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Review article

Microbial-processing of fruit and vegetable wastes for production of vital enzymes and organic acids: Biotechnology and scopes

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ABSTRACT

Wastes generated from fruits and vegetables are organic in nature and contribute a major share in soil and water pollution. Also, green house gas emission caused by fruit and vegetable wastes (FVWs) is a matter of serious environmental concern. This review addresses the developments over the last one decade on microbial processing technologies for production of enzymes and organic acids from FVWs. The advances in genetic engineering for improvement of microbial strains in order to enhance the production of the value added bio-products as well as the concept of zero-waste economy have been briefly discussed.

1. Introduction

With the rise in population on the planet, researchers are working towards increasing the yield of food materials, especially fruits, vegetables and cereals to meet the demand. The global production of fruits and vegetables is in the increasing trend and having been recorded as 1.74 billion tons in 2013; 9.4% more than in 2012, when it amounted to 1.59 billion tons (World Farmers Organization, 2014). For example, India is the second largest producer of fruits and vegetables with a global production share of 10% and 14%, respectively (Das and Mondal, 2013; Ingale et al., 2014). The fruit production of India rose from 0.04 billion tons in 2001–2002 to 0.08 billion tons in 2012–2013. Similarly, the vegetable production has increased from 0.08 billion tons in 2001–2002 to 0.16 billion tons in 2012–2013 (Indian Horticultural Database, 2013). Out of the total fruit and vegetable production in India, 30–40% of the total weight (about 50 million tons) is discarded as waste due to various reasons which has a worth of US $483.9 million (Sridevi and Ramanujam, 2012). Likewise, South Africa produces 8.2 million tons of fruits and vegetables per annum (FAO FAOSTAT, 2010a, 2010b). In South Africa it has been estimated that fruit and vegetable wastes (FVWs) contribute 47% of the total food waste generated which is around 4.2 million tons per annum (Oelofse and Nahman, 2013). In the United States, 40% of the food (including fruits and vegetables) which is equal to $165 billion/annum, are wasted uneaten (Gunders, 2012). Although food wastes are used as one of the major components for land filling in the US, it is not cost effective as the waste's main constituent is moisture (Gunders, 2012). FVWs are generated in different stages of supply chain, from farm to fork. In US, 7% of the planted crops including fruits and vegetables are not harvested every year (Kantor et al., 1997). As per the collective data obtained for USA, Canada, Australia and New Zealand, the total loss of fruits and vegetables is 52% of the total production. The major loss (20%) has been reported during production and harvesting (FAO, 2011). Significant amount of FVWs are also generated from processing units such as pickle, sauces, puree and juice. FVWs generated from industrial processing account for 30–50% of the input materials (Di Donato et al., 2011).

Pollution of soil and water caused by the disposal of bulk amount of FVWs is a matter of serious concern. Additionally, the food wastes emit significant amount of green house gas that has been estimated to be 4.14 t of CO_2 equivalent per ton of food wasted (Oelofse and Nahman, 2013). However, FVWs are rich in moisture, carbohydrates and other compounds depending upon

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their origin. Apart from moisture and carbohydrates, some FVWs contain considerable quantities of proteins, fats, natural colorants and in some cases, antioxidants and other bioactive compounds (Wijngaard et al., 2009). Because of the size of the FVWs generated throughout the world and their biochemical characteristics, several studies have been carried out to transform the waste to value added products. The studies were mostly based on microbial processing. Products like enzymes, organic acids, flavoring compounds, food colorants, bio-ethanol, bio-methane, etc are known to be successfully developed from FVWs *via* microbial applications (Laufenberg et al., 2003). Several researchers have developed genetically modified microorganisms by inserting the genes of interest for over-production of such metabolites. Metabolic engineering, protoplast fusion and biochemical pathway modification have been proved successful in over-expression of biological products like enzymes, organic acids, colorants, bio-fuels, etc.

The current review aims at discussing the overall applications of microbial processing of FVWs into essential enzymes and organic acids. The developments in genetic and metabolic engineering of microorganisms for enhanced bio-production have also been briefly discussed. However, the upscaling of these technologies has not been included in this article; as another review article is in preparation that will exclusively focus on the upscaling procedures of enzymes and organic acids from FVWs.

2. Microbial bio-processing

Microbial bio-processing of organic wastes has been proven to be a potential tool for cleaning up the environment while also producing of value added products. Several groups of microorganisms are known to be used in transformation of FVWs to novel bio-products. *Saccharomyces cerevisiae* is used to enrich fruit waste with protein by bio-processing technology to prepare feed (Correia et al., 2007). *Aspergillus* sp. is known to produce organic acids such as citric and lactic acid from FVWs and *Bacillus* sp. is popular strain for producing enzymes such as cellulase, amylase and protease (Mussatto et al., 2012). Similarly *Streptomyces* produces bioactive compounds like bafilomycin, oxytetracylin and cephamycin from FVWs (Mussato et al., 2012). Depending upon the physical condition of the FVWs, different technologies are adopted for extraction of the desired products. Microbial bio-processing may broadly be classified into (i) solid state fermentation (SSF) and (ii) submerged fermentation (SmF) (Ray and Ward, 2006). SSF is defined as the fermentation process in which microbes grow on solid materials generated from agricultural/horticultural residues without the presence of free liquid (Bhargav et al., 2008; Mohanty et al., 2009). SmF is about culturing of microorganisms in liquid broth (Pandey, 2003).

3. Types of FVWs

FVWs may be categorized based upon its quality and the point of generation at the Food Supply Chain (FSC). The important stages of FSC are: (a) production, (b) distribution and transportation, (c) processing and retailing, and (d) consumer level (FAO, 2011). In the organized sector of India, losses and wastage of fruits and vegetables are 25%, 10% and 7% during processing, distribution and consumption, respectively (Rais and Sheoran, 2015). In South Africa, loss of fruits and vegetables have been estimated at different stages of the supply chain such as during production, 10%; post harvest handling and storage, 9%; processing and packaging, 25%, distribution, 17% and at consumer level, 5% (FAO, 2011).Similarly in the organized sectors of both China and U.S.A, the losses and wastage are 2% during the processing stage of FSC and 8% and 12% during distribution and 15% and 28% during the consumption, respectively (Wadhwa and Bakshi, 2013). In mid-sized Brazilian cities, 16.6% (weight) of the total FVWs are generated during the marketing stage whereas only 3.4% (weight) was produced at the consumer level (Fehr and Romao, 2001). Wastes generated from fruits and vegetables are generally in solid or semi-solid forms except for the effluents generated from processing units.

4. Microbial processing of FVWs

Several studies have been conducted to bio-valorize the solid FVWs to specific high end finished products. SSF is carried out with low moisture level. The hydro-content of the solid mash taken as substrate varies from 40–80% (Ali and Zulkali, 2011). SSF is advantageous over SmF as it produces no foam and reduces control over parameters such as pH, aeration, temperature during fermentation (Couto, 2008). Selection of microorganisms for a particular type of waste and optimization of physico-chemical parameters plays a vital role in the production of value added biological products (Panda and Ray, 2015).

5. Enzymes

Enzymes are biological catalysts responsible for various metabolic processes (Chapman-Smith and Cronan, 1999). Most enzymes are purely proteins; some of the enzymes require either a co-factor (one or more inorganic ions) or a co-enzyme (organic or a metallo-organic molecule) along with the amino acid sequence for their activity (Nelson and Cox, 2004). Now a day's enzymes are used in different industries. For example, amylases and pectinases are used in food industries, cellulases are used in bio-fuel industries and tannase is used to reduce tannic acid concentration in tannery effluent. The knowledge over the production and stability of enzymes has led researchers to develop technologies for production from cheaper substrates. The current section discusses the impending quality of the FVWs and their microbial processing to produce valuable enzymes (Table 1).

5.1. Amylases

Amylolytic enzymes hydrolyze starch and similar oligo- and poly-saccharides into low molecular weight sugars like glucose, fructose, maltose etc. The major constituents of starch are amylose (linear chain of unbranched D-glucose residues connected by α 1–4 linkages) and amylopectin (highly branched D-glucose residues connected by α 1–6 linkages). Based on the approach of hydrolysis, amylases may be classified into exo-amylase and endo-amylase. Exo-amylase attacks α 1–4 bonds and some exo-amylases such as glucoamylase target both α- 1–4 bonds and α- 1–6 bonds to produce simpler sugars (maltose and glucose) (Kar and Ray, 2008). Endo-amylase cleaves α- 1–4 bonds in starch and does not impinge on α- 1–6 linkage in amylopectin and related complex polysaccharides. Alpha- amylase is the best illustration of endo-amylase and is known to produce varying fragments of oligosaccharides from starch. Alpha- amylase can act on random locations on the starch whereas glucoamylase can target the non-reducing ends (Horvathova et al., 2000).

Kernels from fruits are regarded as low valued sugar waste. Erdal and Taskin (2010) verified the production of α-amylase by *Penicillium expansum* in SSF using loquat (*Eriobotrya japonica* Lindley) kernels, a waste generated from loquat fruit (popular South Asian fruit). Under the optimized conditions (initial moisture content, 70%; particle size, 1 mm; pH, 6.0; incubation temperature, 30 °C; starch and peptone as supplements) the