chamber.[45]

– The height of the substrate in the tray can range from 5–15 cm.[46]

For laboratory scale of this group of bioreactors there are several types of equipment used such as petri dishes, jars, plastic bags and Erlenmeyer flasks, which offer the advantages of simplicity. Godoy *et al.*[47] used tray-type lab scale bioreactors for lipase production from castor bean waste using *Penicillium simplicissimum* fungus. Diaz *et al*.[48] produced xylanase and exo-poligalacturonase using *Aspergillus awamori* that was grown on grape pomace using a petri dish. In addition, Hashemi *et al.*[49] used a petri dish for the production of α-amylase from wheat bran substrate using *Bacillus sp.* They estimated a mathematical model for the biomass and product. They used this model to describe the different phases of the bacterial growth curve during the fermentation time. For the production of fungal pectinases, Patil and Dayanad[50] used deseeded sunflower as a substrate and *Aspergillus niger*. The fermentation was carried out in a 250 mL shallow glass container with a flat bottom. Mohanty *et al.*[51] used 1000 mL Roux bottles (132 mm × 275 mm) for bioethanol production from mahula flowers using *Saccharomyces cerevisiae*.

Table 1 – *Types of bioreactors and products that developed through solid-state fermentation*

Bioreactor	Substrate	Capacity	Microorganism	Products	Yield	Ref
Tray (Erlenmeyer flask)	Wheat straw	5 g	*Aspergillus ellipticus*	Cellulases	130.92 U g^{-1} substrate	17
Tray (Erlenmeyer flask)	Coffee husk	15 g	*Ceratocystis fimbiiata*	Fruity Flavour	8.29 mmol / 1 per gram	18
Tray (tray bioreactor)	Wheat bran & bean cake powder	70 m^3	*Bacillus thuringiensis (Bt)*	Cultivation of (Bt)	18,000 IU mg^{-1}	19
Tray (Erlenmeyer flask)	Tea waste	5 g	*Aspergillus niger*	Gluconic Acid	82.2 g L^{-1}	20
Tray (Erlenmeyer flask)	Poplar wood	5 g	*Trametes trogii*	Laccase Endooxylanase	510 U g^{-1} 780 U g^{-1}	21
Tray (Erlenmeyer flask)	Wheat bran & sesame oil cake	5 g	*Zygosaccharomyces rouxii*	L-glutaminase	11.61 U g^{-1} substrate	22
Tray (Erlenmeyer flask)	Jackfruit seed powder	5 g	*Monascus purpureus*	Pigments	25 OD U g^{-1} dry substrate	23
Tray (Erlenmeyer flask)	Wheat bran	10 g	*Rhizopus oligosporous*	Lipase	48.0 U g^{-1} substrate	24
Tray (Erlenmeyer flask)	Wheat bran & sesame oil cake	5 g	*Mucor racemosus*	Phytase	32.2 U g^{-1} dry substrate	25
Tray (Erlenmeyer flask)	Soy bran	4 g	*Fomes sclerodermeus*	Laccase Manganese Peroxidase	520 U g^{-1} 14.5 U g^{-1}	26
Tray (Erlenmeyer flask)	Wheat flour & wheat bran	10 g	*Rhizopus chinensis*	Lipase	24.447 U kg^{-1} substarte	27
Tray (conical flask)	Swine manure	20 g	*Bacillus subtilis*	poly-c-glutamicacid	6.0 %	28
Packed-bed bioreactor	Whole rice	60 g	*Monascus sp.*	Biopigments	500 AU g^{-1} dry substrate	29
Packed-bed bioreactor	Tapicoca starch	10 g	*Rhizopus oligosporus*	Cultivation		30
Packed-bed bioreactor			*Aspergillus niger*	Protease	Modeling according to N-tank in series	31
Packed-bed bioreactor	Sugarcane bagasse	12 g	*Bacillus subtilis*	Penicillin	Respiration Study	32
Packed-bed bioreactor	Sugarcane, cassava bagasse and coffee		*Aspergillus niger*	Citric acid	88 g kg^{-1} dry substrate	33
Rotating drum bioreactor	Pineapple waste	600 g	*Aspergillus niger*	Citric acid	194 g kg^{-1} dry substrate	34
Rotating drum bioreactor	Soil bioremediation	7.5 kg	*Microbial cell*	Cultivation		35
Rotating drum bioreactor	Polyurethane foam		*Penicillium glabrum*	Fungal Tannase		36
Continuous mixing, forcefully aerated	Glucose	2.5 L	*Phanerochaete chrysosporium*	Ligninolytic enzymes	239 U day^{-1}	37
Intermittent mixing, forcefully aerated	Corn		*Aspergillus niger*	Biomass		38
Continuous mixing, forcefully aerated	Whole wheat grains	35.3 L	*Aspergillus oryzae*	Cultivation		39

Papinutti and Forchiassin[52] used 500 mL Erlenmeyer flasks to produce lignocellulolyticenzymes from soy bran and wheat bran using *Fomes sclerodermeus*. Asaff *et al.*[53] studied the fermentation kinetics and carbon distribution in SSF and SmF on the metabolism of *Paecilomyces fumosoroseus* and evaluated a mathematical model that described the substrate consumption and biomass. Ustok *et al.*[54] used 500 mL Erlenmeyer flasks as a tray bioreactor for producing polygalacturonase by using *Aspergillus sojae*.

Since the air is not blown forcefully between the trays, O_2 and CO_2 can only move within the bed and the headspace by diffusion, but in opposite directions. The surface temperature of the bed in the tray depends on the heat transfer coefficient of the bed-to-air component, which is affected by the velocity of air between the tray surfaces. Chen *et al.*[55] studied the effect of the aeration on the temperature gradient within the bed height using a novel tray bioreactor. Through the change in internal air circulation and air pressure pulsation through the trays, they evaluated the temperature gradient. The benefit of internal circulation of air was to accelerate the heat transfer between the substrate surface and the outside air. Under optimized conditions the maximum temperature gradient was 0.12 °C cm^{-1} with a 9.0 cm bed height. Generally, aeration is an effective method to control the temperature inside the substrate bed instead of agitation, which might damage or disrupt the fungus hyphae.

The modeling is very important because the information that could be achieved from it would lead to a more comprehensive understanding of this complicated system. Rahardjo *et al.*[56] studied the rate of oxygen transfer during culturing of *Aspergillus oryzae* on wheat-flour. They showed that modeling is an important device used in the scale-up of the process and helps control the process in industrial applications. Ikasari *et al.*[57] used a modified two-phase growth model to describe the effect of the temperature gradient during the fermentation time. They used a petri dish as a simple kind of SSF bioreactor and put it in an incubator at a specific sequence of three temperatures in order to mimic the temperature gradient that actually happened during real SSF.

Lareo *et al.*[58] have been studying the effect of water content (moisture level) and nutrient concentration on the growth and sporulation of *Mucor bacilliformis*. They used logistic and exponential model to estimate the specific growth rate (μ_{max}). Through the experimental work, high (μ_{max}) was evaluated from the exponential model. Santos *et al.*[59] evaluated the scientific growth rate (μ) and fungal growth (X) of the *Thermoascus aurantiacus* in producing xylanase in Erlenmeyer flasks from sugar cane bagasse and rice bran extract.

Through the experiments and researches that considered the heat, O_2 transfer within the bed and the layer of substrate in trays, it was found that the limit of the bed height was around 5 cm. Gutarra *et al.*[60] used lab-scale tray-type bioreactor for lipase production from babassu cake using the *Penicillium simplicissimum* fungus. Each tray contained 10 g of the babassu cake forming a 1 cm-deep layer to achieve a good aeration and heat transfer between the cake and the surrounding space.

The scale-up of the process could not be achieved by increasing the bed height but by increasing the area of the trays. Shanker and Mulimani[61] proved this fact through their study of α-galactosidase production from red gram plant waste with wheat bran using *Aspergillus oryzae*. They found that with increasing the amount of used substrate (100–500 g) the α-galactosidase yields decreased from 4.5 U g^{-1} to 3.08 U g^{-1}. This decrease in enzyme production was due to the increase in the substrate bed height in the tray, which would affect the amount of the aeration through the bed. Thus, the scale-up can be done either by using wider trays or simply by increasing the number of the trays; in other words, the large-scale process must use a larger number of trays of the same size that are used in the laboratory. It is also difficult to apply this technology to sterile processes, except if sterile rooms are built and if procedures and equipment for the employees were provided, which would be prohibitive, therefore these fermenters would be characterized as highly costly.[62]

Group 2 (Packed-bed bioreactors)

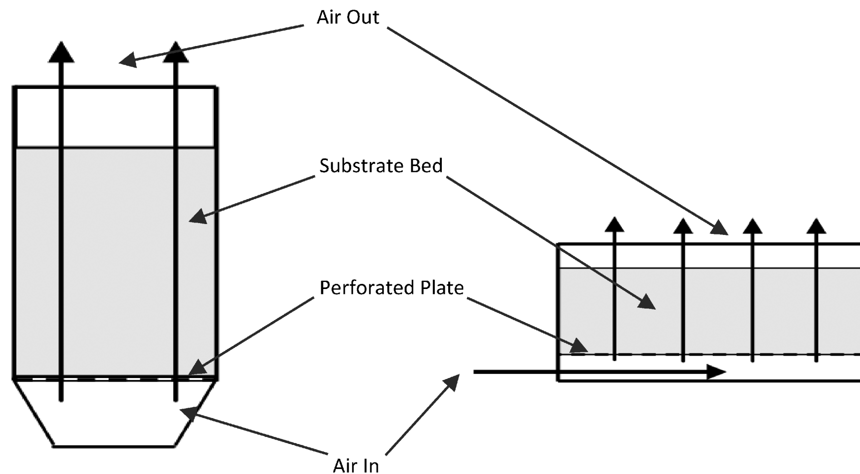
This type of bioreactor is operated under conditions where forced aeration is used, in which air is blown through a sieve, but the substrate bed is not mixed. This mode of operation is appropriate for those SSF processes in which it is not desirable to mix the substrate bed at all during the fermentation due to deleterious effects on either microbial growth or the physical structure of the final product.

The basic design features of a packed-bed bioreactor are as shown in Figs. 2 and 3:

– The column may have a cross-section other than circular.

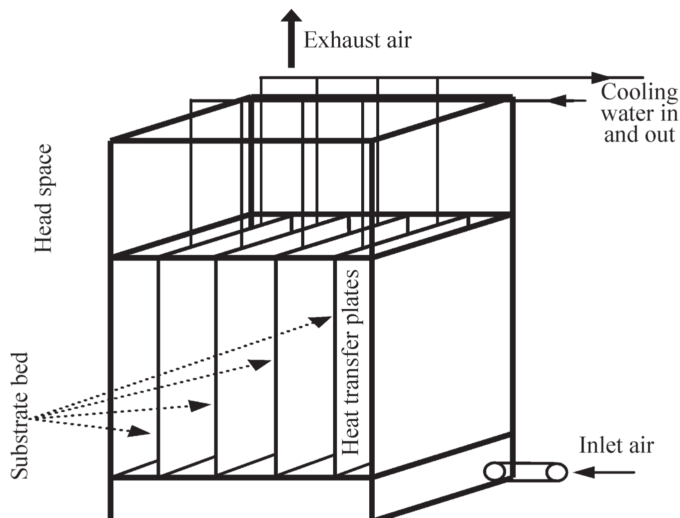
– The column can lie vertically, horizontally or at any angle, depending on the direction of the force effects due to gravity.

– The column can be aerated from any point, and for a vertical column, the air may enter from either top or bottom.



F i g . 2 – *Schematic diagram for "traditional packed-bed bioreactor" as 'vertical' and 'horizontal'*

– On the basis of heat removal considerations, the column may be covered with a water jacket that would be called a "traditional packed-bed bioreactor" (Fig. 2), or use a heat transfer plate inserted into the bed, which is called a "Zymatis packed-bed bioreactor" (Fig. 3).



F i g . 3 – *Zymotis packed bed bioreactor with internal heat transfer plates*

The temperature and the O_2 concentration of the air that flows within the bed in a traditional packed-bed bioreactor will change along the bed towards the outlet. The excessive temperature is always the greater problem than the O_2 supply to the microorganism within packed-bed bioreactors. The temperature appears to increase linearly with the increase in bed height, and it also increases linearly as the air flow rate was decreased, except at the lowest bed height.[63] Sangsurasak and Mitchell[64] studied the validation of the developed model in describing the SSF process and evaluating the temperature gradients in the axial and radial dimensions. It was found that the growth rate was very sensitive to the temperature gradients more than other parameters, including the substrate density. According to the model, increasing the height of the bed will lead to an increase in the rate of aeration to keep the temperature at a certain degree level through the increase in evaporation.

Aloui *et al.*[65] used a traditional packed-bed bioreactor. They studied the decolorization of olive mill wastes using four kinds of strains; *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pycnoporus cinnabarinus* and *Aspergillus niger*; the result showed good prospects for using *P. chrysosporium* for the decolorization of the olive mill wastes. Sella *et al.*[66] had used a packed-bed bioreactor (similar to the bioreactor that had been developed by Raimbault[67]) for spore production of *Bacillus arrophaeus*. The solid state culturing was carried out in glass columns with a 4 cm diameter and a 20 cm length. The maximum spore production was reached ($3.3 \cdot 10^{10}$ CFU g dry matter^{-1}) at 80 % initial humidity and no aeration. As the *Bacillus* does not prefer aeration during sporulation, a confirmation was done in an Erlenmeyer flask (tray-type bioreactor). The best sporulation production was ($4.7 \cdot 10^{10}$ CFU g dry matter^{-1}) with 91–93 % initial moisture level. A comparison was made between the spores collected from the Solid State Culture and the usual culture when they germinated in tryptone soy agar (TSA). It was noted that the SS Culture had mucoid colonies, were large and were not delimited (pic showed in the original paper). In addition, Banos *et al.*[68] used a Raimbault-type set-up bioreactor for the production of lovastatin using high density polyurethane foam as an inert support. The dimension of the glass column fermenter was (15 cm high × 2.1 cm ID), and the culture was aerated with pre-humidified air. The re-

sults showed the highest production rate was reached at a low aeration rate. It was found that forced aeration was not beneficial for lovastatin production in such a system. This system differs from other SSF systems in that the liquid phase is more vulnerable to drying, since the liquid medium in the polymeric structure is in direct contact with the air flow. The maximum production was 19.95 mg g^{-1} dry culture. With this result, the production yield of lovastatin by using polyurethane foam was two-fold higher than that of the system of bagasse support. Mitchell *et al.*[69] studied the strategies of scale-up for the packed bed. They compared two approaches, one based on a heat transfer model, and the other based on a modified Damköhler number. Due to their work, a geometric similarity can be used to scale-up bioreactors using laboratory scale results, but until the critical height, further increases in height can be achieved by increasing the width of the bed. Weber *et al.*[70] studied the solid cultivation of *Coniothyrium minitans* fungus using a laboratory scale packed-bed bioreactor. They evaluated a mathematical model using material and energy balance to simulate the temperature and moisture gradients in the bed. They assumed that the heat loss through the reactor wall was negligible, so that there would be no radial gradients inside the bed. They found from the experiments and resolving the mathematical models, that moisture control is the limiting factor for the cultivation. A recent attempt of Weber *et al.*[71] was made to evaluate a mathematical model for describing the operation of packed-bed bioreactors and for optimum operation during scale-up to industrial applications. They used two kinds of microorganisms, *Coniothyrium minitans* and *Aspergillus oryzae*, and four kinds of solid substrate; hemp, oats, perlite and bagasse. The results showed that to achieve a successful process it is essential to choose the proper solid substrate. The comparison was made according to the physical properties of the substrate during fermentation, such as shrinkage and channeling. Carranco *et al.*[72] compared the production of pectinase from *Aspergillus niger* using SmF and SSF. They used a packed-bed bioreactor (2.0 cm diameter and 15 cm height). The results showed the pectinase that was produced by SSF was more thermostable than those produced by SmF. Shojaosadati and Babaripour[73] studied the production of citric acid from apple pomace as a substrate by *A. niger* using a packed-bed with four stages. Under the optimised conditions, 124 g of citric acid was produced from 1 kg dry apple pomace with a yield of 80 %, based on total sugar.

There will be evaporation of the water in the packed-bed as a result of the temperature gradients and air flow through the bed, so it is impossible to prevent evaporation from occurring in packed-bed bioreactor, even if the air supplied to the bed is saturated. To replenish the water lost in the evaporation process, water to the bed can be replenished, so it is possible to use unsaturated air to aerate the bed of the bioreactor. Rojas *et al.*[74] studied the mechanisms of heat removal (conductive, convective and evaporative) in SSF using a packed-bed reactor. Average temperature gradients obtained during the culture were 1.3 and 0.42 °C cm^{-1} in the radial and axial directions, respectively. During maximal metabolic activity of *Aspergillus niger* the medium temperature rose from 32 to 48 °C, and it was indicated that conductive heat transfer was the least efficient mechanism (8.65 %) when compared with the convective (26.65 %) and evaporative (64.7 %) mechanisms.

The basic feature of this group of bioreactors is that it is "un-mixed", so its operation is static, which means that the hyphae that grow into the inter-particle spaces are not disrupted or squashed onto the particle surface; therefore, it would be an impediment to air flow and increasing the drop in pressure. The pressure drop will affect the bioreactor and decrease the fermentation due to the bed pulling away from the walls, leaving a gap through which the air can pass. Hence, it will lead to heat and mass transfer limitations within the bed and wall of the bioreactor.

Mitchell *et al.*[75] have suggested some modifications on the classical packed-bed bioreactor. They suggested dividing the bed into multi-layer trays and put them one above the other to take the classical shape of the packed-bed. This would allow moving the layers continuously at certain time intervals. They used a mathematical model based on the N-tank-in-series theory to evaluate the benefits of this modification upon the classical type. Through the experiments, and using the mathematical model, they were able to evaluate the heat generation in the modified and classical operation. They found the heat generation in the modified operation was 60 % of the value in the classical operation. As a result, the maximum bed temperature in the multi-layer packed bed process is 4.5 °C lower than the classical operation. The advantage of this modification was to reduce the axial temperature gradients that occur in the classical operation, especially near the air outlet point. This was solved by moving the upper trays down in certain time intervals to make sure all the trays underwent the same operational conditions.

In order to reduce the need for a strong aeration, another concept was recently developed, the Zymatis packed-bed bioreactor that was developed by Roussos *et al.*[76] In this design, heat removal is prompted by the insertion of closely-spaced internal

heat transfer plates into the bed, so the temperature gradients in Zymatis bioreactor is less than the traditional packed-bed bioreactor.[77] Mitchell and Meien[78] used a mathematical model based on the energy balance for only the Zymatis bioreactor during the growth of *A. niger* on starchy substrate. This developed model provided a useful guidance for optimum design and operation of this bioreactor. According to the experiments and developed model, the key for highest performance and higher productivity was the spacing between the internal cooling plates; 5 cm was the best distance between the internal cooling plates to achieve a faster growth of *A. niger*. Although little quantitative data was provided for the Zymotis bioreactor, enzyme levels achieved with 12 kg of dry matter were comparable to those obtained with column bioreactors of 20 cm height and 2.2 cm diameter.

In traditional packed-bed bioreactors, there is a problem of heat removal on a large scale. This design has been successful on a small scale because the small diameter allows reasonable heat removal through the bioreactor walls. If the substrate bed must remain static, then the best bioreactor design for SSF is the Zymatis packed-bed. To achieve a high productivity on a large scale, the spacing between the internal cooling spaces that are used must be the same as on a laboratory scale, the same as in tray bioreactors.[71,79,80]

Group 3 (Rotating-drum and stirred-drum bioreactors)

The bed in this kind of bioreactor is mixed either continuously or intermittently with a frequency from minutes to hours, and the air is circulated through the headspace of the bed, but not blown forcefully through it. Two bioreactors that have this mode of operation, using different mechanisms to achieve agitation, are the "stirred-drum bioreactors" and the "rotating-drum bioreactors".[62]

The basic design features are as shown in Fig. 4:

– These typically consist of a drum of cylindrical cross section lying horizontally

– The drum is partially filled with a bed of substrate, and air is blown through the headspace

– In rotating drums, the whole drum rotates around its central axis to mix the bed

– The rotating drum might include the use of baffles as "lifters"

– In stirred drum, the bioreactor body remains stationary with paddles or scrapers mounted on a shaft running along the central axis of the bioreactor rotating within the drum.

The intermittent mixing bioreactor operates like a tray bioreactor during the static period and like a continuous rotating bioreactor during the period of rotation; because of the conditions of the static period, it is necessary to limit the height of the substrate bed in order to achieve a good transfer of O_2 and CO_2 and avoid the accumulation of heat generation in the substrate, otherwise continuous mixing would be necessary. The metabolic heat generation during the fermentation will remove and transfer from the bed culture either to the headspace by diffusion or through the conductive wall to the surroundings. Kalogeris *et al.*[81] studied the production of cellulases and himecellulases in an intermittent agitation rotatable drum bioreactor by using *Thermoascus aurantiacus* fungus. They studied the effect of the initial moisture content, the growth temperature rate and the air flow to find the optimum conditions for best production. A mathematical model (the Le Duy model[82]) was used to explain and show the relationship between the fungus growth and the enzyme production.

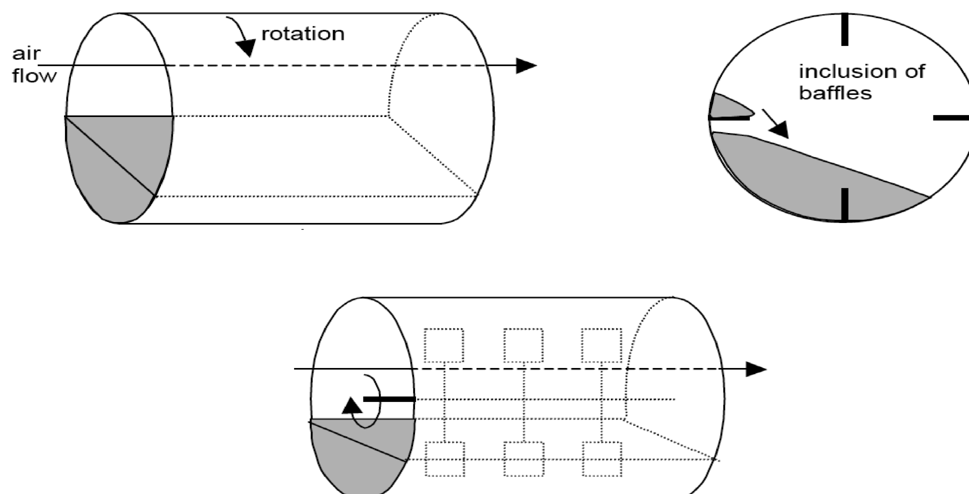


Fig. 4 – *Diagram of the "rotating-drum bioreactor" and "stirred-drum bioreactor"*

The growth of the microorganism in intermittent rotation is considered more uniform than in the tray fermentation, where it prevents the mycelium from knitting the bed together, which is an important difference from trays. Intermittent rotation is potentially less damaging to fungal mycelium than the continuous rotation.[83,84]

The performance of rotating and stirred-drum bioreactors will depend strongly on the effectiveness of the exchange of water that evaporates and the heat transfer between the bed and headspace. Wang *et al.*[85] developed a mathematical model to describe the redial temperature gradients in the substrate bed during the anaerobic SSF of sweet sorghum to produce ethanol fuel. Validation experiments were conducted in a 5000 L pilot-plant fermenter. The mathematical model agreed with the experimental data. Nagel *et al.*[86] estimated a mathematical model that described the moisture content of wheat flour during SSF using *Aspergillus oryzae*. This model was developed according to the data that was obtained from the growth of *A. oryzae* on membrane-based system, which mimics the growth of *A. oryzae* as used in mixed bioreactor. The developed model was tested in a 1.5 L rotatable drum bioreactor and a 35 L horizontal paddle bioreactor to figure its validation. The model was able to predict the experimental moisture content very well. Mitchell *et al.*[87] studied the axial temperature gradient in a 24 L rotating drum bioreactor through the growth of *Aspergillus oryzae*. They used a heat transfer model to evaluate the temperature gradient in the substrate bed. From the experimental results, and using the developed model, it was found that the initial velocity needed for the 24 L bioreactor was 0.0023 m s^{-1} and 15 % RH. The developed model predicted that for the scale-up of a 204 L bioreactor, the initial velocity would be 1 m s^{-1} at 90 % RH. For a 2200 L bioreactor the initial velocity will be 0.4 m s^{-1} and 15 % RH. These results show the need for less humidity air in order to increase the cooling capacity within the bed, which is the most important factor, especially during the scale-up. They suggest that to improve the axial mixing of the bioreactor, angled lifters should be used.

In a rotating-drum bioreactor, if the rotational rate is greater than l0 % of the critical speed used, then it might not be essential to include baffles within the drum. If a baffle drum is used to promote the mixing, then it should use quite low rotation rates. Schutyser *et al.*[88] studied different conditions of using baffles for developing a mathematical model describing the mixing gradient in three dimensions, as before this was attempted it was developed for only 2-dimensional models. In order to predict the radial and axial mixing they used three different drum designs: 1) without baffles; 2) with straight baffles; 3) with curved baffles. It was found from the experimental results, analyzed from video and image-analysis techniques, that the curved baffles were better than other designs due to the complete radial and axial mixing that was achieved after three or four rotations. In later work, Schutyser *et al.*[89] attempted to develop a mathematical model for industrial applications (scale-up). The two-phase model that was developed for the 28 L rotatable drum bioreactor consists of a solid phase and a gas phase. The experimental results and the temperature simulation according to the developed model show the validation of keeping the temperature within the optimum value. Several simulations were conducted for different fermenter sizes. The results show that the temperature gradients within the bed increased with the increase in fermenter size.

There is a relation between the rotational speed and the fermentation, where increased rotational speed increased fermentation to a critical speed, following which the fermentation decreased with the increasing of the rotational speed, where it is unlikely that the bioreactor would be well-mixed. There are two ways to avoid this problem: *i)* to use high rotational rates, but note that high rotational rates can affect the growth of microorganisms due to the shear effects *ii)* incorporate baffles. The rotation affects the fermentation when all substrate particles within the bed are regularly brought to the surface in order for the transformation of heat, water and O_2 between the bed and headspace.[90] Stuart *et al.*[91] studied the operating variables of the rotating drum bioreactor through the growth of *Aspergillus oryzae*. It was found that the growth rate decreased with the increasing of the rotational speed due to shear forces. The growth in this type was much better than the tray bioreactor due to the agitation, which leads to an increase in the amount of heat and mass transfer between the substrate bed and the headspace. Lagemaat *et al.*[92] studied the continuous production of enzyme tannase using a laboratory scale rotatable cylindrical bioreactor. The results showed that the rate of enzyme production was less than the batch production when they used a rotation speed of 0.7 rpm. They suggested that to improve the production rate, the rotation must be reduced to a specific speed to keep a well-mixed condition in the vessel.

Fractional filling allows the use of as much of the drum volume as possible without compromising the mixing too much; it should not be more than 40 % and may need to be less. It should be determined experimentally for each particular combination of substrate and microorganism.[42]

Group 4 (Mixed, forcefully-aerated bioreactors)

In this group, the bed of the bioreactors is agitated and air is blown forcefully through the bed. This type of bioreactor can typically be operated in one of two modes, according to the way of mixing, so it is useful to identify the two subgroups; bioreactors mixed continuously or bioreactors mixed intermittently. The combination of mixing and forced aeration can help in avoiding the temperature and moisture gradients that occur in other bioreactor types. With the feature mixing in this type it is possible to add water to the bed and reduce the need for using a high evaporation rate as a cooling mechanism. The choice between continuous and intermittent mixing will depend on the sensitivity of the microorganism to shear effects during mixing and the properties of the substrate particles such as their mechanical strength and stickiness. Various designs fulfill these criteria, "gas-solid fluidized beds", "the rocking drum", and various "stirred-aerated bioreactors".[16]

Continuous mixing, forcefully-aerated bioreactors

There are numerous different ways in which bioreactors can be agitated; therefore, bioreactors in this group may have quite different appearances on how the agitation is achieved as shown in Figs. 5, 6, and 7. The efficiency of mixing and aeration will vary significantly with various designs.[62] The bioreactors that are designed for continuous mixing can also be used in intermittent-mixing mode. In this case, the aeration should be considered; if a bed is to remain static for long periods, then it is important to design the aeration system to aerate the bed evenly during the static periods. Some bioreactors have been designed so the air enters at specific points, not over a cross-section of the bed. Therefore, the efficiency of aeration for these bioreactors

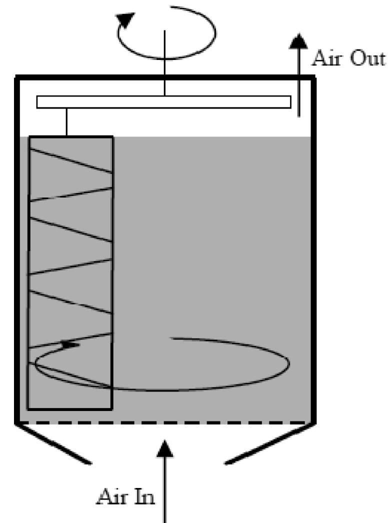


Fig. 5 – *Stirred-aerated bioreactors*

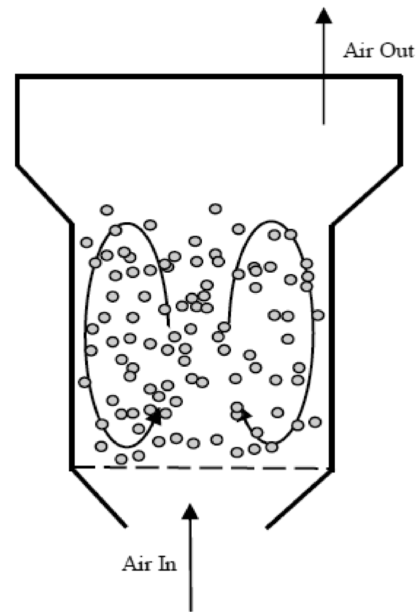


Fig. 6 – *Gas-solid fluidized bed*

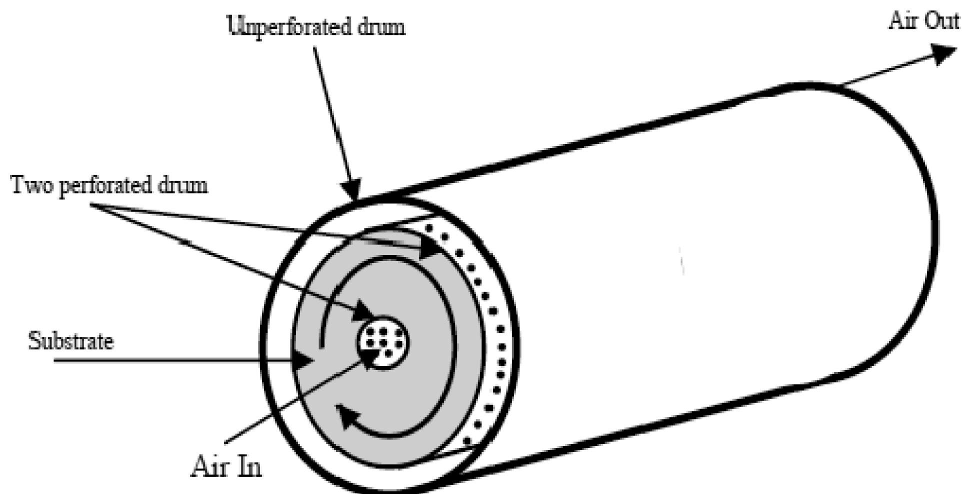


Fig. 7 – *Rocking drum*

will depend on the degree of mixing achieved by the agitation technique; this kind of bioreactor is not recommended for intermittent mixing. Santos *et al.*[93] studied the effect of aeration and continuous mixing on the enzyme (protease) production from *Penicillium fellutanum*. They used a packed-bed bioreactor with a water jacket to maintain the temperature and that the bed is well-mixed. A mathematical model was developed based on the results obtained from enzyme activities to help in predicting the temperature profile during the scale-up of the bioreactor. It was proved by the results that the enzyme production using SSF was better than SmF, especially when higher yields were obtained. Lillo *et al.*[94] developed a mathematical model to describe the water content and the bed temperature of a 200 kg pilot-scale solid substrate cultivation bioreactor during the growth of *Gibberella fujikuroi* on wheat bran as a case study. The model used the CO_2 production rate and inlet air condition to estimate the bed water content and bed temperature. The feature design of this bioreactor was the sequential process of agitation of the bed and the automatic manipulation of the dry or moisture inlet air to maintain the temperature gradient in the specified range and the water content at a constant amount.

The laboratory bioreactors that have cooling through a water jacket will have problems at scale-up and design for larger scale operations with similar proportions (the length-to-diameter ratio is maintained constant), the effectiveness of the water jacket would decrease due to the decrease in the surface area for heat transfer to the volume of the substrate bed.[95]

Takashi *et al.*[96] used a new solid state fermentation reactor (SSFR) for the production of Iturin A using *Bacillus subtilis* from okara as a solid substrate. The new features of this design is that it has two kinds of mixing; they used an ordinary impeller rotating clockwise and the floor of the machine rotates at the same time counter-clockwise, and vice versa, to achieve a higher rate of agitation without needing to use a higher rotation speed. The results show the effectiveness of using SSFR when a comparison was made between the static state and agitation; the production rate in a static condition was low due to the heat generation and temperature rise inside the reactor. The temperature must be maintained within a range of 25–30 °C to have higher production rates. This was achieved through the significant improvement in the manner of agitation. Miron *et al.*[97] used a newly designed bioreactor for the production of glucose oxidase. The bioreactor consisted of a cylindrical tube of glass (16 cm ID and 100 cm L) with a working volume of 20 L, covered with a band of electric resistance to control and maintain the temperature of the bioreactor. The design features of this bioreactor were the two disks that were located inside the tube and installed with scrapers to allow them to move along the tube but in opposite directions to achieve a higher degree of agitation. The results showed that the production rate of glucose oxidase improved 13 times than in the batch and regular SSF.

The rocking drum bioreactor has a different design that allows a gentle continuous mixing as shown in Fig. 7. It consists of substrate held between two perforated drums lying horizontally, encased in an unperforated outer bioreactor shell. The two outer drums are rotated back and forth relatively slowly in relation to the inner drum (e.g. 0.2 rev min^{-1}).[98]

The ability and effectiveness of gas-solid fluidized bed bioreactors depends on the substrate properties, where if the substrate is sticky it will form large agglomerates that cannot be fluidized. The substrate must have the same particle size in order to fluidize all; when there are differences in size then some of the particles might fluidize (small size) and others might not (large size). There is no problem with controlling the temperature and cooling the substrate bed, since the high flow rates required for fluidization should provide sufficient convective cooling capacity.[99,100]

Intermittent mixing, forcefully-aerated bioreactors

For this kind of bioreactor the "packed-bed bioreactor", the most recommended, has the same design and operating variables as a packed-bed bioreactor, the only addition being a type of agitation, where the bed may be mixed by a mechanical stirrer or by the air flow. It has advantages in intermittent mixing that prevent the pressure drop from becoming too high within the bed and added water to the bed in a reasonably uniform manner. For the microorganisms that can tolerate continuous mixing such as filamentous fungi, the intermittent-mixing is a more suitable technique. Velera *et al.*[101] studied the production of lovastatin in different types of bioreactors: petri dish (tray type), forced aeration, and a diffusive aeration mixing bioreactor. The reactor (B-Bruan, Germany) that was used for lovastatin production was modified in a way to achieve a higher rate of mixing by replacing the impeller with one specially designed. They studied the effect of aeration on the rate of production. The results show that forced aeration with intermittent mixing gives the highest lovastatin production of 16.65 mg g^{-1} dry solid. Schutyser *et al.*[102] studied the problem of channeling in packed-bed bioreactors that were caused by the evaporation of water during cooling and the formation of a mycelium network. A mathematical model was developed to

predict the effect of mycelium bonds on the mixing of the bed. In order to mimic the conditions of mixing of the SSF inside the bioreactor, an experimental setup of SS culture of *Aspergillus oryzae* between two wheat-dough disks was done to estimate the tensile strength of the aerial mycelium. The developed model was successful in describing the mixing process in a 28 m^3 rotating drum bioreactor. It was observed from the experimental results and the mathematical model that the disruption of the mycelium network, in the early steps of the SSF, was more essential than the added water to compensate the evaporation losses to avoid the aggregation of the bed. Meien *et al.*[103] tested different control strategies for an intermittently stirred, forcefully aerated solid-state fermentation bioreactor. The study was based on the analysis of a distributed parameter model; the temperature and water content of the substrate bed. They suggest that there would be a remarkable improvement in bioreactor productivity when control strategies are used. They suggest that reasonable control of the bed temperature and moisture content can be achieved by manipulating inlet air temperature, while maintaining the bioreactor at 100 % humidity, and using a mixing event triggered by a drop in the humidity of the outlet air to replenish water. This is easier to achieve in practice than providing air at both a specified temperature and a specified relative humidity, with these specifications changing over time. Khanahmadi *et al.*[104] developed a method for predicting the moisture content of the fermenting solids in an intermittently mixed packed-bed bioreactor on the basis of measurements of the inlet and outlet gas stream temperatures. They provide a useful and cheap tool for making decisions about when to add water to the bed and how much to add during each mixing and water-addition event. In conclusion, this type of bioreactor is considered better than other types due to the heat and mass transfer characteristics.

References

1. *Shuler, M. L., Kargi, F.,* Bioprocess Engineering: Basic principle. 2nd ed., Prentice Hall PTR, USA, 2002.
2. *Rahardjo, Y. S. P., Tramper, J., Rinzema, A.,* Biotech. Adv. **24** (2006) 161.
3. *XIE, G.,* Ph.D. Thesis. University of South Dakota, USA, 2006.
4. *Asagbra, A. E., Sanni, A. I., Oyewole, O. B.,* W. J. Micro. Biotech. **21** (2005) 107.
5. *O'Toole, D. K.,* Fungal Solid State Fermentation. In: *Kun, L.Y.,*(Ed.) Microbial Biotechnology: Principles and Applications. 2nd ed. World Scientific Publishing Co. Pte. Ltd., Singapore, 2006, pp. 335–349.
6. *Singhania, R. R., Patel, A. K., Soccol, C. R., Pandey, A.,* Biochem. Eng. J. **44** (2009) 13.
7. *Couto, S. R., Sanroman, M. A.,* J. Food Eng. **76** (2006) 291.
8. *Holker, U., Lenz, J.,* Eco. Ind. Micro. **8** (2005) 301.
9. *Mitchell, D. A., Meien, O. F., Krienger, N., Dalsenter, F. D.,* Biochem. Eng. J. **17** (2004) 15.
10. *Sun, S. Y., Xu, Y.,* Biores. Tech. **100** (2009) 1336.
11. *Long, C., Ou, Y., Guo, P., Liu, Y., Cui, J., Long, M., Hu, Z.,* Ann. Micro. **59** (2009) 517.
12. *Camilios-Neto, D., Bugay, C., Santana-Filho, A., Joslin, T., Souza, L. M., Sassaki, Mitchell, D. A., Krieger, N.*, Appl. Micro. Biotech. **89** (2010) 1395.
13. *Chen, H., Qiu, W.*, Biotech. Adv. **28** (2010) 556.
14. *Couto, S. R., Sanroman, M. A.*, Bioch. Eng. J. **22** (2005) 211.
15. *Brand, D., Soccol, C. R., Sabu, A., Roussos, S.*, Micro. Appl. Int. **22** (2010) 31.
16. *Mitchell, D. A., Berovic, M., Kriger, N.*, Introduction to Solid-State Fermentation Bioreactors. In: *Mitchell, D. A., Kriger, N., Berovic, M.*, (Eds.). Solid-State Fermentation Bioreactors, Fundamentals of Design and Operation. Springer, Berlin, Germany, 2006, pp. 33–44.
17. *Acharya, B. K., Mohana, S., Jog, R., Divecha J., Madamwar, D.*, J. Env. Manag. **91** (2010) 2019.
18. *Soares, M., Christen, P., Pandey, A., Soccol, C. R.*, Proc. Biochem. **35** (2000) 857.
19. *Hongzhang, C., Fujian, X., Zhonghou, T., Zuohu, L.*, J. Biosci. Bioeng. **93** (2002) 211.
20. *Sharma, A., Vivekanand, V., Singh, R. P.*, Biorec. Tech. **99** (2008) 3444.
21. *Levin, L., Herrmann, C., Papinutti, V. L.*, Biochem. Eng. J. **39** (2008) 207.
22. *Kashyap, P., Sabu, A., Pandey, A., Szakacs, G., Soccol, C.*, Proc. Biochem. **38** (2002) 307.
23. *Babitha, S., Soccol, C., Pandey, A.*, Biores. Tech. **98** (2007) 1554.
24. *Haq, I., Idrees, S., Rajoka, M. I.*, Proc. Biochem. **37** (2002) 637.
25. *Roopesh, K., Ramachandran, S., Nampoothiri, K. M., Szakacs, G., Pandey, A.*, Biores. Tech. **97** (2006) 506.
26. *Papinutti, V. L., Forchiassin, F.*, J. Food Eng. **81** (2007) 54.
27. *Sun, S. Y., Xu, Y.*, Proc. Biochem., **43** (2008) 219.
28. *Chen, X., Chen, S., Sun, M., Yu, Z.*, Biores. Tech. **96** (2005) 1872.
29. *Carvalho, J. C., Pandey, A., Oishi, B. O., Brand, D., Leon, J. A. R., Soccol, C.*, Biochem. Eng. J. **29** (2006) 262.
30. *Nopharatana, M., Mitchell, D. A., Howes, T.*, Biotech. Bioeng. **81** (2003) 438.
31. *Sahir, A. H., Kumar, S., Kumar, S.*, Biochem. Eng. J. **35** (2007) 20.
32. *Dominguez, M., Mejia, A., Gonzalez, J. B.*, J. Biosc. Bioeng. **89** (2000) 409.
33. *Vandenberghe, L. P. S., Soccol, C., Pandey, A., Lebeault, J. M.*, Biores. Tech. **74** (2000) 175.
34. *Tran, C. T., Sly, L. I., Mitchell, D. A.*, W. J. Micro. Biotech. **14** (1998) 399.
35. *Troquet, J., Larroche, C., Dussap, C. G.*, Biochem. Eng. J. **13** (2003) 103.
36. *Lagemaat, J., Pyle, D. L.*, Biotech. Bioeng. **87** (2004) 924.
37. *Couto, S. R., Barreiro, M., Rivela, I., Longo, M. A., Sanroman, A.*, Proc. Biochem. **38** (2002) 219.
38. *Meien, O. F., Mitchell, D. A.*, Biotech. Bioeng. **79** (2002) 416.
39. *Nagel, F. J., Tramper, J., Bakker, M. S. N., Rinzema, A.*, Biotech. Bioeng. **72** (2001) 219.

40. *Tokuoka, M., Sawamura, N., Kobayashi, K., Mizuno, A.*, J. Biosc. Bioeng. **110** (2010) 665.
41. *Couri, S., Terzi, S. C., Pinto, G. A. S., Freitas, S. P., de Costa, A. C. A.*, Proc. Biochem. **36** (2000) 255.
42. *Mitchell, D. A., Berovic, M., Krieger, N.*, Biochemical Engineering Aspects of Solid State Bioprocessing. In: *Scheper, T.* (Ed.) New Products and New Areas of Bioprocess Engineering. Springer, New York, USA, 2000, pp. 61–138.
43. *Shih, I., Kuo, C., Hsieh, F., Kao, S., Hsieh, C.*, J. Chin. Ins. Chem. Eng. **39** (2008) 635.
44. *Hsieh, C., Yang, F.*, Biores. Tech. **91** (2004) 105.
45. *Yang, Z., Zhang, B., Chen, X., Bai, Z., Zhang, H.*, J. Anal. Appl. Pyro. **81** (2008) 243.
46. *Shankar, S. K., Mulimani, V. H.*, Biores. Tech. **98** (2007) 958.
47. *Godoy, M. G., Gutarra, M. L. E., Castro, A. M., Machado, O. L. T., Freire, D. M. G.*, J. Ind. Micro. Biotech. (2010).
48. *Diaz, A. B., Caro, I., Ory, I., Blandio, A.*, Enzy. Micro. Tech. **41** (2007) 302.
49. *Hashemi, M., Mousavi, S. M., Razavi, S. H., Shojaosadati, S. A.*, Biochem. Eng. J. **53** (2010) 159.
50. *Patil, S. R., Dayanand, A.*, Biores. Tech. **97** (2006) 2340.
51. *Mohanty, S. K., Behera, S., Swain, M. R., Ray, R. C.*, Appl. Ener. **86** (2009) 640.
52. *Papinutti, V. L., Forchiassin, F.*, J. Food Eng. **81** (2007) 54.
53. *Asaff, A. A., Rojas, C. M., Gonzalez, G. V., Torre, M.*, Proc. Biochem. **41** (2006) 1303.
54. *Ustok, F. I., Tari, C., Gogus, N.*, J. Biotech. **127** (2007) 322.
55. *Chen, H., Xu, J., Li, Z.*, Biochem. Eng. J. **23** (2005) 117.
56. *Rahardjo, Y. S. P., Weber, F. J., Comte, E. P., Tramper, J., Rinzema, A.*, Biotech. Bioeng. **78** (2002) 539.
57. *Lkasari, L., Mitchell, D. A., Stuart, D. M.*, Biotech. Bioeng. **64** (1999) 722.
58. *Lareo, C., Sposito, A. F., Bossio, A. L., Volpe, D. C.*, Enzy. Micro. Tech. **38** (2006) 391.
59. *Santos, E., Piovan, T., Roberto, I. C., Milagres, A. M. F.*, Biotech. Lett. **25** (2003) 13.
60. *Gutarra, M. L. E., Gogoy, M. G., Maugeri, F., Rodrigues, M. I., Freire, D. M. G., Castilho, L. R.*, Biores. Tech. **100** (2009) 5249.
61. *Shankar, S. K., Mulimani, V. H.*, Biores. Tech. **98** (2007) 958.
62. *Mitchell, D. A., Krieger, N., Stuart, D. M., Pandey, A.*, Proc. Biochem. **35** (2000) 1211.
63. *Fanaei, M. A., Vaziri, B. M.*, Chem. Eng. Proc. **48** (2009) 446.
64. *Sangsurasak, P., Mitchell, D. A.*, Biotech. Bioeng. **60** (1998) 739.
65. *Aloui, F., Abid, N., Roussos, S., Sayadi, S.*, Biochem. Eng. J. **35** (2007) 120.
66. *Sella, S. R. B. R., Guizelini, B. P., Vandenberghe, L. P. S., Medeiros, A. B. P., Soccol, C. R.*, Braz. Arch. Biol. Tech. **52** (2009) 159.
67. *Raimbault, M.*, Elctro. J. Biotech. **1** (1988) 174.
68. *Banos, J. G., Tomasini, A., Szakacs, G., Gonzalez, J. B.*, J. Biosci. Bioeng. **108** (2009) 105.
69. *Mitchell, D. A., Pandey, A., Sangsurasak, P., Krieger, N.*, Proc. Biochem. **35** (1999) 167.
70. *Weber, F. J., Tramper, J., Rinzema, A.*, Biotech. Bioeng. **65** (1999) 447.
71. *Weber, F. J., Oostra, J., Tramper, J., Rinzema, A.*, Biotech. Bioeng. **77** (2002) 381.
72. *Carranco, A. M., Aguilar, B. A., Aguilar, G., Gonzailez, G. V.*, Enzy. Micro. Tech. **21** (1997) 25.
73. *Shojaosadati, S. A., Babaeipour, V.*, Proc. Biochem. **37** (2002) 909.
74. *Rojas, M. G., Hosn, S. A., Auria, R., Revah, S., Tones, E. F.*, Proc. Biochem. **3l** (1996) 363.
75. *Mitchell, D. A., Cunha, L. E. N., Machado, A. V. L., Luz, L. F., Krieger, N.*, Biochem. Eng. J. **48** (2010) 195.
76. *Roussos, S., Raimbault, M., Prebois, J. P., Lonsane, B. K.*, Appl. Biochem. Biotech. **42** (1993) 37.
77. *Pandey, A.*, Biochem. Eng. J. **13** (2003) 81.
78. *Mitchell, D. A., Meien, O. F.*, Biotech. Bioeng. **68** (2000) 127.
79. *Pandey, A.*, Proc. Biochem. **26** (1991) 355.
80. *Mitchell, D. A., Meien, O. F., Krieger, N.*, Biochem. Eng. J. **13** (2003) 137.
81. *Kalogeris, E., Iniotaki, F., Topakas, E., Christakopoulos, P., Kekos, D., Macris, B. J.*, Biores. Tech. **86** (2003) 207.
82. *Brown, D., Vass, R.*, Biotech. Bioeng. **15** (1973) 321.
83. *Kim, J.*, Ph.D. Thesis. University of McGill, Quebec, Canada, (2004).
84. *Alam, M. Z., Mamun, A. A., Qudsieh, I. Y., Muyibi, S. A., Salleh, H. M., Omar, N. M.*, Biochem. Eng. J. **46** (2008) 61.
85. *Wang, E. Q., Li, S. Z., Tao, L., Geng, X., Li, T. C.*, Appl. Ene. **87** (2010) 2839.
86. *Nagel, F. J., Tramper, J., Bakker, M. S. N., Rinzema, A.*, Biotech. Bioeng. **72** (2001) 231.
87. *Mitchell, D. A., Tongta, A., Stuart, D. M., Krieger, N.*, Biotech. Bioeng. **80** (2002) 114.
88. *Schutyser, M. A. I., Weber, F. J., Briels, W. J., Boom, R. M., Rinzema, A.*, Biotech. Bioeng. **79** (2002) 284.
89. *Schutyser, M. A. I., Briels, W. J., Boom, R. M., Rinzema, A.*, Biotech. Bioeng **86** (2004) 405.
90. *Durand, A.*, Biochem. Eng. J. **13** (2003) 113.
91. *Stuart, D. M., Mitchell, D. A., Johns, M. R., Litster, J. D.*, Biotech. Bioeng. **63** (1998) 383.
92. *Lagemant, J., Pyle, D. L.*, Chem. Eng. J. **84** (2001) 115.
93. *Santos, M. M., Rosa, A. S., Dal'Boit, S., Mitchell, D. A., Krieger, N.*, Biores. Tech. **93** (2004) 261.
94. *Lillo, M. P., Correa, R. P., Agosin, E., Latrille, E.*, Biotech. Bioeng. **76** (2001) 44.
95. *Couto, S. R., Barreiro, M., Rivela, I., Longo, M. A., Sanroman, A.*, Proc. Biochem. **38** (2002) 219.
96. *Takashi, A., Yuan, J. G., Shinji, M., Shahedur, R. M., Kasumasa, O., Makoto, S.*, J. Env. Sci. Supp. (2009) S162.
97. *Miron, J., Vazques, J. A., Gonzalez, P., Murado, M. A.*, Enz. Micro. Tech. **46** (2010) 21.
98. *Ryoo, D., Murphy, V. G., Karim, M. N., Tengerdy, R. P.*, Biotech. Tech. **5** (1991) 19.
99. *Foong, C. W., Janaun, J., Krishnaiah, K., Prabhakar, A.*, Ind. Crops Prod. **30** (2009) 114.
100. *Jang, H. D., Yang, S. S.*, Biores. Tech. **99** (2008) 6181.
101. *Valera, H. R., Gomesa, J., Lakshmi, S., Gururaja, R., Suryanarayan, S., Kumarc, D.*, Enz. Micro. Tech. **37** (2005) 521.
102. *Schutyser, M. A. I., Briels, W. J., Rinzema, A., Boom, R. M.*, Biotech. Bioeng. **84** (2003) 29.
103. *Meiena, O. F., Luz, L. F. L., Mitchell, D. A., Perez-Correa, J. R., Agosin, E., Fernandez, M. F., Arcase, J. A.*, Chem. Eng. Sci. **59** (2004) 4493.
104. *Khanahmadi, M., Roostaazad, R., Mitchell, D. A., Miranzadeh, M., Bozorgmehri, R., Safekordi, A.*, Chem. Eng. Sci. **61** (2006) 5654.