American Journal of Food Technology 2 (1): 1-11, 2007
ISSN 1557-4571

Production of Thermostable α-amylases by Solid State Fermentation-A Review

Rahul Regulapati, Prem N. Malav and Sathyanarayana N. Gummadi
Department of Biotechnology, Indian Institute of Technology-Madras,
Chennai 600 036, India

Abstract: α-amylases are digestive enzymes which hydrolyze α-1,4-glycosidic bonds in starch. α-amylases have a lot of potential applications in food processing industries such as sugar, baking, brewing and preparation of digestive aids. Thermostable α-amylases have received a lot of importance because of their usage under harsh industrial conditions. α-amylases can be produced by microorganisms using submerged and solid state fermentation. However, solid state fermentation is attractive because of usage of cheap substrate, high yields and avoids substrate inhibition. This study reviews the microbial sources, fermentation characteristics and different substrates used for the production of thermostable α-amylases. In addition, the downstream processing of thermostable amylases and its properties is discussed in this study.

Key words: α-amylases, thermostable, solid state fermentation, starch hydrolysis

Introduction

α-amylases (EC 3.2.1.1, endo-1,4-α-D-glucan glucohydrolase) are endo-acting enzymes which randomly hydrolyze α-1,4-glycosidic bonds between adjacent glucose units in a starch polymer leading to the formation of linear and branched oligosaccharides. They are usually used in synergy with glucoamylases or β-amylases or pullunases for the complete hydrolysis of the starch (Poonam and Dalel, 1995). These starch hydrolytic enzymes comprise 30% of the world's enzyme consumption (Van der Maarel *et al*., 2002). Thus, α-amylases are extensively used in sugar, textile, alcohol and paper industries (Fogarty and Kelly, 1983; Emanilova and Toda, 1984; Plant *et al*., 1987; Zamost *et al*., 1991; Mamo *et al*., 1999; Pandey *et al*., 2000a; Bruins *et al*., 2001; Hakri and Rakshit, 2003; Gupta *et al*., 2003). Further, they are employed in processed food industry such as baking, brewing, preparation of digestive aids, production of cakes, fruit juices and starch syrups (Cornelis, 1987; James and Simpson, 1996; Rosell *et al*., 2001; Gupta *et al*., 2003; Coutoa and Sanromán, 2006). In addition, they are used in sewage treatment for reducing the disposable solid content of sludge (Karam and Nicell, 1999; Parmar *et al*., 2001) and as detergents (Kumar *et al*., 1998).

In sugar processing, the first step includes gelatinization of the starch slurry which is achieved by heating starch with water at temperatures around 100°C, due to insolubility of starch at lower temperatures (Rakshit, 1998). This step involves dissolution of starch granules thereby forming a viscous suspension. Because of this high viscosity it poses serious problems with mixing and pumping (Muller, 2000; Soni *et al*., 2003, 2005; Kunamneni and Singh, 2005). To overcome these viscosity-associated problems, gelatinization is coupled with liquefaction which involves partial hydrolysis and loss in viscosity. This action is brought about by thermostable α-amylases (Weber *et al*., 1990; Crabb and Mitchinson, 1997), which can act at temperatures around 70-100°C depending on the temperature profile of the α-amylase (Soni *et al*., 2003). Further steps in sugar processing include saccharification,

Corresponding Author: Sathyanarayana N. Gummadi, Department of Biotechnology, Indian Institute of Technology-Madras, Chennai 600 036, India Tel/Fax: 91-44-2257-4102

involving the production of glucose and maltose via further hydrolysis. As a result, thermostable α-amylases are gaining wide industrial and biotechnological interest because their enzymes are better suited for harsh industrial processes and in addition, their application reduces contamination risk and reaction time (Leuschner and Antranikan, 1995; Fredrich and Antrakian, 1996; Diane *et al*., 1997; Zeikus *et al*., 1998).

Both fungal and bacterial systems are widely used in the production of α-amylases under submerged fermentation (SmF). However, bacterial systems are preferred because of the several characteristic advantages it offers (Pandey *et al*., 2000a). With the advent of Solid State Fermentation (SSF), production of industrial enzymes received lot of importance. Though, the usage of bacteria is very limited in SSF, production of thermostable α-amylases using bacteria has received lot of importance. The reasons are (i) *Bacillus* sp. are most widely used bacterial strain for α-amylase production, (ii) *Bacillus* sp. is the most studied bacterial strain in SSF because of its survival at low moisture content and (iii) isolating thermostable microorganisms is easier in bacteria. Most research on thermostable α-amylases systems has so far concentrated only on thermophiles and extreme thermophiles. There exists considerable potential for research on enzyme production by facultative thermophiles, which can grow at a range of temperatures. In this review, we deal with the various aspects of SSF when applied to the production of thermostable α-amylases. We review the various microorganisms used successfully for the production of thermostable α-amylases by SSF and its recovery and properties.

Why SSF is Preferred for Production of Thermostable α-Amylases?

Solid state fermentation is of special importance where the crude fermented product can be directly used as the enzyme source (Tengerdy, 1998), as is the case for thermostable α-amylases. In addition, it has been well established through comparative studies that higher yields of the product are obtained in SSF when compared to SmF (Hesseltine, 1972; Aidoo *et al*., 1981; Steinkraus, 1984). Thermostable α-amylases have been produced under both submerged (Okalo *et al*., 1996; Malhotra *et al*., 2000; Dey *et al*., 2001; Stamford *et al*., 2001; Tsurikova *et al*., 2002) and solid state fermentations (Ramesh and Lonsane, 1987a,b; Ramesh and Lonsane, 1991; Babu and Satyanarayana, 1995; Soni *et al*., 2003, 2005; Kunamneni and Singh, 2005). Production of thermostable α-amylases via SmF systems is known to cause potential problems (Ramesh and Lonsane, 1987a): presence of the product in low concentration, handling and reduction and disposal of large volume of water during down-stream processing. These unit operations are also cost intensive and are poorly understood (Datar, 1986). Usage of SSF systems effectively overcomes these problems as the yield is several times higher and is cost-effective (Arima, 1964; Ghildyal *et al*., 1985). Moreover, the product is recovered from fermented products in lower volume of solvent (Ramakrishna *et al*., 1982) and thereby achieving a much simpler and cheaper downstream process.

Further, production of thermostable α-amylases by SSF processes significantly overcomes catabolite repression than SmF systems (Emanuilova and Toda, 1984; Ramesh, 1989a; Ramesh and Lonsane, 1991). This ability of the SSF system aids the overall economy of the process as it eliminates the need to use low substrate concentrations and costly operation strategies. During one such comparative study, Ramesh and Lonsane (1991) reported values of 19,550 U mL^{-1} of enzyme extract in a SSF system even when the medium contained 15% glucose. In contrast, the production was negligible and completely inhibited in the submerged system even when the concentration of glucose was as little as 1%. Solid state fermentation for the production of bacterial thermostable α-amylases have been hailed as potential tools for achieving economy in enzyme production and starch hydrolysis (Ramesh and Lonsane, 1990a). In addition, the equipment for SSF is simpler, uses cheap naturally available raw materials as substrates, but yielding higher volumetric productivities and

higher concentration of products (Hesseltine, 1972; Aidoo *et al*., 1981; Pandey, 1991, 1992; Nigam and Singh, 1994). Since the starch processing industry requires a cost-effective method for production of thermostable α-amylases, SSF processes are gaining prominence as they are capable of economically producing higher titres of α-amylases (Pandey *et al*., 2000b, 2001b).

Production of Thermostable α-Amylases

The production of thermostable α-amylases in SSF systems has so far been limited chiefly to the *Bacillus* genus (Ramesh and Lonsane, 1990a): *B. amyloliquefaciens*, *B. gavealeus*, *B. mesentericus*, *B. myocides*, *B. polymyxa*, *B. vulgatus*; *B. aterrimus* (Beckford *et al*., 1945); *B. subtilis* (Beckford *et al*., 1945; Baysal *et al*., 2003); *B. coagulans* (Babu and Satyanarayana, 1995); *B. licheniformis* (Ramesh and Lonsane, 1990b); *B. megaterium* (Ramesh and Lonsane, 1987a). Production levels were observed to be very low when *Streptomyces* strains were used under SSF (Dey and Agarwal, 1999). Among fungal microorganisms, a strain of *Rhizopus* has shown ability to produce thermostable α-amylases in SSF systems, although it has not been exploited as yet (de Souza *et al*., 1996). It has been reported that 500 U g^{-1} bacterial bran of α-amylase can be produced from the thermophilic fungus *Thermomyces lanuginosus* (Kunamneni *et al*., 2005a,b). This yield is very poor when compared to yields from commercial strains of *Bacillus*. It was concluded after the study that *T. langunosus* is a poor producer of α-amylase and strain manipulation methods like mutagenesis, protoplast fusion, cloning and transformation, as well as media optimization may be required to achieve higher enzyme titres (Kunamneni *et al*., 2005b). Although thermostable α-amylases can be produced by a variety of microorganisms, it is a challenging task to obtain a strain that can produce at commercially acceptable yields. Only *B. amyloliquefaciens* and *B. licheniformis* strains have hitherto been employed in commercial applications. Various methods to reduce the cost of production, with regard to the enzyme production by these two strains have been reviewed in literature (Ramesh and Lonsane, 1990a).

In majority of the cases Wheat Bran (WB) has been the preferred choice of substrate for the production of thermostable α-amylases in SSF. As early as in 1945, thermostable α-amylases were produced from *B. subtilis* (Beckord *et al*., 1945) on WB moistened with dilute phosphate buffer. Usage of tapioca or mustard cake or rice husk (Baysal *et al*., 2003) as a substitute to WB decreases the production of α-amylase (Babu and Satyanarayana, 1995). Thermostable α-amylase production using maize bran is comparable to that with WB, although the extraction is reported to be much more difficult with maize bran (Babu and Satyanarayana, 1995). On screening 10 different kinds of substrates, which included various types of brans, cereals, maize etc., it was found that the production on WB was appreciably higher (Kunamneni *et al*., 2005b). Although, the most apt medium for the production of thermostable α-amylases may vary from one microorganism to another, depending on the organism's physico-chemical requirements (Ramesh and Lonsane, 1990a; Babu and Satyanarayana, 1995), WB is popularly used by researchers in most bacterial systems, primarily because this well-studied medium contains sufficient nutrients and is loose even in moist conditions (Feniksova *et al*., 1960).

Based on certain comparative studies conducted on a variety of substrates (which included different combinations of wheat bran, rice bran and corn bran) for production from *Bacillus* sp. PS-7 under SSF, it was reported that the maximum production occurred on a medium containing WB optimized with external supplements (Soni *et al*., 2005). This maximum production was 464,000 U g^{-1} dry bacterial bran after incubating at 37°C when WB was supplemented with 1% (w/w) each of glycerol and soyabean meal, 0.1% (w/w) of L-proline and 0.01% (w/w) of vitamin B-complex. This maximum production occurred after 48 h when the organism had established itself well in the deeper layers of the solid medium. In another study using *Bacillus* sp. AS-1, a maximum production of 198,950 U g^{-1} dry bacterial bran was obtained when WB media was supplemented with 1% (w/w) glucose, 0.5% (w/w) soyabean meal, 0.01 M $MnSO_4$ after 48 h at 37°C (Soni *et al*., 2003).

Using *B. subtilis* as the microbial source and moistened WB as the substrate, Kunamneni and Singh observed appreciable yields (167,395 U g^{-1} dry bacterial bran) within 72 h at 50°C (Kunamneni and Singh, 2005b). In comparison, Babu and Satyanarayana obtained 25,692 U g^{-1} dry bacterial bran of thermostable α-amylase from *B. coagulans* on WB moistened with salt solution after incubating at 50°C (Babu and Satyanarayana, 1995). Prolonged incubation is also not favorable because it may cause loss of moisture at these high temperatures. The incubation time is mainly governed by the characteristics of the culture organism and the enzyme production pattern (Park and Rivera, 1982).

Ramesh and Lonsane obtained 21,000 U g^{-1} dry bacterial bran of thermostable α-amylase from *B. licheniformis*, when WB was enriched with 3.3% of di-ammonium hydrogen phosphate (Ramesh and Lonsane, 1989a). In this study, the commercial WB of 0.2-0.8 cm particle size was supplemented with 2.2% $Na_2HPO_4.2H_2O$, 1.22% $Na_2H_2PO_4.2H_2O$, 0.6% KCl and 0.02% of $MgSO_4.7H_2O$. The above authors also obtained considerable yields when the WB was supplemented with up to 25% (w/v) glucose, significantly overcoming catabolite repression (Ramesh and Lonsane, 1991).

Although the nature of the moistening agent does not affect the production appreciably, the ratio of substrate to the moistening agent has been singled out to be a crucial factor in this production (Babu and Satyanarayana, 1995). It was generally expected that the increase in moisture content of the medium would lead to increased productivity of the bacterial SSF system. However, this is usually true only for the first 24 h (Ramesh and Lonsane, 1990b). It was shown by these authors that the enzyme production is highest in basal WB medium having initial moisture content of 1:1.85 (weight by volume) with a peak in enzyme titre at 48 h. They had also observed a large reduction (30-78%) in the production of the enzyme when the moisture content was raised beyond this standardized value. It has been reported by several researchers that the optimum value of the ratio of WB and tap water (or buffer), lies in the range between 1:1.5 and 1:2 (Beckford *et al*., 1945; Ramesh and Lonsane, 1987a, 1990b, 1991; Babu and Satyanarayana, 1995; Krishna and Chandrasekaran, 1996; Soni *et al*., 2005). However, the precise value depends on many factors including the nature of the organism and moistening agent. In bacterial SSF systems, the effects of higher moisture content over critical values were reported to involve decreased porosity, loss of WB particle structure, development of stickiness, reduction in gas volume, decreased gas exchange and lowered oxygen transfer due to decreased diffusion (Feniksova *et al*., 1960; Nishio *et al*., 1979; Silman *et al*., 1979; Lonsane *et al*., 1985; Ramesh and Lonsane, 1990a,b). Table 1 enumerates the different ratios of WB media to moistening agent that have been employed for this production.

In another study, a maximum yield of 5345 U g^{-1} min^{-1} was reported when banana fruit stalk was used instead of WB (Krishna and Chandrasekaran, 1996). However, the thermostability and characterization of α-amylase and the usage of other strains of *Bacillus* for production on this medium remains to be studied. Supplementing the WB medium with glucose, maltose or even starch (Ramesh and Lonsane, 1987b; Babu and Satyanarayana, 1995) does not increase α-amylase production for many strains. It has been reported that the supplementation of soluble starch in dilute quantities might increase the production for certain strains (Feniksova *et al*., 1960). A couple of studies also reported an increase in production by supplementing WB with castor seed cakes or peanut cakes (Lulla and Subrahmanyan, 1954; Qadeer *et al*., 1980). However, no further analysis on the usability of these supplements has been reported in literature.

Bioreactor Studies

Various bioreactor types including packed-beds, rotating drums, gas-solid fluidized beds and various stirred bioreactors have been used in SSF processes (Pandey, 1991; Mitchell *et al*., 2000). Conversely, SSF processes for production of thermostable α-amylases have popularly used either shake flasks or metallic trays presumably because most of these processes have been conducted at the laboratory scale. (Babu and Satyanarayana, 1995; Kunmaneni *et al*., 2005a,b; Soni *et al*., 2005).

Table 1: Production of bacterial thermostable α-amylases by solid state fermentation using wheat bran as the substrate

Organism	Ratio of WB to moistening agent (w/v)	Nature of moistening agent	Production (U g^{-1} dry bacterial bran)	Production conditions: pH; Temp(°C); pProduction vessel	Authors
Bacillus sp PS-7	1:1.5	Tap water	464,000	6.0; 37°C; Erlenmeyer flasks	Soni *et al.* (2005)
Bacillus subtilis ATTC 23350	1:4	Distilled water	167,395	6.5; 50°C; Erlenmeyer flasks	Kunamneni and Singh (2005b)
Bacillus sp. AS-1	1:1.25	Distilled water	198,950	NA ; 37°C; Enamel coated metallic trays	Soni *et al.* (2003)
Bacillus coagulans	1:1.85	Salt solution	26,350	7.0; 50°C; Aerated Reactor	Babu and Satyanarayana (1995)
Bacillus licheniformis M27	1:1.85	Phosphate Buffer	21,500	7.0; 35°C; Erlenmeyer flasks	Ramesh and Lonsane (1991)
Bacillus licheniformis M27	1:1.85	Tap water	24,531	7.0; 35°C; Erlenmeyer flasks	Ramesh and Lonsane (1990b)
Bacillus licheniformis M27	1:2	Distilled water	21,000	7.0; 35°C; Erlenmeyer flasks	Ramesh and Lonsane (1989a)
Bacillus megaterium 16M	1:2	Sodium phosphate Buffer	30,000	7.0; 40°C; Erlenmeyer flasks	Ramesh and Lonsane (1987a)

Bioreactor design aspects especially at the industrial level have not been given enough attention by researchers and it is not possible to currently indicate an ideal type of bioreactor for these SSF systems.

In one study, it was observed that the production in shake flasks was higher than in static conditions and the time of maximum production reduced from 72 to 48 h (Babu and Satyanarayana, 1995). Production was even higher in an aerated reactor, because of better aeration and increased surface area (Table 1). Usage of enamel coated metallic trays, which contained the mositened WB too produced high yields and the cells, in this case, were observed to have colonized well on the wheat bran based solid media (Soni *et al.*, 2003). Recently, Soni *et al.* (2005) have reported high production levels in both Erlenmeyer flasks and enamel coated metallic trays. Fermentations were done in both flasks and trays of various sizes and they found that the maximum production occurred after 48 h in case of flasks and 72 h in case of trays. The production levels in both flasks and metallic trays were observed to increase with an increase in its volume and the quantity of bran. This result must be encouraging for large scale production of the enzyme, although the yields do tend to exhibit a slight decline at high substrate quantities. The authors have speculated this decline to possible low levels of aeration at higher volumes.

Downstream Studies

Most applications of thermostable α-amylases do not require them to be of high purity and the usage of crude or partially purified enzyme preparations is popular. However, it is important to obtain enzymes with high specific activity. Purification in SSF systems is done in several steps including centrifugation after extraction, followed by ultrafiltration, selective precipitation by ammonium sulphate and may be followed by affinity or ion exchange chromatography or gel filtration (Tengerdy, 1998; Pandey *et al.*, 2001 a,b; Soni *et al.*, 2005). In one such study, α-amylase produced from *Bacillus* sp PS-7 under SSF was partially purified 12.7 fold by subjecting the cell free supernatant to ammonium sulphate precipitation, gel filtration on Sephadex G-75 followed by Phenyl agarose hydrophobic interaction chromatography (Soni *et al.*, 2005). In another study, α-amylase was extracted from the bacterial bran with 10 mM phosphate buffer at pH 7 and was then centrifuged at 15,000 rpm (Babu and Satyanarayana, 1995). In contrast, Ramesh and Lonsane (1989a) purified the thermostable α-amylase through two concomitant precipitation steps. Foremost they precipitated the α-amylase with ammonium sulphate and followed it by centrifuging at 8000 rpm. Next, after dissolving in phosphate buffer and subsequent dialysis, they reprecipitated the α-amylase from the supernatant by

Table 2: Characteristics of thermostable α-amylases produced by solid state fermentation

Organism used for production	Optimal temperature (°C)	Optimal pH	References
Bacillus sp. PS-7	60	6.0	Soni *et al.* (2005)
Bacillus sp. AS-1	50	6.5	Soni *et al.* (2003)
Bacillus licheniformis M27	85-90	6.5-7 and 9.5	Ramesh and Lonsane (1989a)
Bacillus HOP-40	85	5.0, 8.5-9.0 and 7.0	Ramesh and Lonsane (1987b)
Bacillus megaterium 16M	70	6.0	Ramesh and Lonsane (1987a)

cold acetone. The purification was 10 fold after the first precipitation and 19 fold after reprecipitation. The outcome of such a strategy was that the specific activity increased from 240 to 4582 U mg^{-1} protein.

In SSF, the products are formed at or near the surfaces of the solid materials with low moisture content (Selvakumar and Pandey, 1999). Hence, selection of an optimal solvent for leaching out the α-amylase from the fermented mass is a crucial step. An optimization study was conducted for the extraction of α-amylase in SSF of wheat bran by *Bacillus cirulans* GRS313 (Palit and Banerjee, 2001). They found that maximum extraction was achieved when 2.5% (v/v) glycerol was added. The optimum conditions were observed to be 2.5 h soaking time at 30°C under recirculation and agitated condition.

One step rapid purification methods using ammonium sulphate precipitation (Stamford *et al.*, 2001) or acetone precipitation (Malhotra *et al.*, 2000) are also popular. Usage of ammonium sulphate as a precipitant not only reduces processing cost but also has a high recovery rate. In food processing industries α-amylase recovered only through ammonium sulphate precipitation is normally recommended (Couto *et al.*, 2006). The extraction of α-amylase from bacterial bran in SSF systems is also reported to be temperature dependent (Padmanabhan *et al.*, 1992). In this study, the found that recovery at 50°C is much higher compared to 30°C. This result could be of strategic and economic importance. However, the α-amylases recovered from solid state cultivation are said to be more stable with respect to temperature and pH compared to those produced by submerged cultivation (Yang and Wang, 1999).

Characterization of Thermostable α-Amylases Produced by SSF Systems

The marketability of bacterial α-amylases is determined by its characteristics (Aunstrup, 1983). It is important to judicially select the sources as thermostable α-amylases from different sources could have differing product profiles (Ramesh and Lonsane, 1989b). As starch liquefaction is carried out at high temperatures, usually between 60-80°C, it is desirable to have the extracted α-amylase be optimally active at these temperatures. The optimal temperature range for activity differs, depending on the kind of organism used for the production: 85-90°C (Ramesh and Lonsane, 1989a), 85°C (Ramesh and Lonsane, 1987b), 70°C (Ramesh and Lonsane, 1987a), 60°C (Soni *et al.*, 2005), 50°C (Soni *et al.*, 2003) (Table 2). In higher concentrations of starch, the enzymes are stable even at higher temperatures (Ramesh and Lonsane, 1987b).

Metal ions, monovalent, divalent or polyvalent exert a significant influence on the activity and stability of any enzyme. Ca^{2+}, at low concentrations, is reported to increase the stability of most thermostable α-amylases at higher temperatures (Beckford *et al.*, 1945; Krishnan and Chandra, 1983; Hayashida *et al.*, 1988; Ramesh and Lonsane, 1989a; Soni *et al.*, 2003; Kunamneni and Singh, 2005b; Soni *et al.*, 2005). Ca^{2+} probably forces α-amylase to adopt a compact structure, by salting out the hydrophobic residues of the enzyme, thereby increasing the stability of the enzyme at higher temperatures (Soni *et al.*, 2005). Notably, a study informs that the presence of zinc sulphate increases the stability of the α-amylases produced by *Bacillus* sp. HOP-40 effectively than Ca^{2+} salts (Ramesh and Lonsane, 1987b). However, the effect of Zn^{2+} on thermostability has not been studied for α-amylases produced from other bacterial species. It is also not clear if metal ions appreciably affect the activity of thermostable α-amylases and conflicting observations have been reported (Soni *et al.*, 2003, 2005; Ramesh and Lonsane, 1987a).

In starch liquefaction, α-amylases are often used in synergy with amyloglucosidases; hence, in order for the combined system to be active, the temperature and pH optima of these two enzymes should be compatible. However, the pH optimum of most amyloglucosidases is at or near 5.0 and as a result, one would seek to have α-amylases to be active and stable at this low pH. Most of the thermostable α-amylases reported in literature show maximal activity at pH 6.5-7 in 0.5-1% starch solution (Beckford *et al*., 1945; Ramesh and Lonsane, 1987a,b; Ramesh and Lonsane, 1989a; Soni *et al*., 2003, 2005). These alkaline α-amylases exhibits a sharp drop in activity in acidic medium (Beckford *et al*., 1945; Ramesh and Lonsane, 1987a,b, 1989a). Importance should be given to resourceful α-amylase systems, which exhibit more than one pH optimum, with one on either side of pH 7. The α-amylase produced by *B. licheniformis* (Ramesh and Lonsane, 1989a) from SSF showed pH optima at 6.5-7 and 9.5. A novel thermostable α-amylase produced by *Bacillus* sp. HOP-40 under SSF gave three peaks at pH 5.0, 8.5-9.0 and 7.0 (Ramesh and Lonsane, 1987b). Another α-amylase from *Bacillus* sp. PS-7 was stable in a distributed pH range of 5.0-8.0 with maximum stability at a pH of 6.0 (Soni *et al*., 2005). Such candidates can be effectively acted in synergism with the commercial amyloglucosidase during direct malt starch hydrolysis for ethanol fermentation (Soni *et al*., 2005).

Conclusions

A detailed analysis of the literature reveals that the production of thermostable α-amylases by SSF has several advantages: production at high enzyme titres, stability in a range of temperature and pH being some of them. These, along with the economics of the production make SSF the ideal choice for the production of α-amylases. Needless to say, these techniques should be of valuable importance to various amylase intensive industries like food-processing, textiles and detergents. However, major hurdles remain in the commercialization of the technology such as online measurement and control of temperature, pH, aeration and moisture content because the production of enzymes in SSF is highly dependent on these environmental factors.

References

Aidoo, K.E., R. Hendry and B.J.B. Wood, 1981. Estimation of fungal growth in a solidstate fermentation system. J. Applied Microbiol. Biotechnol., 12: 6-9.

Arima, K., 1964. Microbial enzyme production. In: Global Impacts of Applied Microbiology. Ed. M.P. Starr, New York: John Wiley and Sons, pp: 279-294.

Aunstrup, K., 1983. Enzymes of Industrial Interest-Traditional Products. In: Annual Reports on Fermentation Processes, Ed. Tsao, G.T. New York: Academic Press, 6: 175-201.

Babu, K.R. and T. Satyanarayana, 1995. α-Amylase production by thermophilic *Bacilluscoagulans* in solid state fermentation. Process Biochem., 30: 305-309.

Baysal, Z., F. Uyar and C. Aytekin, 2003. Solid state fermentation for production of α-amylase by a thermotolerant *Bacillus subtilis* from hot-spring water. Process Biochem., 38: 1665-1668.

Beckford, L.D., E. Kneen and K.H. Lewis, 1945. Bacterial amylase production on wheatbran. Ind. Eng. Chem., 37: 692-696.

Bruins M.E., A.E.M. Janssen and R.M. Boom, 2001. Thermozymes and their applications-A review of recent literature and patents. Applied Biochem. Biotechnol., 90: 155-186.

Cornelis, P., 1987. Microbial amylases. Microbiol. Sci., 4: 342-343.

Coutoa, S.R. and M.A. Sanromán, 2006. Application of solid-state fermentation to food industry-A review. J. Food. Eng., 76: 291-302.

Crabb, W.D. and C. Mitchinson, 1997. Enzymes involved in the processing of starch tosugars. Tibtech, 15: 349-352.

Datar, R., 1986. Economics of primary separation steps in relation to fermentation and genetic engineering. Process Biochem., 21: 19-26.

de Souza, E.L., E.H.E. Hoffmann, V.M. Castilho, V.A. de Lima and M.Z. Bellini, 1996.Production and characterization of α-amylase from *Rhizopus* sp. Arq. Biol.Technol., 39: 831-839.

Dey, S. and S.O. Agarwal, 1999. Characterization of a thermostable alpha-amylase froma thermophilic *Streptomyces megasporus* strain SD12. Ind. J. Biochem. Biophys., 36: 150-157.

Dey, N., R. Soni and S.K. Soni, 2001. A novel thermostable α-amylase from thermophilic *Bacillus* sp. SN-1 and its application in the liquefaction of sorghum starch for ethanol fermentation. Asian J. Microbiol. Biotechnol. Environ. Sci., 4: 1-6.

Diane, W., R. Stephan and Z. Wolfgang, 1997. Application of thermostable enzymes for carbohydrate modification. In: Contribution of the Fourth International Workshop on Carbohydrate as Organic Raw Materials, WUV-Universitatverlag, Vienna, March 20-21.

Emanuilova, E.I. and K. Toda, 1984. α-Amylase production in batch and continuouscultures by *Bacillus caldolyticus*. J. Applied Microbiol. Biotechnol., 19: 301-305.

Feniksova, R.V., A.S. Tikhormirova and B.E. Rakhleeva, 1960. Conditions for formingamylase and proteinase in surface culture of *Bacillus subtilis*. Mikrobiologia, 29: 745-748.

Fogarty, W.M. and C.T. Kelly, 1983. Amylases, Amyloglucosidases and Related Glucanases. In: Microbial Enzymes and Biotechnology. Fogarty, W.M. (Ed.). London: Applied Sci., pp: 115-170.

Fredrich, A. and G. Antrakian, 1996. Keratin degradation by *Fervidobacterium pennavorans*, a novel thermophilicanaerobic species of the order Thermotogales. Applied Environ. Microbiol., 62: 2875-2882.

Ghildyal, N.P., B.K. Lonsane, K.R. Sreekantiah and V.S. Murthy, 1985. Economics of submerged and solid state fermentations for the production of amyloglucosidase. J. Food Sci. Technol., 22: 171-176.

Gupta R., P. Gigras, H. Mohapatra, V.K. Goswami and B. Chauhan, 2003. Microbialalpha-amylases: A biotechnological perspective. Process Biochem., 38: 1599-1616.

Haki, G.D. and S.K. Rakshit, 2003. Developments in industrially important thermostable enzymes: A review. Bioresource Technol., 89: 17-34.

Hayashida S., Y. Termato and T. Inoue, 1988. Production and characterisation of rawstarch degrading α-amylase from *B. subtilis*. Applied Environ. Microbiol., 54: 1516-1522.

Hesseltine C.W., 1972. Biotechnology report: Solid state fermentations. Biotechnol. Bioeng., 14: 517-532.

James J. and B.K. Simpson, 1996. Applications of enzymes in food processing. Crit. Rev. Food. Sci. Nutr., 36: 437-463.

Karam, J. and J.A. Nicell, 1999. Potential applications of enzymes in waste treatment. J. Chem. Technol. Biotechnol., 69: 141-153.

Krishna C. and M. Chandrasekaran, 1996. Banana waste as substrate for α-amylase production by *Bacillus subtilis* (CBTK 106) under solid-state fermentation. Applied Microbiol. Biotechnol., 46: 106-111.

Krishnan T. and A.K. Chandra, 1983. Purification and characterization of α-amylase from *B. licheniformis* CUMC 305. Applied Environ. Microbiol., 46: 430-437.

Kumar, C.G., R.K. Malik and M.P. Tiwari, 1998. Novel enzyme-based detergents: An Indian perspective. Curr. Sci. India, 75: 1312-1318.

Kunamneni, A. and S. Singh, 2005. Response surface optimization of enzymati chydrolysis of maize starch for higher glucose production. Biochem. Eng. J., 27: 179-190.

Kunamneni, A.K. Permaul and S. Singh, 2005a. Amylase production in solid state fermentation by the thermophilic fungus *Thermomyces lanuginosus*. J. Biosci. Bioeng., 100: 168-171.

Kunamneni, A. K.S. Kumar and S. Singh, 2005b. Response surface methodological approach to optimize the nutritional parameters for enhanced production of α-amylase in solid state fermentation by *Thermomyces lanuginosus* Afr. J. Biotechnol., 4: 708-716.

Leuschner, C. and G. Antranikan, 1995. Heat stable enzymes from extremely thermophilic and hyperthermophilic microorganisms. World J. Microbiol. Biotechnol., 11: 95-114.

Lonsane, B.K., N.P. Ghildyal, S. Budiatman and S.V. Ramakrishna, 1985. Engineering aspects of solid state fermentation. Enzyme Microbiol. Technol., 7: 258-265.

Lulla, B.S. and V. Subrahmanyan, 1954. Influence of culture media on the development of bacterial amylase. J. Sci. Ind. Res. Sect. B., 13: 410-412.

Malhotra, R., S.M. Noorwez and T. Satyanarayana, 2000. Production and partial characterization of thermostable and calcium-independent α-amylase of anextreme thermophile *Bacillus thermooleovorans* NP54. Lett. Applied Microbiol., 31: 378-384.

Mamo, G., B.A. Gashe and A. Gessesse, 1999. A highly thermostable amylase from a newly isolated thermophilic *Bacillus* sp. WN11. J. Applied Microbiol., 86: 557-560.

Mitchell, D.A., N. Krieger, D.M. Stuart and A. Pandey, 2000. New developments insolid-state fermentation: II. Rational approaches to the design, operation and scale-up of bioreactors, Process Biochem., 35: 1211-1225.

Muller, R., 2000. A mathematical model of the formation of fermentable sugars from starch hydrolysis during high temperature mashing, Enzyme Microbiol. Technol., 27: 337-344.

Nigam, P. and D.J. Singh, 1994. Recent process developments in solid state substrate systems and their applications in biotechnology. Basic Microbiol., 34: 405-422.

Nishio, N., K. Tai and S. Naga, 1979. Hydrolase production by *Aspergillus niger* in solid state cultivation. Eur. J. Applied Microbiol. Biotechnol., 8: 263-270.

Okalo, B.N., L.I. Ezeogu and C.O. Ebisike, 1996. Raw starch digesting amylase from *Thermoactinomyces thalophilus* F13. World J. Microbiol. Biotechnol., 12: 637-638.

Padmanabhan, S., M. Ramakrishna, B.K. Lonsane and M.M. Krishnaiah, 1992. Enhanced leaching of product at elevated temperatures: Alpha-amylase produced by *Bacillus licheniformis* M27 in solid state fermentation system. Lett. Applied Microbiol., 15: 235-238.

Palit, S. and R. Banerjee, 2001. Optimization of extraction parameters for recovery of α-amylase from the fermented bran of *Bacillus circulans* GRS313. Braz. Arch. Biol. Technol., 44: 107-111.

Pandey, A., 1991. Aspects of fermenter design for solid-state fermentations. Process Biochem., 26: 355-361.

Pandey, A., 1992. Recent Process Developments in Solid-state Fermentation. Process Biochem., 27: 109-117.

Pandey, A., P. Nigam, C.R. Soccol, V.T. Soccol, D. Singh and R. Mohan, 2000a. Advances in microbial amylases-Review. Biotechnol. Applied Biochem., 31: 135-152

Pandey, A., C.R. Soccol and D. Mitchell, 2000b. New developments in solid state fermentation: I-bioprocesses and products. Process Biochem., 35: 1153-1169.

Pandey, A., C.R. Soccol, J.A. Rodriguez-Leon and P. Nigam, 2001a. History and development of Solid State Fermentation. In: Solid-State Fermentation In biotechnology: Fundamentals and Applications., Ed. Pandey, A. New Delhi: Asiatech Publishers, pp: 3-7.

Pandey, A., C.R. Soccol, J.A. Rodriguez-Leon and P. Nigam, 2001b. General Considerations about Solid-State Fermentation Process. In: Solid-state Fermentationin Biotechnology: Fundamentals and Applications., Ed. Pandey, A. New Delhi: Asiatech Publishers, pp: 8-17.

Park, Y.K. and B.C. Rivera, 1982. Alchol production from various enzyme converted starches with or without cooking. Biotechnol. Bioeng., 24: 495-500.

Parmar, N., A. Singh and O.P. Ward, 2001. Enzyme treatment to reduce solids and improve settling of sewage sludge. J. Ind. Microbiol. Biotechnol., 26: 383-386.

Plant, A., R.M. Clemens, R.M. Daniel and H.W. Morgan, 1987. Purification and preliminary characterization of an extracellular pullulanase from *Thermoanaerobium* Tok6-B1. J. Applied Microbiol. Biotechnol., 26: 427-433.

Poonam, N. and S. Dalel, 1995. Enzyme and microbial systems involved in starch processing. Enzyme Microbiol. Technol., 17: 770-778.

Qadeer M.A., J.I. Anjum and R. Akhtar, 1980. Biosynthesis of enzymes by solid substrate fermentation. Part II. Production of α-amylase by *Bacillus subtilis*. Pak. J. Sci. Ind. Res., 23: 25-29.

Rakshit, S.K., 1998. Utilization of Starch Industry Wastes. Bioconversion of Waste Materials to Industrial Products, 2nd Edn., Ed. Martin, A. London: Blackie Academic and Professional.

Ramakrishna, S.V., T. Suseela, N.P. Ghildyal, S.A. Jaleel, P. Prema, B.K. Lonsane and S.Y. Ahmed, 1982. Recovery of amyloglucosidase from mouldy bran. Indian J. Technol., 20: 476-480.

Ramesh, M.V. and B.K. Lonsane, 1987a. Solid state fermentation for production of α-amylase by *Bacillus megaterium* 16M. Biotechnol. Lett., 9: 323-328.

Ramesh, M.V. and B.K. Lonsane, 1987b. A novel bacterial thermostable alpha-amylase from bacterial bran produced under solid state fermentation. Biotechnol. Lett., 9:505-508.

Ramesh, M.V., 1989. Production of heat stable bacterial α-amylase. Ph.D. Thesis, University of Mysore, Mysore, India

Ramesh, M.V. and B.K. Lonsane, 1989a. Solid state fermentation for production of higher titres of thermostable α-amylase with two peaks for pH optima by*Bacillus licheniformis* M27. Biotechnol. Lett., 11: 649-652.

Ramesh, M.V. and B.K. Lonsane, 1989b. End product profiles of starch hydrolysis bybacterial α-amylases at different temperature and pH values. Biotechnol. Lett., 9: 649-52.

Ramesh, M.V. and B.K. Lonsane, 1990a. Production of bacterial thermostable α-amylase by SSF. A potential tool for achieveing economy in enzyme production and starch hydrolysis. Av. Applied Microbiol., 35: 1-56.

Ramesh, M.V. and B.K. Lonsane, 1990b. Critical importance of moisture content in α-amylase production by *Bacillus licheniformis* M27 in solid state fermentation. Applied Microbiol. Biotechnol., 33: 501-505.

Ramesh, M. V. and B.K. Lonsane, 1991. Ability of a solid state fermentation technique to significantly minimise catabolite repression of α-amylase production by *Bacillus licheni formis* M27. Applied Microbiol. Biotechnol., 35: 591-593.

Rosell, C.M., M. Haros, C. Escriva and C.M. de Barber, 2001. Experimental approach to optimize the use of α-amylases in breadmaking. J. Agric. Food Chem., 49: 2973-2977.

Selvakumar, P. and A. Pandey, (1999), Solid State fermentation for the synthesis of inulinase from *Staphylo coccus* sp and *Kluyveromyces marxiams*. Process Biochem., 34: 851-855.

Silman, R.W., H.F. Conway, R.A. Anderson and E.B. Bagley, 1979. Production of a flatoxin in corn by a large scale solid-substrate fermentation process. Biotechnol. Bioeng., 21: 1799-1808.

Soni, S.K., A. Kaur and J.K. Gupta, 2003. A solid state fermentation based bacterial α-amylase and fungal glucoamylase system and its suitability for the hydrolysis of wheat starch. Process Biochem., 39: 185-192.

Soni, S.K., H. K. Sodhi, K. Sharma and J.K. Gupta, 2005. Production of a thermostable α-amylase from *Bacillus* sp. PS-7 by solid state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production. Process Biochem., 40: 525-534.

Stamford, T.L., N.P. Stamford, L.C. Coelho and J.M. Araujo, 2001. Production and characterization of a thermostable α-amylase from *Nocardiopsis* sp. endophyte of yam bean. Bioresource Technol., 76: 137-141.

Steinkraus, K.H., 1984. Solid-state (solid-substrate) food/beverage fermentations involving fungi. Acta Biotechnol., 4: 83-88.

Tengerdy, R. P., 1998. In: Advances in Biotechnology Ed. Pandey, A. New Delhi: Educational Publishers and Distributors, pp: 13-16.

Tsurikova, N.V. *et al*., 2002. Preparation of an active strain of *Bacillus licheniformis* producer of thermostable alpha-amylase. Prikl. Biokhim. Microbiol., 38: 502-508.

Van der Maarel, M., B. Van der Veen, H. Uitdehaag, H. Leemhuis and L. Dijkhuizen, 2002. Properties and applications of starchconverting enzymes of the α-amylase family. J. Biotechnol., 94: 137-155.

Weber, H., H.J. Schepers and W. Trosch, 1990. Starch-degrading enzymes from anaerobic non-clostridial bacteria. Applied Microbiol. Biotechnol., 33: 602-606.

Yang S.-S. and J.-W. Wang, 1999. Protease and amylase production of *Streptomyces rimosus* in submerged and solid state cultivations, Bot. Bull. Acad. Sin., 40: 259-265.

Zamost, B.L., H.K. Nielsen and R.L. Starnes, 1991. Thermostable enzymes for industrial applications. J. Ind. Microbiol. Biotechnol., 8: 71-81.

Zeikus, J., C. Lee, Y. Lee and B. Saha, 1998. Studies on the solid-state production of thermostable endoxylanases from *Thermoascus aurantiacus*: Characterization of two isoenzymes. J. Biotechnol., 60: 155-163.