MICROBIAL PRODUCTION OF XYLITOL AND ITS APPLICATION – A REVIEW

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Introduction

Xylitol is a sugar alcohol with an increasing global market with many applications and widely used in food, agricultural and pharmaceutical industries (Salli*et al.*, 2016). It is a naturally occurring five-carbon polyol that is commercially used as a sweetener in food products. During mammalian metabolism of carbohydrate, it is a normal metabolic intermediate produced at a range of 5-15 g per day in an adult human (Winkelhausen*et al.*, 1998). Xylitol has potential application in food industry, due to its cost and lack of availability the volume of xylitol used is small in the food industry, it is mainly used as a sweetner in confectionery (Povelainen*et al.*, 2008). Xylitol is used in personal health products like mouthwash and toothpaste (Affleck, 2000). In pharmaceutical products xylitol is used as a sweetener or coating agent in the pharmaceutical industry (Pepper *et al.*, 1998).

The purified xylose obtained by acid hydrolysis of lignocellulosic substrate by chemical method using metal catalyst at extreme pressure and temperature is the conventional production process of xylitol. Hemicellulosichydrolysate from biomass is used as raw material with the conversion of pentose sugars using microorganisms such as bacteria or yeast is the biotechnological alternative method. Using microorganism fermentation of the pentose sugars is an eco-friendly process that is done under mild conditions such as ambient temperature and atmospheric pressure; this avoids the purification step of xylose, which is the expensive step in conventional catalytic process (Prakasham*et al.*, 2009). Production of xylitol by enzymatic technology is an attractive alternative to chemical and fermentation process (Rafique*et al.*, 2012).

Xylitol production from agricultural residues has great potential due to the presence of high xylan content in the form of hemicellulose (Ur-Rehman*et al.*, 2015). Biotechnological production of xylitol from agricultural wastes such as rice husk (Hickert*et al.*, 2013), corn cobs (Wei *et al.*, 2010), soybean hull (Cortivo*et al.*, 2018), sugar cane bagasse (Vaz de Arruda*et al.*, 2017), sorghum bagasse (Ledezma-Orozco *et al.*, 2018).

Xylitol producing microorganisms

Bacteria

A small amount of xylitol can be produced by *Corynebacterium* sp., *Enterobacterliquefaciens*.,*Mycobacterium smegmatis*, and *Gluconobacteroxydans*,*Enterobacterliquefaciens*, *Corynebacterium* sp. The *Enterobacter* strain used D-xylose was by NADPH-dependent XR and produced xylitol extracellularly (Yoshitake*et al.*, 1973). This proved that not only fungi and yeast, but bacteria also can undergo enzymatic conversion. The xylitol yield was 33.3 g/L from this strain in a production medium containing 100 g/L of initial xylose for 4 days with a productivity of 0.35 g/L h. Only in the medium containing both D-xylose and gluconate, *Corynebacterium* species produced xylitol. Among 17 cultures of facultative bacteria were screened, *Corynebacterium* sp. B-4247 produced high amount of xylitol (Rangaswamy*et al.*, 2002). Using an initial concentration of xylose 75 g/L, within 24 hours maximum xylitol yield 0.57 g/g was produced. Xylitol production of about 80% was reported using D-xylose as the substrate and immobilizing D-xylose isomerase and *Mycobacterium smegmatis*. Based on xylitol production from D-arabinitol, 420 bacterial strains were tested and *Gluconobacteroxydans* was found to be the best xylitol producers among the isolates with an yield of 29.2g/L of xylitol from 52.4 g/L D-arabinitol after incubation with intact cells as the enzyme source for 27 hours (Suzuki *et al.*, 2002). The xylose metabolism by bacteria

Fungi

Xylitol production from filamentous fungi such as *Rhizopus, Penicillium, Myrothecium, Aspergillus, Gliocladium, Byssochlamys, Neurospora* sp.produced small quantities of xylitol in xylose-containing media (Chiang *et al.*, 1960). *Mucor*sp on fermentation of sugarcane bagasse hemicellulose hydrolyzate, the amount of xylitol produced was less (Ueng*et al.*, 1982). Xylitol production from *Fusariumoxysporum* was reported to be 1g/L when grown in aerobic conditions for two days in a medium containing 50g/L of xylose (Suihko*et al.*, 1984). The fungi *Petromycesalbertensis* showed significant production of xylitol with an yield of 0.4 g/g xylose after 10 days of incubation in a fermentation medium containing 100 g/L D-xylose (Dahiya., 1991).

Yeast

Yeasts are said to produce xylitol efficiently than compared to bacteria of fungi. The metabolism of D-xylose occurs in yeast in which xylitol production occurs as a natural intermediate. A two step bioconversion from xylose to xylulose occurs for the accumulation of xylitol. Reduction of xylose to xylitol conversion by the enzyme D-xylose reductase occurs in the first step. In the next step, the intermediary xylitol is oxidized to xylulose D-xylitol dehydrogenase (Pal *et al.,* 2013).Xylitol bioproduction from *Candida boidinii, Candida pelliculosa, Candida guilliermondii, Candida tropicalis* and *Pachysolentannophilus*has been reported (Onishi*et al.,* 1969). Xylose bioconversion was analysed for fifteen yeast strains in which a mutant strain of *Candida tropicalis* HPX2 showed highest yield of xylitol of 0.90 g/g from 20% D-xylose (Gong *et al.,* 1981). Twenty different strains of eleven species of *Candida*, twenty one strains of eight species of *Saccharomyces*, and 8 strains of *Schizosaccharomycespombe*(Gong *et al.,* 1983).Most of the *Candida* sp. produced xylitol of about 10 – 15% *w/v*. The ability to convert xylose to xylitol was assessed for fourty-four yeast strains from five genera of *Candida, Hansenula, Kluyveromyces*, *Pachysolen*, and *Pichia* (Barbosa *et al.*, 1988).

Production of Xylitol using different biomass

The raw materials such as lignocellulosic biomass and their potential xylitol production was investigated (Santos *et al.,* 2005). The composition of lignocellulosic material includes cellulose, hemicellulose and lignin and their composition differs according to each material. In the lignocellulosic raw material, the composition of sugarcane bagasse was found to be 45 % of cellulose and 25.8 % of hemicellulose and 19.1 % of lignin content was investigated. (Canilha*et al.,*2011). The raw material sugarcane straw contains 33.6 % cellulose, 28.9 % of hemicellulose and 31.8 % of lignin was investigated (Silva *et al.*, 2010). The raw material rice straw contains 43.4 % cellulose, 22.9 % of hemicellulose and 17.2 % of lignin was investigated (Roberto*et al.,* 2003). The raw material corn stover contains 34.4 % cellulose, 22.8 % of hemicellulose and 18.0 % of lignin was investigated (Kumar *et al.*, 2009). The raw material corncob contains 38.8 % cellulose, 44.4 % of hemicellulose and 11.9 % of lignin was investigated (Pointner*et al.,* 2014). The raw material wheat straw contains 40.1 % cellulose, 32.8 % of hemicellulose and 14.1 % of lignin was investigated (Sun *et al.*, 2000). In Brazil, the most abundant agricultural crop is sugarcane which is produced 600 million tons annually. Choosing a raw material that is abundant is an important factor for choosing a profitable biomass to be used in a bioprocess. In Brazil, sugarcane juice is used as carbon source for generation of bioethanol and in sugar industry. More amount of sugarcane bagasse is generated per ton of processes sugarcane (Canilha*et al.*, 2010).

The raw material wheat straw was used and the xylitol yield was 0.9 g/g (Canilha*et al.*, 2003) which was higher compared to the yield 0.023 g/g from sunflower stalks (Martínez*et al.*, 2012). Xylitol production from hemicellulosichydrolysate of sugarcane bagasse obtained by hydrolysis of dilute sulphuric acid using *Candida guilliermondii*, the yield of xylitol was observed to be 0.59 g/g from 46 g/L of initial xylose concentration in the hydrolysate (Silva *et al.*, 2007). In another study, the author was able to observe xylitol production from acid hydrolysate of sugarcane bagasse using the yeast *Meyerozymaguilliermondii*, isolated from sugarcane juice; it also produced ethanol as a byproduct (Martini *et al.*, 2016).

Fermentation methods for Xylitol production

Xylitol can be produced using different fermentation methods such as batch, fed-batch and continuous fermentation process. Batch fermentation of xylitol is the most often used method since it is easy to control contamination. Stirred tank reactor, basket-type stirred tank reactor and fluidized bed reactor has been used in batch fermentation method. Using horticultural waste hemicellulosichydrolysates, xylitol production has been done using *Candida athensensis* SB18. The batch fermentation was done using stirred tank reactor method with an agitation speed of 200 rpm, temperature 30ºC and air flow rate of 0.7 $L.min^{-1}$. Maximum xylitol production (100 g L^{-1}) and productivity (0.98 g L^{-1} h^{-1}), with an efficiency of 89% of the theoretical yield (0.917 g of xylitol.g^{-1} of xylose.(Zhang *et al.*, 2012).

Production of xylitol from corncob hydrolysates using immobilized *Candida tropicalis* was done using 5L Stirred tank reactor at a temperature of 30ºC at an agitation speed of 200 rpm. Use of immobilized cells had better yield of xylitol (5-10%) than using free cells (Wang *et al.*, 2012). Fluidized bed reactor was used for xylitol production using immobilized cells. In the study, sugarcane bagasse hydrolysate was fermented using the yeast *Candida guilliermondii* FTI 20037 immobilized in porous glass beads. The air flow rate was increased from 25 to 140 $mL.min^{-1}$ that increased the xylitol productivity from 0.19 to 0.28 gL^{-1} h^{-1} (Santos *et al.*, 2003). A comparative study of basket-type stirred tank reactor and stirred tank reactor was reported with an yield higher in stirred tank reactor than basket-type stirred tank reactor (Carvalho*et al.*, 2003).

The fed-batch fermentation method showed good yield of xylitol production and other microbial metabolites(Gong *et al.*, 1981). The use of *Candida tropicalis* in fed-batch fermentation with an aeration of 0.5 min. The yeast used was a promising strain that can be used even under non-sterile conditions. The fed-batch fermentation process was started when the biomass reached the exponential phase and xylose concentration dropped to 40 g L^{-1}. The initial xylose concentration 60 – 80 g L^{-1}, at pH 5.5 at 37ºC, with 90% of xylitol yield (Tamburini*et al.*, 2015). Fed-batch production of xylitol was done repeatedly in three stages by *Candida magnoliae* TISTR 5663 where xylitol production in the feed-batch I, II and III were 235 g L^{-1} , 284 g L^{-1} and 280 g L^{-1} , respectively.

Continuous fermentation method is advantageous compared to batch processes where a significant reduction in time, easier instrumental control, reduction in equipment size can be achieved using stirred tank reactor, membrane systems and packed bed reactor (Rao*et al.*, 2016). Using hemicellulosichydrolysates and *Candida guilliermondii* FTI 20037, the yield of xylitol was observed to be (0.63 g g^{-1}) (Martínez*et al.*, 2003). Immobilized recombinant *Saccharomyces cerevisiae* S641 was used in a continuous packed-bed reactor by using xylose and glucose as substrate, the xylitol yield was observed to be (0.6 g g^{-1}) under anaerobic condition (Roca *et al.*, 1996).

Separation and purification of xylitol

The recovery of product by downstream methods plays a significant role after fermentation process. To obtain in pure form for human consumption, the xylitol developed after fermentation process must undergo purification steps(De Faveri*et al.*, 2004). When xylose is used in non-purified form, other polyols present along with xylitol must be eliminated initially by ion-exchange chromatography. The obtained xylitol rich fraction is concentrated and crystallized from aqueous solution and then separated (Sampaio*et al.*, 2006). The purification and recovery of xylitol is the most difficult step after fermentation process since the product obtained is in a low concentration, the complexity of the fermentation medium (Faveri*et al.*, 2004) and the byproducts of fermentation (Martínez*et al.*, 2007).

To purify and recover xylitol present in fermentation broth, the method of crystallization can be used. The method also includes in a sequential manner of centrifugation, adsorption, precipitation of ethanol, centrifugation, evaporation, and crystallization (Rivas *et al.*, 2006). To separate the cell biomass, the fermented broth is centrifuged and the obtained supernatant is treated with activated charcoal to remove impurities such as proteins, colored substances, uronic acid and other non-volatile components. To precipitate non-volatile components ethanol is added leaving xylitol. The precipitate is removed by centrifugation and the clear supernatant is concentrated and crystallized at freezing temperature. At the end xylitol crystals obtained by vacuum filtration are rinsed with methanol. The sugarcane bagasse hydrolyzate on fermentation used charcoal for xylitol purification and found to be 20% loss of xylitol (Gurgel*et al.*, 1995). The process of crystallization produced good yield of xylitol, but the solution obtained after concentration was viscous and colored, making the process of crystallization difficult and time consuming.

The optimum xylitol supersaturation value and cooling temperature was found to be 728 g/L and −6.0˚C using response surface methodology and xylitol-xylose pure solutions, the yield of xylitol after crystallization was 0.54 (g/g) with 0.97 degree of purity (Faveri*et al.*, 2004). Using corncob hydrolyzate fermentation, xylitol was purified and crystallized which showed regular shape, crystals that are homogenous with 98.9% (w/w) of xylitol with an yield of 0.47 g/g ofxylitol crystals (Rivas *et al.*, 2006). Xylitol crystals were obtained from two steps of crystallization with 92-94% purity using fermented hemicellulosichydrolyzate (Martínez*et al.*, 2007).To separate and purify xylitol from fermented broth, an alternative technique of membrane filtration was employed since it is an energy saving method and the product was highly pure (Affleck *et al.*, 2000). For the separation and purification of xylitol polysulfone membrane was found to be effective with a purity of 90.3% of xylitol crystals. The downstream processing methods for purification and recovering xylitol is still not an economically feasible method (Mussatto*et al.*, 2005).

Applications of xylitol

Xylitol is used in food sources, drugs, toothpaste, chewing gums, syrups, and candies (Barathikannan*et al.*, 2016). To control glucose level for diabetic patients, to decrease lipid stage, to control weight, xylitol is used as a better alternative sweetener (Huttunen*et al.*, 1982). The white sugar is being replaced with xylitol to stabilize blood sugar levels and to decrease overall lipid storage (Islam *et al.*, 2012). Xylitol and erythritolinhibited the growth of clinical strains of mutans streptococci and *Scardoviawiggsiae*which is a newly recognized cariogenic bacterium. Also biofilm formation of mutans streptococci was strongly inhibited (Kõljalg*et al.*, 2020). The properties like moisture retention, remineralization, microbial stability, high solubility, non-fermentability are possessed by xylitol (Mäkinen, 2000). Xylitol has tooth rehardening and anti-cariogenic property hence it is used in developing toothpaste and mouth washes (Janket*et al.*, 2019). The oral pathogenic organisms like *Streptococcus mutans* and *Helicobacter pylori*are responsible for causing plaque formation, tooth erosion, tooth decay, gingival inflammation, xerostomia. These organisms feed on sugars present in the teeth and mouth and metabolize them, but they cannot metabolize xylitol and hence the presence of xylitol gives a cooling and refreshing effect in toothpaste and mouthwashes, also preventing dental caries. In medicines given for childrens, xylitol is used as sweetening agent which is recommended to be given after brushing (Feigal*et al.,* 1981). The anti-bacterial property of xylitol inhibits the growth of pathogenic microorganism causing tooth decay by preventing its attachment to the teeth surface and reducing its corrosive activity on teeth (Nayak*et al.*, 2014).

The pathogen causing ear and lung infection includes *Streptococcus pneumoniae*and*Haemophilusinfluenzae*. Theyare responsible for causing acute otitis media. Xylitol with its anti-bacterial and anti-inflammatory property reduces middle ear infection and respiratory tract infections by inhibiting the growth of these organisms (Vernacchio*et al.*, 2014). Xylitol containing syrups and chewing gums have been displayed to ensure from the middle ear infections in children (Uhari*et al.,* 1996).

In bakery products, the characteristic flavour and color in baked products can be improved by adding xylitol. Hence xylitol can act as a low energy sweetener and can be a better substitute in sugar cakes (Winkelhausen*et al.,* 1996). The presence of xylitol enhanced the taste, color, flavour, and texture in cookies and there was no major effect on flavour and texture even after long-term storage of cookies (Mushtaq*et al.*, 2010).

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