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Bioconversion of Pineapple Wastes into Biotechnological Products: A Review

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There is huge accumulation of agro-industrial waste-materials generated by the milling, brewing and various agriculture and food based industries which in total amount to about 500 million tons per year in India. Most of these by-products contain three major structural polymers such as cellulose, hemicellulose and lignin. A high proportion of this waste material is carbohydrate and phenolic in nature. The vast accumulation of this kind of biomass not only results in the deterioration of the environment, but also a huge amount of potentially important materials are lost. Biological degradation, now-a-days, has become an increasingly popular alternative for the treatment of agricultural, industrial, organic as well as toxic wastes. Pineapple by-products resulting from the processing of pineapple being about 60% of the weight of the original pineapple fruit in the form of peeled skin, core, crown end, etc are not utilized and discharged as wastage causing problems of disposal and pollution. The dry matter content of pineapple waste is around 10%, composed of about 96% organic matter and 4 % inorganic matter. Cellulose and hemicellulose being the major constituents can be referred to as valuable resources for a number of reasons, mainly due to the fact that they can be bio-converted easily into valuable products. Pineapple waste can be used as the important source for bioconversion process towards the production of various value-added products such as ethanol, phenolic anti-oxidants, Phenolic flavor compounds, organic acids, biogas and fiber production.

Key words: Pineapple waste, Bioconversion, Bioethanol, Biogas.

The pineapple (*Ananas comosus*) is the most important horticultural produce of the family *Bromeliaceae* positioning as the important fruits in the world. The plant develops to a cone-shaped juicy and fleshy fruit with crown at the top (Morton, 1987; Tran, 2006) growing up to a height of 75-150 cm. The major pineapple growing countries in the world are Brazil, Thailand, Philippines, Costa Rica, China and India. The total area under pineapple cultivation in the world is 909.84 thousand ha with production around 19412.91 thousand tons. Apart from the use as fleshy raw fruit, these are also used as processed food items like juices, concentrates, and jams. After orange and apple juices, the pineapple juice occupies the third most preferred position worldwide (Cabrera *et al*., 2000). Pineapple (*Ananas comosus*) is one of the commercially important fruit crops of India. It is abundantly grown in almost entire North East region, West Bengal, Kerala, Karnataka, Bihar, Goa and Maharashtra states. There is sizeable increase in acreage and production of pineapple in India. In acreage, there is an increase from 87 thousand ha in 2006-07 to 89 thousand ha in 2010-11.Similarly the production has increased from 1,362.00 thousand tons in 2006-07 to 1,415.00 thousand tons in 2010-11. Maximum area under pineapple cultivation is in Assam (14 thousand ha), where productivity is of medium scale. Total

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production is higher in West Bengal, where productivity is also high.

North-Eastern India having total geographical area of 2.62 million km^2, comprising total 8 states has a high potential for pineapple cultivation. This region gets immense climatic diversity, fertile and organic soil, sufficient rainfall thereby having scope for cropping variation, About more than 40% of the total pineapple production of the country was made from the NE region. and 90 to 95% of the produce is organic. 'Giant Kew' and 'Queen'.are the common cultivars grown this region. About 95 % of pineapple produce from this region are regarded as organic in nature. NE is considered as the major producer of pineapple in India about more than 40% of national production. The fruits are getting high attraction due to high TSS and have less fiber content thus having much sweeter than other region. Various scientific and academician have much attention towards the exploration of processing and preservation of such an important produce of this region.

Pineapple by-products consist basically of the residual pulp, peels, stem and leaves. The increasing production of pineapple processed items, results in massive waste generations mainly due to the elimination of components unsuitable for human consumption. Apart from these, rough handling of fruits and exposure to adverse environmental conditions during transportation and storage can cause up to 55% of product waste (Nunes *et al.*, 2009). These wastes are usually prone to microbial spoilage thus limiting further exploitation. Further, the drying, storage and shipment of these wastes are cost effective and hence efficient, inexpensive and eco-friendly utilization is becoming more and more necessary (Upadhyay et al 2010). Owing to the above facts, the biotechnological approaches for efficient use of lignocellulosic materials like pineapple by-products having enormous availability in NE region may be focussed as cheap sources of phytochemicals, antioxidants, biofuels and value-added phenolic compounds. This paper presents brief scenario about the various value-added products from pineapple waste byproducts by the adoption of different technologies.

Bioconversion of pineapple waste

Agricultural residues play important role in meeting the growing energy demand of the society. Lignocellulosic agricultural crop residues have vast energy generation potential which has been reported to be unutilized so far. Lignocellulosic biomass is a renewable resource on earth and it has attracted continuing efforts to produce fuels and chemicals for future uses. Lignocellulosic biomass contains three major structural polymers: cellulose, hemicellulose and lignin. Specific sugar residues may be released from the cell-wall by the action of carbohydrases. Many species of microorganisms are able to degrade agricultural by-products releasing valuable products which may otherwise would be locked in plant for secondary metabolites. Several studies have been carried out for exploration of different possible value –addition of the pineapple by products. The wastes from pineapple canneries have been used as the substrate for the production of bromelain, , ethanol, citric acid, lactic acids etc. (Larraurietal, 1997; Nigam, 1999a, b; Dacera *et al.*, 2009). The following value-added products are the some bioconverted products from pineapple by-products utilizing different microbial was well as biotechnological technologies.

Bioethanol

First generation biofuels ferment starches and sugars from food crops (e.g. sugar beet/cane, corn and cereal grains) into liquid biofuels, leading to widespread concern over competition with food production highlighting about food security. In this response, second generation technologies are being developed which use lignocellulosic feedstocks like agricultural residues and straws. Ethanol is the most promising biofuels, as in principle those could be derived from any material containing simple or complex sugars. Industrial ethanol production has been reported using sugar cane and various starchy materials. Bioethanol is one of the most well-known promising alternatives to fossil fuels which can be produced through biological process from a variety of lignocelluloses biomasses. Pineapple waste having high level of sucrose is regarded as the best source for the bioethanol production. Organisms like *Saccharomyces cerevisiae* and *Zymomonas mobilis* were used for ethanol fermentation (Ban-Koffi and Han, 1990). In this case fermentable sugars which included sucrose, glucose and fructose were relatively low and proper pretreatment of the substrate with enzymes were necessary for maximum alcohol production. Both organisms were capable of producing about 8% ethanol from pineapple waste in 48 h after pretreating with enzymes cellulase and hemi-cellulase. The study conducted by Zain et al 2012, Bakers' yeast (*Saccharomyces cerevisiae*) was immobilized in modified PVA-alginate beads to produce bioethanol from liquid pineapple waste. The results revealed that by using 10 % (w/v) of the immobilized cells highest overall productivity of 0.0752 g/L/h and maximum production of 5.4179 g/L of bioethanol. Maximum productivity of immobilized yeast was 0.0752 g/L/h was lower than that of the free cells. *Saccharomyces cerevislae* and *Zymomonas mobilis* were grown on pineapple waste and their alcohol production characteristics compared. Pretreatment of pineapple waste with cellulase and hemi-cellulase and then fermentation with *S. cerevlslae* or *Z. mobllls* produced about 8% ethanol from pineapple waste in 48h. In the study conducted by Tanaka *et al*., 1999, *Zymomonas mobilis* ATCC 10988 as fermenting organisms for ethanol production . The raw material used here was pineapple cannery waste as well as the juice of rotten or discarded fruit. Ethanol production was 59.0 g/l without supplementation and regulation in pH. Respiration deficient strain *Saccharomyces cerevisiae* ATCC 24553 for continuous ethanol production from pressed juice of pineapple cannery waste was performed by Nigam (1999b). No Pretreatment of juice was done and the liquid effluents collected from various stages of processing were added. At a dilution rate of 0.05 h^{-1}, the ethanol production was 92.5% of the theoretical value.

Ferulic acid

Ferulic acid is a phenolic compound universally found in plant tissues and relatively abundant in cell walls. These are found covalently linked to polysaccharides by ester bonds and to components of lignin by ester or ether bonds. Some important functions of ferulic acid are: (1) inhibition or prevention of cancers of the colon, lung, stomach, and tongue (2) prevention of brain damage by Alzheimer's proteins (3) inhibiting prostate growth (4) strengthening of bone (5) prevention of diabetes-induced free radical formation (6) expansion of pancreatic islets. A number of microorganisms have shown to be capable of degrading ferulic acid into vanillate derivatives (Walton et al., 2003; Preifert et al., 2001; Lomascolo et al., 1999). The catabolic routes of ferulic acid degradation differ from organism to organism (Peng et al., 2003). This phenolic antioxidant is widely used in the food and cosmetic industry. Pineapple peel has been used for the alkali extraction of ferulic acid (Tialy *et al*., 2008). More researchable issues can be came out from the bioconversion of pineapple wastes utilization for ferulic acid production by utilizing various suitable microorganisms.

Lactic acid

Lactic acid has an important position in the family of carboxylic acids because of its application in both food and non-food industries. It is used as a preservative and acidulant in food industries. Researchers have used pineapple syrup, a food processing waste, as low cost substrate for the production of lactic acid using *Lactobacillus lactis* and enzyme invertase to hydrolyze sucrose into glucose and fructose having the yield of 20 and 92 g/l from 20 and 100 g total sugars/l (Ueno *et al*., 2003). Liquid pineapple waste as substrate to ferment to lactic acid using *Lactobacillus delbrueckii* under anaerobic conditions for 72 h. (Idris and Suzana , 2006) They used calcium alginate as the immobilization matrix to produce maximum yield of 0.7822-0.8248 g lactic acid/g glucose under different conditions of temperature and pH. By the application of fungal strain *Rhizopus arrhizus* and *R. oryzae* lactic acid production from pineapple waste resulted in 19.3 and 14.7 g/L (Jin *et al*., 2005). The experiments carried out in batch fermentation using the liquid and solid pineapple wastes to produce lactic acid by Abdullah, 2007. The anaerobic fermentation of lactic acid were performed at 40^{o} C, pH 6, 5% inocolum and 50 rpm. Initially results show that the liquid pineapple waste by using *Lactobacillus delbrueckii* can be used as carbon source for lactic acid fermentation. The production of lactic acid are found to be 79 % yield, while only 56% yield was produced by using solid waste. Canned pineapple syrup, a food processing waste, was utilized as a substrate for l-lactic acid production

(Nakanishi, et al 2010) To optimize the utilization of sucrose in pineapple syrup, in the presence of glucose and fructose, *Lactobacillus paracasei* NRIC 0765 was characterized and selected from 158 lactic acid bacteria strains. The highest lactic acid concentrations achieved were 19 and 93 g l-1 from 20 and 100 g l-1 total sugar in pineapple syrup, without a lag period for sucrose consumption. They demonstrated that *Lb. paracasei* NRIC 0765 has a great capacity to produce l-lactic acid from sugar mixtures containing high sucrose concentrations.

Citric acid

This commercially valuable product is widely used in food, pharmaceutical and beverage industries as substrate to acidify and enhance flavor. Production of citric acid by *A. niger* under solid state fermentation conditions using pineapple waste (from juice extractor) as substrates was performed by Kumar *et al.*, 2003. They also investigated the effect of methanol on the fermentation, which increased the yield from 37.8% to 54.2%. Production of citric acid by *Yarrowia lipolytica* under solid state fermentation conditions using pineapple waste as the sole substrate was conducted by Imandi *et al.*, 2008. They optimized the culture conditions and the citric acid production was 202.35 g/kg dried pineapple waste. Tran and Mitchell, 1995 used wet pineapple waste as the substrate for the production of citric acid. It was found that solid state fermentation using *Aspergillus foetidus* ACM 3996 produced higher amount of citric acid than from other waste sources. Four species of *Aspergillus* were used to for the production of citric acid under solid state fermentation (Tran *et al.*, 1998). Under optimized conditions, a yield of 19.4 g citric acid/100 g dry fermented pineapple waste was obtained. *Aspergillus niger* and *Saccharomyces cerevisiae* were grown on pineapple waste and their citric acid production characteristics compared (Femi-Ola et al 2009). The effects of pH and methanol on the production were investigated. The highest concentration of citric acid was produced by *A. niger* at initial pH of 4.5 in the presence of methanol.

Bromelain

Bromelain is the most valuable and important component from the pineapple waste having usesful as anti-inflammatory, antithrombotic (Bhui *et al.*, 2009), fibrinolytic activities and anticancer agent (Chobotova *et al.*, 2009). Although it is present in stem and fruit, small amount of bromelain is also found in pineapple waste. Hebbar *et al*, 2008 used reverse micellar systems to extract and purify bromelain from aqueous extract of pineapple wastes like core, peel, crown and extended stem. Murachi *et al.*, 1964) have purified the bromelain by successive use of ion-exchange chromatography, gel filtration, and ammonium sulfate fractionation. Purification of bromelain was also extensively studied by Babu *et al.*, 2008 and Nie *et al.*, 2008.

Biogas

Agro-industrial fruit waste consists of high moisture content and full of carbon source. Biogas production is the most effectively solution for these waste management. which has been accepted as the best alternatives renewable energy. The biogas production mechanisms are com-posed of acidogenesis and methanogenesis processes through anaerobic digestion of organic matter. Biogas production from pineapple peel was studied by Chulalaksananukul *et a*, 2012. They have performed in a lab-scale of 6 liters of bioreactors at ambient temperature. The conditions affected biogas production, such as type of microorganism, pH value, carbon to nitrogen ratio, as well as the organic loading rate, were investigated. The results showed that pineapple peel solid waste can be generated successfully to be a methane gas of an attractive concentration of 48% at 20 days by using the indigenous microorganism. According to the study conducted by Bardiya *et al.*, 1996 production of methane using semi-continuous anaerobic digestion produced up to 1682 ml/day of biogas with methane content of 51% in maximum. Pineapple peels gave biogas yields ranging from 0.41-0.67 m3/kg volatile solids with methane content of 41-65%. as reported by Rani and Nand , 2004. Pineapple waste, was used as one of the substrates along with other fruit wastes, has been utilized for biogas generation (Lane, 1984; Prema *et al.*, 1992). When using 15% pineapple peel in the mixed fruit peel waste, bio-hydrogen gas was generated at 0.73m^3/kg of volatile solid destroyed (Vijayaraghavan *et al.*, 2007).

Single cell protein

The single cell protein is a dehydrated cell consisting of mixture of proteins, lipids, carbohydrates, nucleic acids, inorganic compounds and a variety of vitamins. There is exploration for novel exploitation of non-conventional food sources as potential alternatives due to the huge growing population leading to the acute food shortages in the world and food protein deficiency. Single-Cell Protein (SCP) is the most promising alternative in this context by using agro residues as substrates. Bioconversion of pineapple in to single cell protein (SCP) by using two yeast strains; *Saccharomyces cerevisiae* and *Candida tropicalis* was studied by Hanasekaran et al, 2011. They have used pineapple waste as sole carbon source in five concentrations for preparation of fermentation media on which two strains of yeasts, *Saccharomyces cerevisiae* and *Candida tropicalis* were grown. The increased concentration of pineapple hydrolysate enhanced the biomass yield and the protein formation within the yeast cells. The sugars contained in pineapple cannery effluent have been utilized for the production of single cell protein using continuous cultivation (Nigam, 1999b). The dilution rate had significant effect on biomass as well as protein content. There was an increase in biomass and protein content of *Candida utilis* with increasing dilution rate.

Phenolic antioxidant

Agro-industrial by-products are extensively being explored for the production of natural antioxidants.. The low cost of these residues, discarded as waste in the environment, can be taken as the useful and valuable sources for different phenolic antioxidants recovery. The methanol extraction yield and total phenolic contents of pineapple residue like pulp, seeds and peel were 30.2% and 10 mg/g GAE (Oliveira *et al*., 2009). They co-related the antioxidant activities of the phenolic compounds using DPPH free radical scavenging activity and superoxide anion scavenging activity. Larrauri *et al*., 1997 identified myricetin, salicyclic acid, tannic acid, *trans*-cinnamic acid and *p*-coumaric acid from pineapple shell. Phenolic compounds from pineapple wastes have been enhanced using certain bioprocesses when the fungus *Rhizopus oligosporus* was incubated for 12 days in 1:1 pineapple: soybean flour mixture (Correia *et al*., 2004a). In the another study Correia *et al*., 2004b) conducted experiments showing mixture of pineapple residue and soy flour (9:1 and 5:5) using *R. oligosporus* has revealed that extracts obtained after 2 days with 9:1 treatment showed potent ±-amylase inhibition while the extract obtained after 10 days with 5:5 treatment exhibited *Helicobacter pylori* inhibition. According to Babel *et al*., 2004, solid pineapple waste has been also used to produce volatile fatty acids and methane. They reported that at higher alkalinity, up to 53 g volatile fatty acids were produced from one kg of pineapple waste.

Lignocellulosic fibers and Biocomposites

The global population growth, corroborated with the average individual consumption increase has led to very high global consumption levels of raw materials and finished products. Moreover, there is an increasing search for "green" or "environmentally friendly" materials and methods.

Such trend and awareness to environmentally friendly behaviour and increased demand have progressively driven the primary sector to seek substitutes for materials with polluting attributes and/or from non-renewable sources throughout the production process (Leao et al., 2009). Lignocellulosic fibers have emerged as a reinforcement alternative in polymer matrices to obtain composites for a wide variety of applications (Sanadi et al., 1995; Leao et al., 2009) There are some advantages over synthetic fibers, namely: low densities and abrasiveness (relatively), high possible filling levels resulting in high stiffness and high specific properties, recyclability, high bending resistance, biodegradability, wide variety of fibers available worldwide, rural financial income generation and low cost (Sanadi et al., 1994; Sanadi, 2004; Santos, 2006; Shanks et al., 2006; Chattopadhyay et al., 2009). Pineapple leaves yield a strong, white, silky fiber which was extracted by Filipinos before 1591. Fibers production from pineapple fruit has been stated by several researchers. Pineapple leaf is a source of high quality natural fiber but has not been properly explored. Pineapple leaf fiber (PALF) has many potential applications similar to other natural fibers such as plastic reinforcement, sound and thermal insulations Sreenath *et al*., 1996 conducted Alkaline pulping methods to be superior over semi-chemical mechanical pulping with yields below 40%. A yield of 2.1g fiber/100 g pineapple pulp waste. Due to high cellulosic content, and abundance, pineapple leaf fibers are used in making fiber-reinforced polymeric composites (Devi *et al*., 1997; Luo and Netravalli, 1999; Arib *et al*., 2006).

CONCLUSION

Due to huge amount of agro waste accumulation, there is globally problem for proper utilization of these into value added biotechnological products using various microorganisms as well as chemical conversion. These create not only the ecological pollution problems but also there are losses of huge value-added compounds due to non-proper bioconversion procedures. There are various value added food additives and medicinal important compounds remained unexplored inside the pineapple waste having about 50-60% as these wastes materials. Thus high exploitation potential ability of these wastes can be emphasized by various academicians and researchers for future biofuel and valuable food additives. Having immense potential and ready available compounds can be biochemically degraded into all sectors of compounds including pharmaceuticals, food, beverages and chemical fibers industries. Hence improved bioconversion technologies utilizing recombinant microorganisms will definitely help in the production of important compounds thereby supplementing into renewable energy sources and the food security problems in future. Different biocatalytic routes during the bioconversion technologies can be explored for evolution of novel compounds of global importance meeting different problems in food and pharmaceutical areas. Biocatalyst having novel actions utilizing pineapple wastes can be explored for sustainable betterment of life.

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