Application of solid-state fermentation to food industry—A review

Susana Rodríguez Couto a,b, Mª Ángeles Sanromán a,*

a Department of Chemical Engineering, Isaac Newton Building, University of Vigo, Lagoas Marquesende, 36310 Vigo, Spain
b Department of Chemical Engineering, Chemical Engineering School, Rovira i Virgili University, 43007 Tarragona, Spain

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Abstract

Solid state fermentation (SSF) has become a very attractive alternative to submerged fermentation (SmF) for specific applications due to the recent improvements in reactor designs. This paper reviews the application of SSF to the production of several metabolites relevant for the food processing industry, centred on flavours, enzymes (α-amylase, fructosyl transferase, lipase, pectinase), organic acids (lactic acid, citric acid) and xanthan gum. In addition, different types of biorreactor for SSF processes have been described.

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1. Introduction

Microorganisms have long played a major role in the production of food (dairy, fish and meat products) and alcoholic beverages. In addition, several products of microbial fermentation are also incorporated into food as additives and supplements (antioxidants, flavours, colourants, preservatives, sweeteners, ...). There is great interest in the development and use of natural food and additives derived from microorganisms, since they are more desirable than the synthetic ones produced by chemical processes.

Solid-state fermentation (SSF) reproduces the natural microbiological processes like composting and ensiling. In industrial applications this natural process can be utilised in a controlled way to produce a desired product. SSF is defined as any fermentation process performed on a non-soluble material that acts both as physical support and source of nutrients in absence of free flowing liquid (Pandey, 1992). The low moisture content means that fermentation can only be carried out by a limited number of microorganisms, mainly yeasts and fungi, although some bacteria have also been used (Pandey, Soccol, & Mitchell, 2000a). Some examples of SSF processes for each category of microorganisms are reported in Table 1.

SSF offers numerous advantages for the production of bulk chemicals and enzymes (Hesseltine, 1977; Pandey, Selvakumar, Soccol, & Nigam, 1999a; Soccol, Iloki, Marin, & Raimbault, 1994). This process is known from ancient times and different fungi have been cultivated in SSF for the production of food. Typical examples of it are the fermentation of rice by Aspergillus oryzae to initiate the koji process and Penicillium roquefortii for cheese production. Also, in China, SSF has been used extensively to produce brewed foods (such as Chinese wine, soy sauce and vinegar) since ancient time (Chen, 1992). Also, in Japan SSF is used commercially to produce industrial enzymes (Suryanarayanan, 2003). Since 1986 in Brazil a series of research projects for the value-addition of tropical agricultural products and sub-products by SSF has been developed due to the high...
Table 1
Main groups of microorganisms involved in SSF processes (extracted from Raimbault, 1998)

<table>
<thead>
<tr>
<th>Microflora</th>
<th>SSF process</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>Composting, matto, amylase</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>Composting</td>
</tr>
<tr>
<td>Serratia sp.</td>
<td>Composting</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td>Composting</td>
</tr>
<tr>
<td>Lactobacillus sp.</td>
<td>Ensiling, food</td>
</tr>
<tr>
<td>Clostridium sp.</td>
<td>Ensiling, food</td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td></td>
</tr>
<tr>
<td>Endomycopsis bartonii</td>
<td>Tape cassava, rice</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>Food, ethanol</td>
</tr>
<tr>
<td>Schwannomyces castelli</td>
<td>Ethanol, amylase</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
</tr>
<tr>
<td>Alternaria sp.</td>
<td>Composting</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>Composting, industrial, food</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>Composting, gibberellins</td>
</tr>
<tr>
<td>Monilia sp.</td>
<td>Composting</td>
</tr>
<tr>
<td>Mucor sp.</td>
<td>Composting, food, enzyme</td>
</tr>
<tr>
<td>Rhizopus sp.</td>
<td>Composting, food, enzymes, organic acids</td>
</tr>
<tr>
<td>Phanerochaete chrysosporium</td>
<td>Composting, lignin degradation</td>
</tr>
<tr>
<td>Trichoderma sp.</td>
<td>Composting, biological control, biosepecticide</td>
</tr>
<tr>
<td>Beauveria sp., Metharizium sp.</td>
<td>Biological control, biosepecticide</td>
</tr>
<tr>
<td>Amylomyces rouxii</td>
<td>Tape cassava, rice</td>
</tr>
<tr>
<td>Aspergillus oryzae</td>
<td>Koji, food, citric acid</td>
</tr>
<tr>
<td>Rhizopus oligosporus</td>
<td>Tempeh, soybean, amylase, lipase</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Feed, proteins, amylase, citric acid</td>
</tr>
<tr>
<td>Pleurotus ostreatus, sajor-caju</td>
<td>Mushroom</td>
</tr>
<tr>
<td>Lentinus edodes</td>
<td>Shiitake mushroom</td>
</tr>
<tr>
<td>Penicillium notatum, roquefortii</td>
<td>Penicillin, cheese</td>
</tr>
</tbody>
</table>

amounts of agricultural residues generated by this country (Soccol & Vandenbergh, 2003). Thus, the production of bulk chemicals and value-added fine products such as ethanol, single-cell protein (SCP), mushrooms, enzymes, organic acids, amino acids, biological activity secondary metabolites, etc. (Hölker, Höfer, & Lenz, 2004; Pandey, 1992; Pandey, 1994; Pandey, Azmi, Singh, & Banerjee, 1999b; Pandey et al., 1999c; Pandey, Nigam, & Vogel, 1988; Pandey et al., 2000b; Pandey et al., 1999d; Vandenbergh, Soccol, Pandey, & Lebeault, 2000) has been produced from these raw materials by means of SSF technique.

In recent years, SSF has received more and more interest from researchers, since several studies for enzymes (Pandey et al., 1999a), flavours (Ferron, Bonnarame, & Durand, 1996), colourants (Johns & Stuart, 1991) and other substances of interest to the food industry have shown that SSF can give higher yields (Tsuchiya et al., 1994) or better product characteristics (Acunia-Arguelles, Gutierrez-Rojas, Viniengra-González, & Favela-Torres, 1995) than submerged fermentation (SmF). In addition, costs are much lower due to the efficient utilisation and value-addition of wastes (Robinson & Nigam, 2003). Castillo, Alves, and Medronho (2000), have performed a detail economic analysis of the production of Penicillium restrictum lipase in both SmF and SSF. They found that for a production scale of 100 m³ lipase concentrate per year, total capital investment needed for SmF was 78% higher than that needed for SSF. Also, SSF unitary product cost was 47% lower than the selling price. These studies pointed out that the great advantage of SSF processes is the extremely cheap raw material used as main substrate. Therefore, SSF is certainly a good way of utilising nutrient rich solid wastes as a substrate. Both food and agricultural wastes are produced in huge amounts and since they are rich in carbohydrates and other nutrients, they can serve as a substrate for the production of bulk chemicals and enzymes using SSF technique.

The nature of the solid substrate employed is the most important factor affecting SSF processes and its selection depends upon several factors mainly related with cost and availability and, thus, may involve the screening of several agro-industrial residues. In SSF process the solid substrate not only supplies the nutrients to the culture but also serves as an anchorage for the microbial cells. Among the several factors, which are important for microbial growth and activity in a particular substrate, particle size and moisture level/water activity are the most critical (Auria, Palacios, & Revah, 1992; Barrios-Gonzalez, Gonzalez, & Mejia, 1993; Echevarria, Leon, Espinosa, & Delgado, 1991; Liu & Tseng, 1999; Pandey, Ashakumary, Selvakumar, & Vijayalakshmi, 1994; Pastrana, Gonzalez, Pintado, & Murado, 1995; Roussos, Raimbault, Prebois, & Lonsane, 1993; Sarrette, Nout, Gervais, & Rombouts, 1992; Smail, Salhi, & Knapp, 1995; Zadrazil & Punia, 1995).

Generally, smaller substrate particles provide a larger surface area for microbial attack but if they are too small may result in substrate agglomeration as well as poor growth. In contrast, larger particles provide better aeration but a limited surface for microbial attack. Therefore, a compromised particle size must be selected for each particular process (Pandey et al., 1999a).

Research on the selection of suitable substrates for SSF has mainly been centred around agro-industrial residues due to their potential advantages for filamentous fungi, which are capable of penetrating into the hardest of these solid substrates, aided by the presence of turgor pressure at the tip of the mycelium (Ramachandran et al., 2004). In addition, the utilisation of these agro-industrial wastes, on the one hand, provides alternative substrates and, on the other, helps in solving pollution problems, which otherwise may cause their disposal (Pandey et al., 1999a).

SSF offers numerous advantages over SmF such as simpler technique and lower cost (Table 2). However, there are few designs available in the literature for bioreactors operating in solid-state conditions. This is princi-
Several researchers have studied the production of aroma compounds by SSF from several microorganisms such as *Neurospora* sp. (Pastore, Park, & Min, 1994), *Zygosaccharomyces rouxii* (Sugawara, Hashimoto, Sakurai, & Kobayashi, 1994), *Aspergillus* sp. (Ito, Yoshida, Ishikawa, & Kobayashi, 1990), using pre-gelatinised rice, miso and cellulose fibres, respectively. Bramorski, Soccol, Christen, and Revah (1998) compared fruity aroma production by *Ceratocystis fimбриата* in solid-state cultures using several agro-industrial wastes (cassava bagasse, apple pomace, amaranth and soybean), determining that the media with cassava bagasse, apple pomace or soybean produced a strong fruity aroma. Soares, Christen, Pandey, and Soccol (2000) also reported the production of strong pineapple aroma when SSF was carried out using coffee husk as a substrate by this strain. Bramorski, Christen, Ramirez, Soccol, and Revah (1998) and Christen, Bramorski, Revah, and Soccol (2000) described the production of volatile compounds such as acetaldehyde and 3-methylbutanol by the edible fungus *Rhizopus oryzae* during SSF on tropical agro-industrial substrates.

*Kluyveromyces marxianus* produced aroma compounds, such as monoterpenes, alcohols and isomyl acetate (responsible for fruity aromas), in SSF using cassava bagasse or giant palm bran as a substrate (Medeiros et al., 2001).

Esters are the source of the aromas and among them pyrazines, which possess a nutty and roasty flavour, are used as a food additive for flavouring (Seitz, 1994). Beson, Creuly, Gros, and Larroche (1997) and Larroche, Besson, and Gros (1999) studied the production of 2,5-dimethylpyrazine (2,5-DMP) and tetramethylpyrazine (TTMP) using *B. natto* and *B. subtilis*, respectively, on soybeans in SSF. They found that SSF was very suitable for the production of these compounds.

### 2. Some examples of applications of SSF to food industry

#### 2.1. Flavours

Flavours comprise over a quarter of the world market for food additives. Most of the flavouring compounds are produced via chemical synthesis or by extraction from natural materials. However, recent market surveys have shown that consumers prefer foodstuff that can be labelled as natural. Plants have been major sources of essential oils and flavours but their use depends on natural factors difficult to control such as weather conditions and plant diseases. An alternative route for flavour synthesis is based on microbial biosynthesis or bioconversion (Janssens, de Pooter, Vandamme, & Schamp, 1992). Several microorganisms, including bacteria and fungi, are currently known for their ability to synthesise different aroma compounds. Attempts to use these microorganisms in SmF resulted in low productivity of aroma compounds (Yamguchi et al., 1993), which hampered their industrial application. SSF could be of high potential for this purpose (Berger, 1995). Thus, Ferron et al. (1996) reviewed the prospects of microbial production of food flavours and the recommended SSF processes for their production.

### Table 2

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher productivity</td>
<td>Difficulties on scale-up</td>
</tr>
<tr>
<td>Better oxygen circulation</td>
<td>Low mix effectively</td>
</tr>
<tr>
<td>Low-cost media</td>
<td>Difficult control of process parameters (pH, heat, moisture, nutrient conditions,...)</td>
</tr>
<tr>
<td>Less effort in downstream processing</td>
<td>Problems with heat build-up</td>
</tr>
<tr>
<td>Reduced energy and cost requirements</td>
<td>Higher impurity product, increasing recovery product costs</td>
</tr>
<tr>
<td>Simple technology</td>
<td></td>
</tr>
<tr>
<td>Scarce operational problems</td>
<td></td>
</tr>
<tr>
<td>It resembles the natural habitat for several microorganisms</td>
<td></td>
</tr>
</tbody>
</table>

pally due to several problems encountered in the control of different parameters such as pH, temperature, aeration and oxygen transfer and moisture. SSF lacks the sophisticated control mechanisms that are usually associated with SmF. Control of the environment within the bioreactors is also difficult to achieve, particularly temperature and moisture.

The aim of this paper is to review the potential application of SSF for the production of several metabolites of great interest to the food industry. In addition, different types of bioreactor for SSF processes are described.

#### 2.2. Enzyme production

Recently, dos Santos, Souza da Rosa, Dal’Boit, Mitchell, and Krieger (2004) evaluated whether SSF is the best system for producing enzymes. They found that SSF is appropriate for the production of enzymes and other thermolabile products, especially when higher yields can be obtained than in SmF.

#### 2.2.1. α-Amylase

α-Amylases (endo-1,4-α-D-glucan glucanohydrolase EC 3.2.1.1) are extra-cellular endo enzymes that randomly cleave the 1,4-α linkages between adjacent glucose units in the linear amylose chain and ultimately generates glucose, maltose and maltotriose units. Since the 1950s, fungal amylases have been used to manufacture sugar syrups containing specific mixtures of sugars...
that could not be produced by conventional acid hydrolysis of starch. Amylases are extensively employed in processed-food industry such as baking, brewing, preparation of digestive aids, production of cakes, fruit juices, starch syrups, etc.

The production of α-amylases has generally been carried out using SmF; however, SSF systems appear as a promising technology. Recently, Francis et al. (2003) used spent brewing grains in SSF for the production of α-amylase and determined that the supplement of fermentation media with Tween-80 or calcium ions enhanced α-amylase activity.

Krishna and Chandrasekaran (1996) used banana fruit stalk as a substrate in SSF with Bacillus subtilis. Different factors such as initial moisture content, particle size, thermal treatment time and temperature, pH, incubation temperature, additional nutrients, inoculum size and incubation period on the production of α-amylase were characterised. Results obtained for the optimisation of process parameters clearly shown their impact on the gross yield of enzymes as well as their independent nature in influencing the organism’s ability to synthesise the enzyme. It is known that particle size (specific surface area) is a critical factor in SSF. Banana fruit stalk particles of 400 μm favoured maximal α-amylase production compared to larger particles. A similar trend was reported for the production of glucoamylases with wheat bran (Pandey, 1991) and cellulases with coir pith of small particle size (Muniswaran & Charyulu, 1994).

Nowadays, gelatinisation is coupled with liquefaction, which is possible by the action of thermostable amylases, which have been reported in both SmF (Stamford, Stamford, Coelho, & Araujo, 2001) and SSF (Babu & Satyanarayana, 1995). Sodhi, Sharma, Gupta, and Soni (2005) determined that the productivity of thermostable amylases from Bacillus sp. was affected by the nature of the solid substrate (wheat bran, rice bran, corn bran and combination of two brans), nature of the moistening agent, level of moisture content, incubation temperature, presence or absence of surfactant, carbon, nitrogen, mineral, amino acid and vitamin supplements. Maximum enzyme production was obtained on wheat bran supplemented with glycerol (1.0%, w/w), soyabean meal (1.0%, w/w), L-proline (0.1%, w/w), vitamin B-complex (0.01%) and moistened with tap water containing 1% Tween-40.

Recently, Ramachandran et al. (2004) reported the use of coconut oil cake (COC) as a substrate for the production of α-amylase by A. oryzae under SSF conditions. Raw COC supported the growth of the culture, resulting in the production of 1372 U/gds α-amylase in 24 h. Supplementation with 0.5% starch and 1% peptone to the substrate positively enhanced the enzyme synthesis producing 3388 U/gds, proving COC a promising substrate for α-amylase production.

2.2.2. Fructosyl transferase

Fructosyl transferase (EC 2.4.1.10) catalyses the formation of fructo-oligosaccharides from sucrose. Fructo-oligosaccharides are present in various commonly consumed foods like fruits, vegetables, cereals and honey in trace amounts. The production of fructosyl transferase derived from microorganisms has attracted attention in recent years by SmF using Aspergillus spp. Penicillium spp and Aureobasidium spp (Prapulla, Subhaprada, & Karanth, 2000) and SSF using both Aspergillus foetidus and A. oryzae (Hang, Woodams, & Jang, 1995; Sangeetha, Ramesh, & Prapulla, 2004).

Recently, Sangeetha et al. (2004) have studied the production of fructosyl transferase by A. oryzae employing a great variety of agricultural by-products as substrates: Cereal brans (wheat bran, rice bran and oat bran), corn products (corn cob, corn bran, corn germ, corn meal, corn grits and whole corn powder), coffee-and tea-processing by-products (coffee husk, coffee pulp, spent coffee and spent tea), sugarcane bagasse and cassava bagasse. They found that, among them, the best results were obtained when rice bran, wheat bran, corn germ, spent coffee and tea were used supplemented with yeast extract and complete synthetic media.

2.2.3. Lipase

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are well known as efficient biocatalysts for the hydrolysis of water-insoluble fatty-acid esters, being triacylglycerols of long chain fatty acids their natural substrates. Lipases are nowadays widely used at industrial scale with applications in food, detergent, cosmetic and pharmaceutical industries (Jaeger & Reetz, 1998).

Most studies on lipolytic enzymes production by bacteria, fungi and yeasts have been performed in submerged cultures; however, there are few reports on lipase synthesis in solid state cultures. In recent years, increasing attention has been paid to the conversion of processing industry wastes in lipase by solid state cultures.

There are several reports dealing with extracellular lipase production by fungus such as Rizopus sp., Aspergillus sp., Penicillium sp. on different solid substrates (Christen, Angeles, Corzo, Farres, & Revah, 1995; Cordova et al., 1998; Gombert, Pinto, Castilho, & Freire, 1999; Kamini, Mala, & Puvanakrishnan, 1998; Miranda et al., 1999) under submerged conditions. However, few researchers have investigated the synthesis of lipase by yeasts using SSF technique. Among them, Rao, Jayaraman, and Lakshmanan (1993) determined that the C/N ratio of the medium is an important parameter for lipase production by the yeast Candida rugosa.

systems for lipase production. All they found that enzyme yields were higher and stable in the latter.

Several factors can affect extracellular lipase production such as pH, temperature, aeration and medium composition. Furthermore, the presence of triglycerides or fatty acids has been reported to increase lipolytic enzyme secretion by a certain number of microorganisms (Marek & Bednarski, 1996). Therefore, in SSF the type of substrate could be used to enhance the production of enzymes, as several food and agroindustrial wastes are rich in fatty acids, triglycerides and/or sugars.

Dominguez, Costas, Longo, and Sanroman (2003) have reported the great potential of food-agroindustrial wastes (ground nut and barley bran) as support-substrates for lipase production in solid state cultures of the yeast Y. lipolytica, since they led to much higher activities than those found using an inert support.

2.2.4. Pectinases

They constitute a heterogeneous group of enzymes that catalyse the degradation of pectins, which are the structural polysaccharides present in vegetable cells and are responsible for maintaining the plant tissues integrity (Alkorta, Garbisu, Llama, & Serra, 1998). Pectinases are widely used in the food industry to clarify fruit juices and wine, to improve oil extraction, to remove the peel from citrus fruit, to increase the firmness of several fruits and to degum fibres (Baker & Wicker, 1996; Chang, Siddiq, Sinha, & Cash, 1994).

Commercial pectinase preparations are produced from fungal microorganisms, mainly by Aspergillus niger strains. The use of SSF for pectinase production has been proposed using different solid agricultural and agro-industrial residues as substrates such as wheat bran (Castilho, Alves, & Medronho, 1999; Singh, Plattner, & Diekmann, 1999), soy bran (Castilho et al., 2000), cranberry and strawberry pomace (Zheng & Shetty, 2000), coffee pulp and coffee husk (Antier, Minjares, Roussos, & Viniegra-Gonzalez, 1993), husk (Antier, Minjares, Roussos, Raimbault, & Viniegra-Gonzalez, 1993; Boccas, Roussos, Gutierrez, Serrano, & Viniegra, 1994), cocoa (Schwan, Cooper, & Wheals, 1997), lemon and orange peel (Garzón & Hours, 1991; Ismail, 1996; Maldonado, Navarro, & Callieri, 1986), orange bagasse, sugar cane bagasse and wheat bran (Martins, Silva, Da Silva, & Gomes, 2002), sugar cane bagasse (Acuña-Arguelles, Gutierrez-Rojas, Viniegra-González, & Favela-Torres, 1994) and apple pomace (Hours, Voget, & Ertola, 1988a, 1988b). Also, Bai, Zhang, Qi, Peng, and Li (2004) produced pectinase from A. niger by SSF using sugar beet pulp as a carbon source and wastewater from monosodium glutamate production as nitrogen and water source. This allowed not only reducing production costs but also decreasing the pollution source.

It was found that SSF was more productive than SmF and, in addition, the pectinases produced by SSF showed more stable properties: they had a higher stability to pH and temperature and they were less affected by catabolic repression than pectinases produced by SmF (Acuña-Arguelles et al., 1995).

2.3. Organic acids

Organic acids have been utilised for long time by the food industry as food additives and preservatives for prevent deterioration and extending the shelf life of perishable food ingredients. Here, two common organic acids widely used on the food industry have been considered.

2.3.1. Lactic acid

Lactic acid fermentation has received extensive attention since long time (Benninga, 1990; Vickroy, 1985). It has wide applications in food, pharmaceutical, leather and textile industries and as a chemical feed stock. It has two enantiomers L(+) and D(−) of which L(+) is used by human metabolism due to the presence of L-lactate dehydrogenase and is preferred for food. Nowadays, lactic acid is in great demand due to its use as starting material to produce biodegradable polymers used in medical, industrial and consumer products (Bohllmann & Yoshida, 2000; Gross & Kalra, 2002; Lichtfield, 1996; Malhotra, Raina, & Sanjay, 2000).

Soccol, Marin, Rimbault, and Labeault (1994) studied the production of L(+)-lactic acid by Rhizopus oryzae in solid-state conditions operating with sugarcane bagasse as a support. They obtained a slightly higher productivity than in submerged cultivation. Also, Richter and Träger (1994) investigated the L(+)-lactic acid production by Lactobacillus paracasei in solid-state conditions using sweet sorghum as a support. More recently, Naveena, Altai, Bhadrayya, Madhavendra, and Reddy (2005) and Naveena, Altai, Bhadriah, and Reddy (2005) have reported the production of L(+) lactic acid by Lactobacillus amylophilus GV6 under SSF conditions using wheat bran as both support and substrate.

2.4. Citric acid

Citric acid is one of the most commonly used organic acids in food and pharmaceutical industries. The food industry is the largest consumer of citric acid, using almost 70% of the total production, followed by about 12% for the pharmaceutical industry and 18% for other applications (Shah, Chattaw, Baroda, & Patiala, 1993). Its pleasant taste, high solubility and flavour-enhancing properties have ensured its dominant position in the market. Although citric acid can be obtained by chemical synthesis, the cost is much higher than using fermentation. It is mainly produced by SmF, by the filamentous fungus A. niger. Recently, in order to increase the
efficiency of citric acid production using *A. niger*, SSF has been studied as a potential alternative to SmF.

The production of citric acid depends strongly on an appropriate strain and on operational conditions. Oxygen level is an important parameter for citric acid fermentation. Several researchers (Pintado, Lonsane, Gaime-Perraud, & Roussos, 1998; Prado et al., 2004) have studied the influence of forced aeration on citric acid production and the metabolic activity of *A. niger* in SSF by respirometric analysis. They showed that citric acid production was favoured by a limited biomass production, which occurred with low aeration rates. Both works showed the feasibility of using the strain *A. niger* for citric acid production by SSF.

Different agro-industrial residues such as apple pomace, coffee husk, wheat straw, pineapple waste, mixed fruit, maosmi waste, cassava bagasse, banana, sugar beet cosset and kiwi fruit peel have been investigated for their potential to be used as substrates (Hang & Woodams, 1985; Khare, Krishna, & Gandhi, 1995; Kumar, Jain, Shanker, & Srivastava, 2003a, 2003b; Shojaosadati & Babarpour, 2002). In addition, SSF gave high citric acid yield without inhibition related to the presence of certain metal ions such as Fe$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, etc. (Gutierrez-Rozas, Cordova, Auria, Revah, & Favela-Torres, 1995), although Shankaranand and Lonsane (1994) reported that addition of these minerals to the production media to a certain level enhanced citric acid production by 1.4–1.9 fold with respect to SmF. Therefore, SSF is a good way of using nutrient rich solid waste as a substrate.

### 2.5. Xanthan gum

Xanthan gum is a hetero-polysaccharide produced industrially by the bacterium *Xanthomonas campestris*, fermenting commonly glucose or sucrose. It is the most important microbial polysaccharide from the commercial point of view, with a worldwide production of about 30,000 tons per year, corresponding to a market of $408 million (Demain, 2000; Sutherland, 1998). This water-soluble microbial polysaccharide gives aqueous solutions with several industrial applications in the food, cosmetic, textile and pharmaceutical industries due to their rheological properties. Because of these properties, they have been used as emulsifiers, as stabilisers and as texture enhancers in the food industry.

Recently, the feasibility of this exopolysaccharide production using SSF has been reported by the group of Stredansky and Conti (1999), Stredansky, Conti, Navarini, and Bertocchi (1999). *X. campestris* strains were cultivated on a great variety of solid substrates or by-products such as spent malt grains, apple pomace, grape pomace and citrus peels, easily available and low cost substrates, in order to evaluate their ability to produce the exopolysaccharide xanthan. With most of the substrates, xanthan yields were comparable to those obtained from conventional submerged cultivation. In addition, the products were analysed by NMR spectroscopy, revealing a composition consistent with that of commercial xanthan.

### 2.6. SSF bioreactors

The design of an efficient industrial-level reactor for SSF is of significance because SSF is more environmentally friendly than SmF. However, it shows considerable drawbacks such as transfer resistance, steep gaseous concentration and heat gradients that develop within the medium bed, which may adversely affect solid-state fermentor performances (Ghildyal, Ramalaishna, Lonsane, & Karantb, 1992; Lonsane, Saucedo-Castuneda, & Raimbault, 1992; Sargantanis, Karim, Murphy, Ryoo, & Tengerdy, 1992). Agitation and rotation in SSF were often carried out to improve mass and heat transfers, but the shearing force caused by agitation and rotation has adverse effects on medium porosity and disrupts fungal mycelia.

There are four types of reactors to perform SSF processes and each in their own design tries to make conditions more favourable for fermentation under solid state conditions. The bioreactors commonly used, which can be distinguished by the type of aeration or the mixed system employed, include the following:

- **Tray**: It consists of flat trays. The substrate is spread onto each tray forming a thin layer, only a few centimetres deep. The reactor is kept in a chamber at constant temperature through which humidified air is circulated (Fig. 1). The main disadvantage of this configuration is that numerous trays and large volume are required, making it an unattractive design for large-scale production (Pandey, Soccol, Rodriguez-Leon, & Nigam, 2001).

- **Packed-bed**: It is usually composed of a column of glass or plastic with the solid substrate retained on a perforated base. Through the bed of substrate humidified air is continuously forced (Durand et al., 1993; Raimbault, 1998; Rodríguez Couto, Rivela, Muñoz, & Sanromán, 2000). It may be fitted with a jacket for circulation of water to control the temperature during fermentation (Fig. 2). This is the configuration usually employed in commercial koji production. The main drawbacks associated with this configuration are: difficulties in obtaining the product, non-uniform growth, poor heat removal and scale-up problems.

- **Horizontal drum**: This design allows adequate aeration and mixing of the substrate, whilst limiting the damage to the inoculum or product. Mixing is performed by rotating the entire vessel or by various agitation devices such as paddles and baffles (Domínguez, Rivela, Rodríguez Couto, & Sanromán, 2001; Nagel, Tramper, Bakker, & Rinzema, 2001a, Nagel, Tramper, Bakker, & Rinzema, 2001b; Prado et al., 2004; Stuart,
Mitchell, Johns, & Litster, 1999) (Fig. 3). Its main disadvantage is that the drum is filled to only 30% capacity, otherwise mixing is inefficient.

Fluidised bed: In order to avoid the adhesion and aggregation of substrate particles, this design supplies a continue agitation with forced air (Fig. 4). Although the mass heat transfer, aeration and mixing of the substrate is increased, damage to inoculum and heat build-up through sheer forces may affect the final product yield.

The different disadvantages detected in the above-mentioned bioreactor designs to perform SSF processes have promoted the necessity of developing new bioreactor configurations or modifying the already existing designs. These bioreactor configurations should be able to operate in continuous mode with high productivity for prolonged periods of time without operational problems as well as permit the scale-up of the process. Our research group has been working in this field, resulting in the design of a new bioreactor, called immersion bioreactor. This bioreactor consists of a jacketed cylindrical glass vessel with a round bottom, inside which several wire mesh baskets filled with support colonised by the fungus are placed. They moved upwards and downwards by means of a pneumatic system, remaining more time outside than inside the medium (Rivela, Rodríguez Couto, & Sanromán, 2000) (Fig. 5). It is noteworthy that this bioreactor configuration was also able to run in continuous mode without operational problems, attaining high ligninolytic enzyme activities (Rodriguez Couto, Barreiro, Rivela, Longo, & Sanroman, 2002).

Different studies were carried out for the production of natural food and additives derived from microorganisms in different bioreactor configurations. For
example, the production of aroma compounds by \textit{K. marxianus} grown on cassava bagasse in solid state fermentation using packed bed reactors, testing two different aeration rates was studied by Medeiros \textit{et al.} (2001). Headspace analysis of the culture by gas chromatography showed the production of 11 compounds. The predominant compounds were ethyl acetate, ethanol and acetaldehyde. The fruity aroma was attributed to the productions of esters.

Recently, Navarrete-Bolanos, Jiménez-Islas, Botello-Alvarez, Rico-Martínez, and Paredes-López (2004) have employed a modular rotating drum bioreactor (equipped with inlet air injection, variable speed pumps, humidifier, and gas analyser) for xanthophylls extraction from marigold flowers. Marigold extracts have been commercialised internationally and are used as additives for poultry feed, as they provide bright colours in egg yolks, skin, and fatty tissues. For this reason, they are used as an additive in several food and pharmaceutical industries. Based on experimental design strategies, optimum operation values were determined for aeration, moisture, agitation and marigold-to-inoculum ratio in SSF of marigold flowers by mixed culture of three microorganisms (\textit{Flavobacterium IIb}, \textit{Acinetobacter anitratus}, and \textit{Rhizopus nigricans}), leading to a xanthophylls yield of 17.8-g/kg dry weight. This value represented a 65% increase in relation to the control.

Milagres, Santos, Piovan, and Roberto (2004) have shown that \textit{Thermoascus aurantiacus} was able to produce a high level of thermostable xylanase when sugar cane bagasse was used as a substrate in a glass-column reactor with forced aeration. The airflow rate had a significant effect on enzyme activity, whereas initial mass of bagasse had none. The highest yield of xylanase (1597 U/g) was obtained operating in the bioreactor at the optimal conditions: airflow rate (6 l/h g) and substrate (8 g).

A packed-bed bioreactor with four stages was constructed and operated for microbial production of citric acid by \textit{A. niger} using apple pomace as a substrate. Under the optimised conditions, 124 g citric acid was produced from 1 kg dry apple pomace with yield of 80% based on total sugar (Shojaosadati & Babaripour, 2002).

3. Conclusion

Critical analysis of the literature shows that production of relevant compounds for the food processing industry by SSF offers several advantages. It has been well established that enzyme titres produced in SSF systems are much higher than the achieved in SmF ones. Although the reasons for this are not clear, this fact is kept in mind while developing novel bioreactors for SSF processes.

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