From Wastes to High Value Added Products: Novel Aspects of SSF in the Production of Enzymes

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Solid-state fermentation (SSF), a process that occurs in the absence or near absence of water, has been used for the production of various high value added products such as enzymes and other organic components. This paper reviews the recent studies reported on the use of SSF for the production of enzymes: lipases, proteases, cellulases, hemicellulases, ligninases, glucoamylases, pectinases, and inulinases. The microorganisms used for fermentation are mostly fungi, and substrates are waste materials from the agriculture and food industry. This shows the advantages of SSF from an economic and environmental viewpoint. The paper provides an update on several issues, viz. wastes, microorganisms, and issues of scaling up and controlling the process of fermentation in solid state.

KEY WORDS: cellulases, enzymes, lipases, proteases, process scale up, solid-state fermentation.

1. INTRODUCTION

Solid-state fermentation (SSF) is a process of fermentation performed on non-soluble materials, namely the substrate, in the absence or near absence of water (Salihu et al., 2012). The substrate acts mainly as source of nutrients for the microorganisms responsible for the fermentation. There are various
TABLE 1. A comparison between SSF and SmF for the production of enzymes, showing the main advantages and disadvantages

| Factor                        | SSF                        | SmF                        |
|-------------------------------|----------------------------|----------------------------|
| Substrate                     | No-cost materials, e.g.    | Very expensive media       |
|                               | waste products             | ingredients                |
| Inoculum                      | Not necessary              | Essential                  |
| Aseptic conditions            | Not needed                 | Essential                  |
| Moisture                      | No free water              | Liquid media required      |
| Agitation                     | Very difficult             | Easy                       |
| Process control (T, pH)       | Difficult                  | Easy                       |
| Contamination                 | Less chance                | High risk                  |
| Enzyme yield                  | Very high                  | Low                        |
| Downstream processing         | Easy, cheap, not           | Very difficult, very       |
|                               | time-consuming             | expensive                  |
| Liquid waste                  | Not produced               | High quantities            |
| Scale up                      | Difficult, new design      | Easy, industrial equipment|
|                               | equipment needed           | available                  |
| Volume and costs of equipment | Small reactors can be used,| Large-scale reactors required, |
|                               | low costs                  | very high costs            |

groups of microorganisms used in SSF depending on the final product considered necessary to be obtained from the fermentation. Among these, filamentous fungi like species of *Aspergillus* and *Rhizopus* are the best adapted microbial species reported in most recent studies (Belmessikh et al., 2013; Cunha et al., 2012; Dhillon et al., 2011a, b; Thanapimmethaa et al., 2012). The SSF process has been extensively used for the production of high value added products such as enzymes, biofuel, biosurfactants, and biopesticides (Singhania et al., 2009).

In particular, for the production of enzymes, the fermentation is commonly conducted in a liquid medium containing the required dissolved nutrients (Colla et al., 2010). This process of fermentation is known as submerged fermentation (SmF), which presents the benefit of homogeneity of the culture media used and possibility of controlling the parameters like temperature and pH. However, there are several advantages of SSF over the use of SmF (Mitchel et al., 2006). A comparison between SSF and SmF is presented in Table 1. SSF allows for the production of enzymes with higher activity and stability with lower water and energy demands. Additionally, from the environmental and economical perspectives, the main advantage of SSF is related to lower volume of effluent produced, compared to SmF, and the possibility of carrying out the process under non-sterile conditions (Subramaniyam and Vimala, 2012). SSF uses low-cost waste products mainly from the sector of agriculture and food industries such as wheat bran and peels of fruits and vegetables. These wastes can be used as ideal substrates for the microbial fermentation due to their rich contents of organic components, which are considered as essential sources for carbon, nitrogen, and
many micronutrients that are important for the production of metabolites. Despite these advantages of SSF, the use of this type of fermentation in industrial processes is not widely applied due to challenges and limitations concerning monitoring, controlling, and scaling up of the process (Salihu et al., 2012; Sukumaran et al., 2010). For example, one of the critical issues regarding the latter is the inability to remove the heat excess generated by microbial metabolism during the fermentation. Another important disadvantage of SSF is the handling of solids on large scales, as 200 kg is reported to be the maximum weight of solids to be used in the fermentation on industrial scales. Both are considered being the main disadvantages of SSF for industrial applications.

The objective of this paper is to review the recent studies reported on the use of SSF of waste materials for the production of enzymes, as high value added products. The paper provides an update on various aspects: wastes, microorganisms, and SSF process scale up and control.

2. WASTES USED IN SSF

The wastes used in the processes of SSF for the production of enzymes are mainly of animal and plant origin from the food industry. Table 2 outlines the wastes and microorganisms used in the processes of SSF and also the enzymes produced. In this section, a detailed description of the wastes of animal and plant origin used is also presented.

2.1. Wastes of Animal Origin

The waste materials of animal origin, including tannery solid wastes, cow dung, chicken feather, and fish flour, have been mainly used in the production of only proteases through SSF processes (Table 2).

Tannery solid wastes, in the form of raw hide trimmings and splits, limed and green animal fleshings, chrome shavings, and hair wastes, are produced with huge quantities during leather manufacturing and are not usually used or underused (Nalawade et al., 2009). Therefore, these waste materials are creating a solid waste disposal problem in tanneries. Kumar et al. (2009) studied the use of animal fleshings, the proteinaceous part of tannery solid wastes, as the substrate for the production of aspartic protease by a *Synergistes* sp. It was suggested that there is a possibility to produce this enzyme by SSF using a cheap substrate and moreover, the enzyme obtained exhibited high stability in various organic solvents. Hair wastes have been also used in SSF for the production of proteases (Abraham et al., 2014). This waste mixed with raw sludge from wastewater treatment has been valorized by SSF without the inoculation with a pure microorganism. Alkaline protease was produced as a consequence of the degradation of hair by the microbial
TABLE 2. Types of various waste materials used in the SSF processes for the production of enzymes, including the microorganisms if present in these processes.

| Category of waste | Types of waste          | Microorganisms                      | Enzymes              | References                                                                 |
|-------------------|-------------------------|-------------------------------------|----------------------|---------------------------------------------------------------------------|
| Wastes of animal  | Fish flour              | *Aspergillus niger*                 | Proteases           | Garcia-Gomez et al. (2009), Rai et al. (2009)                               |
| origin            | Chicken feather         | *Aspergillus subtilis*              |                     | Vijayaraghavan and Vincent (2012)                                          |
|                   | Cow dung                | *Halomonas sp.*                     |                     | Abraham et al. (2013, 2014)                                               |
|                   | Hair waste              | *nc*                                |                     | Kumar et al. (2009)                                                       |
|                   | Tannery solid waste     | *Synergistes sp.*                   |                     |                                                                           |
|                   | Oil wastes              | *Aspergillus niger*                 | Lipases, Amylase     | Colla et al. (2010), Chaturvedi et al. (2010), Edwinoliver et al. (2010), |
|                   |                         | *Bacillus subtilis*                 |                     | Godoy et al. (2011)                                                      |
|                   |                         | *Penicillium spp.*                  |                     |                                                                           |
|                   |                         | *Aspergillus oryzae*                |                     |                                                                           |
| Wastes of plant   | Wheat wastes            | *Rhizopus oryzae*                   | Protease            | Thanapimmethaa et al. (2012)                                              |
| origin and food   |                         | *Aspergillus spp.*                  | Lipases             | Garlapati and Banerjee (2010)                                             |
| industry          |                         | *Trichoderma reesei*                | Cellulases, Xylanase | Dhillon et al. (2011), Bansal et al. (2012), Pirola et al. (2013)         |
|                   | Rice wastes             | *Aspergillus sojae*                 | Pectinase           | Demir and Tari (2014)                                                    |
|                   |                         | *Aspergillus spp.*                  | Cellulase           | Soni et al. (2010), Dhillon et al. (2011)                                 |
|                   |                         | *Aspergillus niger*                 | Proteases           | Paranthaman et al. (2009)                                                |
|                   |                         | *Aerigillus fumigatus*              | Cellulase           | Liu et al. (2011)                                                        |
|                   | Soy wastes              | *Aspergillus terreus*               | Cellulase           | Narra et al. (2012)                                                      |
|                   |                         | *Bacillus pumilus*                  | Xylanase            | Kapilan and Arasaratnam (2011)                                            |
|                   |                         | *Aspergillus niger*                 | Lipases             | Rigo et al. (2009), Colla et al. (2010)                                   |
|                   |                         | *Penicillium sp.*                   | Prostates           | Abraham et al. (2013)                                                    |
|                   |                         | *Aspergillus oryzae*               | Cellulases, Xylanase | Brijwani et al. (2010, 2011)                                             |
| Source                          | Organism                  | Enzymes                  | References                                                                 |
|--------------------------------|---------------------------|--------------------------|-----------------------------------------------------------------------------|
| Peels of fruits and vegetables | *Aspergillus niger*      | Cellulase, xylanase,     | Rodriguez-Fernandez, et al. (2011) 2012, 2013, Mamma et al. (2008)         |
|                                | *Bacillus subtilis*       | pectinase, phytase,      |                                                                             |
|                                | *Bacillus firmus*         | Proteases                | Mukherjee et al. (2008, 2009)                                              |
|                                |                           |                          | Elayaraaja et al. (2011)                                                   |
| Sugarcane bagasse              | *Aspergillus niger*       | Cellulases               | Cunha et al. (2012), Mekala et al. (2008)                                   |
|                                | *Kluvyromyces marxianus*  | Inulinases               | Astolfi et al. (2011)                                                      |
| Cotton wastes                  | *Aspergillus spp.*        | Hydrolytic & oxidative   | Csizar et al. (2007), Liu et al. (2011)                                     |
|                                |                           | enzymes                  |                                                                             |
| Pomace of fruits and vegetables| *Aspergillus spp.*        | Proteases, cellulases     | Belmessikh et al., 2013, Dhillon et al. (2012a,b)                           |
|                                | *Bacillus licheniformis*  |                          | Chaari et al. (2012)                                                       |
|                                | *Pleurotus eryngii*       |                          |                                                                             |
| Grape, apricot waste           | *Aspergillus fumigatus*   | Lichenase                | Akpinar and Urek (2012, 2014)                                              |
| Oil palm trunk                 |                           |                          |                                                                             |
| Waste bread                    | *Aspergillus awamori*     | Lignolytic enzymes       |                                                                             |
| Agrowastes                     | *Bacillus subtilis*       |                          |                                                                             |
| Winterization residues, sludge | *nc*                      | Cellulases, Xylanses     | Ang et al. (2013)                                                           |
|                                |                           |                          |                                                                             |
| nc: not controlled             |                           |                          |                                                                             |

2003
populations developed. Stabilized compost was another by-product of the process.

Cow dung, as an inexpensive waste material, has been evaluated as a substrate for the production of protease by *Halomonas* sp. through SSF (Vijayaraghavana and Vincent, 2012). A high production of halo-tolerant alkaline protease was obtained when compared with a substrate of wheat bran under the same process conditions. Accordingly, cow dung, which is characterized by its increased availability and low costs, might be used in future research as a key substrate in the production of protease. Keratin wastes such as chicken feather has been utilized in SSF by a feather degradating strain of *Bacillus subtilis* (Rai et al., 2009). The process conditions were optimized in order to maximize the yield of β-keratinase, which is a type of protease. This was one of the important studies that show the successful use of a keratin waste material in the production of enzymes. Fish flour, a fish processing by-product, mixed with polyurethane foam has been used by *Aspergillus oryzae* for the production of a proteolytic extract (Garcia-Gomez et al., 2009). This extract showed a higher enzymatic activity, that is, a higher degree of protein hydrolysis, when tested on fish muscle compared to a commercially available enzyme. Therefore, it was concluded from the results of this research that it was highly feasible to use fish flour as a substrate in the production of proteolytic enzymes.

2.2. Wastes of Plant Origin and Food Industry

There are various types of wastes of plant origin and also of food industry that have been used in processes of SSF for the production of enzymes (Table 2). These waste materials include wastes of wheat and rice such as wheat bran and rice husk, peels and pomace of fruits and vegetables, sugarcane bagasse, soy and cotton wastes, waste bread, and brewery spent grain. Therefore, by using such a wide range of waste materials, it was possible to obtain through the process of fermentation several types of enzymes, that is, lipases, proteases, cellulases, xylanases, pectinases, amylases, and inulinases.

2.2.1. Wastes of Vegetable Oil

Wastes of vegetable oil (oil cakes) have been used for the production of lipases, proteases and xylanases through SSF. Lipase has been produced when cakes of edible oil have been used as substrate. For instance, Colla et al. (2010) have used soybean oil cake as a substrate after adding about 10% rice husk for increasing the porosity of the media that allows for oxygen transfer, in the presence of *Aspergillus* spp. as inoculum. In comparison to SmF, SSF led to higher enzymatic activity of lipase. This was due to the fact that in solid substrates the nutrients are more concentrated than those in liquid medium. This resulted in excellent cell–substrate interaction that consequently led to a higher enzyme production. Another oil cake used for
the production of this enzyme was ground nut oil cake (Chaturvedi et al., 2010). It was shown that the enzyme production through the fermentation by *Bacillus subtilis* was highly affected by various process conditions such as pH and moisture levels. It was found that the maximum yield of lipase was at a moisture of 70% and pH of 8.0. Interestingly, the oil cake of *Jatropha curcas*, a major energy crop in Thailand, has been used for the production of several enzymes through SSF as mentioned in recent literature (Mahanta et al., 2008; Joshi and Khare, 2011; Ncube et al., 2012). The outcome of the research performed was desirable, as it was possible to obtain enzymes such as proteases, lipases, and xylanases by using various microorganisms of *Pseudomonas*, *Scytalidium*, and *Aspergillus*. It is of high importance to establish a beneficial disposal of this waste material, as it is characterized by high contents of toxic compounds such as antineutrinos and phorbol esters (Ahmed and Salimon, 2009).

2.2.2. WASTES OF WHEAT, RICE, SUGARCANE, AND PALM TRUNK

Wastes of wheat and rice, sugarcane bagasse, and oil palm trunk have been mainly used as substrates for the production of cellulases through SFF. Therefore, it can be observed that there is a view to developing low-cost production systems for cellulase enzymes.

Wheat bran materials, as a lignocellulosic material, among various kitchen and agro-industrial wastes, such as corn cobs, peelings of fruits, and sawdust, appeared to be the best-suited substrate producing appreciable yields of cellulase enzyme, in the presence of an inoculum of *Aspergillus niger* and *Trichoderma reesei* (Bansal et al., 2012; Dhillon et al., 2011). Interestingly, there was no need for a supplementation of exogenous nutrients and therefore this research highlights the potential of wheat bran as possible raw material for the enzyme production. Wheat bran and *A. niger* were also evaluated for the production of cellulases and xylanase under SSF by Dhillon et al. (2011), and enzyme yields were compared with SSF where the inoculation and substrate were mixed with *T. reesei* and rice husk with a ratio of 1:1 and 2:3, respectively. In this case, it was reported that mixed microbial cultures and waste materials led to the production of higher amounts of enzymes. In addition, the inclusion of rice straw provided an additional source for the carbon required by the microorganisms used. This is in agreement with another research work that optimized the production of cellulase by *Aspergillus fumigatus* under SSF (Soni et al., 2010). Moreover, wheat bran has been also used as a supplement to soybean hulls for the production of cellulase using a mixed microbial culture of *T. reesei* and *A. oryzae* (Brijwani et al., 2010). Mixed cultures clearly showed their compatibility for hyper enzyme production.

Sugarcane bagasse, a waste product that is generated from the sugarcane industry in huge amounts, has been used evaluated as a substrate for
the production of cellulase through SFF. Mekala et al. (2008) addressed the optimization of environmental parameters and media for the fermentation by using *T. reesei* for enhancing the yield of the enzyme. A suitable SSF process has been developed for cellulase production with this cheap biomass resource as substrate. In addition, Cunha et al. (2012) have evaluated sugarcane bagasse as a substrate for the production of cellulase through SSF and SmF. The fungus *A. niger* has been used as an inoculum in both methods. It was shown that SSF was superior compared to SmF, as in this first case the cellulase production was threefold higher. This was due to the fact that in SSF, the nutrients are more concentrated than those in liquid media used in SmF, as previously explained (Section 2.2.1). On the other hand, cellulase production is controlled by feedback, that is, the more the substrate availability, the higher the enzyme yield. The advantage of SSF of this waste material is: first, this method is an economical process for the use of a lignocellulosic waste that exhibits a long-standing difficulty in the costs associated with the enzymatic hydrolysis of this material by other methods and second, SSF developed can go a long way in bringing down the cost of cellulases production, which will eventually help to develop economical processes for biofuel production.

Direct utilization of complex untreated oil palm trunk, a cheap and abundant material, for cellulases and xylanase production by lignocellulosic degrading fungi such as *Aspergillus fumigatus* was evaluated under SSF (Ang et al., 2013). The palm trunk, which was isolated from cow dung, was used as sole carbon source for the fungus during the fermentation process. The ability to produce xylanase with high levels of cellulases was also shown. However, in future studies, there is still a need for statistical optimization of all the parameters involved in the fermentation process.

### 2.2.3. WASTES OF FRUIT AND VEGETABLE INDUSTRIES

The industry of fruits and vegetables is producing a high amount of wastes and, therefore, it is interesting to use these materials in processes of SSF. Peels and pomace have been used for the production of enzymes. These enzymes include mainly cellulases, xylanases, pectinases, and proteases.

Potato peels have been determined as one of the best substrates among various agro-industrial wastes for the production of alkaline proteases by *Bacillus subtilis* (Mukherjee et al., 2008). Interestingly, citrus peels were chosen as a substrate for the production of enzymes because it is an important agro-industrial by-product that offers several carbon sources required for the growth of microorganisms and for the production of phytases, pectinases, and xylanases (Mamma et al., 2008). Microorganisms readily use this waste in fermentations due to its rich composition, especially due to its high content of organic matter, which is about 80%, being total dietary fibers (above 50%) free sugars and pectin the main compounds. This composition
justifies the use of citrus peels as inducing substrate for the production of multienzyme complexes, without the need for the addition of pectic materials as inducers to the media used in the fermentation (Kang et al., 2004). In addition, citrus peel is the major solid waste that is generated by the citrus processing industry, which represents approximately more than the half of the fresh fruit weight. Accordingly, the disposal of this by-product poses a big challenge to the fruit industry, where this waste is mostly pelletized and employed as animal feed or pectin precursor. This waste has been successfully used by Rodriguez-Fernandez et al. (2011) for producing pectinase and xylanase by *A. niger* through SSF. The kinetics of microbial growth related to the synthesis of the enzymes has been determined. Moreover, citrus waste has been also utilized for the production of phytase by the same fungus, and a scale-up process was achieved (Rodriguez-Fernandez et al., 2012, 2013).

Pomace of fruits and vegetables has been recently used as substrates for the production of protease and cellulase through the process of SSF. Apple pomace was the substrate for obtaining cellulase through the fermentation by *A. niger* (Dhillon et al., 2012a, b). Results showed a rapid bio-production of fungal cellulase using this low-cost waste material especially with a supplementation of inducers such as lactose. Tomato pomace has been also used as a substrate in SSF for the production of protease by the same genus of fungi (Belmessikh et al., 2013). The use of this tomato waste constitutes an efficient and inexpensive substrate for the enzyme production and a suitable mean for the waste valorization toward an attempt for reducing the ecological impact.

### 3. MICROORGANISMS USED IN SSF

This section deals with the research work performed on the microorganisms used in the processes of SSF and the substrates and end products obtained (Table 2) The microorganisms used in the SSF processes for the production of enzymes are fungi and bacteria, mainly *Aspergillus* spp. and *Bacillus* spp., respectively, which will be discussed in detail.

#### 3.1. Fungi

Fungi are the best adapted microbial species reported in most recent studies for the production of enzymes through SSF. This is due to the ability of these microorganisms to grow on surfaces of solid wastes and penetrate into the interparticle spaces of the substrates. The fungal hyphae can also penetrate some solid structure of the matrix. The fungal genera used are *Aspergillus*, *Penicillium*, and *Rhizopus* (Table 2). The fungal genus *Aspergillus* has a broad range of species that have been used in the processes of SSF. These
species include *A. niger*, *A. oryzae*, *A. terreus*, *A. fumigatus*, *A. sojae*, and *A. candidus*, where the most frequently used fungus is SSF is *A. niger*, a filamentous mesophilic fungus. This fungus was used to produce a multienzyme preparation containing pectinolytic, cellulolytic, and xylanolytic enzymes under SSF process on citrus peels (Mamma et al., 2008). This process was enhanced by the optimization of initial pH of the culture medium and moisture levels. Most importantly is the water activity, which limits the microbial growth. After the SSF process, the fermented substrate was either directly exposed to auto hydrolysis or new materials were added, and the in situ produced multienzyme systems were successfully used for the partial degradation of orange peel polysaccharides. Fermentable sugars were liberated, which could be converted to bioethanol. In a more recent study on SSF using the same substrate and fungus, the production of these enzymes were optimized based on aeration conditions to allow for a sufficient amount of oxygen that is required for the growth of the microorganism and the removal of CO2 and metabolic heat (Rodriguez-Fernandez et al., 2011). In addition, a mathematical model was applied to determine the different kinetic parameters related to SSF.

*A. niger* was used in SSF for the production of citric acid and cellulase enzyme, by using apple pomace and apple pomace ultrafiltration sludge, which are by-products from the apple processing industry (Dhillon et al., 2011; 2012a). The addition of 3–4% of ethanol and methanol to the apple pomace substantially increased the values of the citric acid attained. The cellulase obtained, after its recovery being optimized using various extraction solvents, was used for the saccharification of apple pomace and brewer’s spent grain. Sugarcane bagasse was also used for the production of this enzyme by SSF, where a combination of SSF and SmF was shown to be superior to the conventional submerged method due to the improved assimilation of sugarcane bagasse and fungal growth morphology (Cunha et al., 2012). The concentration of the substrate was fundamental in the comparison. The germination of the fungi on a solid-state medium allowed for the development of a dispersed filamentous form, which resulted in superior cell–substrate interaction and accordingly a higher production of the enzyme. In addition, cellulase was produced by using SSF of various wastes, agricultural and kitchen wastes such as corn cobs, carrot peelings, composite, grass, leaves, fruit peels, rice husk, sugarcane bagasse, saw dust, wheat bran, and wheat straw (Bansal et al., 2012). Of all the substrates tested, it was found that wheat bran is the most suited substrate for a high production of cellulase. *A. niger* was also used in the production of proteases and lipases (Paranthaman et al., 2009; Colla et al., 2010; Edwinoliver et al., 2010). For instance, Paranthaman et al. (2009) studied the production of protease using rice broken and rice mill wastes as substrates in SSF. The protease obtained could be commercially used in detergents and leather industry.
A. oryzae was used in SSF for the production of cellulases, proteases, and xylanase. Thanapimmethaa et al. (2012) showed that it is feasible to use deoiled Jatropha curcas, a major energy crop in Thailand used for biodiesel, seed cake as a substrate in the process of SSF after the optimization of the conditions of moisture, inoculum, and temperature. Recently, Pirota et al. (2013) used a new strain of A. oryzae isolated from the Amazon rain forest in SSF processes in the production of xylanase. The substrate used was wheat bran and the production of xylanase was on a lab scale with a possibility of scaling up of the process. A. oryzae was also used in mixed cultures in the production of enzymes by SSF. This fungus was used with Aspergillus giganteus, Phanerochaete chrysosporium, and T. virens in SSF on cotton seed-coat fragment waste as substrate (Csizar et al., 2007). The enzyme complexes produced were composed of hydrolytic and oxidative enzymes, such as cellulases and xylanase. A. oryzae was also used with Aspergillus awamori or T. reesei in the production of glucoamylase and protease or cellulase enzymes via SSF, using wheat bran that is a waste product of the wheat milling industry, or soybean hulls as substrate, respectively (Brijwani et al., 2010; Du et al., 2008).

3.2. Bacteria

In general, bacteria are not widely used in the production of enzymes through SSF. The bacteria are mainly of the genus Bacillus (Table 2), specifically its species subtilis, licheniformis, pumilus, and firmus, which have been used in the production of amylases, proteases, lichenases, and xylanases (Chaari et al., 2012; Kapilan and Arasaratnam, 2011; Mukherjee et al., 2008, 2009; Nimkar et al., 2010). The waste materials used as substrate in the SSF were mainly agrowastes, such as potato peel and pea pomace, and chicken feather that is considered an animal waste by-product. B. subtilis was most often used in SSF processes. This bacterial species was used in SSF for the production of proteases and α-amylases.

Amylase was successfully produced using wheat and rice bran as substrate materials for the SSF process after optimization of the various parameters such as pH and temperature (Nimkar et al., 2010). In addition, Mukherjee et al. (2009) found that potato peel, which is considered as a novel inexpensive substrate, was the best waste material among agro-industrial waste residues to be used for the production of amylase due to its high starch contents and the absence of monosaccharides. This waste material was combined with other agrowastes such as grass and protein sources, which allowed for the production of protease by using the same species of Bacillus (Mukherjee et al., 2008). Recently, potato peel was also utilized by the bacterium Bacillus firmus, isolated from marine sediment of Parangipettai coast, to produce thermostable alkaline amylase by SSF process at optimized process conditions (Elayaraja et al., 2011).
4. ENZYMES PRODUCED BY SSF, THEIR PROCESS CONDITIONS, AND APPLICATIONS

In general enzymes have been extensively produced by SmF and have been commercially available since many decades (Anwar and Saleemuddin, 1998; Pandey et al. 2003; Queiroga et al., 2012). Recently, the production of enzymes on SSF has been implemented in order to reduce the costs involved, especially if residues are used as substrates, and enhance the field of application (Kumar et al., 2009; Sandhya et al., 2005). However, there is indeed few research work developed on bench-scale solid substrate fermentation, as the majority of this research was conducted on few grams of substrate materials, that is on a lab-scale. In addition, some research work was performed in media with high moisture contents of more than 70%, which might be due to some solid substrates that are able to retain high moisture levels, whereas SSF is defined as fermentation being performed in the absence or nearly absence of free water (Pandey et al. 2003).

This section is discussing in detail the enzymes such as lipases, proteases, cellulases, and xylanases and other enzymes such as fucoidanase and pectinases that are obtained through the process of SSF. In addition, there will be a detailed description on the conditions of the fermentation process and the various applications of lipases, proteases, cellulases, and xylanases.

4.1. Enzymes
4.1.1. LIPASES

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are enzymes capable of catalyzing the hydrolysis of triacylglycerols to glycerol and fatty acids at an oil–water interface and reactions of esterification, transesterification, and interesterification of lipids (Sharma et al., 2001). In recent years, there has been an increasing interest in the study of lipases mainly due to their potential applications to a wide range of industrial sectors (Hasan et al., 2006, 2009). In the chemistry and pharmaceutical industries, lipases are used in the production of surfactants, detergents, and antibiotics; whereas in the food industry these enzymes are used to synthesize emulsifiers and develop flavors. Commercially useful lipases are typically obtained as microbial extracellular enzymes. However, since lipases are products of industrial interest, their production must be coupled with low-cost processes. These enzymes would be economically manufactured in processes of SSF that utilize residues as substrates and that give high yields.

The substrates used, which varied in amount from few grams up to 3 kg, in the processes of SSF for the production of lipases are oil wastes, wheat bran, and sludge (Table 3). The activity of the lipase varies to a very high extent; the range of this activity is from 4.5 to 120,000 U/g. Wastes of oil, such
| Substrate                                      | Amount (g) | Process conditions | Microorganisms       | Enzyme activity (U/g) | Reference                        |
|-----------------------------------------------|------------|--------------------|----------------------|-----------------------|----------------------------------|
| Coconut oil cake, wheat bran, wheat rawa     | Up to 3,000| 30                 | nc                   | 60                    | *Apergillus niger* 745.7         | Edwinoliver et al. (2010)        |
| Mix of oil cakes                             | 10         | 30                 | 8                    | 70                    | *Bacillus subtilis* 4.5          | Chaturvedi et al. (2010)         |
| Niger seed oil cake                          | 10         | 30                 | 6.4–6.8              | 60                    | *Yarrowia lipolytica* 26.42      | Imandi et al. (2010)             |
| Babassu cake                                 | 10         | 30                 | nc                   | 70                    | *Penicillium simplicissimum* 314 | Gutarra et al. (2009)            |
| Castor bean waste                            | 20         | 30                 | nc                   | nc                    | *Penicillium simplicissimum* 80.24 | Godoy et al. (2011)              |
| Soybean meal                                 | 10         | 20                 | 7                    | 75                    | *Penicillium sp.* 317            | Rigo et al., (2009)              |
| Soybean meal, rice husk                      | 50         | 30                 | 4.5                  | 60                    | *Apergillus niger* 25.22         | Colla et al. (2010)              |
| Jatropha curcas seed cake                    | 5          | 30                 | 7                    | 50                    | *Pseudomonas aeruginosa* 312.5   | Mahanta et al. (2008)            |
| Wheat bran                                   | 4          | 35                 | 5.28                 | 60                    | *Rhizopus oryzae* 96.52          | Garlapati and Banerjee, (2010)   |
| Winterization residue, sludge                | 2,500      | Higher than 45     | 7                    | 50                    | nc                               | 120,000 Santis-Navarro et al. (2011) |

nc: not controlled

*Initial moisture content of the substrate(s) used
as solid wastes from the production of vegetable oils (oil cakes), are one of the common waste materials used as substrates for the production of lipase by SSF. Oil cakes are good supports for microbial growth necessary during the process of SSF, because this waste has excellent sources of proteinaceous nutrients needed for the microbial fermentation, that is, requiring low or no supplementation (Ramachandran et al., 2006). This waste material has also another advantage. It is inexpensive and available in high amounts from the oil industry.

Oil cakes of coconut, ground nut, mustard, linseed, and neem has been used as substrates in SSF in the presence of *B. subtilis* for the production of extracellular lipase on a lab-scale using 10 g of waste materials (Chaturvedi et al., 2010). It was observed that the nature of the substrate significantly influenced the impact of initial moisture content and therefore affected the process of SSF. The physical nature and water holding capacity of the substrate are important criteria for its use in SSF process because the moisture content is an important factor that determines the microbial growth and activity of the enzyme. In another study of Edwinoliver et al. (2010), coconut oil cake was also used and mixed with wheat bran and rawa for the production of lipase through SSF in the presence of *A. niger*. The scaling up of the process was possible from lab-scale to bench-scale using up to 3 kg of wastes as substrates. The SSF process led to a maximum activity of the lipase of 745.7 U/g. In addition, babassu cake supplemented with sugar cane molasses as a substrate and the fungus *Penicillium simplicissimum* were used for the production of lipase, on a lab-scale, with a maximum activity of 314 U/g through SSF (Guturra et al., 2009). This fungus has been also used in SSF for the production of lipase, but by using other oil wastes such as soybean cake and castor bean waste showing an enzymatic activity of 317 and 80.24 U/g, respectively (Godoy et al., 2011; Rigo et al., 2009).

The oil cake of biodiesel crops, which contain about 50% oil called biocrude that can be converted into biodiesel by esterification, are also used as substrates for the production of lipase enzymes through SSF. Lipase was produced by using Niger oil cake, as it is rich in various nutrients such as fatty acids and sugars, through SSF on a lab-scale, where 5 to 10 g of wastes were used as substrate (Imandi et al., 2010). The marine yeast *Yarrowia lipolytica* was used as an inoculum for the fermentation. There was a low enzyme activity obtained, with a maximum of 26.42 U/g. Another biodiesel crop called *Jatropha curcas*, a major energy crop in Thailand, was used by Mahanta et al. (2008) for the production of lipase through SSF by *Pseudomonas aeruginosa*. The seed cake supported good bacterial growth and enzyme production of an activity of 625 U/g, due to the composition of this cake that contains a high content of fat and fibers.

Winterization residue from oil refinery and raw sludge were used as solid matrices for the processes of SSF for lipase production on a bench-scale using 2.5 kg of waste materials, where the fermentation was dependent on
the microbial consortium present (Santis-Navarro et al., 2011). Winterization residue was used a source of fat and the sludge was added as co-substrate and inoculum. It was reported that the lipolytic activity of the enzyme obtained reached a maximum of 120,000 U/g in the fermented solid, which is substantially higher than activities reported in other research on SSF. This highlights the possibility to work with solid wastes as effective biocatalysts, a topic that has been scarcely treated in SSF literature.

4.1.2. PROTEASES

Proteases (EC 3.4.21-24), which are hydrolases that catalyze the cleavage of peptide bonds in proteins, are a highly complex group of enzymes that differ in their substrate specificity and catalytic mechanism (Sumantha et al., 2006; Turk, 2006). These enzymes are classified into three main categories, alkaline, neutral, and acid proteases, on the basis of pH range in which their activities are optimal. Therefore, proteases are the most important industrial enzymes that account for about 60% of the world market of industrial enzymes. The importance of these enzymes is reflected in their tremendous applications in both physiological and commercial fields, for example, in detergent formulations, textile, food, and pharmaceutical industries (Queiroga et al., 2012).

The preferred source of proteases is microorganisms, rather than plant and animal tissues, to their broad biochemical diversity and their susceptibility to genetic manipulation (Ellaiah et al., 2002). Among microbes, fungi as enzyme producers have many advantages, since they could be mostly GRAS (generally regarded as safe) strains and the produced enzymes are extracellular, which makes easy its recuperation from the fermentation broth. Accordingly, the overall cost of the production of a complex group of enzymes is very high, mainly due to low yield of enzymes because most of the costs are related to the recovery and purification. Additionally, there are other high costs associated with the substrates, that is, the commercial media required (Singhania et al., 2009). Therefore, the development of novel processes to increase the yield of proteases coupled with lowering down these costs is highly appreciable. Furthermore, proteases produced by using commercial media possess undesirable flavors, which are unsuitable for applications in food and pharmaceutical industries. Therefore, during the recent years, efforts have been directed to explore the means to reduce the protease production costs through improving the yield and the use of cost-free or low-cost substrates such as agricultural waste materials in processes of SSF for the production of proteases.

The waste materials used as substrates, which highly varied from 5 g to 1.4 kg, in the processes of SSF for the production of proteases are mainly of plant origin, such as potato peel, soy fibers, tomato pomace, and wheat bran or of animal origin like tannery solid wastes, chicken feather, and cow
| Substrate          | Process conditions | Microorganisms     | Max enzyme activity (U/g) | Reference                              |
|--------------------|--------------------|--------------------|---------------------------|----------------------------------------|
| Potato peel, grass | 100 50 8 50        | Bacillus subtilis  | 2,383                     | Mukherjee et al. (2008)                |
| Fish flour, polyurethane foam | 30 30 nc 50 | Aspergillus niger | 120.78                    | Garcia-Gomez et al. (2009)             |
| Soy fiber residues | 1,250 nc 8.5 40–60 | Nc                 | 47,331                    | Abraham et al. (2013)                  |
| Tomato pomace      | 10 30 6.8 60       | Aspergillus oryzae | 21,309                    | Belmessikh et al. (2013)               |
| Jatropha seed cake | 5 30 6 50         | Pseudomonas aeruginosa | 1818                   | Mahanta et al. (2008)                  |
|                     | 25 30 nc 45        | Aspergillus oryzae | 14,273                    | Thanapimmethaa et al. (2012)          |
| Wheat bran, casein | 5 45 nc 60        | Thermomucor indicae-seudaticae | 167.6                | Merheb-Dini et al. (2010)             |
| Wheat bran         | 5 nc nc nc        | Myceliophthora sp. | 19.8                      | Zanphorlin et al. (2011)              |
|                    | 10 24–40 nc 50    | 14 Fungal strains  | 5.05                      | Boyce and Walsh (2012)                |
| Chicken feather    | few grams 27 acidic 60 | Aspergillus oryzae | 8.3 x 10^3               | Vishwanatha et al. (2009)             |
| Cow dung           | 5 50 8 50         | Bacillus subtilis  | 95.3                      | Rai et al. (2009)                     |
| Tannery solid waste | 5 37 8 50      | Halomonas sp.      | 1,351                     | Vijayaraghavan and Vincent (2012)     |
| Hair wastes        | 1,400 nc 8.5 40–60 | Synergistes sp.    | 755                       | Kumar et al. (2009)                   |

nc: not controlled

*Initial moisture content of the substrate(s) used
dung (Table 4). The activity of the enzyme obtained through SSF also highly varied from around 20 to more than 50,000 U/g.

Several studies on the utilization of residues of plant origin in the production of proteases through SSF were carried out on wheat bran. Merheb-Dini et al. (2010) used the microorganism *Thermomucor indicae-seudaticae* in the presence of wheat bran and wheat bran mixed with casein at a ratio of 80:20, respectively, for the production of protease with an enzymatic activity of 168 U/g. In addition, wheat bran has been used as substrate in the production of protease with a maximum activity of 5–20 U/g by a fungal strain of *Schizopyllum commune* and *Myceliophthora* sp. (Boyce and Walsh, 2012; Zanphorlin et al., 2011). Mukherjee et al. (2008) screened various agro-industrial and kitchen waste materials of plant origin, such as oil cake, wheat and rice bran, grass, banana leaves, potato peels and used tea leaves, for the use as substrate for protease production through SSF by *Bacillus subtilis*. It was found that the substrates of potato peel and grass led to the production of proteases with the highest protease activity of up to 2,383 U/g. In another study by Abraham et al. (2013), the effect of three agro-industrial residues was examined; hair waste, coffee husk, and soy fiber. Soy fiber presented the highest yield for protease production showing an enzymatic activity of 47,331 U/g. Recently, tomato pomace was used in a comparative study of protease production by cultivating *A. oryzae* in SSF and SmF (Belmessikh et al., 2013). The results obtained showed a highest enzymatic activity of 21,309 U/g in case of the process of SSF. There were recent few studies on the utilization of residues of *Jatropha curcas* (oil cake), which is a major energy crop that cannot be used in nutrition or animal feed due to its toxicity. Mahanta et al. (2008) and Thanapimmetha et al. (2012) investigated the potential utilization of this oil cake as substrate for protease production by *Pseudomonas aeruginosa* and *A. oryzae*, respectively. The results demonstrated that the utilization of this waste material for the enzyme production was a viable approach, with an activity of about 2,000 up to 14,000 U/g. Moreover, Chutmanop et al. (2008) compared the use of *Jatropha* oil cake with wheat and rice bran as substrates in SSF for the production of proteases under the same fermentation conditions and by using the same inoculum of *A. oryzae*. Interestingly, it was found that the protease activity produced by the oil cake was 30–40% higher than that of wheat and rice bran, due to the fact that this cake has a very high protein content that can be utilized by the microorganism for the production of the enzyme.

Residues of animal origin, tannery waste and cow dung, have been utilized in the SSF process in the research work of Kumar et al. (2009) and Vijayaraghavana and Vincent (2012), respectively. Tannery solid wastes, which consist of hide trimmings and limed animal fleshing, were considered as a proteinaceous substrate for the production of proteases, with activities up to 755 U/g, under SSF by using *Synergistes* sp. Similarly, hair waste from the tanning industry mixed with raw sludge from wastewater treatment,
without the need for inoculations of pure microorganisms, were valorized for the production of protease, where a maximum enzymatic activity of 56,270 U/g was reached (Abraham et al., 2014). Cow dung was used in the presence of an inoculum of *Halomonas* sp. leading to the production of proteases of a relatively high activity of 1,351 U/g, which was substantially higher compared to other waste materials of plant origin that have been used under the same process conditions.

### 4.1.3. Cellulases and Xylanase

Cellulose and xylan are the first two most abundant natural biopolymers, which are most dominating agricultural wastes (Zhang, 2008). The lignocellulosic biomass of most plants consist of mainly cellulose (a homologous polymer of glucose linked by β-1-4 glycosidic bonds); lesser hemicelluloses (a heterologous polymer of 5- and 6-carbon sugars with sugar acids) that contains principally xylan; and finally lignin (a complex aromatic polymer). Cellulose, only its amorphous form, is synergistically hydrolyzed by a complex enzyme system named as cellulases; such as cellulbiohydrodylase or exoglucanase, carboxymethylcellulase or endoglucanase, and cellobiase or β-glucosidase (EC 3.2.1.91, 3.2.1.4 and 3.2.1.21 respectively), while the degradation of xylan requires various enzymes; essentially endo-1-4-β-xylanase (EC 3.2.1.8) and to some extent β-xylosidase, α-glucuronidase, α-L-arabinofuranosidase, and acetylxylan esterases (Maki et al., 2009; Van Dyk and Pletschke, 2012). The lignocellulosic biomass, as it can be degraded, it is a renewable and abundant resource in agricultural industry, with an appropriate treatment, with great potential for bioconversion to value-added bio-products. Therefore, cellulases and xylanasse are now considered as a major group of industrial enzymes that have various industrial applications.

Techniques of fermentation, due to their economic and environmental advantages, have been widely used for a feasible production of cellulases and xylanase (Subramaniyam and Vimala, 2012). The most frequently used techniques are SmF and SSF, where the latter being the most beneficial due to the use or recycle of wastes that are cheap and highly available.

As previously mentioned, cellulose and xylan are present in plants and therefore the substrates used for the production of the enzymes of cellulases and xylan are only of plant origin (Table 5). These substrates are wastes of soybean, wheat, rice, corn, cotton, sugarcane bagasse, and fruits such as apple, as well as residues from wood industries. The yield of the cellulases represented for three enzymes as activities of filter paper (FPase) for cellulbiohydrolase or exoglucanase, carboxymethylcellulase (CMCase) for carboxymethylcellulase or endoglucanase and β-glucosidase (BGase) for cellobiase or β-glucosidase. The yield for xylanase is shown for the activity of endo-1-4-β-xylanase.

Soybean hulls have been used as a substrate for the production of cellulases and xylanase through SSF by a mixed culture of *A. oryzae* and *T. reesei*
TABLE 5. Substrate, process conditions, microorganisms, and enzyme activity of cellulases and xylanase produced by SSF.

| Substrate                      | Process conditions | Enzymes | Reference       |
|--------------------------------|-------------------|---------|-----------------|
|                                | Amount            | Temperature | Moisture | Microorganisms                     | Type | Max activity (U/g) |       |
| Soybean hulls                  | 10,240 g          | 30 °C | 5         | 70          | Aspergillus oryzae, Trichoderma reesei | FPase | 5.39              | Brijwani et al. (2011) |
|                                     |                  |               |  |             |                                         | CMCase | 58.57              |       |
|                                |                  |               |  |             |                                         | Bgase  | 18.36              |       |
|                                |                  |               |  |             |                                         | Bgase  | 242                |       |
| Soybean hulls, wheat bran       | 100 g            | 30 °C | 5         | 70          | Aspergillus oryzae, Trichoderma reesei | FPase | 10.78              | Brijwani et al. (2010) |
|                                     |                  |               |  |             |                                         | CMCase | 100.67             |       |
|                                |                  |               |  |             |                                         | Bgase  | 10.71              |       |
|                                |                  |               |  |             |                                         | Bgase  | 504.9              |       |
|                                |                  |               |  |             |                                         | Xylanase | 3.37              | Soni et al. (2010) |
| Rice straw                     | 5 g              | 45 °C | 7         | 75          | Aspergillus fumigatus                  | FPase | 3.37              |       |
|                                |                  |               |  |             |                                         | CMCase | 98.5               |       |
|                                |                  |               |  |             |                                         | Bgase  | 250.9              |       |
|                                |                  |               |  |             |                                         | Xylanase | 2.782             |       |
| Rice straw, wheat bran         | 10 g             | 30 °C | nc        | nc          | Aspergillus oryzae, Trichoderma reesei | FPase | 35.8               | Dhillon et al. (2011) |
|                                |                  |               |  |             |                                         | CMCase | 132.34             |       |
|                                |                  |               |  |             |                                         | Bgase  | 33.71              |       |
|                                |                  |               |  |             |                                         | Bgase  | 3,106              |       |
| Apple pomace                   | 10 g             | 30 °C | nc        | 70          | Trichoderma sp.                        | FPase | 7.6                | Sun (2010) |
|                                |                  |               |  |             |                                         |        | 133.68             | Dhillon et al. (2012b) |
|                                | 40 g             | 30 °C | nc        | 75          | Aspergillus oryzae                     | FPase |                   | (Continued on next page) |
TABLE 5. Substrate, process conditions, microorganisms, and enzyme activity of cellulases and xylanase produced by SSF. (Continued)

| Substrate Type | Amount (g) | Process conditions | Microorganisms Type | Enzymes Type | Max activity (U/g) | Reference |
|----------------|------------|--------------------|---------------------|--------------|-------------------|-----------|
| Wheat bran     | 5          | 30                 | nc 40-57            | Trichoderma reesei | CMCase 172.3    | Sukumaran et al. (2009) |
| Rice husk, wheat bran | 30       | 30                 | nc nc               | nc           | CMCase 135.44    | Hu et al. (2011) |
| Apple pomace, lactoserum | 40      | 30                 | nc nc               | Aspergillus niger | CMCase 54.7     | Dhillon et al. (2012a) |
| Rice husk, wheat bran & straw, corncob, kitchen wastes | 5       | 30                 | 6.5 60              | Aspergillus oryzae, Trichoderma reesei | CMCase 297 | Bansal et al. (2012) |
| Rice, wheat & cotton straw, corncob | 20      | 50                 | 4 80                | Aspergillus fumigatus | CMCase 526.3 | Liu et al. (2011) |

FPase: Filter paper activity for cellulase
CMCase: Carboxy methyl cellulase
BGase: β-glucosidase
nc: not controlled
*Initial moisture content of the substrate(s) used
(Brijwani et al., 2010, 2011). The maximum enzymatic activity obtained was 101 and 505 U/g for the carboxymethyl cellulase and xylanase, respectively. Results revealed that the additional use of wheat bran in the substrate positively affected the enzymatic activities obtained through the fermentation process. The SSF process was proven to be a valuable technique for producing a system of cellulases and xylanase enzymes with balanced activities, which were able to efficiently saccharify lignocellulosic biomass. Wheat bran, untreated and without any supplements, as a sole substrate has been also evaluated for the production of cellulolytic enzymes through SSF by using the same microbial culture as inoculum. For instance, Bansal et al. (2012) and Dhillon et al. (2011) achieved an enzymatic activity for carboxymethyl cellulase and xylanase up to about 300 and 2,700 U/g.

Wastes of rice, such as the straw and husk, have been recently utilized as substrate materials during the fermentation by *A. oryzae* and *T. reesei* for the production of enzymes. Rice straw supplemented with wheat bran in the ratio of 3:2 resulted in the highest enzymatic activity of up to 132 U/g for carboxymethyl cellulase, whereas the xylanase reached a very high activity of 3,106 U/g (Dhillon et al., 2011). In similar studies, the fungus *A. fumigatus* has been used as inoculum for the process of fermentation, where a cellulolytic activity of up to 251 U/g for β-glucosidase enzyme and 2,782 U/g for xylanase have been reported (Soni et al., 2010).

The feasibility of using apple pomace for cellulase production under SSF was evaluated. The fermentation by *Trichoderma* sp. and a supplement of lactose and corn-steep solid allowed for obtaining of an enzyme activity with a maximum of 7.6 U/g for the exoglucanase (Sun et al., 2010). This activity substantially increased to above 130 U/g and an activity of carboxymethyl cellulase of about 150–170 U/g was also reported, using an inoculum of *A. niger* and especially when lactoserum, which is a source of lactose, was added as a moistening medium (Dhillon et al., 2012a, b). There was also a high activity of xylanase of up to 4,868 U/g obtained.

### 4.1.4. Other Enzymes

In addition to the enzymes of lipase, protease, cellulases, and xylanase that were discussed in detail in previous sections (4.1–4.3), there are other enzymes obtained through SSF processes. These enzymes include mostly glucoamylase, pectinase, and inulinase, which will be discussed in this section. There has been also few research studies performed on the production of certain proteolytic enzyme with a mycotoxin hydrolytic activity, named as ochratoxin A (OTA)-hydrolysing enzyme, and fucoidanase (Abrunhosa et al., 2011; Rodriguez-Jasso et al., 2013, respectively). The latter is able to hydrolyze marine hetero-polysaccharides, called as fucoidans, which have a wide range of biological activity, for example, anticoagulant, antithrombotic, and antiproliferative activities.
4.1.4.1. Glucoamylase. Glucoamylase belongs to the amylases enzymes that hydrolyze polysaccharides, such as starch and its degradation products, into molecules of glucose, maltose and dextrin. Amylases are one of the important enzymes in the industry due to their diverse applications, for example, in the food (bakery products), paper, textiles, pharmaceutical, and detergents industries (Botella et al., 2009). These enzymes are classified into $\alpha$-amyrase (EC 3.2.1.1), $\beta$-amyrase (EC 3.2.1.2), and glucoamylase (EC 3.2.1.3), which is known as amylglucosidase or $\gamma$-amyrase (Norouzian et al., 2006). This enzyme, which hydrolyzes $\alpha$-1,4- and $\alpha$-1,6-glucosidic linkages at the non-reducing ends of polysaccharides, has been recently produced through SSF by using species of the fungal genus Aspergillus. Melikoglu et al. (2013) utilized waste bread as a substrate for the production of this enzyme. At optimum process conditions, such as a moisture of 60% and an incubation period of 144 h, it was possible to obtain an activity of glucoamylase of up to 114 U/g. Moreover, protease enzyme was also obtained through the process of fermentation. Accordingly, this study shows that waste bread could be successfully used as a primary substrate for obtaining enzymes. In another study, the production of glucoamylase was presented by using substrates of agro-residues of rice wastes, wheat bran, cotton seeds, corn steep solids, sugarcane bagasse, and edible oil cakes (Zambare, 2010). The optimization of the SSF process showed that the highest enzyme activity obtained of $\sim$2,000 U/g was with a substrate of wheat bran at a moisture content of 50% and pH of 6, after an incubation of 120 h.

4.1.4.2. Pectinases. Pectinases consist of endo- and exopolygalacturonases (EC 3.2.1.15 and EC 3.2.1.67/82, respectively) and are enzymes that degrade pectin, a complex heteropolysaccharide containing galacturonic acid residues that is a principal component of the middle lamella and primary cell wall of higher plants (El-Sheekh et al., 2009). These enzymes are therefore of great importance to the food industry as they are predominantly used in the clarification of juices, as well as to textile and plant fiber processing industries. In addition, pectinases are applied as food additive for monogastric animals, such as food for pets.

An economical and feasible alternative for the production of pectinases is SSF, where it has been found that species of the fungus Aspergillus are one of the microorganisms that are able to produce these enzymes during the fermentation. Demir and Tari (2014) found that wheat bran, among various agro-industrial wastes, was the most suitable substrate for the production of polygalacturonase using A. sojae. The optimum process conditions that favored the enzyme production were 4 days of fermentation time at a temperature of 37°C and initial moisture of 62% that resulted in an enzyme activity of up to 536 U/g. In addition, waste products of citrus fruits were used as substrates for the production of pectinases by A. niger. The feasibility of using citrus peels was evaluated in a bench-scale bioreactor (Rodriguez-Fernandez et al., 2011). A mathematical model was applied to determine the
different kinetic parameters related to the enzyme production through SSF. The best conditions for pectinase production were at 60% initial moisture and a pH of 5.0 at and 30°C. The maximum activity of pectinase of up to 265 U/g was produced after a fermentation time of 3 days. Ruiz et al. (2012) utilized lemon peel pomace as substrate in a laboratory scale bioreactor at the same condition but with a moisture content of 70%. Results showed that high levels of pectinase activities were obtained, up to a maximum of more than 2,000 U/g, which suggested this process as very promising for pectinase production.

4.1.4.3. Inulinase. Inulinases, most commonly known as endo-inulinase (EC 3.2.1.7), are enzymes that hydrolyze inulin into fructose (Chi et al., 2009). The application of these enzymes are in the production of high fructose syrups and fructoligosaccharides, which are compounds with high nutritional values and therefore can be used in low-calorie diets and as a source of dietary fibers in food preparations. Although the inulinases could be obtained from vegetable and animal sources, microorganisms such as Aspergillus, Kluyveromyces, and Staphylococcus are the best sources for the commercial production of inulinases. This is due to their easy production and high yields obtained. SSF could be one of the useful approaches for the production of these enzymes. Kluyveromyces marxianus was utilized as inoculum for the fermentation in recent studies. Dilipkumar et al. (2013) obtained inulinase using pressmud as substrate, where parameters like air flow rate and particle size were optimized, leading to a maximum enzyme activity of ∼ 300 U/g. Sugarcane bagasse was also used as a substrate for the production of the enzyme with a maximum activity of 590 U/g (Astolfi et al., 2011). The optimized temperature and moisture was 30°C and 65%, respectively, at a fermentation time of 24 h. The study showed the technical feasibility of the process of production of inulinase through SSF.

4.2. Process Conditions
The production of enzymes through SSF required the study of process conditions suitable for the enzyme production such as temperature, incubation time, pH, moisture content, and types and inoculum levels of pure strain bacteria or fungi added or other sources of microorganisms, for example, raw sludge.

4.2.1. Lipase
These conditions for the production of lipase were at temperatures of about 20–30°C or higher than 45°C, neutral pH, and moisture levels of 50% to 75% (Table 3). The microorganisms used as inoculum required for the fermentation are fungi such as Aspergillus and Penicillium, Yarrowia yeast and Bacillus, and Pseudomonas bacteria. Depending on the scale, that is, amount of substrate used, the optimum temperature for the fermentation was 20°C.
up to 45°C for an incubation period of 2–20 days, at pH values of 4.5–8 and moisture levels of 50–70%.

The optimization of the production of lipase through SSF has been studied, mainly on a lab-scale, using experimental designs such as Plackett–Burman and central composite designs. Rigo et al. (2009) studied the lipase production through SSF by using *Penicillium* sp. and soybean meal as substrate. Initially, the effect of different carbon-to-nitrogen ratios (C/N ratios) on lipase production was evaluated and it was considered 6.11 as optimum. Furthermore, the optimum conditions found were at a temperature of 20°C for a fermentation period of 5 days. Higher lipase activities were found in a wide range of pH from 4.0 to 9.0, with a pH of 7 as optimum by using the substrate of soybean and *Penicillium* sp. as inoculum. The moisture level used during SSF was 75%. In another study by Mahanta et al. (2008), the production of lipase through SSF by using oil cake and *Pseudomonas aeruginosa* has been optimized for moisture content (29–80%), incubation time (24–144 hr) and pH (6.0–8.5). It was observed that the optimum moisture level was at 50%. A higher level of moisture content causes a decrease in porosity, development of stickiness, and an increase in the chances of contamination and, accordingly, a decrease in the gas exchange occurs. A lower level of moisture led to sub-optimal microbial growth during the fermentation and a lower degree of the swelling of substrate. The highest yield of the enzyme production was at 120 hr of incubation and there was no significant effect of pH on the lipase production. Imanti et al. (2010) have used *Yarrowia* yeast in the fermentation process and reported that the moisture content was optimized at a level of 60%. The incubation time at which the highest lipase production was obtained was 96 hr as longer periods led to the depletion of nutrients, accumulation of toxic end products, and the change in pH or loss of moisture and shorter incubation times were not sufficient for the microbial growth and hence the lipase production. The effect of pH on the lipase production in the presence of *Bacillus subtilis* as inoculum was studied by Chaturvedi et al. (2010). The lipase activity increased when increasing the pH from 6 to 8 and on further increase of pH to 9 and 10, the lipase activity decreased. This shows that the optimum pH for the lipase production was around a pH of 8.

In another approach of optimization, Garlapati et al. (2010) have used modeling combined with optimization as two vital steps for maximizing the efficacy of SSF. Response surface methodology (RSM), a statistical technique that generates a mathematical model, coupled with differential evolution, which is an optimization technique, has been used. This approach has been used to maximize the lipolytic activity by *Rhizopus oryzae* through SSF. The maximum lipase activity was observed at 35°C, 5.28, 60%, and 116 hr for temperature, pH, moisture, and incubation time, respectively. These obtained results of optimization were experimentally validated, and it was suggested that the developed model and optimization appear to be useful for the design
and control of the extracellular lipase production through SSF by using this microorganism.

4.2.2. Protease

The process conditions for the production of proteases were at mesophilic (30°C) up to thermophilic temperatures (50°C), pH levels of 6–8.5, and moisture levels of about 50% (Table 4). Fungi, such as species of *Aspergillus*, and bacteria, mainly *Bacillus subtilis* were the most predominant microorganisms used for the production of proteases. Concerning the scale of the production of the proteases, most of research work was done on a lab scale by using a maximum of 25 g of substrate, where an Erlenmeyer flask was used as a reactor (Boyce and Walsh, 2012; Merheb-Dini et al., 2010; Vijayaraghavana and Vincent, 2012; Zanphorlin et al., 2011). However, in a recent research work by Abraham et al. (2013, 2014), a 4.5 l air-tight reactor was used, working under near-adiabatic conditions, allowing for the use of 1.25 kg of solid substrate.

The initial moisture content required may vary depending upon the type of substrates and microorganisms used. However, it has to be considered that the keystone in SSF is to remove the metabolic heat produced during the fermentation in order to maintain constant moisture levels during the process, when saturated air is used for the cooling. The optimization of the moisture content has been studied in the processes of SSF for the production of enzymes. For example, Mukherjee et al. (2008) found that 50% initial moisture contents of the substrates of potato peels and grass were optimum for the production of protease by *B. subtilis*, whereas the optimum moisture in the case of wheat bran was 30%. Moreover, when *Jatropha* seed cake was used as substrate in the fermentation by *A. oryzae* at different levels of moisture of 45% to 55%, an optimum moisture content of 45% was reported (Thanapimmethaa et al., 2012).

There has been an optimization for the source of carbon and nitrogen required by the microorganisms used as inoculum for the production of proteases during the process of SSF. In most of the SSF processes, maltose and xylose were the optimum sources of carbon, and yeast and beef extracts were sources of sodium nitrate, and peptone was a source of nitrogen. Mukherjee et al. (2008) tested several sources of carbon and nitrogen required for the growth of *B. subtilis*. The carbon sources were glucose, fructose, galactose, maltose, sucrose, and lactose, being maltose the best source for obtaining the maximum enzyme activity of ~1,100 U/g, whereas the activities were sequentially 50 and 400 for galactose and lactose and glucose, fructose and sucrose. Additionally, it was found that beef extract, followed by yeast extract, rather than ammonium salts and casein, and served as the best nitrogen sources producing enzyme activities of ~1,400, 1,000, 420, and 400 U/g, respectively. In a study by Mahanta et al. (2008) using *Jatropha*
seed cake as substrate and *Pseudomonas aeruginosa* as inoculum, it was also found that the enrichment with maltose compared to other sugars led to an increase in the production of protease. The best nitrogen source was peptone, where ammonium chloride and sodium nitrate were also tested. Recently, carbon sources such as glucose, lactose, trehalose, maltose, xylose, and starch, and nitrogen sources such as gelatin, ammonium nitrate, peptone, yeast extract, urea, and casein were evaluated for the fermentation by *Halomonas* sp. when a substrate of cow dung was used (Vijayaraghavan and Vincent, 2012). The optimum enzyme production was achieved with a combination of xylose and yeast extract.

There have been different statistical methods used for the optimization of various parameters, rather than individual optimization, in the processes of SSF for the production of proteases. By adjusting the conditions to optimum levels, the protease production increased up to 5 times compared to non-optimized experiments. Belmessikh et al. (2013) used the experimental designs of Plackett–Burman and the Central Composite design for the study of the effect of five enrichment factors (wheat bran, casein, ammonium nitrate, sodium chloride and zinc sulfate) on the enzyme production with a substrate of tomato pomace by *A. oryzae*. It was reported that only two factors, casein and sodium chloride, had a significant effect on the production. This was due to the fact that during the fermentation process, casein could provide intact peptides that were necessary in the induction, whereas sodium chloride might have had a role in the protection of the enzyme from denaturation. In addition, the fermentation time was also optimized to 96 hr for the optimum production of protease. The optimized SSF led to a higher production of protease by about 1.5 times than non-optimized processes. Furthermore, optimization via Taguchi method was performed to evaluate the effect of five factors on the protease production by *A. oryzae* (Thanapimmethaa et al., 2012). The effect of three different levels of five factors, including initial moisture content of the substrate used (jatropha seed cake), inoculum size, temperature, type of porous substrate, and fermentation time, were examined. These levels were as followings: moisture content (45%, 50%, and 55%), inoculums size (1%, 5%, 10%), temperature (25°C, 30°C, 35°C), porous substrate (jatropha oil cake, jatropha oil cake mixed with coconut cake, and jatropha oil cake mixed with cassava bagasse, both mixtures with a ratio of 4:1), and time (84, 96, and 108 hr). The optimum conditions for the protease production of up to a maximum of 14,273 U/g were 45% moisture content, 10% inoculum size, 30°C incubation temperature, jatropha cake mixed with cassava bagasse as porous substrate at 84 hr of fermentation time. This statistical approach provided a satisfactory outcome in defining the optimal conditions, as the optimized process led to an increase of 4.6 times in the protease yield. Rai et al. (2009) reported the application of RSM for the optimization of the media composition for
**β**-keratinase production by *Bacillus subtilis* using chicken feather as substrate. The factors studied were the fermentation time (24, 48, 72, 96, and 120 hr), initial moisture content of the substrate (33%, 43%, 50%, 60%, 67%, and 75%), supplementation with co-carbon sources (glucose, fructose, galactose, maltose, sucrose, lactose, and starch at 10%), and co-nitrogen sources (NH₄Cl, NaNO₃, yeast extract, beef extract, casein, and peptone at 1%) were studied. The optimized culture conditions were at a time of 71 hr, 50% moisture and with maltose and sodium nitrate as the best co-carbon and co-nitrogen sources, respectively. The results showed that the optimization led to a fivefold increase in the enzyme obtained, up to 95.3 U/g, compared to non-optimized conditions.

### 4.2.3. Cellulases and Xylanase

The temperatures used for the fermentation were mostly mesophilic (30°C) or slightly thermophilic (45 or 50°C), the pH was 4 to 7, and the moisture content was 50–80% (Table 5). The process conditions of pH and moisture were not controlled in a lot of studies. The enzymes are produced by a variety of microorganisms including bacteria, actinomycetes, and fungi. However, in recent research works, fungi of the genera *Trichoderma* and *Aspergillus* have been reported as the most important microorganisms used as inocula for the process of SSF. Moreover, the effect of pretreatment of the substrate used on the production of enzymes was studied.

Bansal et al. (2012) evaluated various process parameters during the fermentation by *A. niger* of agriculture and kitchen waste residues for the production of cellulase complex. The effect of acid and alkali pretreatment of substrates used was studied. The alkali treatment led to increased yield of enzymes when the wastes, especially potato peels, were utilized as substrate compared to untreated waste materials. This was mainly due to the fact that alkaline pretreatment dissolve lignin present in the lignocellulosic waste and expose the cellulose and hemicellulose fractions for enzyme and microbial actions. Moreover, untreated substrates contain a variety of nutrients may probably have an inhibitory effect on the fermentation process and thus leading to a lesser production of enzymes. However, in the case of using wheat bran as substrate, the untreated waste induced the highest production of enzyme components. In addition, it was demonstrated that appreciable levels of enzymes could be produced over a wide range of temperatures (20–50°C) and pH (3.0–8.0), with an optimum of 30°C and 6.5 respectively, at initial moisture contents of 60%. These results were in agreement with other research work performed with the aim of producing cellulase. The same optimum pH of 6.5 was found to be the best pH for the enzyme production by the *A. niger* and when using municipal solid wastes as substrate (Gautam et al., 2011). The temperature of 30°C was found to be optimum for incubation of the fungus used as inoculum for the production of enzymes. Brijwani et al. (2010) reported 30°C as the optimum
temperature during the fermentation using soybean hulls and wheat bran as substrates by \textit{A. oryzae} and \textit{T. reesei}, for the production of cellulase and \(\beta\)-glucosidase. This temperature, together with the optimized moisture and pH of 70\% and 5 respectively, was used for scale-up processes and a further experimental analysis using novel bioreactor for the production of cellulase complex enzymes (Brijwani et al., 2011). In addition, Sun et al. (2010) found 32\(^\circ\)C as the optimum temperature when evaluating the feasibility of using apple pomace as a substrate for cellulase production by \textit{Trichoderma} sp. Thermophilic temperatures of about 50\(^\circ\)C were also found to be optimum in the process of SSF for the production of cellulase complex enzymes by \textit{A. fumigatus}, where lignocellulosic wastes were used as substrate. For instance, Liu et al. (2011) optimized the cultivation conditions and results showed that for cellulases, both endo- and exoglucanase; the best conditions were at a temperature of 50\(^\circ\)C, in the presence of an initial moisture of 80\% and a pH of 4.0. Soni et al. (2010) reported the optimization of cellulase production at 45\(^\circ\)C, where the culture produced maximal levels of enzyme activity on a medium containing rice straw and beef extract as carbon and nitrogen source, respectively. It was also concluded that optimization of the process of fermentation by mixing different substrates is a strategy for improvement of the production of cellulase enzymes.

The process conditions of SSF for the production of xylanase were similar to those of the cellulases; the temperature, pH and moisture levels were 30 or 45\(^\circ\)C, 5 or 7 and 70\%, respectively (Table 5). It was also observed that mixed substrates of wheat bran and soybean hulls (Brijwani et al., 2010) or rice straw (Dhillon et al., 2011), in the presence of mixed inocula of \textit{A. oryzae} and \textit{T. reesei}, led to an increase in the production of the xylanase enzyme up to a maximum of \(\sim\)3,100 U/g at a fermentation time of 96 h. A higher enzyme activity of \(\sim\)4,900 U/g was obtained by using apple pomace as the sole substrate, with lactoserum as a crude inducer, and an inoculum of \textit{A. niger} (Dhillon et al., 2012a).

4.3. Applications
4.3.1. Lipase

Lipase enzyme produced by SSF has great biotechnological potential applications, mainly due to the thermophilic and thermostable properties. The enzyme has various applications in oil, pharmaceutical, food, and chemical industries (Salihu et al., 2012; Sharma et al., 2011). Recently, lipase produced by SSF was used in synthesis reactions, food applications, and treatment of wastewater (Table 6).

In synthesis reactions, lipases have an important application in the field of bioenergy, especially for the production of biodiesel that is currently an expanding sector in research and on industrial level. Lipase obtained through
TABLE 6. Applications of lipase, protease, cellulases and xylanase enzymes

| Enzyme             | Applications                                                                 | References                                                                 |
|--------------------|------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Lipase             | Oil, pharmaceutical, food and chemical industries: synthesis reactions (biodiesel production), food applications (interesterification of oils) and treatment of wastewater | Salihu et al. (2012), Sharma et al. (2011), Damasceno et al. (2012), Rasera et al. (2012) |
| Protease           | Detergent formulations, dehairing (leather industry), dairy industry          | Venugopal and Saramma (2006), Abraham et al. (2014), Merheb-Dini et al. (2010) |
| Cellulases and xylanase | Paper manufacture, textile industry, bioethanol production                 | Das et al. (2013), Liu et al. (2011), Lever et al. (2013)                      |

SSF of sugarcane bagasse and sunflower oil cake by *Burkholderia cepacia* was used to catalyze the synthesis of biodiesel in a fixed-bed reactor (Liu et al., 2013; Salum et al., 2010). This synthesis was through the ethanolysis of soybean oil in a medium free. It was possible to achieve a biodiesel yield of about 90% after 46 hr of reaction. Compared with some commercial lipases, this process avoids the need for expensive processing steps such as enzyme recuperation and immobilization and co-solvent separation and therefore has potential to decrease the costs associated with enzyme-catalyzed synthesis of biodiesel. In addition, lipase produced by SSF, using *Rhizopus* sp. as a thermotolerant fungus, was used as a catalyst for the enzymatic esterification of oleic acid and ethanol (Martinez-Ruiz et al., 2008). Olive oil and perlite were used as an inducer and inert support, respectively. The results demonstrated that the lipase can be successfully used for the synthesis of ethyl oleate, with high etherification rates and substrate conversion, over short reaction periods under conditions when ethanol is in excess. Similarly, Hernandez-Rodriguez et al. (2009) showed that in addition to the lipase produced by *Rhizopus* sp., the enzyme produced by the thermophilic fungus *Rhizomucor* sp. through SSF can be used in the ethyl oleate synthesis reaction.

In food applications, lipase produced in SSF by *Rhizopus oryzae* and *Rhizopus microsporus*, on a mixture of sugarcane bagasse and sunflower seed meal, was used in interesterification processes of oils to produce fat products with desirable properties (Rasera et al., 2012). This enzyme was able to catalyze the interesterification of a mixture of palm stearin, palm kernel oil, and a concentrate of triacylglycerols enriched with omega-3 polyunsaturated fatty acids. This application could be suitable for the production of edible fat products such as margarines and shortenings with low production costs. Another application of the lipase produced by SSF was in the bioremediation of the waste cooking oil (Kumar et al., 2012). The enzyme was produced by *Penicillium chrysogenum* in the presence of wheat bran and waste grease.
The results showed that the enzyme could be employed for the bioremediation of used cooking oil such as soya, canola, sunflower, and corn oil that contain polyunsaturated oils, which degrade to toxic compounds upon heating.

Wastewater has been treated by a lipase enzymatic preparation, with 0.1% (w/v) of solid enzymatic preparation at 30°C for 24 hr, produced by *Penicillium* sp. during solid state in an anaerobic digester (Rosa et al., 2009). The wastewater that was from the dairy industry contained 1200 mg oil and grease per liter. The oil and grease hydrolysis resulted in a final free acid concentration eight times higher than the initial value. This approach showed the importance of the application of enzymatic preparations obtained by SSF in the treatment of fatty wastewater, with high efficiencies, using anaerobic reactors. In addition, Damasceno et al. (2012) used a lipase produced by SSF with *Penicillium simplicissimum* using babassu cake as substrate. This enzyme, with a concentration of 0.19% (w/v), was combined with a lipid biosurfactant of 114 mg/L, at 33°C, produced from *Pseudomonas aeruginosa* and used for the methane production by anaerobic treatment of a wastewater with a high fat content from a poultry processing plant. These results showed the synergistic effect of these two bio-products on the hydrolysis of fats from the effluent, with the potential to treat a poultry processing effluent rich in oils and greases, without using a flotation step. Thus, this approach allowed for the elimination of the problem of generating solid waste and enhancing the production of methane.

### 4.3.2. Protease

A summary of the applications of the enzyme is presented in Table 6. Alkaline protease produced by SSF processes has been used as an inclusion in detergent formulations, where the suitability of such an enzyme in this application depends on certain factors such as enzyme stability and compatibility with detergent components (Venugopal and Saramma, 2006). In addition, the enzyme should be thermostable and it is preferred to have the ability to act as a detergent component at different temperatures, including room temperature. Another application of this enzyme was in the process of dehairing of goat and cow hides. This enzymatic process of dehairing could lead to the consumption of less water and harmful chemical reagents used in traditional methods. Therefore, the alkaline protease produced through SSF could have potential applications in detergent formulations as well as in the leather processing industry.

Mukherjee et al. (2008) applied the produced alkaline protease by *Bacillus subtilis* through SSF process, as an additive in laundry detergents. The protease showed the ability to function in a broad range of temperatures, that is, high thermal resistance and remained active at room temperature, high stability and compatibility with commercial detergents. It was observed that the enzyme retained 33–90% of its original activity at 37°C in the presence
of commercial detergents. In addition, it was observed that the enzyme obtained was free of any undesirable flavor, which could be advantageous for further applications in food and pharmaceutical industries. Vijayaraghavana and Vincent (2012) evaluated the effect of alkaline protease obtained through SSF, by Halomonas sp., on surfactants, detergents, solvents, and goat hide. The enzyme was remarkably stable on surfactants, such as Tween-20, Triton X-100, and Brij-35 displaying 112%, 202%, and 178% activity, respectively. There was also a high stability observed on various commercial detergents and organic solvents, such as ethanol, acetone, and methanol, with an activity range from 61% to 224% and 49% to 263%, respectively. In addition, the protease effectively dehaired goat hides. This property of the enzyme found as highly significant since most of commercial dehairing proteases are produced by Bacillus bacteria (Subba et al., 2009). Recently, Abraham et al. (2014) have also shown that the protease produced through SSF by the microbial populations developed on the hair solid wastes biodegradation process can be used as a satisfactory alternative for the dehairing of cow hides.

Acid proteases produced by SSF processes have applications in the sector of food science and technology, where recently these enzymes have been used in the field of milk and dairy industry. Merheb-Dini et al. (2010) reported the application of an acid protease, produced from a new and local strain of Thermomucor and using only wheat bran as substrate, in the hydrolysis of bovine casein of milk and the investigation of its peptide profile obtained for a better understanding of the proteolytic activity of the enzyme. Results revealed that the acid protease exhibited high milk-clotting activity and low proteolytic activity. These properties might encourage future experiments by using this microbial enzyme on cheese production where the enzyme could be used as a substitute for animal rennin. The advantages of using such a microbial protease are mainly related to the low-cost production of such an enzyme since in industrial applications the minimization of costs is of a crucial importance. Another application of the protease in the field of dairy industry was investigated by Boyce and Walsh (2012). The enzyme produced by Schizopyllum commune was used to remove an industrial-like milk fouling deposit (containing about 35% minerals) from stainless steel. This experiment imitated the cleaning-in-place operations that use acidic and alkaline solutions in cleaning of various equipment used in the dairy industry, especially heat transfer surfaces used during thermal treatments of milk, where milk deposits are continuously formed. The results of this research work showed that suitable cleaning was achieved using this enzymatic cleaning procedure without the use of environmentally harmful and corrosive chemicals.

4.3.3. CELLULASES AND XYLANASE

Cellulases and xylanase have major and numerous industrial applications, such as in pulp and paper manufacture as well as in the textile industry
for polishing of fabrics and laundry detergents for improving fabric softness (Table 6). For example, Das et al. (2013) used these enzymes, which were produced through optimized processes of SSF, for the deinking of waste pulp of laser printed paper, that is, mainly the removal of chromophores and hydrophobic compounds. In addition, cellulase enzymes are used in the extraction process of fruit and vegetable juices, starch processing and formulations used for animal feeds (Dhillon et al., 2012a, b; Singhania, et al., 2009). Cellulases have found promising applications for non-specific hydrolysis of chitosan to produce chitooligosaccharides with low molecular weight, which showed high antibacterial activity (Xia et al., 2008).

From biotechnological perspectives, the most important and recent application of cellulases and xylanase produced through SSF is in the generation of potentially sustainable energy sources such as sugars and biofuels or, specifically, bio-ethanol. These enzymes are used to hydrolyze cellulosic waste materials to sugars that can be fermented, usually by yeasts, to bioethanol and/or biofuel compounds. It is shown that there is a wide potential to develop a simple biological process to produce ethanol from a variety of lignocellulosic substrates, that is, by hydrolyzing and fermenting carbohydrates, which are considered as waste materials produced in huge amounts especially in the agro-industrial sector. Liu et al. (2011) directly applied the cellulase enzymes, in their crude form, obtained through SSF processes in the hydrolysis of corn stover. The hydrolysates, reducing sugars obtained, were further used as a substrate for the production of ethanol through the fermentation by *Saccharomyces cerevisiae*. The same biofuel was produced through SSF by sequential saccharification of corn fiber where fermentation by the yeast was allowed leading to the production of ethanol (Rasmussen et al., 2010). SSF followed by buffered anaerobic incubation converted a substantial fraction of corn fiber into harvestable reducing sugars, through the action of cellulases and xylanase obtained from the process of fermentation. The sugars released were fermented with or without the yeast to yield bio-ethanol, in the presence of the cellulolytic fungi used for SSF, where the highest yield was obtained in case of utilizing yeast in the process. Several improvements to the production of ethanol were suggested, that is, optimizing the growing conditions such as moisture, pH, temperature, and inoculum used. Similarly, in a study by Sukumaran et al. (2009), it was shown that ethanol can be produced using the saccharification of three different feed stock; rice straw, sugarcane bagasse, and water hyacinth biomass, followed by the yeast fermentation. It was reported that the highest sugar yield and subsequent ethanol production was in the case of using rice straw. Interestingly, crude unprocessed cellulase obtained, which was not high in its yield, was sufficient to produce ethanol from wheat straw in simultaneous saccharification and fermentation by the yeast (Lever et al., 2013). Therefore, the findings of this research could suggest that using SSF of lignocellulosic wastes
TABLE 7. Conditions needed for lab scale vs. large or commercial scale SSF

| Condition                  | Lab scale | Large Scale |
|----------------------------|-----------|-------------|
| pH control                 | Possible through pH adjustment | Not possible |
| T control, Heat Removal    | Easy, possible through controlled temperature water bath | Possible by managing aeration, costly, possible presence of T gradients along the solid matrix in bed reactors. |
| Handling of solid substrates | Very easy | Very difficult |
| Inoculation                | Easy, not expensive | Very high costs, difficult homogenization. |
| Agitation                  | Very easy | Possible in some reactors configurations such as rotatory drums, high energy cost. |
| Aeration                   | Sufficient | Moderate-high, high energy costs |

may be employed instead of commercial enzyme manufacture, which has usually the disadvantage of a production that is associated with high costs.

5. PROCESS SCALE UP AND CONTROL OF SSF

Most research work performed reporting the production of enzymes through SSF use production on a laboratory scale, that is, batch mode in shaken flasks where few grams of substrate is added. There are technological and operational constrains that limit the scaling up of the process of fermentation. These constrains are mainly related to the removal of the excess heat formed and the temperature control during fermentation, and also the agitation of solids and handling techniques required for solid substrates (Singhania et al., 2009). Table 7 summarizes various aspects of lab scale vs. large scale SSF processes. Therefore, large-scale production of enzymes has not yet been proven feasible. However, there are a considerable number of studies focusing on the use of bioreactors in SSF studies at pilot scale for the production of protease and lipase enzymes (Abraham et al., 2014; Edwinoliver et al., 2010; Santis-Navarro et al., 2011). According to a recent review by Thomas et al. (2013), few SSF processes have been developed at an industrial scale: delignification of biomass, dyes bioremediation or *Jatropha* cake detoxification. All these processes have a common objective of enhancing enzymes production, although the enzymes are not targeted as a product, but their effect on the biomass is sought.

In general, there are some basic steps required to scale up the production of enzymes through SSF (Salihu et al., 2012). First, there is a need to choose suitable microorganisms and substrates, which have been reviewed
in the current paper (Sections 2 and 3). Second, it is required to study various process parameters, for example, optimization of moisture, pH, and inoculum used. These were discussed in detail concerning the production of lipase, protease and cellulases (Sections 4.1–4.3). Third, the scale-up process is performed, which depends mainly on operating conditions (aerations, mass, and heat transfer) and process control (Li et al., 2013; Singhania et al., 2009). A last step might be the study of the technical, environmental, and economical viabilities of the process developed.

5.1. Challenges of process scale up

The main aspects of scaling up the production of enzymes through SSF include challenges and recent advances. SSF is difficult to scale up due to the three-phase heterogeneous nature of the substrate and the existing gradients inside the reactor in temperature, pH, moisture, oxygen, and inoculum (Rodriguez et al., 2010, Salihu et al., 2012). In addition, the absence of free water during the fermentation leads to poor heat removal and accessibility of nutrients resulting in slow microbial growth that might lead low or no production of enzymes obtained at the end of the fermentation process. On the other hand, difficult agitation of solid substrates might occur that leads to the heterogeneity of physical and chemical characteristics of the substrates, such as particle size, water retention ability, volumetric heat capacity, and thermal conductivity (Duan and Chen 2012). This inhomogeneity has been proved to substantially affect the fermentation performance. Chen et al. (2011) reported that the variances in the activities of the cellulase enzymes obtained through SSF, in the presence of an inoculum of *T. reesei*, could have been attributed to the heterogeneity of the corn stover that was used as substrate. Moreover, the heat generated due to the metabolic activities of microorganisms is in most cases an inconvenience for biotechnological processes especially when the optimum growth of microorganisms is affected and a large part of the enzymes produced during SSF can be heat-denatured. Another important challenge of scale-up processes is the control of pH within the system during the fermentation, as this control is required to manage the growth of microorganisms and the subsequent production of enzymes. Therefore, the control of heat transfer is one of the major crucial issues in the design and operation of large-scale SSF fermenters. There is also a need to firstly, monitor on-line the parameters throughout the process, such as temperature and pH (Ali and Zulkali, 2011). Most importantly, oxygen consumption and the carbon dioxide evolution are important measurements because they represent the best way of monitoring the growth of microorganisms inside the reactor. As a more sophisticated and no-invasive proposal, Jiang et al. (2012) successfully monitored physical and chemical changes at a 100-l pilot bioreactor using FT-NIR spectroscopy coupled with vector data description, thus avoiding chemical analysis. Second, it is needed to adequately mix the
substrates within the fermenter without negatively affecting the growth of microorganisms as well as the substrates used.

5.2. Recent Advances on Process Scale Up

There are a number of bioreactors that have been designed to overcome the problems of scale up. Commonly used SSF bioreactors are classified into four types based on the pattern of aeration and/or agitation system employed (Mitchel et al., 2006). These types are tray, packed-bed, rotating and stirred-drum and forcefully aerated agitated reactors. Each of these types has its own advantages and disadvantages, which promoted the necessity to develop novel bioreactors with better design in order to solve major problems related to the scale-up processes for the production of enzymes through SSF. There are few recent studies on the overcoming of some of the disadvantages and limitations regarding the scaling-up processes. An extensive analysis on the design and operation of bioreactors in SSF has been published in the recent review by Thomas et al., (2013). Also, Yoon et al. (2014) described in detail the use of bioreactors for cellulase production.

Scaling up of the process of SSF for the production of enzymes from waste material was shown to be successful. Edwinoliver et al. (2010) scaled up the process of lipase production from 10 g up to a level of 3 kg, using *A. niger* as inoculum. The strategy of scaling-up included the transfer of the optimized process conditions developed at laboratory level to pilot production level, where temperature and moisture were online monitored and controlled during the process of fermentation. Another strategy for the scaling-up was mainly depending on control of air flow intensity as a key factor during the production of phytase (Rodriguez-Fernandez et al., 2012). At a 10-fold scale up, from 2 to 20 kg drum bioreactors with a paddle agitation, the control of the air flow intensity was required to maintain the temperature constant during the fermentation, as well as to cool the fermenter at late stages or to allow for the removal of metabolic heat generated. In addition, the air flow was able to provide oxygen that is considered as a crucial factor for the growth of microorganisms.

A new technology of SSF called gas double-dynamic solid-state fermentation (GDD-SSF), which has been proven available on industrial scales for the production of biopesticides, has been tested by He and Chen (2013) for the production of enzymes. A previously invented pilot scale bioreactor of 800 l (Chen and Li, 2007) was used for the production of pectinase, amylase, protease and cellulase using inocula of strains of *Aspergillus* and *Penicillium* and substrates of mainly wheat bran and soy waste. The bioreactor was regulated by a dynamic system of air pulsation for the control of the air pressure during the process of fermentation. The effect of the air pressure and also the liquid–solid ratio of the substrates on enzyme production in different fermentation modes were investigated. Results showed that GDD-SSF offered great
advantages compared to the static or traditional SSF. GDD-SSF led to a three-fold increase in the enzyme activities obtained. Furthermore, a decrease in the fermentation period and temperature gradient were observed.

Different approaches for an easily scalable process have been reported by Santis-Navarro et al., (2011) and Abraham et al. (2014). In these approaches, the temperature was not controlled and enzymes were produced in a batch fermentation process similar to the composting. The temperature rose to thermophilic values due to heat released and decreased to ambient values during the fermentation of the readily biodegradable matter, which has been consumed.

Recently, in other SSF applications, there were also studies on the scaling up of SSF process for the production of bioethanol. Lin et al. (2013) demonstrated that a rotary drum reactor can be directly scaled up to a larger capacity up to 100 l, by using SSF optimized operating conditions obtained at laboratory levels, using flask batch modes and the thermotolerant yeast *Kluyveromyces marxianus* as an inoculum for the fermentation. The results showed that the scale-up process is feasible and has commercial potential, especially when the substrate used, which was sugarcane bagasse, was pretreated with alkali prior to the process of fermentation. The alkali pretreatment of this substrate allows for a direct carbon source for the growth of microorganisms in the SSF system (Chandel et al., 2012). Interestingly, Li et al. (2013) reported an advanced SSF technology, which is capable of overcoming most problems associated with the scale-up and large-scale fermentation processes. An efficient system for the control of mass and heat was connected to a continuous solid-state rotary drum fermentation reactor, developed by the research group. A newly developed microbial strain of *Saccharomyces cerevisiae* was used that allowed for the shortening of the time of fermentation of a substrate of sweet sorghum stems.

6. FINAL REMARKS

In summary, this review provides an update on recent studies that are dealing with the use of SSF for the production of enzymes, and it especially covers issues related to wastes, microorganisms, and scale-up and control of the process of fermentation. The main focus was on the production of lipases, proteases, cellulases, xylanases, glucoamylases, pectinases, and inulinases. For the process of fermentation, the inocula used were mostly fungi, like various species of *Aspergillus*, and substrates were waste materials obtained from the sector of agriculture and food industry. The use or recycle of these wastes, which are very cheap and highly available in big amounts, shows a high benefit of SSF from the economical and environmental perspectives.

Accordingly, SSF presents a substantial advantage over SmF, which has been extensively used for the production of commercially available enzymes.
since many decades. Nevertheless, the majority of research conducted on SSF was on lab-scale, whereas the large scale/commercial production of enzymes is still not developed because of constraints related to the scaling up of the process. For instance, the absence of free water during the process leads to poor mixing and heat removal that results in slow microbial growth and a subsequent low or no production of enzymes. Recent developments are based on transfer of the optimized process conditions developed at laboratory level to larger scales, control of heat and mass transfer, and on-line monitoring of the parameters, such as temperature and pH, where air flow has been shown to be crucial for the maintenance of constant temperatures and microbial growth.

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