

Solid state fermentation (SSF): diversity of applications to valorize waste and biomass

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Abstract Solid state fermentation is currently used in a range of applications including classical applications, such as enzyme or antibiotic production, recently developed products, such as bioactive compounds and organic acids, new trends regarding bioethanol and biodiesel as sources of alternative energy, and biosurfactant molecules with environmental purposes of valorising unexploited biomass. This work summarizes the diversity of applications of solid state fermentation to valorize biomass regarding alternative energy and environmental purposes. The success of applying solid state fermentation to a specific process is affected by the nature of specific microorganisms and substrates. An exhaustive number of microorganisms able to grow in a solid matrix are presented, including fungus such as *Aspergillus* or *Penicillium* for antibiotics, *Rhizopus* for bioactive compounds, *Mortierella* for biodiesel to bacteria, *Bacillus* for biosurfactant production, or yeast for bioethanol.

Keywords Solid state fermentation · Agro-industrial waste · Microorganisms · Metabolites

Introduction

Solid state fermentation (SSF) has been described as the process that takes place in a solid matrix (inert support or support/substrate) in the absence or near absence of free

water (Singhania et al. 2010), but the substrate requires moisture to support the growth and metabolic activity of microorganisms (Thomas et al. 2013). The microbiological process of SSF has generated great interest in recent years because it can be used for a variety of purposes (Thomas et al. 2013), supported by some authors who have even indicated numerous advantages over their liquid counterparts (submerged fermentation) (Singhania et al. 2009). The most important phenomenon attributed to SSF is the resistance of microorganisms (bacterial and fungal cells) to catabolic repression (inhibition of enzyme synthesis) in the presence of abundant substrates, such as glycerol, glucose or other carbon sources (Viniegra-González and Favela-Torres 2006). Another important factor is the possibility of using agro-industrial residues (A-IR) generated by current industrial processes and inclusively using unexploited biotic resources as support/substrate for metabolite production with value-added at low production costs (Bhargav et al. 2008), allowing SSF to be economically viable (Schmidt et al. 2014). Furthermore, reducing environmental problems (Rodríguez Couto 2008), such as A-IR, which may produce odour and soil pollution, represents problems for the industry (Torrado et al. 2011).

SSF was traditionally used for producing metabolites such as enzymes, antibiotics, organic acids, biosurfactants and aroma compounds; however, in reality, SSF received more attention due to the wide number of applications for metabolite production or remediation objectives that can be realized using this system (Wang et al. 2010). Currently, SSF is attracting new interest because of its wide range of applications in valorising unexploited biomass. With environmental problems being generated around the world, SSF has reached great relevance in this context because of the environmental benefits offered with the possibility of using A-IR (Thomas et al. 2013). In this way, a wide

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variety of applications can be achieved, such as bioremediation, production of lipids, biofuels (biodiesel, bioethanol, biobutanol, biohydrogen), aromas and flavours for the food industry, and production/extraction of bioactive compounds, among others. The aim of this work was to summarize the diversity of applications of SSF, from classical, such as enzyme or antibiotic production, to new trends regarding alternative energy, and environmental purposes, such as valorising unexploited biomass of A-IR.

Enzymes

Enzyme production by SSF has been a reference for this type of process, because of the wide variety of enzymes that can be produced, possibly at an industrial scale. The importance of SSF in enzyme production is due to the agro-industrial residues that are generally used for this purpose. Nutrients present in the substrate (A-IR) support growth and due to the natural metabolism of the microorganism, can secrete enzymes, while growing in the solid substrate (Kumar and Kanwar 2012). Indeed, authors have previously mentioned that several enzymes could be produced by the SSF system at pilot scale (He and Chen 2013) and the literature indicates a large variety of enzymes that are produced by SSF (Table 1). For enzyme production at industrial scale it is important to design reactors with control of important aspects of the process such as heat transfer and oxygen as well as moisture. Additionally, in the process design should be considered the search for the substrate and microorganism suitable for the enzyme to be

produced. It is important to mention that in Japan enzymes are already produced at industrial scale using SSF (He and Chen 2013; Rodríguez Couto 2008). In addition, it can also be seen that the range of microorganisms used is very diverse in A-IR revalued for this purpose. Moreover, enzyme production by SSF has been considered the heart of biotechnology because of the importance in market sales of these molecules (Thomas et al. 2013). The market of industrial enzymes has shown gradual growth: for 1998, sales were estimated at \$1 billion (Rao et al. 1998), but for 2015, they were estimated at \$4.4 billion (Thomas et al. 2013). This significant progress demonstrate the importance of SSF in the enzyme market, however, it is important to note that the titers of enzyme activity expressed in SSF are higher than those expressed in submerged fermentation (SF), for example: 5000 and 1600 U l⁻¹ for a pectinase produced by *Aspergillus niger* (Viniestra-González et al. 2003), 7150 and 1714 UI l⁻¹ for an exopectinase produced by *A. niger* C28B25 (Diaz-Godinez et al. 2001), 30 and 8 U/g of dry substrate for a protease produced by *Aspergillus oryzae* (Sandhya et al. 2005), showed the importance of SSF in enzyme production.

Antibiotics

Antibiotic were traditionally produced by SF; however, some authors now indicate that production using SSF is better because fungal strains grow in near natural habitats (Vastrad et al. 2014). Furthermore, the use of A-IR is an important advantage for antibiotic production in SSF

Table 1 Variety of enzymes produced in SSF

Enzyme	Support/substrate	Microorganism	References
Naringinase	Orange and grapefruit rind	<i>Aspergillus foetidus</i> <i>Aspergillus niger</i> <i>Aspergillus niger</i> HPD-2	Mendoza-Cal et al. (2010)
Polygalacturonase	Apple bagasse and wheat bran	<i>Aspergillus niger</i> <i>Penicillium</i> sp. EGC5	Abbasi et al. (2011)
α -Amylase	Rice husk, banana husk, millet, water melon husk, lentil bran, wheat bran and maize oil cake	<i>Anoxybacillus flavithermus</i>	Özdemir et al. (2012)
Manganese peroxidase	Pineapple leaf	<i>Ganoderma lucidum</i>	Hariharan and Nambisan (2012)
Lipase	Sunflower seed and sugarcane bagasse	<i>Burkholderia cenocepacia</i>	Liu et al. (2013)
Protease	Wheat bran and soybean meal	<i>Bacillus subtilis</i>	Imtiaz and Mukhtar (2013)
Cellulase and hemicellulase	Corn straw, rice husk, grass powder, sugarcane barbojo and sugarcane bagasse	<i>Phanerochaete chrysosporium</i>	Saratale et al. (2014)
Ellagitannase	Sugarcane bagasse, corn cobs, coconut husk and candelilla stalks	<i>Aspergillus niger</i> GH1	Buenrostro-Figueroa et al. (2014)
Phytase	Wheat bran	<i>Escherichia coli</i>	McKinney et al. (2015)
Laccase	Poplar sawdust	<i>Ganoderma lucidum</i> <i>Trametes versicolor</i>	Kuhar et al. (2015)

because these may serve as a source of carbon and nitrogen. Moreover, in some cases, nutrients contained in the substrate may be inductors or supplementary nutrients for such production (Adinarayana et al. 2003). To make more evident substrates used in antibiotics production by SSF, some examples are mentioned then: tetracycline by *Streptomyces viridifaciens* ATCC 11989 using sweet potato, rice bran, and soy meal (Yang and Ling 1989), neomycin by *Streptomyces marinensis* using raspberry seed powder, wheat rawa, wheat bran, rice bran (Adinarayana et al. 2003; Ellaiah et al. 2004), cephalosporin C by *Acremonium chrysogenum* C10 using sugarcane bagasse (Cuadra et al. 2008), meroparamycin by *Streptomyces* sp. MAR01 using rice, wheat bran, quaker, bread, and ground corn (El-Naggar et al. 2009), lovastatin by *Penicillium funiculosum* NCIM 1174 using green gram husk, black gram husk, wheat bran and orange peel (Reddy et al. 2011), rifamycin B by *Amycolatopsis mediterranea* MTCC14 and *Nocardia mediterranei* using coconut oil cake, groundnut oil cake, ground nut shell, rice husk and sunflower oil cake (Vastrad and Neelagund 2012; Vastrad et al. 2014), rifamycin SV by *Amycolatopsis mediterranei* OVA5-E7 using ragi bran (Nagavalli et al. 2015). The literature shows that some antibiotics have been produced historically by SSF using A-IR as a support, reflecting the importance of SSF for production of this type of metabolites.

Organic acids

The production of organic acids by SSF emerged as an alternative to SF, which due to the processes of acid production, is generally an expensive process (Kumar et al. 2003). SSF emerges as a cheap alternative because, as mentioned, it may use agro-industrial waste for the same purpose. There are many reports indicating the use of

different A-IR for organic acid production, reducing production cost and environmental problems. Additionally, one important advantage of production by SSF is the feasibility and efficient extraction of acids from fermented matter (Dhillon et al. 2013). Table 2 shows variety of A-IR can be exploited for the production of organic acids, as occurs with the production of citric acid, lactic acid, gluconic and ellagic acid.

Bioactive compounds

Extraction of bioactive compounds from biotic materials has generally been realized using habitual extraction processes (solid–liquid/liquid–liquid). However, SSF has emerged as an alternative for the production/extraction of bioactive compounds (Martins et al. 2013). The implementation of SSF for extraction of bioactive compounds using microorganisms (bacteria, yeast and fungi) is a suitable alternative, due to these microorganisms being able to produce enzymes required for bioactive molecule liberation into cell walls of plants or biotic materials (e.g. pectinases, cellulases, α -amylases, xylanases, β -glucosidase, β -galactosidase, and β -hesperidinase) (Salar et al. 2012; Dey and Kuhad 2014). In the last few years, SSF has been implemented for the production/extraction of molecules with antioxidant activity, as can be seen in Table 3.

Biological control

In recent years, biological control agents has emerged as an alternative to environmental pollution caused by the excessive use of pesticides, these can be replaced by biopesticides for the control of plant pests and plant diseases (Cavalcante et al. 2008). Furthermore, generation of this type of products

Table 2 Organic acids produced in SSF

Acid	Microorganism	Source	References
Citric acid	<i>Aspergillus niger</i> DS 1	Pineapple waste	Kumar et al. (2010)
	<i>Aspergillus niger</i> CECT-2090	Valencia orange peel	Torrado et al. (2011)
	<i>Aspergillus niger</i> PTCC-5010	Sugarcane bagasse	Yadegary et al. (2013)
Lactic acid	<i>Lactobacillus delbrueckii</i>	Sugarcane bagasse as a substrate and cassava bagasse as a carbon source	John et al. (2006)
	<i>Lactobacillus casei</i>	Rice straw	Qi and Yao (2007)
	<i>Lactobacillus amylophilus</i> GV6	Wheat bran	Naveena et al. (2005)
Gluconic acid	<i>Aspergillus niger</i> ARNU-4	Tea waste as a support and molasses as a carbon source	Sharma et al. (2008)
	<i>Aspergillus niger</i>	Sugarcane bagasse	Singh et al. (2003)
Ellagic acid	<i>Aspergillus niger</i>	Pomegranate seeds and husk	Robledo et al. (2008)
	<i>Aspergillus niger</i> GH1	Pomegranate peel	Sepúlveda et al. (2012)

Table 3 Bioactive compounds produced using SSF

Molecule	Activity	Microorganism	Source	References
Phenolic compounds	Antioxidant	<i>Rhizopus oryzae</i>	Rice bran	Schmidt et al. (2014)
Phenolic compounds	Antioxidant	<i>Rhizopus oryzae</i> RCK2012	Wheat	Dey and Kuhad (2014)
Phenolic compounds	Antioxidant	<i>Cordyceps militaris</i> SN-18	Chickpeas	Xiao et al. (2014)
Phenolic peptides	Antioxidant	<i>Bacillus subtilis</i>	Lentils	Torino et al. (2013)
γ -Aminobutyric acid	Antihypertensive			
Phenolic compounds	Antioxidant	<i>Thamnidium elegans</i> CCF 1456	Maize	Salar et al. (2012)
Phenolic compounds	Antioxidant	<i>Phanerochaete chrysosporium</i>	Apple pomace	Ajila et al. (2011)

Table 4 Agents of biological control produced using SSF

Biocontrol agent	Support/substrate	References
<i>Beauveria bassiana</i>	Refused potatoes Coffee husks Sugarcane bagasse	Santa et al. (2005)
<i>Beauveria basiana</i>	Parboiled rice	Taroco et al. (2005)
<i>Trichoderma viride</i>	Winery wastes	Zhihui et al. (2008)
<i>Trichoderma harzianum</i> sp.	Corn bran Wheat bran	Cavalcante et al. (2008)
<i>Trichoderma viride</i> sp.		
<i>Trichoderma koningii</i> sp.		
<i>Trichoderma polysporum</i> sp.		
<i>Beauveria bassiana</i>	Millet grain	Kim et al. (2011)
<i>Metarhizium anisopliae</i>		
<i>Isaria javanica</i>	Sweet potato	Kim et al. (2014)
<i>Isaria fumosorosea</i>	Parboiled rice texturized with water hyacinth	Angel-Cuapio et al. (2015)

can be carried out by SSF as a strategy of A-IR management, impacting the environmental pollution caused by the use of pesticides and the generation of A-IR (Chen et al. 2011; Pham et al. 2010). The use of SSF for this purpose has been increasing due to some advantages compared with SF, e.g. when mycoinsecticides (conidia) are produced in SSF are more tolerant to drying and more stable during the manufacture of complex formulations compared to the conidia produced in SF (Angel-Cuapio et al. 2015). The principal advantage to produce agents of biological control by SSF is that the microorganisms used for these purposes has ability to grow on solid substrates and produce a wide range of extracellular enzymes and conidiospores important for this objective (Prakash et al. 2008). In the literature, several biological control agents produced by SSF are reported, as can be seen in the Table 4.

Lipids—biodiesel

Today with the depletion of fossil fuels and resultant environmental problems from their use, the search for renewable energy has become necessary. Furthermore, the carbon recycle period of biofuels is shorter than the carbon recycle period from fossil energy (Malilas et al. 2013). Recently, the production of biodiesel by trans-esterification of fats and lipids has received increased attention from many researchers because it can be an environmentally friendly and sustainable process (Fei et al. 2011). The problems of biodiesel production are operating costs for both the production of lipids and for the generation of biodiesel itself. In that regard, SSF has recently been considered as an alternative, due to the use of inexpensive substrates or A-IR in this process (Liu et al. 2013; Tsakona et al. 2014).

Microbial lipids have traditionally been produced using physicochemical methods operating at high temperature and pressure (Parfene et al. 2013). In some cases, SF using glucose as carbon source has been used, with the primary objective of being used for biodiesel production (Hui et al. 2010). Some reports indicate that there exist oleaginous microorganisms which can accumulate more than 20% of microbial lipids in oleaginous cells. These microorganisms may be used for lipid production in solid state fermentation, resulting in an alternative process for lipid production that is both cost effective and large scale, and that uses A-IR as a unique carbon source and energy (Liu et al. 2013). In the literature, there are several reports of lipid production using SSF (Table 5). Biodiesel production using oleaginous microorganisms is very attractive for the biofuel industry; because biodiesel may be produced using lipids and lipases produced using SSF.

On the other hand, lipases are important enzymes used for biodiesel production because processes for obtaining biofuel are less polluting than chemical catalysts, are less energy intensive, are more environmentally friendly, and have slight operating conditions (Liu et al. 2013). The main disadvantages of enzyme catalysis are the longer reaction time and the higher cost of the biocatalysts (Freire et al. 2011). In the literature, reports exist on lipase-catalysed biodiesel

Table 5 Lipid production in SSF

Lipid	Microorganism	Source	References
γ -Linolenic acid	<i>Mortierella isabellina</i>	Pear pomace	Fakas et al. (2009)
Gamma linolenic acid	<i>Mucor rouxii</i>	Rice bran	Jangbua et al. (2009)
Oleic acid	<i>Mortierella isabellina</i>	Soy bean meal	Economou et al. (2010)
Palmitic acid		Sorghum	
Lipids	<i>A. oryzae</i>	Wheat straw bran mixture	Hui et al. (2010)
Oleic acid	<i>Mortierella isabellina</i>	Rice hulls	Economou et al. (2011)
Palmitic acid			
Linoleic acid			
Lipids	<i>Mortierella isabellina</i>	Soybean hull	Zhang and Hu (2012)
Lipids	<i>Aspergillus tubingensis</i> TSIP9	Palm empty fruit bunches	Cheirsilp and Kitcha (2015)

Table 6 Lipases produced using SSF and utilized in biodiesel production

Support/substrate	Microorganism	References
Wheat bran	<i>Penicillium camembertii</i> KCCM 11268	Malilas et al. (2013)
Castor bean and sugarcane bagasse	<i>Trichoderma harzianum</i>	Coradi et al. (2013)
Sunflower seed and sugarcane bagasse	<i>Burkholderia cenocepacia</i>	Liu et al. (2013)
Castor oil cake	<i>Aspergillus flavus</i>	Toscano et al. (2013)
Wheat bran and soybean bran	<i>Aspergillus</i> sp., <i>Fusarium</i> sp., and <i>Penicillium</i> sp.	Fleuri et al. (2014)
Olive oil cake	<i>Pseudomonas</i> sp. S1	Sahoo et al. (2014)
Sugarcane bagasse and sunflower seed meal	<i>Burkholderia cepacia</i> LTEB11	Soares et al. (2015)
Plant oil-seed cakes	<i>Lasiodiplodia theobromae</i> VBE-1	Venkatesagowda et al. (2015)

production; nevertheless, the processes are expensive, especially when the catalytic processes include commercial enzymes (Liu et al. 2013). The principal advantage of using lipases for biodiesel production is the feasibility of catalysing several reactions in non-aqueous media, such as that used for biofuel production (Kumar and Kanwar 2012). However, in recent years, SSF has acquired good credibility for metabolite production, including enzymes such as lipases, using cheap raw materials, such as A-IR, which lower production costs (Kumar and Kanwar 2012).

Lipases are enzymes that were traditionally produced in SF; however, in reality, some authors used SSF an alternative method because it presented some advantage over SF, such as the use of A-IR, demanding less water and energy, and easy aeration of medium, representing diminution of production cost (Coradi et al. 2013). Table 6 shows some lipases produced using SSF by different strains of microorganisms.

Bioethanol

Biofuels are important because they replace petroleum fuels. Production of bioethanol reduces consumption of crude oil and production of environmental pollution.

Bioethanol can be produced from different kinds of raw materials. Bioethanol production was traditionally realized by SF; however, in recent years, some researchers have reported the feasibility of production by SSF, because with this process there is the possibility of using A-IR (Mohanty et al. 2009). The use of A-IR combined with SSF for bioethanol production is a sustainable alternative to SF (Rodríguez et al. 2010). These strategies represent efficient bioethanol production using different A-IR and yeast strains for these proposes, e.g. wheat straw (Chen et al. 2007), sweet sorghum (Yu and Tan 2008, Li et al. 2013, Molaverdi et al. 2013), *Madhuca Latifolia* L. (Mohanty et al. 2009), grape and sugar beet pomaces (Rodríguez et al. 2010) rice straw (Roslan et al. 2011), sugarcane bagasse (Shaibani et al. 2011), sweet potato (Swain et al. 2013) using *Saccharomyces cerevisiae*, carob pods using *Zymomonas mobilis* (Mazaheri et al. 2012), and sweet sorghum using *Mucor indicus* (Molaverdi et al. 2013). SSF presents some advantages for ethanol production contrasted with SF, e.g. easy operation, saving of time and energy; also cheap feedstock could be used as carbon source (Rodríguez et al. 2010). Actually SSF technology is used more frequently for ethanol production as an alternative source of energy.

Environmental applications

Biosurfactant production has been improved because it has characteristics such as biodegradability and low toxicity compared to synthetic surfactants, and it can be used in bioremediation, food emulsification and cosmetics, and has stable activity at extremes of pH, salinity and temperature (Kiran et al. 2010; Mukherjee et al. 2006; Bento et al. 2005). In addition, biosurfactants have applications in oil industries due to their capacity to produce surface tension and to disperse one system into another (Neto et al. 2008; Martins et al. 2009). Biosurfactants produced in SF are extensively used in bioremediation purposes (Bento et al. 2005), but one of the principal problems of biosurfactant production is high production costs. For this reason, it is important to use a raw material that has low cost, such as A-IR (Mukherjee et al. 2006), and low-cost culture systems, such as SSF. Although reports exist for biosurfactant production using different microorganisms such as *Bacillus subtilis* SPB1, and substrates such as millet in SSF (Ghribi et al. 2012), more information regarding diversified microorganisms and low-cost substrates are necessary. To go deeper into the basis or the reasons for using the SSF in environmental applications is necessary consider the next factors: (a) in a wider designation, SSF can be seen as including processes during which microorganisms are cultivated in the presence of a liquid phase at maximal substrate concentrations (Hölker and Lenz 2005), exactly the case of non-soluble substrates as hydrocarbons in non-soluble substrates as oil-spills, for example, (b) in SF, aeration and agitation could be an important economic cost (Md 2012) but in SSF this disadvantage is not present: the oxygen transfer from the gas phase are directly in contact with biomass and the bioprocess do not depends of the very low solubility of oxygen in water (less than 10 mg l^{-1}), and (c) biosurfactants can be produced from very cheap raw materials which are available in large quantities (Md 2012), exactly the case of biomass proceeding of agro-industrial wastes.

Conclusion

Solid state fermentation is currently used in an important range of applications, including classical applications such as enzyme or antibiotic production, recently developed applications such as production of bioactive compounds and organic acids, and new applications regarding bioethanol and biodiesel as sources of alternative energy, biosurfactant molecules with environmental purposes, and biological control as an environmental alternative. The success of applying solid state fermentation to a specific process must take into account the nature of specific

microorganisms or specific strains, due to specificity representing an important constraint in solid state fermentation systems, particularly in new trends as biosurfactants with environmental purposes. The SSF an alternative processes for produce some products with industrial interest valorising unexploited biomass.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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