6 Aspects of Design of Bioreactors in SSF

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6.1 INTRODUCTION

It is well known that the solid-state fermentation (SSF) is a new technology with which antibiotics, enzymes, fine chemicals can be obtained by new bioprocesses with improved efficiency (Giovannozzi-Sermanni & Porri 1989; Pandey et al., 2000) if compared to the classical submerged fermentations. SSF processes can be defined as "the growth of microorganisms, mainly fungi, on moist solid materials in the absence of free-flowing water"(Cannel & Moo-Young 1980). In the last few decades SSF has grown quickly in interest and importance and has been used for the production of antibiotics, alkaloids, aroma compounds, plant growth factors, enzymes, biofuel, and also for the bioremediation of polluting compounds.

To understand the importance of the SSF, following must be kept in mind:

- the biological sustainability is associated to the atmospheric content of carbon dioxide.
- the content of carbon dioxide in the atmosphere increases continuously making the chemical energy obtained by the photosynthesis in sufficient to control its rise to which a quote coming from fossil carbon utilization is added (Fig.1).
- the needs of chemical energy requested by human societies are increasing rapidly.
- the availability of water is dependent on environmental conditions and it is decreasing in many countries.
- celluloses, hemicelluloses, lignines, starch and oils could be utilized by new technologies to avoid their loss through wild uncontrolled biological processes.

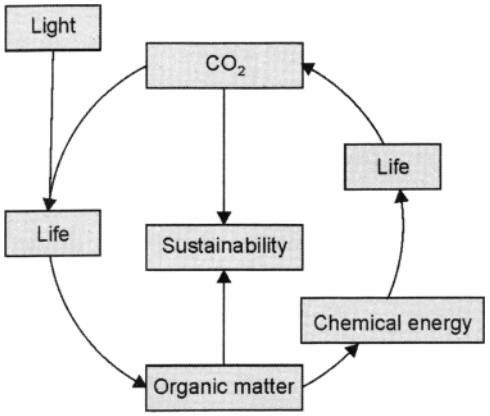


Fig. 1. Atmospheric CO_2 levels arising from the photosynthesis and biological activities.

From the above considerations, it seems that the better controlled biological utilization of lignocelluloses by environmentally friendly applications can deeply contribute to improve the sustainability.

The degradation of water insoluble copolymers has as first steps hydrolytic and oxyreductive reactions, occurring in three-phases (gaseous, liquid and solid phases) systems and can help to efficiently solve problems through SSF, since in recent years studies have demonstrated their superior product yields and simplified downstream processes (Pandey et al., 1999). These factors increase to the possibility of producing more stable products, with less energy requirements, and make SSF a very attractive alternative to the conventional liquid fermentation, especially in the developing countries. The use of solid matter has serious implications in the engineering aspect of bioreactor design and operation.

It is interesting to note that few enzymes, necessary for the biosynthesis and biodegradation of the lignocelluloses have been working in nature at least since 350 millions of years, but until now they are poorly utilized by means of controlled biological processes. These enzymes belong to hydrolytic enzymes (cellulases, hemicellulases, polyphenolases, pectinases, etc.) and oxidative ones (polyphenoloxydases, peroxydases, etc.).

6.2 CHEMICAL, BIOCHEMICAL AND MICROBIOLOGICAL SHORT OUTLINES

Lignocelluloses are copolymers, characterized by highly complex chemical structure. Celluloses, lignins and hemicelluloses, chemically linked each other,

contain large quantities of chemical energy. The agricultural and forestry resources provide immense quantities of such materials but the amount of unutilized lignocellulose can reach easily the 50%. Keeping in mind that more than 3000 million tons of cereal straws roughly are produced per year worldwide and that these residues are mostly disposed of by burning and humification, it results a terrific loss of energy. As a consequence, the chemical energy of unutilized lignocellulosic matter constitute a huge source of energy which could be used for many applications beside the burning and humification.

Cellulose, homopolymer of 1,4-glucose units, hemicellulose, heterogeneous carbohydrate polymer, and lignin, polymer of phenylpropanoidic units interconnected by a great variety of linkages, contain energy which can be utilized by a series of biochemical breakdown reactions, fundamental aspect for efficient applications. Therefore, first of all it is necessary the depolymerization of the water insoluble lignocellulose takes place to produce soluble compounds, and this degradation can start by few enzymes which allow the formation of lower molecular weight water soluble copolymers (Crestini et al., 1998) (Fig.2).

It is interesting to note that simple phenolic compounds can have also effects on redox enzymes activity (Giovannozzi-Sermanni 1981).

6.3 ROLE OF MAIN PARAMETERS IN SSF

6.3.1 General considerations

Targeting a SSF production for a possible industrial interest, two are the ways to approach the problem:

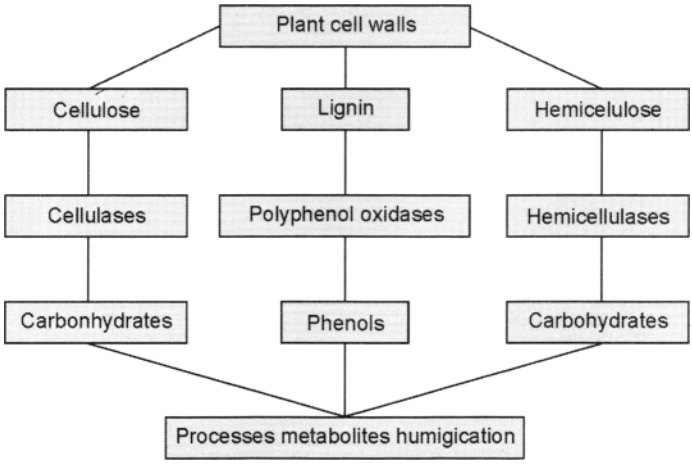


Fig. 2. Pathways of biodegradation of lignocelluloses by enzymes.

- the characteristics of the wanted products determine the choice of the starting matter, generally a low-cost agricultural resource simply available.

- the possibility to use huge amount of available residues encourages the development of new processes to produce value-added goods, by improving also the biological sustainability.

Therefore, the choice of microorganisms is of great importance, which are dependent on parameters such as pH, temperature, moisture content, etc. Consequently the development of devoted bioreactors useful to utilize the set of parameters chosen for the process is of paramount importance. Also, some physical and engineering specifics not directly dependent on the biochemistry of the process must be considered, such as employed materials, analytical controls, economical factors, maintenance, pretreatment and downstream treatments, etc.

6.3.2 Parameters affecting the bioprocess

The most important parameters characterizing an SSF process are the following:

- *water activity and moisture content of the substrate.* This variable has influence on biodegradation processes, biosynthesis and secretion of metabolites (Perez-Guerra et al., 2003).

- *temperature and heat transfer.* This affects directly bioprocesses characteristics, spores germination, cells growth and the efficiency of the process.

- *pH.* It determines the enzyme activities, given that each of them can be dependent on different optimal pH values.

- *aeration.* It determines the O_2 and CO_2 concentration inside the bioreactor, regulating the removal of CO_2 and volatile compounds (such as ethylene), the relative humidity, and it improves the heat transfer.

- *nutrient diffusion.* It affects the nutrient concentration and regulates the actions of enzymes over the solid substrate (normally water insoluble).

- *mixing.* It helps heat removal, gas exchange, water content, uniformity and influences the process conditions.

- *septic conditions.* It is required to avoid the release of the process organism in the environment and the entry of polluting microorganisms.

- *particle size* (density). It regulates the utilization of the substrate by the microorganism at a molecular level, the gas-liquid interfacial area and the thickness of the wet fungal layer. It makes the oxygen utilization dependent on this parameter (a too small particle size may interfere with microbial respiration, causing poor growth; a large particle size provides only a limited surface for microbial attack) (Pandey et al.,1999).

- *physico-mechanical properties of the solid matrix.* The matrix should have wide surface area and should stand gentle stirring or compression if required by the chosen fermentation to avoid sticking behaviour during the process.

- *microbiological inoculum.* Its type, relative humidity, amount, etc. influence the growth rate.

- *biomass uniformity.* It is of fundamental importance to maintain uniform biochemical reactions all over the biomass.

- *morphology of the microorganism.* The most important characteristics are to penetrate into a solid substrate with a low water activity, assimilating complex and variable mixtures of nutrients, the capacity of adherence and i.e. the presence or not of septum in the hyphae for mechanical resistance.

Most of the above parameters are strictly interconnected, but few of them appear to be of major importance in the development of new processes.

6.3.2.1 Temperature and heat transfer

Respiration during the growth, which is dependent to the oxygen consumption and CO_2 formation, is highly exothermic and heat generation is directly related to the level of metabolic activities of the microorganisms. The removal of metabolic heat during large-scale SSF is one of the most critical issues since microbial growth is particularly sensitive to the rise of temperature, given that the heat generation produces thermal gradients (Schutyser et al., 2003; Saucedo-Castañeda et al., 1990).

Many approaches are available using different cooling techniques to enhance the heat transfer and to control the microbial growth, such as mixing, forced aeration, evaporative cooling, utilization of cooling jackets and additional cooling surfaces, that are just the most used systems to achieve the best thermal conditions. These approaches and their role in designing will be considered in detail in par. 6.4.

6.3.2.2 Water activity and moisture content

Water has a solvent function providing nutrients and scavenges wastes, and a structural function which, is involved in the stability and the function of the biological structures (Gervais & Molin 2003). Water in SSF systems is present in a complexed form within the solid matrix or as a thin layer either absorbed on the surface of the particles, or in the capillary region of the solid. The excessive lack of free water does not allow good diffusion of the nutrients and gas exchange, and can cause a loss of the functional properties of some enzymes and a disequilibrium on the metabolic chain of the cells. On the other hand, an excess of water can perturb the transport phenomena at cellular and macroscopical level.

Water requirements should be defined in terms of water activity (a_w), a thermodynamic parameter related to the chemical potential of water. Aw is well correlated to the relative humidity (RH) as follows:

$a_w = RH/100 = p/p_0$

where p = vapour pressure of water in the substrate and p_0 = vapour pressure of pure water at the corresponding temperature (Raimbault 1998).

A reduction in a_w normally extends the lag phase of the microbial growth, causing a low biomass production.

6.3.2.3 pH

The substrate and medium acidity has great importance in the bioprocess, not only because the pH partly screens the microbial growth from a large number of competitors but also because partly gives a right habitat for the microorganism. In fact the metabolic regulation routes, among other factors, may be controlled by pH (Fig. 3) (Giovannozzi Sermanni et al., 1978).

6.3.2.4 Aeration

The rate limiting step in an aerobic submerged liquid fermentation (SmF) is usually the transfer of O_2 from the gas phase to the liquid phase. Therefore the single most important consideration in the design and scale up of bioreactors for submerged liquid fermentations is to provide a sufficiently high rate of O_2 transfer into the liquid medium. Regarding to the SSF processes, especially in packed bed bioreactors, both heat and mass transfer are important, and SSF in packed bed bioreactors, overheating is a major problem (Saucedo-Castaneda et al., 1992; Gowthaman et al., 1993), due to unidirectional flow of air. As the air passes through the column and removes heat from the substrate, the air warms up, loosing its cooling efficiency through the column. Consequently, a

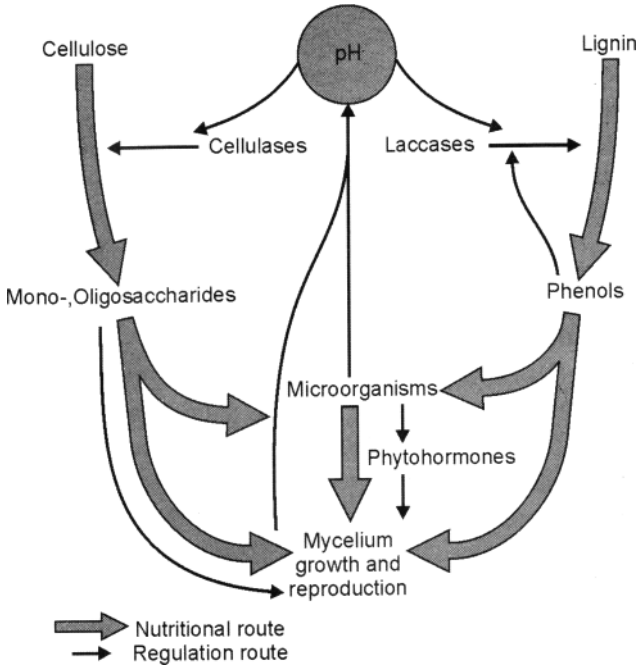


Fig. 3. Influence of pH on the possible regulation of cellulose and lignin degradation by mycelial and microbial metabolism (pH stat) (modified from Giovannozzi et al., 1978).

temperature gradient is established along the column with the highest temperature at the outlet end of the bed.

In a rotating drum bioreactor, the gas exchange is obtained by passing the air through the biomass. In this way, it is possible to maintain a constant chosen O_2 and CO_2 value as shown in Fig. 4, and the total fluxed air during the fermentation and the gas exchange can be recorded continuously to maintain the reproducibility of the process. For example, after few days of fermentation the CO_2 and the O_2 levels can be modified and maintained at a chosen value. In an earlier study, Fung and Mitchell (1995) showed that maximum O_2 uptake rates were higher in a baffled rotating drum than in an unbaffled one, so that O_2 limitation could occur in the unbaffled drum too (Marsh et al., 1998).

6.4 GENERAL ASPECTS OF SSF BIOREACTOR DESIGN

The use of solid matrix, either as an inert support or substrate support, has relevant implications on the engineering aspect of bioreactor design and operation (Robinson & Nigam 2003). Maximization of the rate of formation and the yield of product within the bioreactor are a key of the optimizing the bioprocess. Nevertheless, SSF bioreactor systems have not yet reached a high degree of

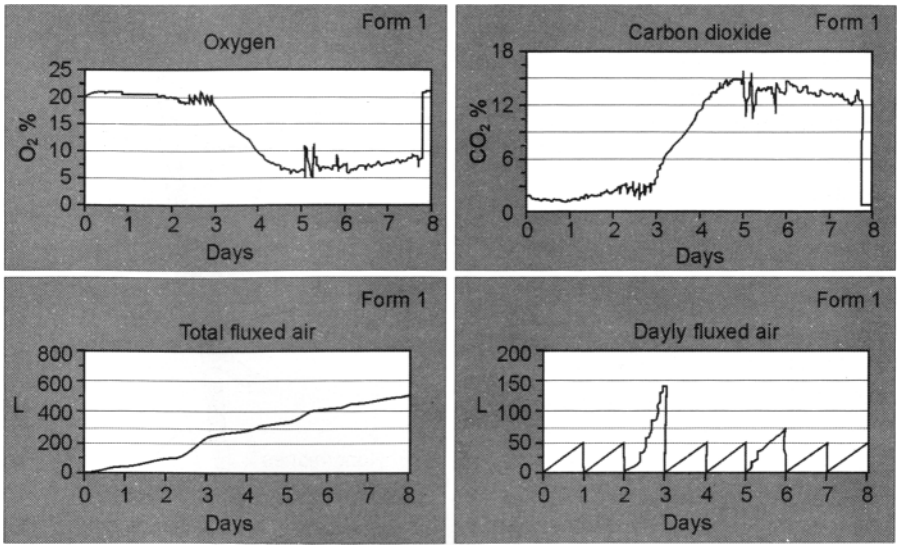


Fig. 4. Control of O_2 and CO_2 levels in a rotating drum bioreactor by using fresh sterile air fluxes.

development, mainly due to the problems associated to the solid beds like poor mixing, heat transfer and material handling. Some of the desired features of a solid-state bioreactor system are the following (Raghavarao et al., 2003):

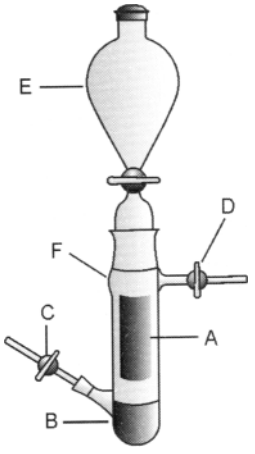
- the constitutive material has to be cheap, inert and resistant to the corrosion and abrasion.
- the system has to be preferably microbiological contamination-free to prevent accidental hazardous due to biological pollution.
- the control and regulation of the operational parameters must be efficient.
- the uniformity of biomass must be provided.
- the bioreactor design has to simplify the maintenance, the loading-unloading and the product recovery (according to the labour cost of the countries where the SSF process is developed).

It is possible to classify the categories of bioreactor for SSF by their size and by the quantity of dry solid substrate involved, so that a lab-scale would process from few grams to few kilograms, a prepilot-scale from few to several kilograms, a pilot-scale several kilograms and an industrial-scale up to several tons. During the scaling-up of the process, the availability of bioreactors designs decreases considerably due to the reasons shown in 6.4.3 and 6.4.4.

6.4.1 Lab-scale

SSF at laboratory-scale shows several biotechnological advantages, if compared to SmF, such as high fermentation productivity, high end-concentration of products, high product stability, low catabolic repression, cultivation of microorganisms able to grow on water-insoluble substrates, and often the demand on sterility is not requested (Hölker et al., 2004). It is also possible at lab-scale to remove properly the metabolic heat generated by the fermentation, keeping the culture vessel in a temperature-controlled environment (water bath, cooling jacket, etc.).

Approximately 90% of all industrial enzymes are produced by SmF, frequently using specifically optimized, genetically manipulated microorganisms. However, almost all these enzymes could also be produced in SSF using wild-type microorganisms (Filer 2001; Pandey et al., 2001). It has been shown that enzyme production in solid state is higher than in submerged fermentation (Viniegra-González et al., 2003). It is very interesting also that the stability of the produced enzymes at high temperature or extreme pH, are better in SSF (Deschamps and Huet 1985; Acuna-Arguelles et al., 1995). Catabolite repression or protein degradation by proteases, severe problems in SmF, were often reduced or absent in SSF (Solis-Pereira et al., 1993; Aguilar et al., 2001). Since 1959, when the first example of laboratory-scale fermentor was described (Giovannozzi, 1959) (Fig. 5), studies on laboratory-scale system represent a powerful aid to develop SSF bioprocesses and a useful front of data for modelling the process.



The solid-state bioreactor **f** contains a metal net basket **a** which is filled with lignocellulosic matter. The funnel **e** may contain substrate useful for the growth of microorganisms. All the apparatus can be sterilized in a steam pressure oven.

The substrate of funnel can be inoculated with microorganisms chosen for the trial. After few days the liquid culture is transferred into the bottom part of the bioreactor so that the matter contained in the basket is inoculated with a single chosen microorganism. Then the liquid phase is pumped away through the **c** tap.

The bottom **b** part of the bioreactor can be utilized to contain different kinds of liquids useful for the trial.

Through **d** and **c** taps equipped with sterile filters it is possible to control the gaseous phase composition of the bioreactor, particularly oxygen and carbon dioxide.

Hence this solid state bioreactor, the first described in the scientific literature, permits to control and to follow the bioconversions of lignocellulosic matter and to test the effects of different microorganisms on the biodegradation of them.

Fig. 5. Lab-scale fermenter (Giovannozzi 1959).

It is significant to note that recent equipments organized in batteries and computer controlled (Fig. 6), useful for a systematic investigation, utilizes single units very similar to the first one already shown. Laboratory equipments such as Petri dishes, jars, wide mouth Erlenmeyer flasks, Roux bottles, roller bottles are of some help for their simplicity and easiness in handling. In the last few decades, more complex lab-scale units have been developed, such as the one patented by an OSTROM team between 1975-1980. It consists of a small column filled of inoculated medium, humidified, and thermoregulated by a water-bath. It is useful for screening studies and for the first step of parameters optimization. The small quantity of used medium (few grams), and the geometry of the glass column guarantee an easy maintenance of the temperature in the reactors. The design of this reactor, however, does not permit sampling during the fermentation and hence, during the process, one entire column has to be utilized for each analysis.

A new generation of small reactors was developed by an INRA-team in France. Such reactors have a working volume of about 1 l, a relative humidity probe, a cooling coil on the air circuit and a heating cover for the vessel (Fig. 7). These changes enhanced the regulation of the water content during the process. Each reactor is automatically controlled by a computer.

Several teams have designed bioreactors based on continuous mixing of the solid substrate/support. Such characteristic is suggested by the aptitude of some filamentous fungi to agglomerate. Therefore, to avoid such behaviour which alter deeply the biological efficiency the mixing can be useful. The bioreactors can be perforated drums (Fig. 8-1), like the one described by Kalogeris et al., (1999), rotating drums (Fig. 8-2), or horizontal paddle mixers (Fig. 9). All those drum bioreactors are designed to allow adequate mixing of the medium and good aeration.

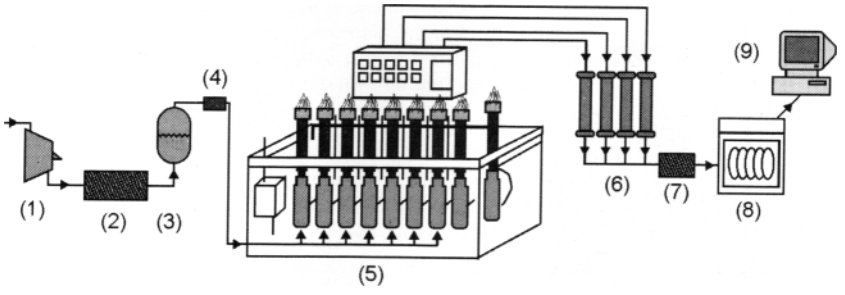


Fig. 6. Schematic packed column bioreactor system. 1) air pump, 2) air filter, 3) humidifier, 4) air distributor, 5) battery of columns in water bath, 6) silica gel columns, 7) gas sampler, 8) chromatograph, 9) PC unit (redrawn from Medeiros et al., 2001).

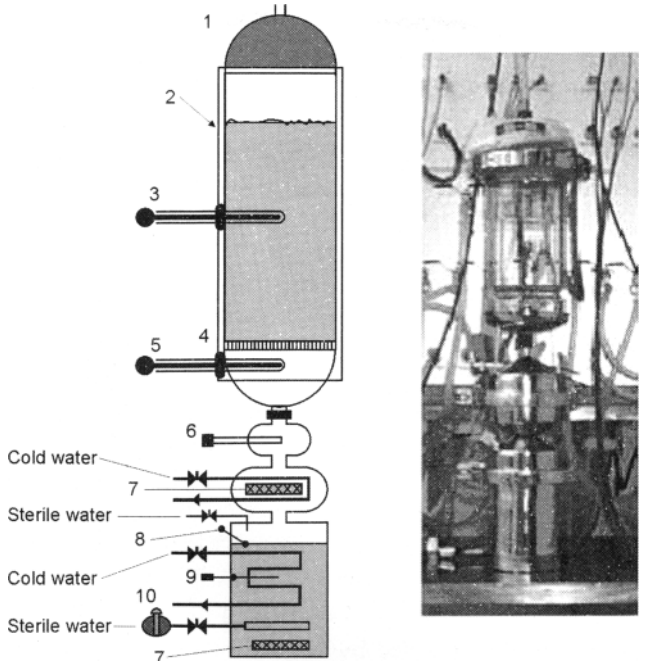


Fig. 7. Schematic and photography of a lab-scale sterile fermenter: 1) heating cover, 2) insulating jacket, 3) biomass temperature probe, 4) stainless steel sieve, 5) air inlet temperature probe, 6) relative humidity probe, 7) heaters, 8) level probe, 9) water temperature probe, 10) massic flow meter (redrawn from Durand 2003).

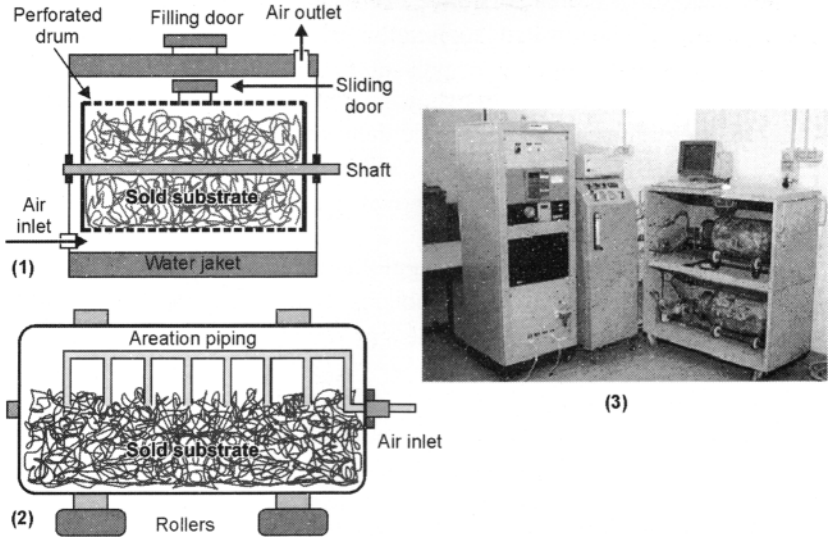


Fig. 8. Schematic of a rocking drum bioreactor (1), a rotating drum bioreactor (2), and photography of a lab-scale battery of drums controlled by a PC unit device (3).

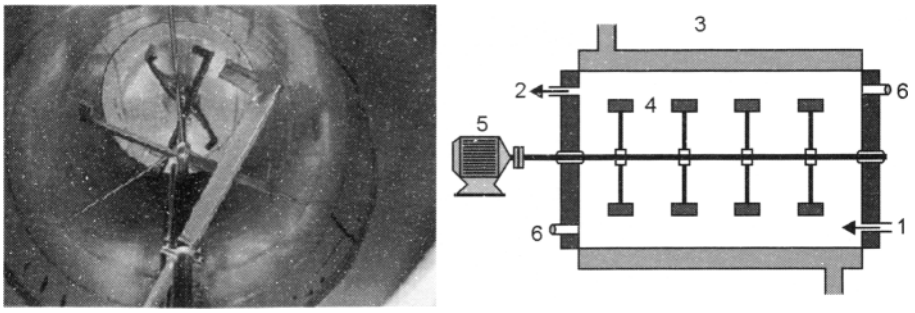


Fig. 9. Photography of a paddle mixer. Schematic of a stirred horizontal bioreactor: 1) air inlet, 2) air outlet, 3) cooling jacket, 4) paddles, 5) motor, 6) temperature probe (redrawn from Durand 2003).

With or without a water-jacket, this type of reactors is required for the continuous mixing to increase the contact between the reactor wall and the solid medium and also to provide oxygen to the microorganisms. For rotating drum bioreactors, as an horizontal cylinder, the mixing is obtained by the tumbling motion of the solid medium which may be aided by baffles on the inner wall of the rotating drum (perforated or not). With these bioreactors, agglomeration of substrate particles during the growth of the mycelium can happen.

6.4.2 Pre-pilot and pilot plants

Only a few designs of bioreactors have been studied and applied to pilot-scale, or industrial level. The limited application of SSF for large-scale is due to some further important parameters present for the scaling-up, such as:

- the mycelium hyphae, which can be damaged by heavy mechanical stirring, particularly if they have no septa, allowing only few possible designs to respect the aeration and heat removal needs.

- the solid medium, which can agglomerate during the process causing shrinkage, air channelling and problems during the operations. That makes difficult the heat removal and the gas transfer and severely restricts the design strategies available.

- the procedures of inoculation, control and the difficulties associated to the sterilization or pasteurization of large volumes as well. Particularly beyond three days of mycelial growth, it could be prohibitive working in non-sterile conditions (Fig. 10).

- the versatility of the reactor for a flexible usage in different conditions.

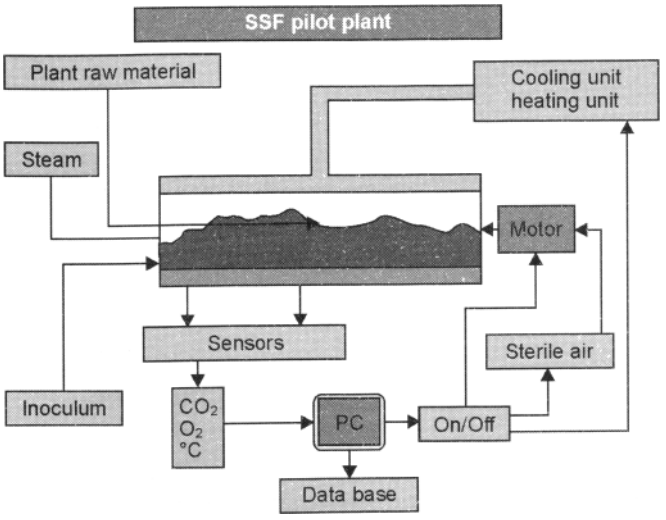


Fig. 10. Scheme of an SSF pilot plant and related controls.

- the maintenance and the procedures of filling, emptying, cleaning of the bioreactor.
- the maintenance of uniformity, difficult for large volumes of biomass.

Few of the previous described bioreactors have been used to develop the industrial and pilot plants that can be divided in categories based on the mixing and aeration strategies adopted (Table1) (Mitchell et al., 2000):

- static, pulsed mixed, continuosly mixed.
- unforced or forced aeration (trough the biomass).

Table 1. Summary of most used bioreactor typologies.

	Static	*Intermittently mixed*	*Continuously mixed*
Without forced aeration	• Tray bioreactor (Koij type)	• Rotating drum	• Rotating drum
With forced aeration	• Packed-bed • Zymotis	• Intermittently-stirred bed • Rocking drum	• Continuously stirred aerated bed • Rotating drum • Gas-solid fluidized bed

6.4.2.1 Static beds without forced aeration (tray bioreactors)

This category is the oldest and the simplest in design, typified by the tray fermenters used in the Koji process. The solid substrate is loaded on trays (made of wood, plastic or metal, perforated or not) in thin layers (typically 5–

15 cm) and placed one above the other with a gap of few centimetres into a thermostated room as shown in Fig. 11. There is fully empirical and experimental evidence of poor heat and mass transfer in trays, limiting the thickness of the substrate bed, that must be not more than a few centimetres. Hence, such reactors are restrictive in the volume of the solid matrix used: only thin layer can be used to avoid overheating and to guarantee aerobic conditions. Temperature and relative humidity are the only external parameters that can be controlled. The scale-up in this case is easy (increasing the number of trays used), but requires large operational area, intensive labour and it is difficult to apply for sterile processes.

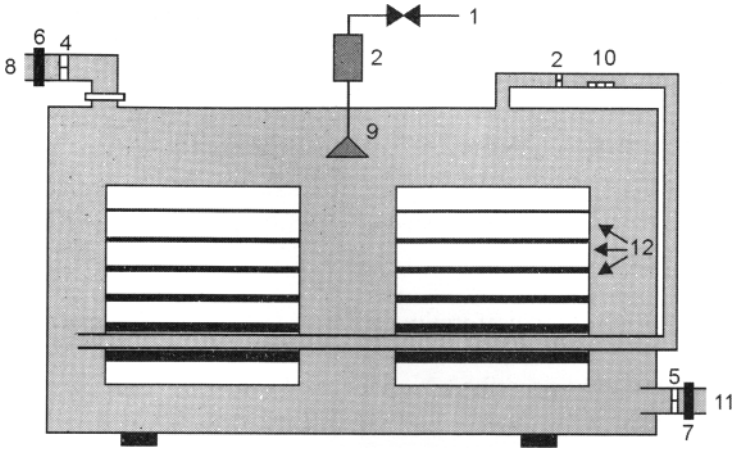


Fig. 11. Schematic of a koji-type reactor: (1) water inlet, (2) UV tube, (3) (4) (5) air blowers, (6) (7) air filters, (8)air outlet, (9) humidifier, (10) heater, (11) Air inlet, (12) Trays, (redrawn from Durand 2003).

6.4.2.2 Static beds with forced aeration (packed beds)

In a packed-bed bioreactor, conditioned air is usually blown through a sieve which supports the substrate. Experimental work demonstrates that the temperature gradients limit considerably the bioreactor performance. These bioreactors have been reported being interesting for their efficient process controls, especially for heat removal, which is due to an ample exchange surface. Strategies to prevent overheating problems have been examined (Ashley et al., 1999), finding more useful the mixing if tolerated by the microorganism than the periodic air reversal. Packed-bed bioreactors show some limitations such as irregular growth and poor heat removal if scaled up (Robinson & Nigam 2003).

Targeting a particularly efficient heat removal, Roussos et al.,(1993) developed the Zymotis bioreactor equipped with vertical rectangular cooling plates closely-spaced and inserted directly into the bed (Fig. 12). The geometry of such type

of bioreactor is particularly promising, but the loading-unloading operations can be uneasy and appears difficult to obtain septic conditions when large volumes are utilized.

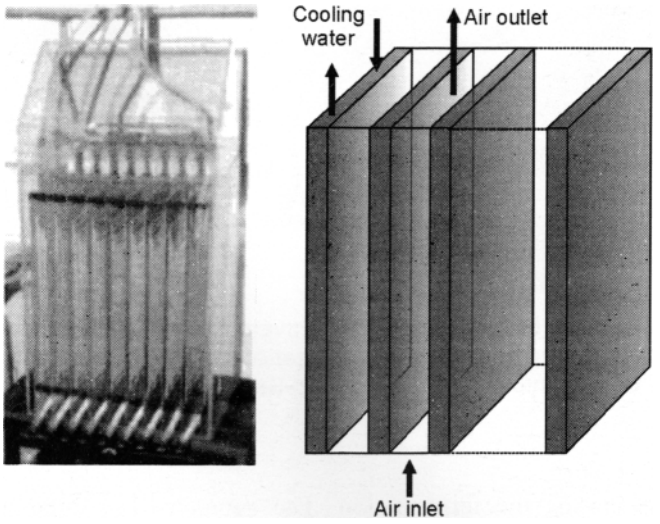


Fig.12. Zymotis bioreactor; photography(Durand 2003) and schematic.

6.4.2.3 Pulsed mixing without forced aeration (discontinuously rotating drum)

The drum bioreactor intermittently rotates operating like a tray bioreactor during the static periods.The pulsed stirring prevents the agglomeration of the mycelium and the shear is less effective on the mycelium than in a continuous rotating drum. This design has been demonstrated scalable up to 4 m^3 (Fig. 13).

This rotating drum can work intermittently or continuously and is equipped by an internal sterile aeration system that allows air circulation trough the substrate if required, a regulation of internal gas phase composition (automatically or manually controlled), a cooling/heating jacket and a sterile inoculation system. By the way it shows some limitations in heat transfer control, that is the greatest barrier in scaling up.

6.4.2.4 Pulsed mixing with forced aeration (intermittently stirred beds)

It is possible to enhance the homogeneity of the cell population and the substrate concentration in the SSF systems adopting sequential mixing and by imposing forced aeration through the solid substrate it is possible to allow the control of water content, water activity, the removal of volatile compounds, CO_2 and

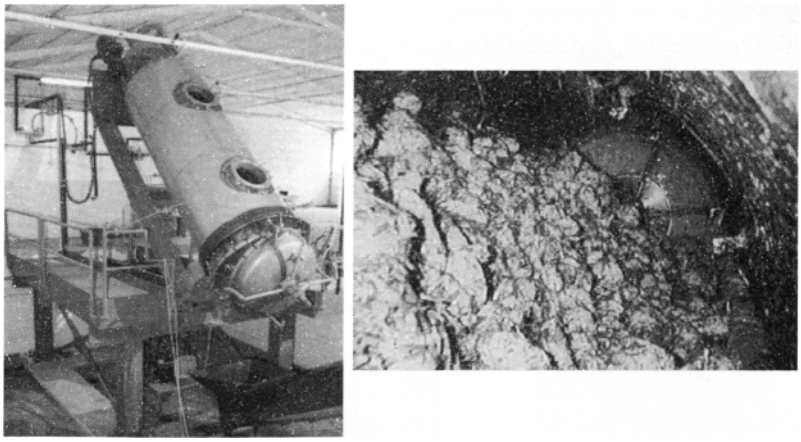


Fig. 13. Rotating drum bioreactor (4m^3) at University of Tuscia; inside view of the bioreactor with complete mycelial colonization at the end of a fermentation before unloading (Giovannozzi-Sermanni et al 1994).

heat generated during the fermentation. The geometry is similar to the one used for packed bed, but it includes a mixing system for the biomass like a screw or an other kind of agitation device (Fig. 14). The capacity of such kind of bioreactor has been scaled up to several tons. The intermittently stirred bed bioreactors are promising for non-sterile processes, but their design can be complex and not simply scalable, if sterile conditions are needed.

6.4.2.5 Continuously mixed without forced aeration (continuously rotating drums)

This type of bioreactor consists of a continuously rotating horizontal drum that holds the substrate bed. It can be equipped with baffles and within the air is not forcefully blown through the bed. The effect of the rotational speed and the other mixing parameters on the fermentation was largely studied by several

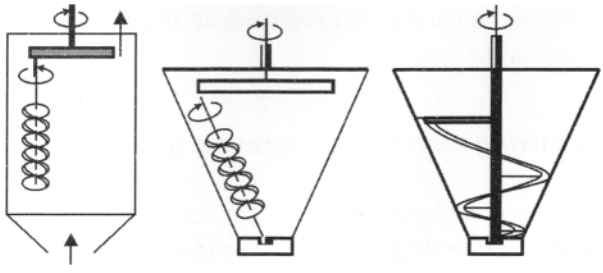


Fig. 14. Schematic of a cylindrical (1), conical (2), screw mixers and an helical blade mixer (3).

teams (Schutyser 2003). To increase the performance of such bioreactor, it is possible to use a baffled geometry and/or use high rotational rates (up to 10-50 rev. min ^{1}).

6.4.2.6 Continuously mixed with forced aeration

The most important types of this category of bioreactor are:

- the rocking drum bioreactor.
- the gas-solid fluidized bed.
- the continuously stirred aerated bed.

Frequently, water is replenished, by spraying it as a fine mist on the biomass or dripping it into the substrate trough perforated tubes. This allows high evaporative cooling. It has been reported that the continuous mixing improves the heat transport to the fermenter wall reducing the need of evaporative cooling and, consequently, the problems related to the substrate drying. The evaporative cooling retains its importance for large scale mixed bioreactors (Nagel et al., 2001).

6.4.2.7 Other designs

In the last few years, some interesting unusual novel designs for SSF bioreactor have been proposed. These designs are based on unusual features and for this reason, they differ visibly from the previous typologies. A patented solid-state fermentation based on periodic air-forced pressure oscillation (SFPAPO) has been described by Chen & Sun (Fig 15). An immersion bioreactor, based on intermittently immersion in a liquid medium has been described (Rivela et al., 2000; Rodríguez-Couto & Sanromán 2006). It consists of a jacketed cylindrical

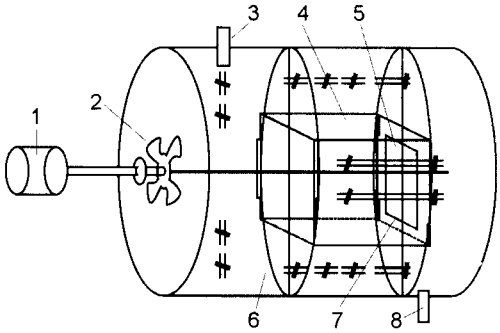


Fig. 15. Schematic of a fermenter with periodic pressure oscillation: (1) variable speed motor, (2) fan, (3) air outlet, (4) tray, (5) air distributor board, (6) fermentation vessel, (7) exhaust pipe, (8) air inlet (redrawn from Chen & Sun Year).

glass vessel with a round bottom, inside which several wire mesh baskets filled with colonized support are placed. They can move upwards and downwards by means of a pneumatic system, remaining more time outside than inside the medium (Fig.16).

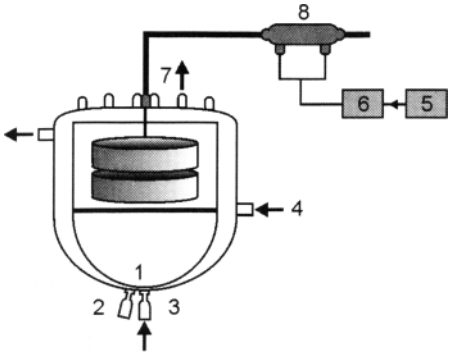


Fig. 16 Scheme of an immersion bioreactor (mechanical agitation; humidified air): (1) medium, (2) sampling port, (3) air inlet, (4) water cooling, (5) compressor, (6) pneumatic system, (7) gas exit, (8) time controller. (redrawn from Rivela et al., 2000).

6.4.3 Scaling-up

SSF scaling-up, essential for uses at industrial scale, raises severe engineering problems due to the increase of temperature, pH, O_2, substrate and moisture gradients. Hence, most published reviews also focus on progress towards industrial engineering. The scale-up of bioreactors is usually based on empirical criteria, related to transport processes (Hsu & Wu 2002), but the application of mathematical modelling techniques to describe the phenomena within the system is the basis of the most significant improvements in designing and scaling-up SSF. Lenz et al., (2004) and Pandey (2003) have shown to these be a powerful aid for the designing and the defining of large-scale bioreactors (Raghavarao et al., 2003). A large-scale prototype of an intermittently stirred bed (1 ton capacity) designed at INRA has been described by Durand & Chereau in 1988 (Fig. 17).

An important concept in the design a bioreactor is the possibility to let take place in one single vessel as many major operations as possible. The pilot plant described by Grant et al., in 1978 is a good example in this respect. In this bioreactor, it was possible to hydrolize (121°C, 30min, 0.5N H_2SO_4, 7:3 liquid:solid) the substrate (100kg of ryegrass straw per batch), to treat it with ammonia to raise the pH (to 5.0), to inoculate it (with *Candida utilis*) and to conduct the fermentation holding the material stationary with air blowing up through it, as shown in Fig. 18.

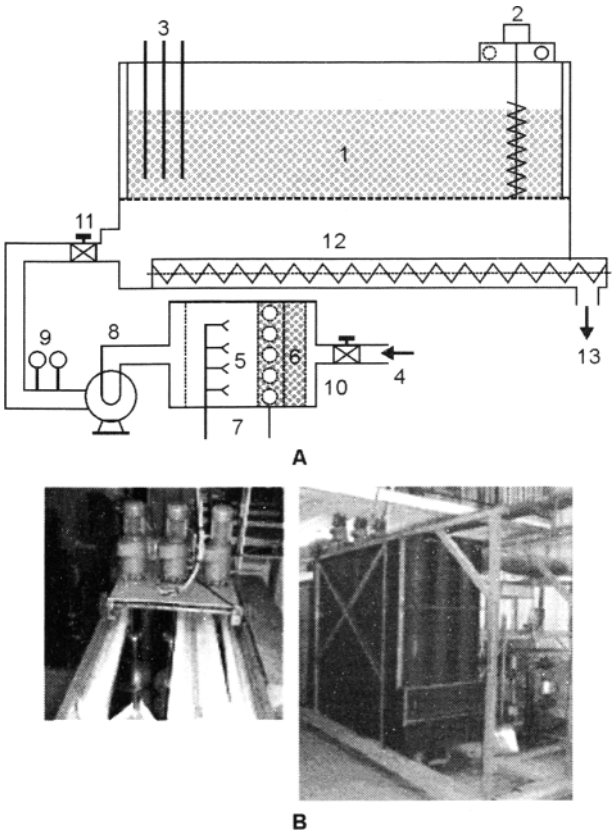


Fig.17 A. Schematic of a large-scale mixed reactor: (1) inoculated biomass, (2) agitation device, (3) probes, (4) air inlet, (5) cooling water, (6) steam, (7) air conditioner, (8) blower, (9) regulator, (10) (11) valves, (12) screw conveyer, (13) outlet (redraw from Xue et al., 1992) B. photographies of a large scale bioreactor (INRA-Dijon, France) (Durand 2003, Durand et al., 1996).

On the same concept, a novel efficient design of integrated matrix bioreactor, called the PLAFRACTOR^{TM}, consisting of a computer controlled device, using complex fermentation control algorithms, has been patented (Suryanarayan & Mazumdar 1999; Suryanarayan 2001). All the operation, such as sterilization, cooling, inoculation, control of fermentation condition, extraction of the product and post-sterilization of the substrate, are all done in one single equipment, maintaining all the advantages of SSF (Fig. 19).

A particularly interesting novel design was scaled-up and reported by Hongzhang et al., (2002). It consists of a cylindrical steel container with the capacity of 70 m^3, used as the fermentation vessel, loaded by trays made up of stainless steel mesh placed horizontally, or vertically. In order to enhance the biological

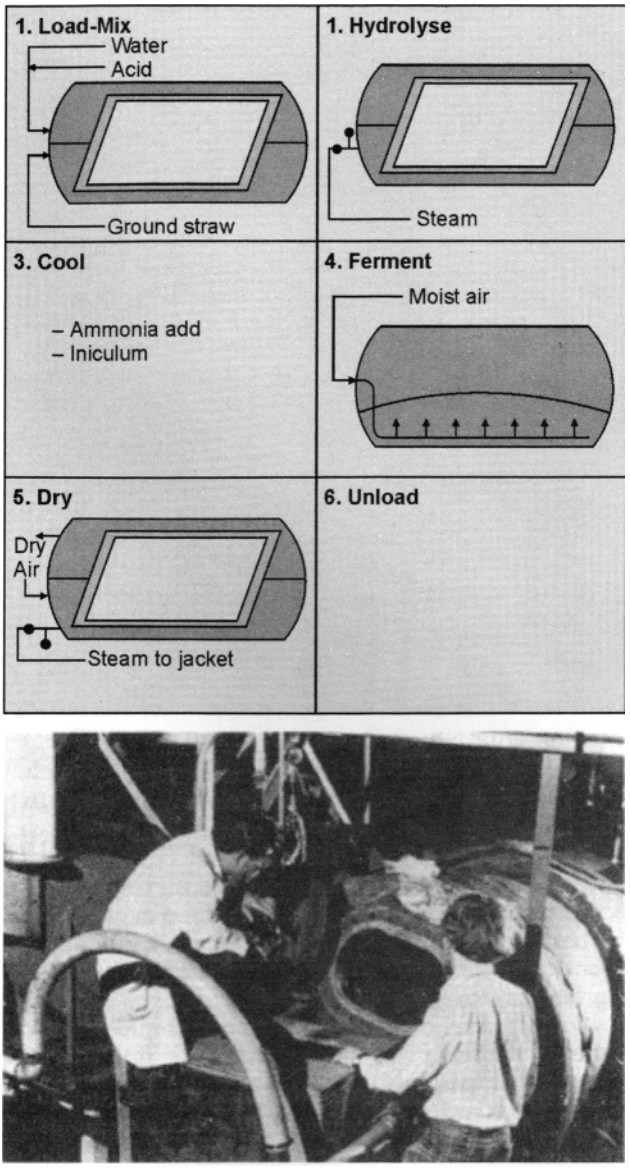


Fig. 18. Pilot plant (2,270 l): operations and photography (Grant et al., 1978).

activity by external stimulation, thermal and mass transfers, the fermentor is supplied by two dynamic changes of air consisting of internal/external circulation and periodic air pressure pulsation (Fig.20). This system provides sufficient gas exchanges, more room for fungal propagation and efficient heat removal without disrupting the mycelia. On these basis, the reactor design was scaled-up from 8 l to 50 l, 25 m^3 and 70 m^3.

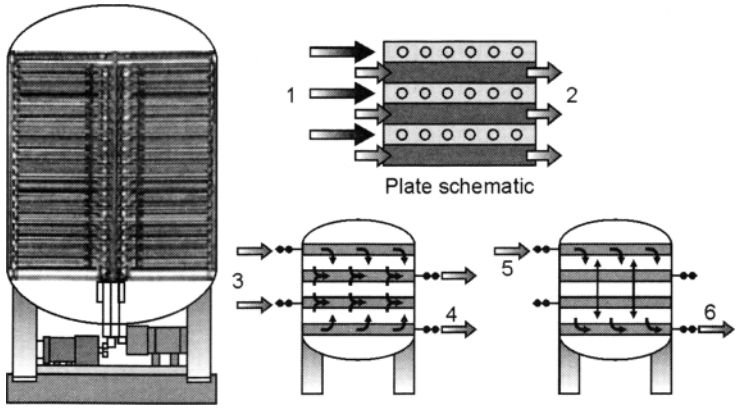


Fig. 19 Plafractor™ bioreactor: schematic with; (1) fluids into communicating channels, (2) fluids out from non-communicating channels, (3) air/steam/ inoculum in, (4) vent, (5) extraction fluid in, (6) extract (Suryanarayan 2003).

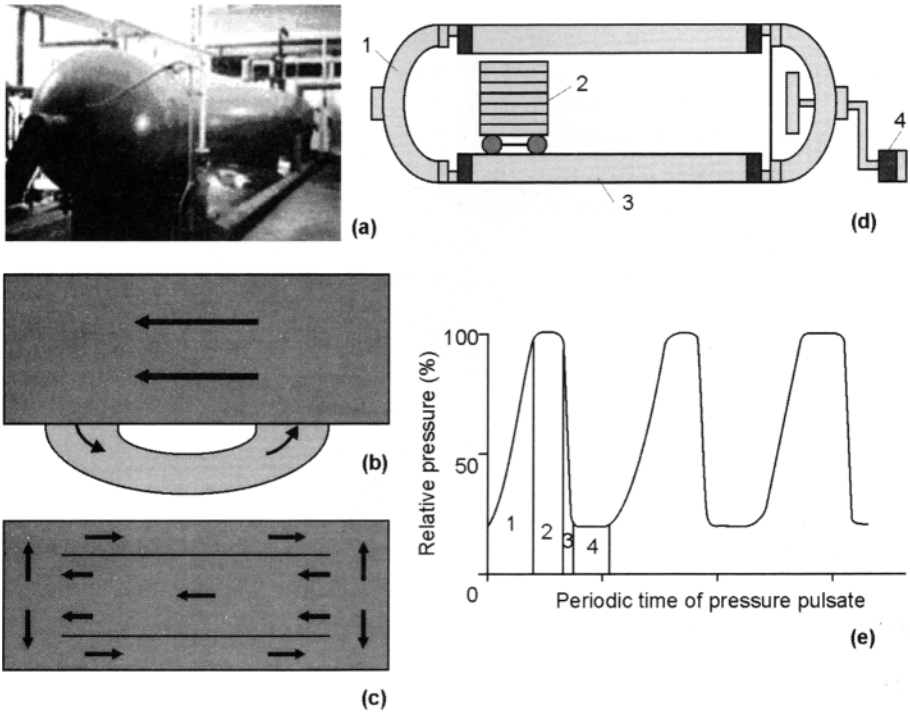


Fig. 20 Photography of of the 70 m³ industrial bioreactor (a); schematic diagram of external (b); and internal (c); circulation of (d): air; schematic of the reactor: 1. door, 2. trays, 3. vessel, 4. fan system; (e); diagram of the periodic pressure changes (drawn from Hongzhang et al., 2002).

6.5 APPLICATION

One interesting example of the application of SSF enzymes production is given by the preparation of cellulose for paper mills, where the opening of the fibres needs large amounts of thermal, mechanical and chemical energy. Enzymes produced by SSF of wheat straw have been used to prepare paper pulps from annual plant residues.

By the rotating drum bioreactor after seven days of fermentation with a *Lentinus edodes* strain, a well colonized biomass is obtained (Fig. 14). With the aid of a press, it is squeezed and the liquid phase is used as a source of enzyme, particularly redox enzymes. Enzymatic mixture applied to annual plant residues have excellent technical properties of high yield paper obtained with kenaf bast strands, cereal straws.

With corn stalks (Giovannozzi Sermanni et al., 1994) remarkable improvements of freeness and strength properties with respect to the control are also obtained, as well as a higher lignin extractability. Previous investigations, carried out on wheat straw and corn stalks, showed that the enzymatic incubations produced chemical modifications, which were not evident after the pre-treatment only. In fact, positive effects, in terms of lignin extractability, were recorded after alkaline cooking. This phenomenon suggested the possibility of a lignin functionalization, which rendered it more extractable under alkaline conditions and/or a physical modification of the biotreated material, such as an enhanced porosity, which facilitated the accessibility of the cooking liquor, thereby improving its effectiveness. The cellulose content of enzyme treated-samples (ET) did not differ from the corresponding controls, confirming the poor cellulolytic capability of the *Lentinus edodes* strain. By incubating recycled fibers with enzymatic cocktails containing cellulases and hemicellulases, the results suggest that the enzymes could act on the surface of the fibers (peeling effect), removing those components with a great affinity for water, poorly contributing to the hydrogen-bonding potential of fibers (Pommier 1989). The biotreatments have poor, or no effects on the opacity and brightness, whereas the latter parameter was reduced by 20 and 15% in wheat straw and corn stalks samples, respectively. The phenomenon increases the strength properties accompanying reduced optical properties during biomechanical pulping of loblolly pine chips (Akhtar et al., 1992; 1993). The enzyme treatment show a significant energy savings during fiberization and refining.

The results obtained by using hexoenzymes allows to obtain the following main results:

- the amounts of alkali employed in the cooking process for enzymatically-treated samples is reduced by 40 % with respect to the untreated controls

(4% vs 7% NaOH on o.d. weight basis). All comparisons are made at the same freeness values of pulps (30-35 S.R. degrees).

- significant energy savings during fiberization and refining operation (50%) are observed in enzyme treated samples.
- no significant differences in the optical properties are observed between the controls and the enzymatically-treated samples, with the exception of kenaf and wheat, being the brightness values of ET samples lower than the corresponding CT samples.

6.6 CONTROLS

Monitoring these processes requires the measurement of environmental parameters (temperature, pH, water content and activity) and the carbon cycle (biomass, substrate concentration, CO_2). However, given the complexity and heterogeneity of the solid medium, these variables are not easily accessible and measurable (Bellon-Maurel et al., 2003). Temperature sensors are generally inserted radially at various distances from the centre of the fermentor and linked to control systems. At the industrial scale, temperature regulation is linked to the moisture content of the solid phase.

Direct measurements of temperature, pH, and water content are considered employing classical sensors, and indirect measurements of the biomass by respirometry or pressure drop (PD). More recent methods have been tested and successfully tested, such as: aroma sensing, infrared spectrometry, artificial vision, and tomographic techniques (X-rays, Magnetic Resonance Imaging or MRI). Therefore, the study and development of novel on-line methods and innovative applications of methods with a potential to measure parameters in SSF are an important task to improve the performance of the bioreactors.

6.7 CONCLUDING REMARKS

The abundance of scientific literature on the solid-state bioreactors makes difficult to organize a comprehensive treatment of the issues given the complexity of the matter. Many solid materials can be utilized for SSF such as soils, animal residues, hides, but without any doubt the most attractive solid-state substrates for new processes are the lignocelluloses, present all over the Planet. By keeping in mind that the amounts of agriculture residues can reach up to the 50% of totally produced biomass, the paramount importance of the SSF is obvious for utilizing the enormous amount of available chemical energy improving at the same time the biological sustainability.

The practical approach for the production of metabolites and enzymes can be related to different areas (paper deinking, paper recycling, agricultural residues utilization, pesticides biodegradation, fodders, olive and seeds oils residues, pruning, fuels, paper pulp production, etc.) and each of them can require some different sets of biotechnological conditions .

As it results from the data of the scientific literature, for obtaining profitable results, it is necessary to have dedicated bioreactors, which can take into account same particular characteristics of the new processes such as hyphen fragility if filamentous fungi are used and/or dissipation of heat due to the metabolic respiration. Hence, the CO_2/O_2 ratios of gas phase inside the bioreactor are important to judge and to define the more useful bioconversion conditions. In fact, these ratios can describe the bioreactor like a living cell, where the biochemical activity can determine different respiratory quotients. Nevertheless, the description in the literature of many different solid state bioreactors suggests that a bioreactor suitable for all the bioconversion conditions seems still under progress, being perhaps the working flexibility the best aspect to achieve.

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