



Innovative Production of Bioproducts From Organic Waste Through Solid-State Fermentation

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Solid-state fermentation (SSF) is, by definition, a technology carried out in absence or near absence of free water. Therefore, it allows the use of solid materials as substrates for further biotransformation. SSF has gained attention in the last years being reported as a promising eco-technology that allows obtaining bioproducts of industrial interest using solid biomass (wastes and by-products). Main advantages over conventional submerged fermentation rely on the lower water and energy requirements, which generate minimum residual streams. However, drawbacks related to poor homogeneity and energy and mass transfer often appear, hindering the process yield and the downstream of the produced bioproducts. Despite the difficulties, many successful processes have been reported on the production of a variety of bioproducts such as hydrolytic enzymes, mostly carbohydrases for bioethanol production, and to a lesser extent, aromas, biosurfactants, biopesticides, bioplastics, organic acids or phenolic compounds. Most of the reported research focuses on process development at small scale; however, the main challenges to overcome in SSF are related to the upscaling and the development of a consistent and continuous operation. In this work, the main advances for the production of valuable/innovative bioproducts are presented and discussed.

Keywords: bioproducts, organic waste, enzymes, biopesticides, biosurfactants, aromas, solid-state fermentation

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BACKGROUND

One of the main interests of the society in the last decades is the valorization of waste. Lately, society has dramatically increase the amount of organic waste generation from different sources. International institutions have promoted the shift in the conception of waste, changing from pollutants to secondary renewable resources. Thus, legislations such as Landfill Directive 1999/31/EC and Waste Directive 2008/98/EC arise in order to reduce the organic waste disposal into landfill and hence promoting a new waste management hierarchy that promotes the use of wastes as secondary raw materials.

In this context, there is a great potential on processing great amounts of waste and by-products and reuse them as energy sources or useful materials. By one side, submerged fermentation is the most conventionally used technology to produce valuable bioproducts. This technology, used generally to valorize liquid wastes, has many advantages such as the possibility to develop a highly controlled bioprocess, as bioreactor's design and implementation are widely reported in both scientific literature and patents releases (Mitchell et al., 2006; Astolfi et al., 2011; Farinas, 2015).

On the other side, strong pretreatments are required in order to treat solid organic wastes. These pretreatments are often environmentally hazardous with high energy and water requirements leading to the generation of a highly diluted product stream with low productivities (Lever, 2005).

A more interesting technology that is gaining attention in the last years is solid-state fermentation (SSF). SSF is defined as a fermentation carried out in absence or near absence of free water. This technology allows using solid organic wastes as substrates without mandatory pretreatment, resembling a natural environment for microorganisms to thrive. Among its benefits are low energy and water requirements, concentrated bioproducts and it is considered as an environmentally friendly process (Mitchell et al., 2006; Thomas et al., 2013).

SSF is an attractive technology, however, there are some constraints hindering its implementation at large scale (scientific literature often cover lab scale experiments), the most relevant being reactor design and upscaling effect on the process productivity (Mitchell et al., 2006; Farinas, 2015).

The main objective of this mini-review is to provide a general overview of the state of the art of the research devoted to bioproducts production through solid-state fermentation.

PRODUCT DEVELOPMENT

The most common bioproducts targeted for production through SSF are hydrolytic enzymes (Table 1). The wide range of application of enzymes make them attractive bioproducts to obtain, specifically those associated with biofuel production, i.e., cellulase and hemicellulase. A wide range of microorganisms produces these enzymes, with the main target to degrade the main components of the cell wall, thus exposing easily metabolizable sugars. Conventionally, several species of *Trichoderma* and *Aspergillus* genus have been consistently used for cellulase and xylanase production from wastes (El-Bakry et al., 2015; Khanahmadia et al., 2018). The highest cellulase production are reported using *Trichoderma* as inoculum and agroindustrial wastes as substrates, with activity production ranging between one and hundreds depending of the operational conditions. For instance, when cellulose content of the substrate is below 30%, low cellulase productivities are obtained (below 3FPU g⁻¹DM), proving that cellulase is highly induced by cellulose (Mejias et al., 2018).

In addition to hydrolytic enzymes, other attractive but less studied bioproducts obtained by SSF are antibiotics, organic acids, biopesticides, aromas, biofuels, bioplastics and biosurfactants (Table 2) (Jimenez-Peñalver et al., 2016; Ballardo et al., 2017; Cerdeja et al., 2017b; Martinez-Ávila et al., 2017). Among those mentioned above, biosurfactants production is lately gaining attention due to the potential substitution of chemically produced surfactants, thus showing less toxicity, higher biodegradability, and resistance to temperature (Claus and Van Bogaert, 2017; Singh et al., 2018). These materials have many applications in the cosmetic industry, soil bioremediation, or even new polymer synthesis (Krieger et al., 2010). The production of different types of biosurfactants by SSF has been studied

(Singh et al., 2019). Sophorolipids are a group of extracellular biosurfactants produced by non-pathogenic strains. Among these strains, *Starmerella bombicola* is the most productive strain, with a volumetric productivity of 3.7 g L⁻¹h⁻¹ in commercial submerged fermentation (Claus and Van Bogaert, 2017). Jimenez-Peñalver et al. (2016) developed an untraditional system able to produce sophorolipids using winterization residues and molasses as substrates for *S. Bombicola* through SSF. These authors reached yield and productivity of 0.21 g g⁻¹ and 0.58 g L⁻¹ h⁻¹, which, however, are low when compared to traditional production systems. Other biosurfactants produced by SSF are rhamnolipids (El-Housseiny et al., 2019). Productivities of 0.19 g L⁻¹ h⁻¹ were obtained using agroindustrial residues as substrates and *Pseudomonas aeruginosa* as inoculum. As with the sophorolipids, the productivities are lower than those observed in traditional production systems. However, it is expected that by devoting more effort to SSF optimization, both reactor design and control parameters, further improvements can be reached (Wang et al., 2018; El-Housseiny et al., 2019). Jimenez-Peñalver et al. (2018) also reported that there is a high influence of the substrates on the type and yield of sophorolipid produced. Therefore, there is a wide range of sophorolipids production yield ranging between 0.06 and 1.07 g g⁻¹DM using agroindustrial sugar and fat sources (Wang et al., 2018).

Another interesting bioproduct produced by SSF are aroma compounds. One of the most used aroma compounds are rose-like scented 2-phenethyl alcohol (2-PE) (Stark et al., 2003) and the floral fragrance 2-phenethyl acetate (2-PEA) (Guo et al., 2017). Martinez-Ávila et al. (2017, 2018) reported a residue-based productive process to obtain fruit-like aromas using *Kluyveromyces marxianus* and sugarcane bagasse via SSF. These authors reported lab scale fermentations to a final production yield up to 12.1 mg_{2-PE} per gram of dry substrate (gTS) and 3.9 mg_{2-PEA} g⁻¹TS. In addition, an increase of 33.6 and 23.8% in the production yield of 2-PE and 2-PEA, respectively, was observed when an external readily metabolizable sugar source was added to the system. According to the published literature, aroma production via SSF allowed working with higher sugar content, using less chemicals and obtaining similar volumetric productivities than those based on submerged fermentations.

Another studied bioproduct are biopesticides, especially those derived from *Bacillus thuringiensis*. There are several reports on this subject, using many different organic wastes as the substrates such as soy fiber (Ballardo et al., 2017), biowaste (Ballardo et al., 2017) or even biowaste digestate (Cerdeja et al., 2019). The most interesting are those using complex substrates such as those derived from biowaste. Ballardo et al. (2017) reported the production of a compost-like material enriched with biopesticide activity derived from the action of *Bacillus thuringiensis* using non-sterile biowaste, thus providing a low-cost alternative for biowaste valorization. As one of the main issues of using biowaste as substrate is its variability, these authors worked at a representative scale of a few kilos of biowaste, thus providing reliability on the obtained results. Other reported biopesticides are those derived from *Beauveria bassiana* from agroindustrial wastes. Particularly, Qiu et al. (2019) reported the production of biopesticides using brewer's spent grain.

TABLE 1 | Summary of enzymes obtained by solid-state fermentation, production yields and process conditions.

Bioproduct	Substrate (s)/Inoculum	Production	Scale/Process configuration/reactor type	References
Cellulases	Biowaste digestate/Autochthonous microbiome	2 FPU/gDM	Lab/Batch (2 d) /Packed bed reactor	Cerde et al., 2019
	Coffee husk/Compost	8 FPU/gDM	Lab/Batch (24 h)/Erlenmeyer flask	Cerde et al., 2017a
	Coffee husk/Specialized consortia	10 FPU/gDM	Bench/Sequential batch operation (RT:2 d)/Packed bed adiabatic reactor	
	Agricultural waste/ <i>Trichoderma</i> or <i>Aspergillus</i> strains	1–400 FPU/gDM	Lab/Batch/Mostly Erlenmeyer flasks	El-Bakry et al., 2015
Xylanases	Biowaste digestate/ <i>Trichoderma reesei</i>	80 UA/gDM	Lab/Sequential batch operation (RT:3.5 d)/Packed bed reactor	Mejias et al., 2018
	Wheat bran/ <i>Aspergillus niger</i>	1,137 ± 104 U/gDM	Lab/Batch (72 h)/Erlenmeyer flask	Khanahmadia et al., 2018
	Sorghum stover/ <i>Aspergillus niger</i>	257 ± 35 U/gDM		
	Corn cob/ <i>Aspergillus niger</i>	380 ± 25 U/gDM		
	Soybean meal/ <i>Aspergillus niger</i>	365 ± 20 U/gDM		
	Wheat bran / <i>Aspergillus niger</i>	2,919 U/gDM	Bench/Batch (72 h)/Tray reactor	
Amylases	Coffee husk /Specialized consortia	48 ± 4 U/gDM	Pilot/Batch (24 h)/Packed bed reactor	Cerde et al., 2017b
	Soy fiber/ <i>Thermomyces lanuginosus</i>	35,000 U/gDM	Lab/Batch(96 h) (Erlenmeyer flask)	Cerde et al., 2016
		41,000 U/gDM	Bench/Batch (96 h)/Packed bed reactor	
228,000 U/gDM		Bench/Sequential batch operation (RT:96 h)/Packed bed adiabatic reactor		

Those findings are in accordance to the reported for bacterial biopesticides, obtaining good results in terms of productivity and setting a good starting point to develop a representative production process.

Other authors have addressed the phenolic compounds production through SSF (Buenrostro-Figueroa et al., 2017; Shin et al., 2019). Shin et al. (2019) developed an SSF process based on the use of black rice bran as substrate and *Aspergillus awamori* as the inoculum for phenolic compounds production. The results showed that the developed process was effective, achieving a production of 1,660 µg pterulic acid g⁻¹ of substrate in a 3-day operation. These authors also pointed the necessity of a pretreatment in order to make the phenolic compounds available for extraction. This is in accordance to the reported by Lee et al. (2019), which suggested aqueous extraction of enzyme components hindering the phenolic compounds production.

Another focus of interest in bioproducts are bioplastics, such as polyhydroxybutyrate (PHB) which production through SSF has been gaining interest. PHB can be produced from industrial, wastewater sludge and agricultural and food waste, as they have been pointed as a suitable feedstock (Rivero et al., 2017).

Finally, other attempts to produce novel bioproducts include pullulan (Singh et al., 2019) or cordycepin (Kunhorm et al., 2019). To summarize, it is virtually possible to produce almost any bioproduct by SSF, simultaneously valorizing solid organic wastes. This way, SSF is an essential tool to fill the gaps in the transition to a circular bioeconomy. However, it is necessary to develop efficient processes in order to SSF be competitive with commercial production systems based on SmF and consolidated end-of-pipe valorization technologies. Following, the current process development for SSF is analyzed.

PROCESS DEVELOPMENT

As detailed above, there are interesting reports on different bioproducts production through SSF; however, most of these studies were performed at a lab scale using small amounts of substrates (1–5 g). Actually, the amount of papers published reporting 5 g fermentations is astonishing, but few authors are devoting efforts to serious process development and upscaling. For instance, Das et al. (2019) revised inulinase production through SSF highlighting the importance of particle size and bioreactor type. However, they cite only two references working with 2.3 kg packed-bed reactors. In this sense, there are still challenges to overcome regarding upscaling and regime of operation (continuous or semi-continuous) in order to achieve a competitive SSF based bioprocess.

There are only a few reports tackling the mentioned operational strategies, but most of them are focused either on inoculation strategies (Cerde et al., 2016; Martinez-Ávila et al., 2017), operational configuration (Cheirsilp and Kitcha, 2015; Cerde et al., 2017a; Martinez-Ávila et al., 2018; Mejias et al., 2018), process control and/or reactor design (Astolfi et al., 2011; Biz et al., 2016; Martinez-Ávila et al., 2018). The last parameter is of great relevance, as it is the main factor hindering the development of large-scale processes (Mitchell et al., 2006; Thomas et al., 2013). Those authors consistently reported that issues regarding heat removal, substrate compaction and limited oxygen transfer are of great relevance when designing a proper SSF system. Interestingly, Rodrigues Pessoa et al. (2019) has developed a mathematical model using computational fluid dynamics (CFD), for heat and mass transfer in a pilot-scale packed-bed bioreactor.

Authors have reported different reactor configurations for substrate bioconversion into valuable bioproducts, such as static reactors (tray or packed bed reactors with forced aeration) or

TABLE 2 | Summary of innovative bioproducts obtained by solid-state fermentation, production yields and process conditions.

Bioproduct	Substrate (s)/Inoculum	Production	Scale/Process configuration/ reactor type	References
Biopesticides	Soy fiber/ <i>Bacillus thuringiensis</i>	1.1·10 ⁸ CFU spores /gDM	Bench/Batch(9 d)/Packed bed reactor	Ballardo et al., 2017
	Biowaste/ <i>Bacillus thuringiensis</i>	2.1·10 ⁷ CFU spores /gDM	Bench/Batch(9 d)/Packed bed reactor	Ballardo et al., 2017
	Brewers's spent grain/ <i>Beauveria bassiana</i>	8.5·10 ⁹ CFU spores /gDM	Lab/Batch(12 d)/Erlenmeyer flask	Qiu et al., 2019
Biosurfactants: Sophorolipids	Sugar beet molasses and winterization oil cake/ <i>Starmella bombicola</i>	0.58 g/L·h	Lab/Batch/Erlenmeyer flask	Jimenez-Peñalver et al., 2018
Biosurfactants: Rhamnolipids	Sugarcane bagasse and sunflower seed meal/ <i>Pseudomonas aeruginosa</i>	0.19 g/L·h	Lab/Batch/Erlenmeyer flasks	El-Housseiny et al., 2019
Aromas	Sugarcane bagasse and sugar beet molasses/ <i>Kluyveromyces marxianus</i>	47.6 mg ester/gDM	Lab/Batch/Erlenmeyer flask	Martinez-Ávila et al., 2017
	Sugarcane bagasse/ <i>Kluyveromyces marxianus</i>	57 mg ester/gDM	Lab/Fed batch/Non-isolated mixed reactor	Martinez-Ávila et al., 2018
Phenolic compounds	Fig residues/ <i>Aspergillus niger</i>	10.19 mg gallic acid/gDM	Lab/Batch (72 h)/Tray reactor	Buenrostro-Figueroa et al., 2017
	Black rice/ <i>Aspergillus awamori</i>	1.7 mg pterulic acid/gDM	Lab/Batch/Erlenmeyer flask	Shin et al., 2019
Bioplastics: PHB	Food waste/ <i>Bacillus</i> spp	0.1–0.53 g/gDM	Lab/Batch(5 d)/Erlenmeyer flask	Rivero et al., 2017
	Agroindustrial waste/ <i>Lactobacillus</i> spp	0.51–0.91 g/gDM	Lab/Batch(5 d)/Erlenmeyer flask	
Pollulan	Cassava bagasse/ <i>Aspergillus pullulans</i>	19–32 g/gDM	Lab/Batch(5 d)/Erlenmeyer flask	Singh et al., 2019
Cordycepin	Agroindustrial waste/ <i>Cordyceps</i> spp	8–25 mg/gDM	Lab/Batch(30–60 d)/Erlenmeyer flask	Kunhorm et al., 2019

mixed reactors (rotatory drum or horizontal paddle) (Durand, 2003; Thomas et al., 2013). Tray reactors are often considered as the most suitable option, as it is a low cost equipment with low maintenance costs. A number of enzymes have been successfully produced using these reactors, especially xylanases, cellulases, laccases, and pectinases (Khanahmadia et al., 2018). Also fungal derived biopesticides (*B. bassiana*) have been obtained in tray bioreactors (Xie et al., 2012). More sophisticated options have been used mostly for enzyme production, such as rotatory drum or packed bed reactors, and to a lesser extent for organic acids, antibiotics and sophorolipid with positive results (Arora et al., 2018). In contrast with tray reactors, rotatory drums and packed bed reactors handles the problems associated with mixing, heat removal or use of the heat.

Some authors have taking special interest in the development of operational strategies toward a self-sustained production process, among them feeding strategies and inoculation of specific or mixed microorganisms. In the case of feeding strategies, there are some reports showing good productivities working in a sequential batch or fed-batch configuration (Astolfi et al., 2011; Cheirsilp and Kittha, 2015; Cerda et al., 2016, 2017a; Martinez-Ávila et al., 2018). Most of these studies aimed to enzyme production, particularly carbohydrases derived from lignocellulosic degradation. These authors observed a consistent enzyme production, however, depending on the bioproducts, different profiles were observed.

Operating SSF as a sequential batch using agroindustrial wastes led to a sustained cellulase production for nearly 15 days in a value of 10 FPU g⁻¹DM (Cerda et al., 2017a). However, the same strategy led to a peak in amylase production after 3 cycles of operation with a 500% increase in production yield. The authors reported that substrate type and inhibitors production throughout the process are conditioners of the effectiveness of the strategy. In this sense, Mejias et al. (2018) reported a sequential batch operation using biowaste digestate as the substrate with no positive results attributing this result to a poor quality of the substrate. Also, the microbial dynamics are determinant, especially when working with a complex microbiome, as they can evolved along the fermentation, and the selected strains can either enhance or reduce the production of the targeted bioproducts (Cerda et al., 2017a).

Xylanase production is widely reported in SSF systems using mostly *Aspergillus* species. Production yield varied between 50 and 6,000 UA g⁻¹DM, depending on the substrate and process conditions (Khanahmadia et al., 2018). The outcomes of Khanahmadia et al. (2018) work were further validated using tray bioreactors (using 100 g of substrate) resulting in a 2–5-fold increment in xylanase production. This contradicts the findings of Cerda et al. (2017b), who observed a reduction in enzyme production when the laboratory fermentations were upscaled to bioreactors (Cerda et al., 2017b). In this sense, overall results on upscaling are contradictory, which hints the fact that each

productive process must be developed individually according to the process requirements.

Most studies on this subject assess enzyme production, but there are a few aiming for other bioproducts. Martínez-Ávila et al. (2018) took a fed-batch approach for aroma production using sugarcane bagasse as the substrate, reaching a maximum of 57 mg_{ester} g⁻¹TS in 60 h, which represented an improvement in comparison with the batch strategy.

Also, Ballardo et al. (2017) succeeded in improving *Bacillus thuringiensis* growth in biowaste operating in sequential batch.

Downstream processes are also a key point in SSF product viability, increasing in importance when developing a large-scale process. Extraction, conservation and remaining activity of the targeted bioproducts can also influence and be influenced by the type of bioreactor and substrate used. Marin et al. (2018) presented a study on the optimization of cellulase extraction from fermented coffee husk after SSF process. Distilled water was successfully used as extracting agent while lyophilization was demonstrated an adequate technology for enzyme conservation.

These findings showed that developing a sustainable, reproducible and continuous SSF process is feasible.

CHALLENGES AND PERSPECTIVES

Bioproducts obtained by SSF is a hot topic and it is constantly evolving. Most of the reported studies highlight the use of organic waste as a substrate for subsequent valorization. Despite this aspect is very important, it is necessary to go beyond that fact and explore the different challenges this type of fermentation has. Clearly, the development of novel bioreactors is one of the main issues that need to be further studied. The improvement of this

matter had a 2-fold aim: (i) to reduce operational constraints such as heat removal or mixing regime and (ii) to obtain a continuous productive process. In this sense, a correct reactor design combined with the most suitable inoculation/feeding strategy can potentially produce a sustainable bioprocess susceptible to simple operational modifications leading to an optimized productive process.

Inoculum requirement for producing targeted bioproducts is a key factor for success. A carefully studied inoculation of a specific strain, isolated from a natural environment, defined microbial consortia or even adapted microbial consortia have been proven fundamental for the configuration of the bioreactor operation. These entwined aspects will determine the productivity of the proposed system. It is also necessary to expand the possibilities for bioproducts generation, focusing not only on enzyme production but on also looking toward more industrially relevant value-added bioproducts. Biosurfactants, bioplastic, and aromas are a few examples of some valuable bioproducts, even though their level of development is still low, there is plenty of room for improvement.

AUTHOR CONTRIBUTIONS

AC wrote the complete manuscript. All the authors contributed to the conception of the manuscript and the interpretation of the literature and critically revised the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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