ENZYMATIC PRODUCTION OF XYLOOLIGOSACCHARIDES FROM FIBRES OF EMPTY FRUIT BUNCH

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Xylooligosaccharides (XO) is a sugar-based product of lignocellulosic material produced from forestry, agricultural or industrial wastes. They possess a variety of beneficial health properties particularly in prebiotic activities and as food additives. Malaysia produces an abundance of oil palm biomass, in which one of it is empty fruit bunch (EFB). EFB is about 21%-22% of the fresh fruit bunch (FFB). Because of the lignocellulosic nature of EFB containing 65% cellulose, 29.2% lignin, 28.8% hemicelluloses and 3.7% extractive (Chang, 2014), this biomass is potentially suitable for the production of XO. Xylans are the most common hemicellulose (which can be extracted from EFB), and can easily be converted into XO via enzymatic hydrolysis. A technology on continuous extraction of XO from xylan extracted from EFB fibres, in a packed bed column reactor (PBCR) with immobilised xylanase was developed. Enzymes can be immobilised by attaching them to a solid surface. In this technology, sodium alginate is used as a carrier for the xylanase enzyme.

FIELDS OF APPLICATION

XO that are available in the market are produced from various xylan extracted from agro-residues using physico-chemical, biological, or combination of various processes. It is a newly developed functional oligosaccharide, having beneficial properties, such as low carcinogenicity, with beneficial properties on the intestinal flora (stimulation of bifidobacteria), dietary fibre-like action, water retention and anti-freezing activity (Alonso *et al.*, 2013; Gupta *et al.*, 2012). XO have also been found effective in the enhancement of immunity, as a source of antioxidant, antibiotic alternative, regulators of blood glucose in diabetics and serum lipids in hyperlipidemics, dental caries prevention and pathogen suppression (Seema and Arun, 2011). In addition, XO are stable over a wide range of pH, temperatures and inhibits starch retro-gradation to improve nutritional and sensory properties of food (Voragen *et al.*, 1998).

DESCRIPTION OF THE PROCESS

The combined chemical and enzymatic extraction of XO is performed in two stages, namely xylan extraction with KOH from EFB fibre, and enzymatic hydrolysis of the xylan into XO. In this process, xylanase enzyme is used, and immobilised onto the carrier (sodium alginate). Immobilised xylanase with a concentration of 8.25 fungal xylanase unit wheat/millilitre (FXUW ml^{-1}) is employed on a PBCR to hydrolyse the xylan at a 55°C and pH 5.5. The XO produced are separated and purified from the mixture of XO using activated charcoal column chromatography.

Enzyme Immobilisation

Immobilisation involves the covalent attachment or entrapment of an enzyme to a carrier. Enzymes can be immobilised by attaching them to a solid surface. In this process, xylanase of *Thermomyces lanuginosus* having an enzyme activity of 2750 FXUW g^{-1} is immobilised on sodium alginate supports and the process is shown as in *Figure 1*.

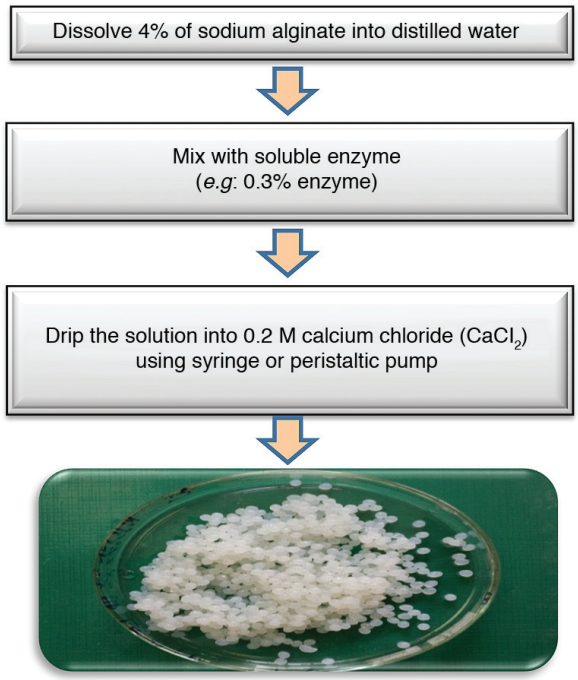

Figure 1. Steps involved in the enzyme immobilisation process.

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Immobilisation allows reuse of enzyme over multiple cycles, hence, reduce production cost. Immobilised enzyme can be packed into columns, use over a long period and easily removed from the product solution. Speedy separation of products may reduce feedback inhibition. Immobilisations also increase thermal stability of the enzyme, thus, allowing higher temperatures to be used. High operating temperature increases the rate of reaction.

Continuous Enzymatic Production

A glass column reactor made from Borosillicate glass measuring 20 cm long and 2 cm diameter was used in this study. Immobilised xylanase with a concentration of 8.25 FXUW ml^{-1} was employed on a PBCR to hydrolyse the EFB xylan at 55°C. A 25 ml of soluble xylan in 100 mM sodium acetate buffer solution at pH 5.5 was fed into the reactor using a peristaltic pump at a flow rate of 60 ml hr^{-1} (*Figure* 2). The reaction temperature was maintained at 55°C using water circulator as this value was the ideal temperature for effective xylanase activity. The XO yields (xylobiose and xylotriose) were determined using HPLC method. The efficiency of immobilised xylanase usage as well as the XO yield in each cycle of treatment is depicted in *Figure 3*.

Purification of Xylooligosaccharides

Products derived from continuous hydrolysis of EFB xylan were separated using activated charcoal column chromatography. Activated charcoal was mixed with hot water and boiled to remove air from grains, which was then used to pack the column avoiding air bubbles and cracking of column. The concentrated XO solution was loaded on the activated charcoal column pre-washed with distilled water. The elution of XO with varying degree of polymerisation was done using increasing gradient of ethanol. The xylobiose and xylotriose were identified as the major end products from the mixture of XO using activated charcoal column separation technique. The mixture of xylobiose and xylotriose was found to exert a stimulatory effect on the selective growth of human intestinal bifidobacteria, and are frequently defined as prebiotics (Chen *et al.*, 1997; Jiang *et al.*, 2004), hence such a mixture can be used as a prebiotic.

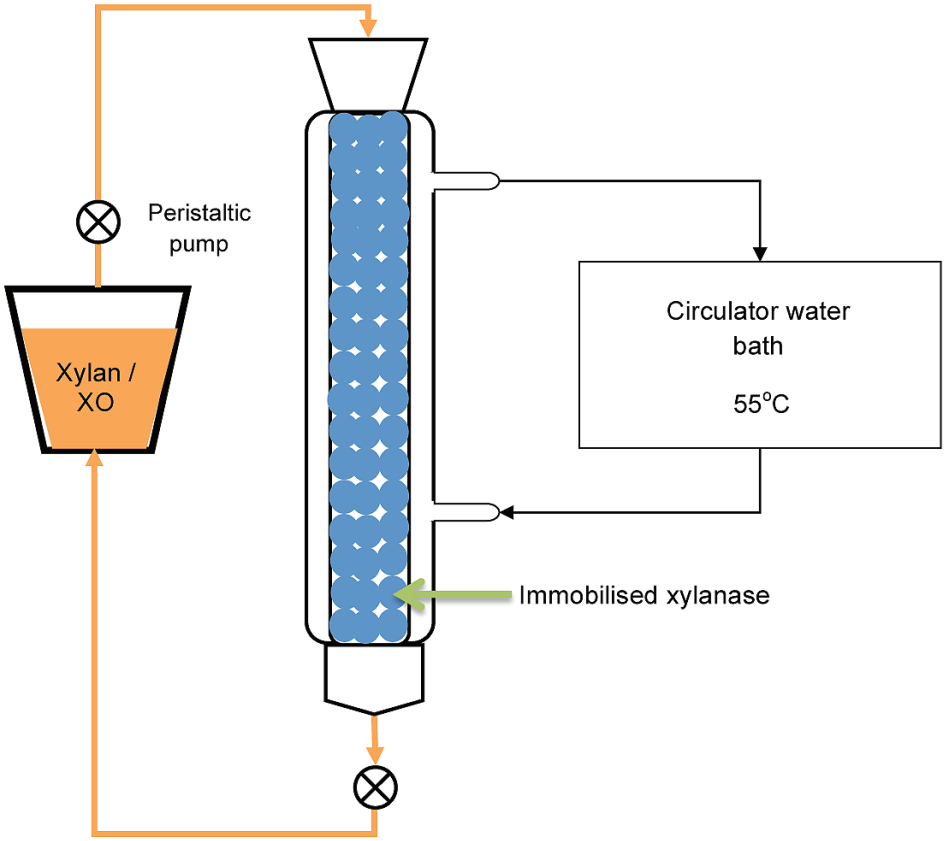

Figure 2. Schematic illustration of enzymatic production of XO from EFB xylan using immobilised xylanase in a PBCR.

FINDINGS

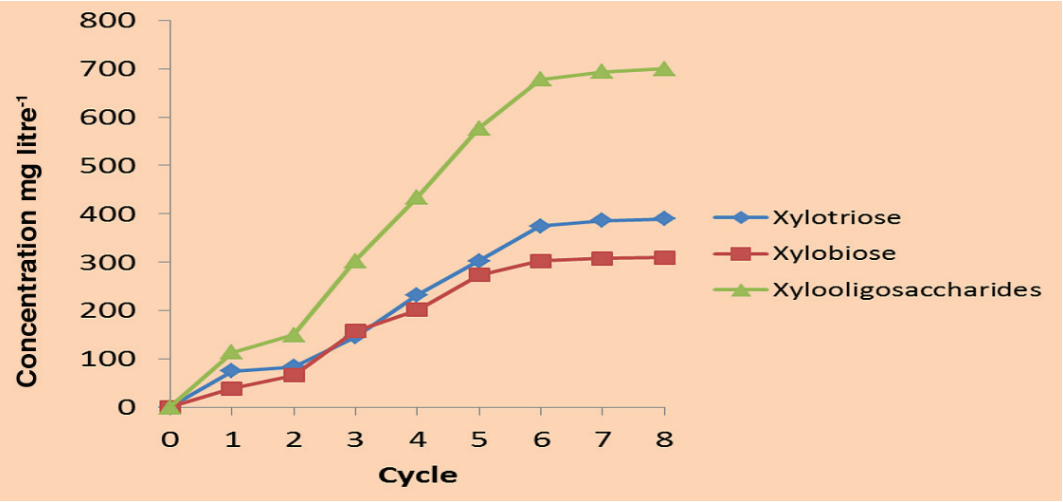

Figure 3. Efficiency of immobilised xylanase usage for the enzymatic XO production from EFB fibres in a PBCR.

ECONOMIC AND MARKET CONSIDERATIONS

XO offer dietary benefits to consumers including fibre-like properties, reducing cholesterol, improving uptake of calcium, and acting as antioxidants (Alonso *et al.*, 2013; Gupta *et al.*, 2012; Seema and Arun, 2011). Palm-based XO can potentially be commercialised, adding value to the production of fine chemicals from oil palm biomass. This can be one of the multi-products derived from bio-refinery of biomass. These products include the extraction of cellulose (glucose) and lignin. In 2015, the price of XO for pharmaceutical and food grades from China is estimated at USD 18-USD 28 kg^{-1}.

CONCLUSION

A high value-added fine-chemical product can be extracted in high amount from EFB fibre using the enzymatic process. The use of immobilised biological enzyme in PBCR to extract XO from palm biomass optimising the cycles of xylanase utilisation for maximum yields and is benign to the environment and profit.

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