REVIEW

Utilization of Agro-Residual Ligno-Cellulosic Substances by Using Solid State Fermentation: A Review

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Summary

Agro-residual wastes contain many usable substrates of high value such as carbohydrates and fibers. Direct disposal of these wastes as burning or landfill will cause serious environmental problems. Thus, designing new methods for exploitation and treatment of these wastes to produce useful products with great economic advantages are substantial. Solid state fermentation (SSF) has become an attractive method in recent years due to its features of using these wastes directly as raw materials or substrates. Thus, SSF is considered as an environmentally-friendly technique for waste treatment. This paper reviews the exploitation of the agro-residual wastes through SSF technology.

Keywords: Agro-residues, Food industry residues, Solid-state fermentation, Environmental–friendly system, Biological active secondary metabolites

1. Introduction

The food, agricultural and forestry industries produce large volumes of wastes annually worldwide, which are causing serious disposal problems. These wastes, which are rich in sugars due to their organic nature, are easily consumed by the micro-organism that consequently makes these wastes very appropriate substances for exploitation as a raw material to produce high value components. During the last two decades many changes have been made in environmental legislation, which has forced the industries to find alternative uses for their residual waste, especially the agro-industrial where they produce thousands of tons of by-products such as sugar cane bagasse, citrus bagasse, fruit peels, corn cobs and cheese whey (Raghavarao et al. 2003 and Graminha et al. 2008).

Solid state fermentation (SSF) is an ancient technique, which was used in Asia to produce some kinds of fermented foods such as koji, cheese, tempeh, Chinese wine, vinegar, and soya sauce (Pandey et al, 2002). Table 1 gives a brief account of the historical developments in SSF (Pandey, 1992). In this technique, the growth and metabolism of microorganisms on moist solids in the absence or near absence of free water, however, the substrate must contain sufficient moisture to support the growth and metabolism of the microorganisms. More attention has been given to the ancient technique during the last two decades according to its ability and success in several fermentation processes, including solid waste management (Shaban, 1999), biomass energy conservation (Kiransree et al. 1999) and in the production of secondary metabolites (Krishna, 1999).

The scope of this paper is to review the utilization of agro-residual and agro-industrial waste for the production of several various metabolic products of great interest to the food industry using the SSF technique.

Table 1. *History and development of solid-state fermentation (Pandey, 1992)*

Period	Development Process
2600 b.c.	Bread making by Egyptians
In Asia (1000 b.c.)	Cheese making by *Penicillium roqueforti*
2500 b.c.	Fish fermentation/preservation with sugar, starch, salts, etc., koji process
7th century	Koji process from China to Japan by Buddhist priests. Vinegar from pomace
18th century	Gallic acid used in tanning, printing, etc.
1860–1900	Sewage treatment, waste-water treatment
1900–1920	Fungal enzymes (mainly amylases), kojic acid
1920–1940	Fungal enzymes, gluconic acid, rotary drum fermenter, citric acid
1940–1950	Fantastic development in fermentation industry. Penicillin production by SSF and submerged Fermentation
1950–1960	Steroid transformation by fungal cultures
1960–1980	Production of mycotoxins, protein enriched feed
1980–present	Various other products like alcohol, gibberellic Acid

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2. Agro-residual and Agro-industrial waste survey

The solid biological waste that comes from agricultural processes and the food industries causes a serious environmental problem due to the industries' way of dealing with and treatment of these wastes, either by burning or leaving it in the field to decay naturally or by burying it under the ground. A noticeable increase has been seen during the last two decades in the quantity of the agro-wastes due to the increase in agricultural activities and the activities of the food industry. Table 2 shows a list of waste quantities that are produced in some countries of the world (Laufenberg et al, 2003). Therefore, investigation and development for useful processes and techniques to dispose of these wastes and upgrade their value is highly attractive.

The major advantages of solid-state fermentation over submerged fermentation systems are (Reddy et al, 1999 and Kim et al, 2003a):

1. The small volume of fermentation mash or reactor volume, resulting in lower overall operating costs.
2. A lower chance of bacterial contamination due to low moisture levels.
3. Easy product separation.
4. Economic use of energy.
5. The product yield is, for the most part, higher.
6. Oxygen is typically, freely available at the surface of the particles.

Pandey (2003), Lonsane et al. (1990), and Sun et al. (2009), described several other features of (SSF) that are advantages over submerged fermentation, and they are:

Table 2. *The amount of waste quantities that produced in different countries (Lanufenberg, 2003)*

Country	Quantity and waste type
Germany, 1997	380 000 t/a organic waste only from potato, vegetable and fruit processing. 1 954 000 t/a spent malt and hops (breweries), 1 800 000 t/a grape pomace (viniculture). 3 000 000 t/a crude fibre residues (sugar production)
Belgium, 1992	105 000 t/a biowaste (vegetable, garden and fruit waste) 280 000 t/a estimations due to legislation of separate household collection
Thailand, 1993	Palm oil production: 386 930 t/a empty fruit bunches, 165 830 t/a palm press fibre, 110 550 t/a palm kernel shells. 1 000 000 t/a cassava pulp
Spain, 1997	>250 000 t/a olive pomace
Portugal, 1994	14 000 t/a tomato pomace
Jordan, 1999	36 000 t/a olive pomace
Malaysia, 1996	Palm oil production: 2 520 000 t/a palm mesocarp fibre, 1 440 000 t/a oil palm shells, 4 140 000 t/a empty fruit bunches
Australia, 1995	400 000 t/a pineapple peel
USA	300 000 t/a grape pomace in California only. 9525 t/a cranberry pomace. 200 000 t/a almond shells. 3 300 000 t/a orange peel in Florida
Sarawak (Malaysia), 2006	50–110 t/d sago hampas
Greece, 2007	35 000 t/a (dry weight) of citrus peels

3. Bioengineering of SSF

SSF is defined as fermentation of a solid substrate at low moisture levels or water activities. The water content of a typical submerged fermentation is more than 95%, the water content of a solid mash in SSF often varies from between 40% and 80% (Shuler, 2002). The process was well-known from ancient times in the Middle East, but it has been ignored in Western countries due to the development of submerged fermentation (SmF) in becoming a model and acceptable technology for the production of many items that are produced by fermentation as a result of penicillin production using SmF. Recently, SSF has gained renewed attention, and interest has shown SSF to be particularly suitable for the production of many compounds with little cost and higher yields than SmF.

1. No waste production in the case of enzyme koji fermentation.
2. A longer production phase in amyloglucosidase production.
3. An absence of co-produced carbohydrates.
4. Use of waste or spent low value raw materials to produce high value products.
5. No foam generation.
6. Reduces the need for rigorous control of many parameters during fermentation.

There are several important aspects, which should be considered in general, for the development of any bioprocess in SSF. These include selection of suitable microorganisms and substrates, and optimization of process parameters (Couto, 2008b).

The definition of SSF is based on water activity; only fungi and yeast were considered as suitable microorganisms for SSF, based on the fact that the culture conditions of SSF are closer to the natural habitat of these microorganisms, so it will lead to an increase in their activity (Kun, 2006).

The selection of a proper substrate is essential in SSF, where the substrate will act as a physical support and nutrient source for the micro-organism. There are two major considerations for the selection of substrate; the first one about the specific substrate that requires suitable value-addition and/or disposal. The second could be related to the goal of producing a specific product from a suitable substrate. It is necessary to screen various substrates for microbial growth and product formation (Soccol, 2003).

Many process parameters, physicochemical and biochemical, that affect the fermentation process should be selected and optimized. These parameters vary depending upon the kind, level and application of experimentation. These parameters are: particle size, initial moisture, pH and pre-treatment of the substrate, relative humidity, temperature of incubation, agitation and aeration, age and size of the inoculum, supplementation of nutrients such as sources of sodium, phosphates and trace elements, supplementation of additional carbon source and inducers (Pandey, 1991).

Various bioreactor types have been used in SSF processes depending on how they mixed and aerated the fermentation culture, such as packed-beds, rotating drums, gas-solid fluidized-beds, rocking drums and other stirred bioreactors; however, the available information does not indicate an ideal bioreactor for solid-state processes. The commercial application of SSF is limited due to the scant knowledge on the design and operation of large scale bioreactors. It is difficult to scale up some bioreactors due to the difficulties in temperature and pH control processes (Mitchell et al. 2006; and Scheper, 2000).

4. Applications of SSF to the industry

The researchers and industries focus on the opportunity to use the agro-residual and agro-industrial waste as suitable substrates for SSF due to the potential advantages for some micro-organism, especially filamentous fungi that are capable of penetrating into the hardest of these solid substrates and also the higher yield that could be achieved when compared with submerged fermentation (Pandey et al. 2000). Many kinds of wastes have been used in SSF due to its efficient utilization and to value addition of these wastes, which can be classified according to basic content:

• Ligno-cellulosic: Wheat straw, Wheat bran, Sugar cane bagasse, Sugar beet pulp, Coffee pulp, Coffee husk, corn meal, rice flour, barley

• Starchy: Rice bran, Cassava bagasse, Buckwheat seeds, Soybean, Banana wastes

• Others: Citrus peel, Apple pomace, Kiwifruit peel, Amaranth grain, kumara, spent brewing grains, coconut oil cake, carob pod, oil palm, banana skin

Various secondary metabolic products can be produced by using the above classified wastes, which can be grouped into the following categories: enzymes, organic acids, antibiotics, bio-pesticides, biofuels, and aroma compounds.

4.1 Organic acids

Organic acids represent the third largest category among the biological products. Citric acid, acetic acid, lactic acid, tartaric acid, malic acid, gluconic acid, propionic acid and fumaric acid are some of the organic acids that are produced commercially either by chemical synthesis or fermentation. The production by SSF is related with the development of SSF. Organic acids have been utilized in many industrial applications, most of all in food industries as food additives and preservatives (Pandey, 2008). Recently, researchers have focused on efficient utilization and value addition of agro-industrial wastes to reduce the environmental problems that are caused by these wastes; also these wastes are considered as suitable substrates for the SSF due to their cellulosic and starchy nature. Different kinds of agro-industrial wastes that are used in SSF to produce different kinds of organic acids are such as cassava bagasse, coffee husk, apple pomace, wheat brain, sugarcane press-mud as tabulated in Table 3 (Singhania, 2009).

Citric acid is a commercially valuable microbial product that is produced in tonnage and is widely used in food, pharmaceutical and beverage industries as an acidifying and flavor-enhancing agent. It is produced commercially by submerged fermentation of sucrose- or molasses-based media using *Aspergillus niger*. Recently, researchers used alternative carbon sources through solid state fermentation techniques, such as agricultural residue and agro-industrial wastes that are considered as suitable substrates for the SSF due to their cellulosic and starchy nature. Leangon et al. (2000) have shown through their work the effectiveness of using the fungi *A. niger* as a microorganism for producing citric acid in solid state fermentation. Adham (2002) has compared surface and submerged fermentations for citric acid production from beet-molasses by *A. niger*. The results showed that surface fermentation was more efficient than submerged, where the concentration of citric acid after 12 days of incubation was 72.9 g/L and 5.3 g/L for surface and submerged, respectively. Pallares et al. (1996) compared the fermentation in solid state and submerged systems. They used glucose as a raw material and A. niger. The results showed that the concentration of citric acid that was produced in SSF was more than in SmF (21.24g/L and 20.58 g/L) and the productivity was 3.54 and 1.47 g/L.d, respectively. The SSF reduced the fermentation time from 14 to 6 days. A probable explanation for the behavior was that conditions in SSF were more related to the natural environmental conditions. Kumar et al. (2003b) used sugarcane bagasse with sucrose and molasses. It was observed from the results that the amount of citric acid produced, and compared between sucrose and molasses, was 202 and 198 g/kg dry solid, respectively. They tested different kinds of agricultural wastes (pineapple, mixed fruit and maosmi) as the sugar source (Kumar et al. 2003a). The results showed different citric acid yields were obtained (54.2, 46.5 and 50 g/kg sugar consumed), respectively. Vandenberghe et al. (2000) used three kinds of agro-industrial residue, sugar cane bagasse, coffee husk and cassava bagasse in SSF with *A. niger*. The cassava bagasse has shown the best support during the mould growth and gave the highest yield of citric acid among the tested substrates, where the maximum yield obtained was 88g/kg for dry cassava bagasse. The difference in the amount of citric acid produced was related to the amount of carbohydrates in the used substrate. Bari et al. (2009) studied the production of citric acid from oil palm empty fruit bunches (EFB) as a substrate in SSF and *A. niger*. The maximum yield that was achieved with an initial sucrose concentration of 6.4% w/w was 337.94g/Kg dry EFB. This rate of production was 3 times higher than the theoretical yield according to the initial sucrose used, which showed that the fungi seemed to convert some of the cellulose in the oil palm empty fruit bunches to sugar and using it to produce citric acid. Jamal et al. (2007) and Alam et al. (2008) have used the residue from the oil palm industry for production of citric acid but in a liquid phase. The maximum citric acid concentration was 5.24 g/L after optimizing the fermentation conditions. Kurbanoglu et al. (2004) used ram horn peptones in submerged fermentation. They showed that the pre-treatment of ram horns with H_2SO_4 seemed to increase the concentration of citric acid from 62 g/L to 84 g/L after 6 days of fermentation. Imandi et al. (2008) used pineapple waste as a sole substrate and using *Yarrowia lipolytica* to produce citric acid. A maximum yield of 202.35 g citric acid/kg dry substrate was obtained after optimizing the composition of the media culture. Barrington et al. (2008) and Kim et al. (2006b) used peat moss as an inert support material for *A. niger* to grow and produce citric acid. The use of experimental design seemed to increase the yield about 2.7fold compared with that obtained from the control experiment before optimization, where the maximum yield achieved after 6 days of fermentation was 82 g/kg dry peat moss. Tran et al. (1998) compared citric production from pineapple waste in different bioreactors; flask, tray and drum bioreactors. They reported the best production in flasks (140 g/kg dry pineapple waste) and lower in rotating drum bioreactors (80 g/kg dry pineapple waste). Shojaosadati et al. (2002) used apple pomace as a substrate in a multi-layer packed bed. They reported that by using optimized conditions, 124 g of citric acid was produced from 1kg dry apple pomace. Jianlong et al. (2000) improved the traditional packed-bed bioreactor by adding ion-exchange resin adsorption when they used beet molasses as a substrate. The experimental results indicated that, as compared with a conventional batch, the packed-bed techniques increased the citric acid productivity from 0.338 g/l h to 0.543 g/l h. Rozas et al. (1996) compared three different mechanisms of heat removal (conductive, convective and evaporative) from packed-bed bioreactors in SSF for citric acid production with an inert support. The results showed that the conductive heat transfer was the least efficient mechanism (8.65%) when compared with convective (26.65%) and evaporative (64.7%).

Lactic acid has an important position in the family of carboxylic acids because of its applications in both food and non-food industries. It is used as a preservative and acidulant in foods. The lactic acid production using SSF has been carried out using fungal as well as bacterial strains. Application of agro-industrial wastes in bioprocesses provides an alternative way to replace the refined and costly raw materials; in addition, the bulk use of such materials helps to solve many environmental hazards. John et al. (2009b) reviewed recent developments in lactic acid fermentation by presenting a brief summary on fermentation techniques (focus on simultaneous saccharification), microorganisms and the benefits from utilization of agro-industrial byproducts and crop residue as a substrate. Naveena et al. (2005) studied the production of lactic acid in SSF by *Lactobacillus amylophilus* using wheat bran as a support-substrate. They obtained a high yield of lactic acid by optimizing the parameters that were affecting the fermentation process, which increased the yield to 100% and rose to 360 g/kg dry wheat bran. John et al. (2006a) used two kinds of agro-industrial waste; cassava bagasse and sugar cane bagasse as a substrate for lactic acid fermentation by *Lactobacillus delbrueckii*. A maximum of 249 mg/gds lactic acid was obtained after 5 days of fermentation under the optimized conditions with a conversion efficiency of about 99% of the initial reducing sugars. A strain of *Rhizopus oryzae* was used by Soccol et al. (1994) to evaluate lactic acid production in SmF and SSF. For SSF, an inert solid support (sugarcane bagasse) was used. Both production level and productivity were higher in SSF. Lactic acid production was of 93.8 and 137.0 g/L in SmF and SSF, respectively. The productivity was 1.38 g/L.h in a liquid medium and 1.43 g/L.h in a solid substrate. However, the fermentation yield was about 77%, regardless of the fermentation technique. Richter et al. (1994) also compared SSF and SmF for lactic acid production using a bacterial strain of *Lactobacillus paracasei*. Lactate concentrations and yields were 88–106 g/l and 91–95% for SmF, and 90 g/kg and 91–95% for SSF, respectively.

Oxalic acid is an organic acid produced by white and brown rot fungi. Its compounds have widespread industrial applications in several fields such as oil refining, catalysts, pharmaceuticals, dyes, explosives, straw bleaching, and printing (Guru et al. 2001). Hakala et al. (2005) used white-rot fungus *Physisporinus rivulosus* to produce oxalic acid when it was grown on spruce wood chips through solid state fermentation. During solid-state cultivation, the fungus produced oxalic acid (28μmol/g dry wood). Oxalic acid has a significant role in the degradation of cellulosic compounds; Lee et al. (2009 and 2010) used it in the pretreatment of corncobs when it was used as a substrate in their research for ethanol production. Guru et al. (2001) produced oxalic acid from sugar beet molasses chemically by forming nitrogen oxides using three reactors connected in series. The maximum yield obtained by the developed method was 78.9% of the theoretical yield. Andre et al. (2010) used the glycerol waste produced from a bio-diesel process as the sole carbon source. They used two fungi strains; *Lentinula edodes* and *A. niger* in liquid phase fermentation. *A. niger* showed significant production of oxalic acid in nitrogen limited culture that reached 21.5 g/L.

4.2 Enzymes

Enzyme production is a growing field of biotechnology with an annual world sales of billions of dollars (Godinez et al. 2003). The obvious increase is due to its applications in pharmaceuticals, food, the paper industry and in animal feeding as feed ingredients, when it serves as partial degradation for agro-industrial residue (Couto et al. 2005a). SSF shows large potential for enzyme production more than SmF, and it is particularly suitable for lingo-cellulosic enzymes production from various agro-biotechnological wastes; also, the cost of production in SmF is high and it is uneconomical to use many enzymes in several processes (Sekhar et al. 2005).

Table 3. *Enzymes that are produced from agro-residual waste by SSF and their applications*

Enzymes	Application in industry	Microorganism	Substrate [wastes]	Ref
Xylanase	Increased digestibility of silage Pulp and paper Food and animal feed industries	*Aspergillus awamori* *Aspergillus terreus* *Thermoascus auriantacus* *Thermomyces lanuginosus*	Grape pomace Sugarcane bagassse Sugar cane bagasse Shorgum straw	Diaz et al, 2007 and Botella et al, 2007 Gawande et al, 1999 Milagres et al, 2004 Sonia et al, 2005
Lignocellulosic	Degradation of cellulosic components	*Fomes sclerodermeus* *Fusarium oxysporum*	Soy and wheat bran Orange peels	Papinutti et al, 2007 Mamma et al. 2007
Polygalacturonase	Beverage industry Processing of fruits and vegetables Extraction of olive oil Fermentation of tea and coffee	*Aspergillus sojae*	Crushed maize	Ustok et al, 2007
α-Amylase	Food industry such as baking, brewing Preparation of digestive aids Production of chocolate cakes, moist cakes, fruit juices, starch syrups	*Aspergillus oryzae* *Aspergillus oryzae* *Bacillus subtilis* *Aspergillus oryzae* *Bacillus sp.*	Wheat-flour Spent brewing grains Wheat bran Coconut oil cake Wheat bran, rice bran	Rahardjo et al, 2005 Francis et al, 2003 Baysal et al, 2003 Ramachandran et al, 2004 Sodhi et al, 2005
Protease	Food industry, Beverage Detergents Tanning	*Penicillum sp.* *Aspergillus parasiticus* *Bacillus sp.* *Bacillus sp.* *Pseudomonas aeruginosa*	Soybean defatted cake Wheat bran Wheat bran, lentil husk Green + red gram husk Jatropha curcas seed	Germano et al, 2003 Tunga et al, 2003 Uyar et al, 2004 Parkasham et al, 2006 Mahanta et al, 2008

Many investigations have been carried out to evaluate the efficiency of enzyme processes. Biocon India Limited (Bangalore, India), in comparing minor metabolite profiles of similar enzymes produced by SSF and by SmF, came to the conclusion that commercially available solid-state produced enzymes were richer in side activities compared to commercially available SmF enzymes, when normalized to the same primary activity (Couto et al. 2008a). Patil et al. (2006) reported the production of pectinase from deseeded sunflower heads using *A. niger*. The solid state fermentation gave superior results compared to submerged conditions for pectinase production; under the optimum conditions SSF showed results 2.7fold higher than SmF.

Recently, many enzymes have been produced using SSF technique from different agro-industrial waste as shown in Table 3. It lists some of the enzymes that are produced and the agro-waste used as a substrate.

4.3 Antibiotics

Antibiotics can be defined as low molecular-weight natural organic products (secondary metabolites) produced by microorganisms (primarily bacteria and fungi) that are active against other microorganisms at low concentrations. It is produced usually in a late phase in the growth cycle of the microorganism. Many studies have been carried out on the production of various antibiotics in SSF. These include penicillin, cephalosprin, tetracyclines, chlorotetracyclines, oxytetracyclines, iturin, surfcatin, actinorhodin, methylenomycin and monorden. These components have been found to have many applications and uses in different industrial fields, mainly: pharmaceutical, cosmetics, food and agriculture (Pandey et al. 2008).

Antibiotics are usually produced using SmF. Recently; requirements for huge amounts with minimum costs are needed for the continual fight against bacterial diseases. The potential development of SSF has been used in production of these secondary metabolic components due to several production advances like higher product yield, often shorter time, higher product stability, lower energy requirements and the use of agro-industrial waste as low cost carbohydrate sources when they are used as a substrate.

Table 4 is a tabulation of some studies on the application of agro-industrial residues as a substrate for antibiotic production.

Table 4. *Antibiotics that are produced by SSF*

Antibiotic	Microorganism	Substrate [wastes]	Ref
Cephalosporin	*Acremonium chrysogenum*	wheat bran, wheat rawa, bombay rawa, barley rice bran	Adinarayana et al 2003a
Tetracycline	*Streptomyces viridifaciens* *Streptomyces strains* *Strepfomyces rimosus*	Sweet potato residue Corncob, cassava peels Corncob	Balakrishman et al, 1996 Asagbra et al, 2005 Yang 1996
Penicillin	*Penicillium chrysogenum*	Sugarcane bagassea	Gonzalez et al, 1993
Iturin A	*Bacillus subtilis*	Soya bean curd residue	Mizumoto et al, 2006
Neomycin	*Streptomyces marinensis* *Streptomyces marinensis*	Wheat bran, rice bran Wheat rawa	Ellaiah et al, 2004 Adinarayana et al, 2003b
Surfactin	*Bacillus subtilis*	Okara	Nakayama et al, 1997

5. Conclusion

The current improvements in the food industry have led to the production of a large amount of agro-residual wastes, which causes serious environmental problems. The development of environmentally-friendly methods for treatment and dealing with the agro-industrial residues are needed. SSF offers several advantages for using and consuming these residues as a substrate to produce various value-added products. There has been much development in the application of SSF in various areas such as organic acids, enzymes, antibiotics, bio-pesticides, biofuels and aromatic compounds.

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