SOLID STATE FERMENTATION

Mrs. Neetu Das
Assistant Professor (Microbiology)
Govt. V.Y.T. P.G. Autonomous College , Durg , C.G.

Introduction

Solid state fermentation (SSF) is a process in which microorganisms grow in an environment without free water, or with very low content of free water.

Considering the last century and the recent decades it is still used for production of important biomolecules and products for many industries, including food, pharmaceutical, textile, biochemical and bioenergy, among others .

The main counterpart for SSF is the submerged fermentation (Smf), a process in which microorganisms grow in liquid medium, with high content of free water. Biological processes carried out in Smf have notable advantages regarding instrumentation and control (monitoring of pH, dissolved oxygen, temperature, concentration of water soluble molecules), separation of biomass after the fermentation, mixing, aeration and scaling up.

Contrarily, SSF mimics the natural habitat of most part of microorganisms (mainly fungi and mold); demand less energy for sterilization (because of lower water activity);is less susceptible to bacterial contamination; regarding the products, it enables higher enzymatic productivity for many enzymes, it is less susceptible to substrate inhibition , and hence it allows higher final concentration of products; has several environmental advantages, since it allows the use of solid agro-industrial wastes as substrate and/or energy source in their natural form and facilitates the solid waste management, besides lesser wastewater production.

Other aspects cited as positive concerning SSF are: the higher quality and higher activity of extracts; no need of organic solvents (which generally confer some level of toxicity for the extract); lower capital and operating costs; reduced down-stream processing and reduced stirring .

Microorganisms

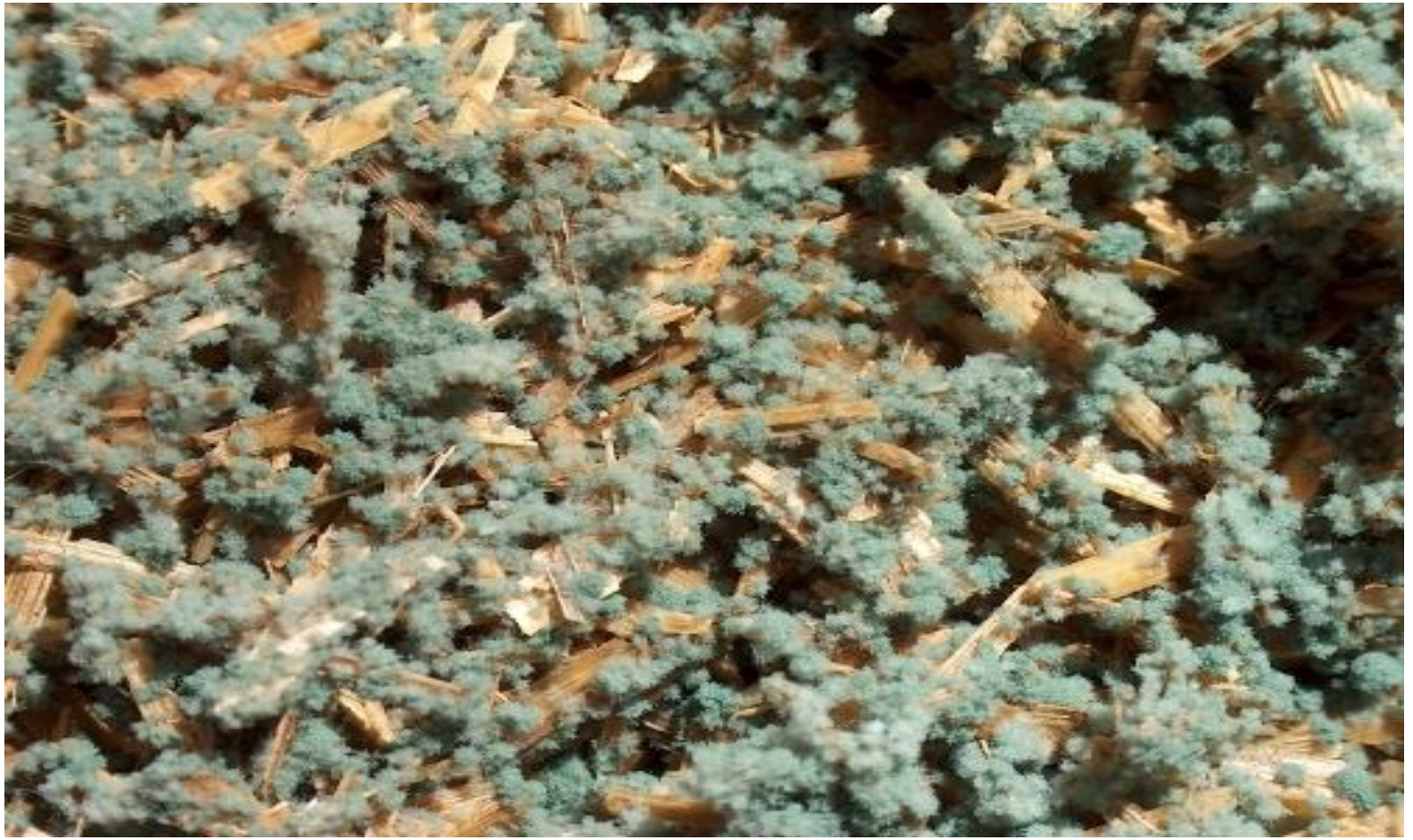
The most important factors to be considered during the development of a SSF are the choice of microorganisms and the choice of substrates. Microorganisms that are particularly suitable for SSF are the filamentous fungi, since the technique simulates their natural habitat.

In this condition, they are able to synthesize considerable amounts of enzymes and other metabolites.

Although filamentous fungi are considered the most appropriate microorganisms for SSF, and secondly the yeasts, which are also able to grow in a low water activity environment, there are also some species of bacteria (e.g. *Bacillus subtilis*, *Bacillus thuringiensis* and *Lactobacillus* sp.) which have been reported to successfully produce enzymes in solid-state condition .

Actinomycetes such as *Streptomyces* sp. are also indicated for SSF processes since they present characteristics such as abundant colonization of solid residues, production of a wide range of degradative enzymes and high resistance to extreme conditions.

Solid state fermentation (SSF) in Erlenmeyer-flask



Growth of Trichoderma asperellum on wheat straw
in solid state fermentation (SSF)

Substrates

The most promising residues for SSF include agricultural and forestry residues, which are very abundant and normally under utilized.

Agro residues that can be used as substrates for SSF include sugarcane bagasse, cassava bagasse, cereal brans such as wheat bran, rice bran, oat bran and soybean bran, coffee pulp and husks, fruit peels and pulps, corn cobs, straws and husks of different origins .

These materials are basically composed by cellulose, hemi-cellulose, lignin, starch, pectin and other fibers The choice of the most appropriate microorganisms to be cultivated in the agro residue depends much on its composition.

In the case of lingo cellulosic residues, the wood rotting fungi are usually the most indicated. Wood rotting fungi can be classified in white-rot fungi (Basidiomycetes and some Ascomycetes), which preferentially degrade lignin, brown-rot fungi (Basidiomycetes) , which degrade cellulose and hemicelluloses, and the soft-rot fungi (e.g. *Aspergillus niger* and *Trichoderma reesei*) , which secrete a complete system of cellulases.

Usually, these agro residues are not only a solid support for nutrients absorption and biomass growth, but they are also a source of carbon and nutrients. Sometimes, supplementation is needed in order to provide all necessary nutrients for optimum growth. Macro and micronutrients that are usually added to the medium include phosphorus , sulfur, potassium, magnesium, calcium, zinc, manganese, copper, iron, cobalt, and iodine.

Cost and availability are the main factors to be considered in the choice of a residue as substrate or support in SSF. However, other characteristics such as crystallinity, accessible area, surface area, porosity, and particle size are important aspects to be considered for SSF process.

Applications of SSF

Enzymes- Production ,mainly cellulases and xylanases production.

The class of the cellulases is formed by endoglucanases (EC 3.2.1.4) that cleave randomly the cellulose chain, exoglucanases (EC3.2.1.74 and EC 3.2.1.91), which catalyze the hydrolysis of chain ends, and glucosidases (EC 3.2.1.21), also called cellobiases, that hydrolyse the product of the exoglucanases releasing glucose monomers.

Xylanases are a class of enzymes that catalyze the hydrolysis of 1,4- -D-xylosidic linkages in xylans, one of the components of the hemicellulose fraction of plant cell walls.

Carboxymethyl cellulase (CMCase) activities between 172 U/g and545 U/g were obtained, which represents the activity of endoglucanases, produced from substrates such as wheat bran, corn stover and apple pomace by filamentous fungi(*A. niger*, *Aspergillus fumigatus* and *Rhyzopus oryzae*). For xylanase, activities as high as 73,000 U/g or 98,000 U/g were reported, this time by *Bacillus* species cultivated on wheat bran. Both classes of enzymes have important applications in the conversion of biomass to products such as ethanol, and also other important applications in the textile, paper, food and beverages industries .

Proteases were the third most studied group of enzymes produced by SSF in the recent literature. They were produced from various agro industrial and also from tannery industry wastes, especially by fungi of the genus *Aspergillus*. Activities higher than 3000 U/g and higher than 50,000 U/g were reported .Proteases are considered the most significant industrial enzymes, representing around 60% of the global market, with applications in detergents, leather processing, food and feed processing, pharmaceuticals, chemicals and waste treatment .

Amylases (E.C. 3.2.1.) were produced from residues such as wheat bran and straw, apple pomace, soy , bread and date waste, some of them enriched with starch , and different microorganisms were employed (*Aspergillus oryzae*, *Bacillus* spp., *Thermomyces* sp. and *Macrophomina phaseolina*). Highest activity (39,900 U/g in 4 days,)was obtained with soy and bread waste fermented by *Thermomyces* sp. Amylases are widely employed in the food industry, for ethanol production from starchy materials , and in pharmaceuticals, paper and detergents, representing around 30% of the global market of enzymes.

Biopulping

Biopulping is a kind of biomass pretreatment that promotes the delignification of cellulosic material through the action of ligninolytic exoenzymes produced ‘‘*in situ*’’, which is normally performed by fungi that grow onto the solid ligninolytic material or a solid process fermentation.
These enzymes act on the solid substrate, breaking the chemical bonds of the lignin molecule, depolymerizing the whole structure into smaller structures .

The fungus must be selected according its enzymatic activities, presenting a high laccase and peroxidase activity and a low cellulasic activity to preserve the cellulosic fraction. This leads to preferably digestion of the lignin that is present in the vegetable cell wall. Due to the mild conditions used, biopulping produces cellulose pulp demanding less energy and chemical products in a lesssevere process compared to the chemical or mechanical conventional pulping. Also, biopulping improves the cellulose recovering yield and the quality of the paper fiber.

The most common and efficient fungi used are those of the wood decomposition, known as the white rot fungi and the brown rot fungi, that during their secondary metabolism secret these enzymes .*Trametes and Pleurotus* genus are the most studied such as *Trametes pubescens, Trametes versicolor , Trametes hirsuta* , *Pleurotus ostreatus* ,but it can also be cited *Coriolus hirsutus*, *Neurospora crassa.*

The disadvantage of the biopulping is the time demanding for the process including time necessary to the fungus growth and the substrate colonization, so an alternative to turn the process faster is the application of the enzymatic ligninolytic ‘‘pool’’, produced in an optimized fermentative process.

This enzymatic pretreatment was proposed as an alternative to the biological pretreatments that use the fungus application onto the material to be treated, slower due to the time demanding to the fungus growth and the difficulties to keep the process aseptic for long time periods.

The enzymatic pulping besides replacing the fungus direct application and reducing the time necessary to the process, can also replace the chemical catalysts of the traditional thermochemical pulping, promoting the material’s digestion in milder conditions. The production of the ligninolytic enzymatic pool is carried out in bioreactors under controlled and optimized conditions. The enzymatic broth produced can be composed by the desired enzymes according to the material to be treated and the fraction of the material is supposed to be preserved.

Bioreactor design for SSF

SSF bioreactors can be classified based on the mixing system that is employed: **static bioreactors** (fixed bed, perforated trays) or **stirred bioreactor** (horizontal drum or stirred drum).

Other classifications can be also found **according to the type of aeration (with or without forced aeration) or employed mixing system .**

These are the tray, packed-bed, horizontal drum and fluidized bed bioreactors having their own advantages and disadvantages, which promoted the necessity to develop novel bioreactors with better design.

Durand (2003) has given relevant information on various designs of bioreactor for SSF. The design of bioreactors must take into account some peculiarities of the materials that are used as growth media, and their characteristics such as composition, size, strength, porosity and water holding capacity.

SSF occurs in the absence of free water and filamentous fungi are the microorganisms that are naturally adapted to this fermentation technique. In this case, some details must be considered in bioreactor design such as fungus morphology, with respect to the presence of septate hyphae or not, which influences the choice of agitation (without stirring, stirring occasionally or continuously). Another point to consider is the aeration by diffusion or forced aeration. All these details certainly raise bioreactors complexity.

Static bioreactors

Different types of static bioreactors are used for SSF from laboratory to industrial scale. Erlenmeyer flasks, small perforated trays, fixed bed bioreactors or Raimbault columns, Petri dishes, jars, Roux bottles and roller bottles offer the advantage of simplicity and the possibility to work with small volumes. The main characteristic of these bioreactors is the absence of agitation (Durand, 2003). Erlenmeyer flasks are very simple and are used in laboratory scale for initial studies and processes' conditions optimization. They are made of glass and have limited size. These flasks are closed with cotton plugs, which allow the aeration by diffusion. Some advantages are: easy of handle, low cost and allow multiple simultaneous tests. Different types of static bioreactors for SSF are presented below.

Fixed bed bioreactors --- the Raimbault columns

The Raimbault columns, packed bed or fixed bed bioreactors are filled with the solid substrate or supports. The column bioreactors are closed systems with forced aeration. Columns are connected to air bubblers, and introduced into a water bath with controlled temperature. Aeration, made by saturated air that is pumped through the columns, is adjusted to the desired airflow and controlled with the help of a flow meter attached to the column air outlet.

This model of bioreactors allows the study of the influence of forced aeration on growth through the evaluation of respirometry (O_2 consumed and CO_2 produced) of the microorganism for the understanding of their metabolism. These bioreactors are the most employed in laboratory scale. A model of columns reactors made of glass coupled with a system that analyses the oxygen and carbon dioxide composition in the exhaust airflow from the column. Limited bacterial contamination is observed due to the close system.

The use of column reactors minimizes problems with temperature gradients, due to the convection caused by air entering the reactor. These bioreactors have been considered interesting due to process control, especially by removing heat (which does not happen efficiently in large-scale processes).

Furthermore, the CO2 released during metabolic reactions can be eliminated, allowing its replacement by air, which is an advantage for some processes.

Temperature control is done by placing the reactor in a bath or using jackets with circulating coolant fluid. However, the reduction in porosity of the bed, with the progress of fermentation, is a problem to overcome in this type of bioreactor .

So the basic design feature of packed-bed bioreactors is the introduction of air through a sieve which supports the substrate. In this way, a bioreactor was developed at pre-pilot scale for defining the control strategy and optimizing the air-inlet temperature, the airflow rate, the addition of water and agitation during a SSF process.

Located in a clean room, the reactor can be pasteurized in situ by steam generated by the water-bath used for the air humidification. This reactor is very simple and can process a few kilograms of dry solid medium. An adaptation of packed bed bioreactors would be the insertion of mixing coupled with forced aeration, which could increase the homogeneity of the cell population.

The geometry of the stirred bed reactor is similar to that used in fixed bed, but includes a mixing system. In this case, the great advantage of this system would be the possibility to work with higher scales .

Perforated trays bioreactors

Tray bioreactors are simple systems in which the substrate is laid out on trays, made by wood or stainless steel, which are perforated to facilitate air convection.

The substrate is placed in the tray in thin layers (from 5 to 15 cm) that are arranged with a space between them to allow the aeration.

Trays are arranged in a chamber or room with controlled temperature and humidity. This model is easy to scale-up, but requires large areas for operations, intensive labor and difficulties with contamination control (non sterile processes).

Problems can also occur with oxygen transfer, which depends on tray characteristics and the height of the substrate layer.

When the substrate is inoculated, the oxygen concentration is uniform. Then, the formation of the mycelium changes the porosity and thus the effective diffusivity.

The release of CO2and heat also limit the transport of O2and the creation of higher O2 gradients that are inevitable, especially for higher substrate layers.

Agitated bioreactors

Another concept of bioreactors for SSF, which was based on continuous or intermittent agitation of the solid medium, was also developed .The bioreactors can be a rotating drum or horizontal paddle mixer with or without a water-jacket. When working with continuously mixing, it is expected to increase the homogeneity of the solid medium and better oxygen transfer to the microorganism.

Koji bioreactors

An example of static non sterile bioreactor is represented by the rotary type automatic *Koji* making equipment marketed by Fujiwara in Japan .The treated substrate is heaped up on a rotary disk. Depending on the diameter of this disk, different working volumes are available but always with a layer of maximum thickness 50 cm. This non-sterile reactor operates with a microcomputer which controls all the parameters (temperature of the air-inlet, air flow rate and agitation periods) . The main drawback of this equipment is the need to prepare and inoculate the substrate in other equipment before filling the reactor. Nevertheless , this type of design is widely used in Asian countries.

Horizontal drum

Horizontal drum bioreactors are built in drum-shape and contain baffles for agitation of the medium . The agitation in this type of reactor can be continuous or sporadic, and may lead to problems of shear and damage of the mycelium structure.

Mixing bioreactor

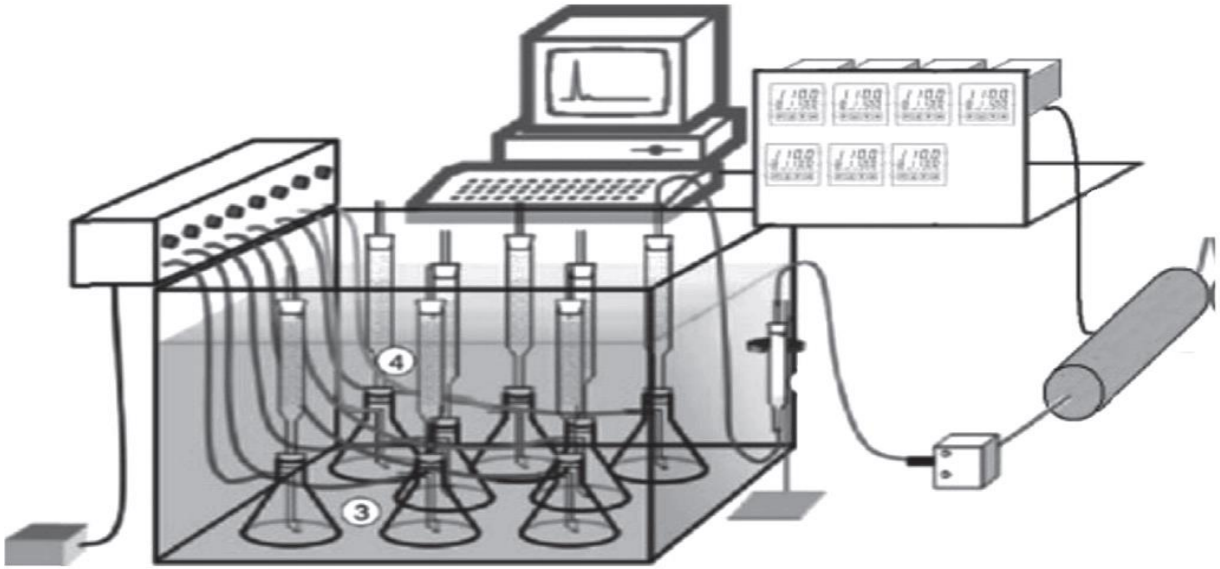
A 50 L bed bioreactor was patented by Durand (2003). This reactor, which has a planetary mixing device and is completely automatic for the different process steps : sterilization of the bioreactor, sterilization of the medium , process control during fermentation and data acquisition.

Rotating drum

Rotating drum bioreactors (RDB) consist of an horizontal cylinder, where the mixing is provided 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).

However, in all these reactors, the mixing is less efficient than with a paddle mixer. RDBs provide relatively gentle and uniform mixing by improving baffle design, since there is no agitator within the substrate bed. The engineering principles of RDB have received interest for biofuels production using cellulosic materials.

Rotating drum bioreactors with air circulation and continuously mixing are commonly used in pilot or lab scale process. According to Durand (2003) the largest reactor cited in the literature was a 200 L stainless steel rotating drum, which used 10 kg of steamed wheat bran as substrate for kinetic studies of *Rhizopus* .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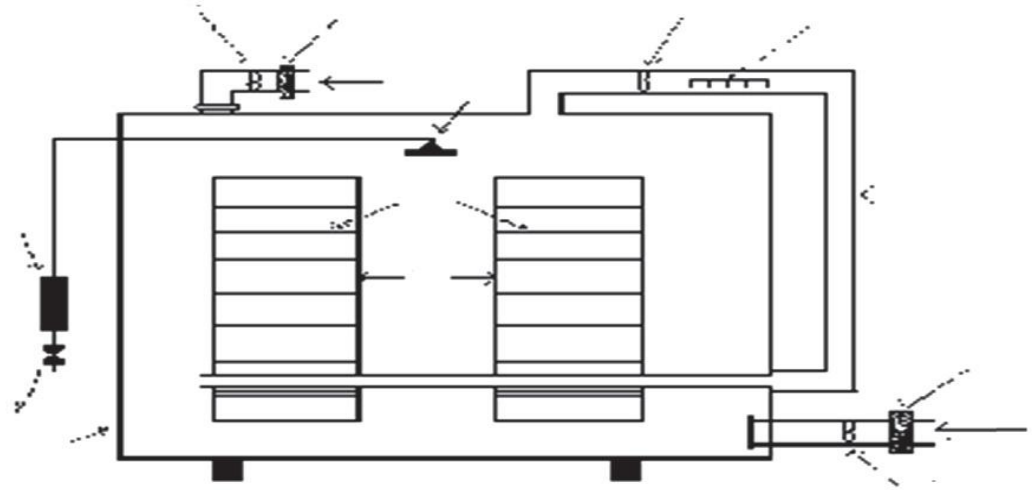


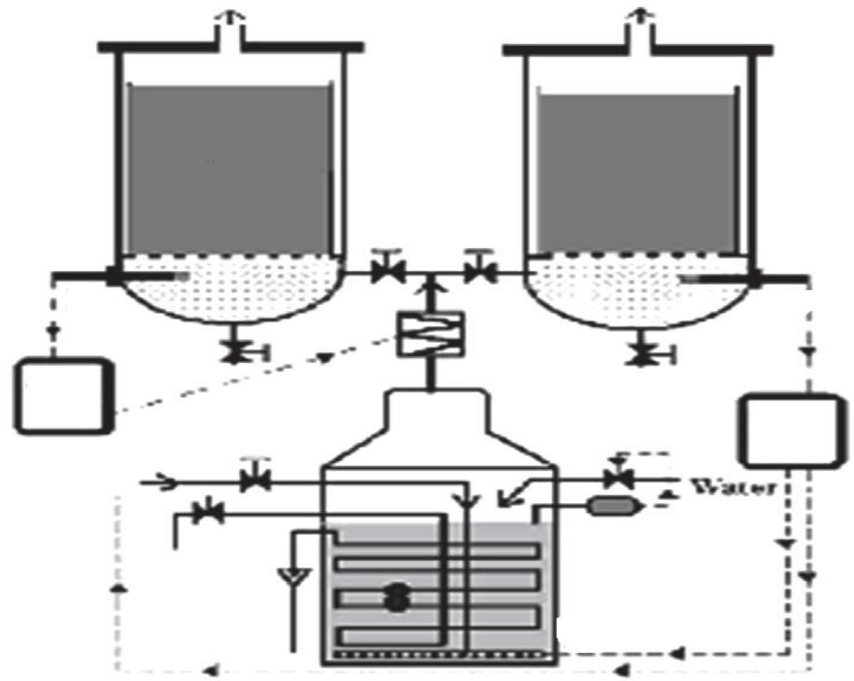
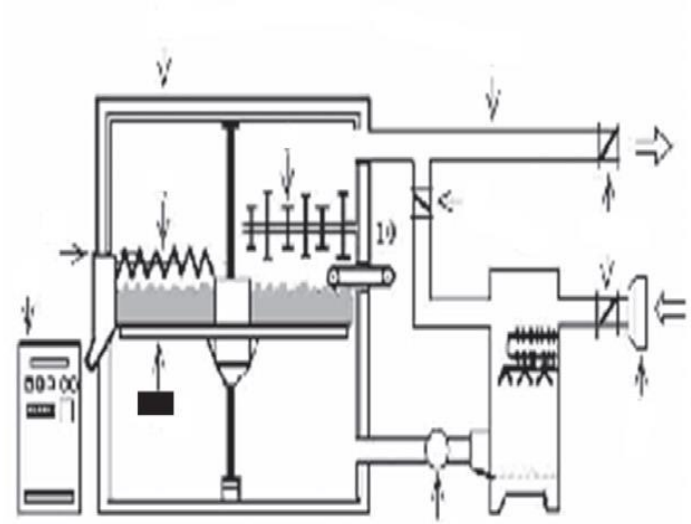

Water

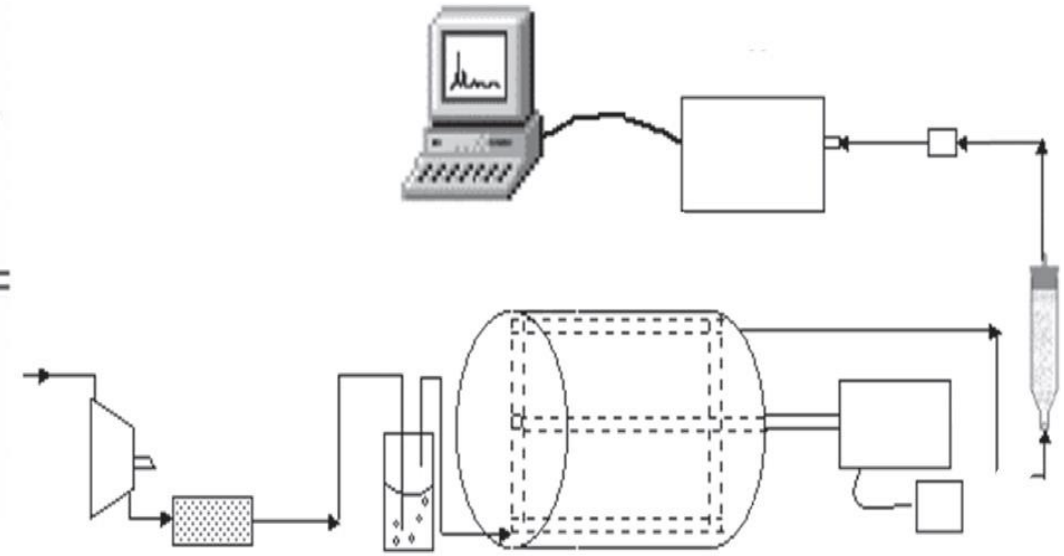
Figure 1

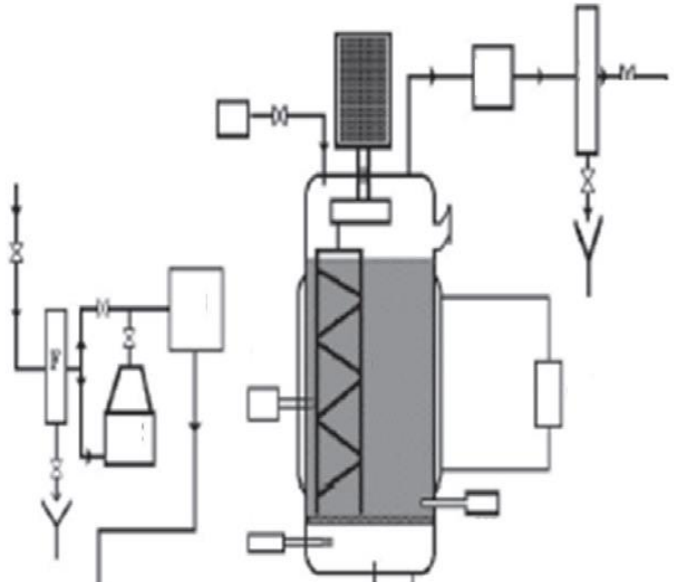
(A) Lab-scale fixed bed bioreactors apparatus for solid state fermentation: (1) air pump; (2) air distribution system; (3)humidifiers; (4) fermentation columns immersed in a water bath with controlled temperature; reactor; (5) filter; (6) flow sensor;(7) controllers display; (8) computer with data acquisition and control software; (9) cylindrical sensor base, where the following sensors are installed: CO2and O2, humidity and outlet temperature.

(B) (B) Perforated trays bioreactor.

(C) (C) Unmixed bioreactors with forced aeration. (1) Basket containing the solid medium, (2) valves for airflow adjustment, (3) air temperature probe, (4) relative humidity probe, (5) drain cocks, (6) heating box, (7) humidifier, (8) coil for circulation







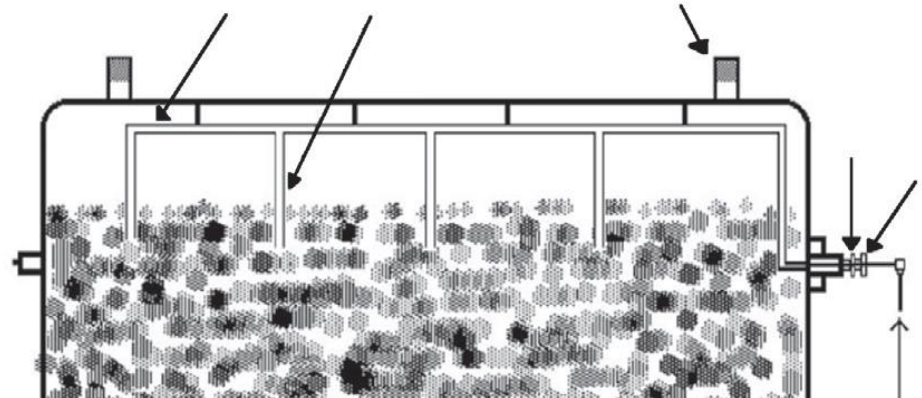


Figure 2

(A) Koji making equipment: (1) Koji room, (2) rotating perforated table, (3) turning machine, (4, 11) screw and machinefor unloading, (5) air conditioner, (6) fan, (7) air outlet, (8) dampers (9) air filter, (10) machine for filling, (12) control board.

(B) (B)Schema horizontal drum: (1) compressor, (2) Air filter, (3) humidifier, (4) horizontal drum, (5) stirrer, (6) motor, (7) speed controller,(8) air discharge, (9) silica gel columns, (10) element name (11) gas chromatograph (12) computer.

(C) (C) Sterile bioreactor developed by the National Institute of Agronomic Research in Dijon (F) Air filter, (HC) humidification chamber, (HB) heating battery, (BP) by-pass, (CB) cooling battery, (HM) probe for air relative humidity measurement, (TP) probe for medium temperature measurement,(WG) weight gauges, (SH) sterile sample handling, (JR) water temperature regulation in the double jacket, (AD) planetary agitation device, (M) motor for agitation, (IS) sterile system for adding inoculum and solutions, (CO) water air condenser. (D) Rotating drum bioreactor. (1) Air-inlet, (2) rotating joint, (3) coupling, (4) air nozzles, (5) air line, (6) rollers, (7) rotating drum, (8) solid medium,(9) rim.

REFERENCE

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