CHAPTER 1

INTRODUCTION

1.1 PREAMBLE

(2R,4S)-Pentane-1,2,3,4,5-pentol is otherwise known as xylitol categorized under sugar alcohol based on the organic chemistry classification. Xylitol is as sweet as sucrose, with ~40% fewer calories. Xylitol is naturally found in low concentrations in the fibers of many fruits and can be pulled from various berries, oats, and mushrooms, as good as a fibrous material such as corn cob, sugar cane bagasse (Rao et al. 2006) and Birchwood (Converti et al. 1999). One gram of xylitol contains 2400 cal, as compared to one gram of sugar, which has 3.87 kcal.

In the recent years the commercial interest for xylitol production has markedly increased because of its enormous viable applications. Marti et al. (2008) claimed that xylitol possess approximately 33% fewer calories and it is primary lower-calorie alternative to table sugar. Xylitol is used as a sweetener in medicines, chewing gum and pastilles (Vasilescu et al. 2011). Multiple studies utilizing electron microscopy have shown that xylitol is effective in inducing remineralization of deeper layers of affected enamel (Scheinin & Makinen 1975). Absorbed more slowly than sugar, it does not contribute to high blood sugar levels rather it results into hyperglycemia which is caused by insufficient insulin response. This characteristic has also shown beneficial for people suffering from a common disorder that includes insulin resistance, high blood pressure, hypercholesterolemia and increased
risk with blood clots (Marti et al. 2008). Xylitol has virtually no aftertaste, and is shown as ‘safe for diabetic mellitus and hyperglycemic patients’. This tolerance is attributed to the lower effect of xylitol on a person's blood sugar level compared to that of regular sugar level as it delivers an extremely low glycemic index (GI) of 7 (GI of glucose - 100).

In the human gut, xylitol is not absorbed as that of glucose or fructose; the unabsorbed xylitol acts as a dietary soluble fiber in helping to defend certain aspects of bowel function. Bacterial fermentation, primarily in the large intestine, partly converts xylitol to short-chain fatty acids that the gut can absorb as fuel for energy production in metabolic oxidative pathways. Xylitol has also been useful in recovery after heavy usage because the human metabolic pathways converts absorbed xylitol to glycogen through glucose 6-phosphate. The conversion is however slow, hence that the xylitol amounts to a low-GI source of energy (Chen et al. 2010). Xylitol has no known toxicity in humans. So far, the xylitol has no known toxicity or carcinogenicity and is considered safe by the U.S. FDA. According to Makinen (1976), the participants consumed a diet incorporating a monthly average of 1.5 kg of xylitol with a maximum daily intake of 430 g with no superficial ill effects.

Yeasts have played significant parts in industrial development for thousands of years. Several species of yeast have been employed in various applications such as in food industries, expression system and as models for the study of higher eukaryotes. ‘Rage’ is a popular traditional yeast ‘cake’ used as a starter in making local Malaysian food such as ‘Taipei’ (fermented rice or manioc) and ‘Tempe’ (soybean fermented into a soybean cake). It has been used in baking, brewing and ethanol production. Yeasts have also been used as one of the powerful expression system. Yeasts are unicellular eukaryote which is simple, to cultivate on inexpensive growth media. Unfolded and misfold protein is less risky in yeast system. Yeasts offer
various advantages for heterologous gene expression. They can grow to ultrahigh cell density, secrete proteins extracellularly, grow under non-selective conditions, and perform post-translational modifications such as glycosylation, multimeric assembly, disulphide bond formation and proteolytic cleavage (Siti et al. 2012).

Xylitol can be produced by biological reduction of xylose, a five-carbon sugar, using microorganisms such as *Candida guilliermondii*, *Pichia stiptis*, *Pachysolentannophilus* and *Candida tropicalis*. Xylose is a major component of hemicellulose. Hemicellulose hydrolyzes into complex mixture of sugars that include xylose, galactose, arabinose, glucose and mannose. These sugars may influence xylitol yield and productivity during xylose fermentation. The hardwoods, corn, sugarcane and soybean are low-cost sources of hemicellulose. The composition of hemicellulosic hydrolyzates varies widely depending on the raw materials, hydrolysis procedure and pretreatment methods are employed (Walter et al. 2002).

Fruits and vegetables, which naturally contain xylitol, are not used for xylitol extraction because of their low content (less than 9 mg /g) makes manufacturing expensive. Ergonomics provide an alternative through microorganisms such as bacteria and yeasts that can convert xylose into xylitol, a highly specific chemical process since 80% of the sugar is transformed into sugar alcohol (Gong et al. 1983). It is especially attractive because of its sweetening power comparable to that of sucrose, but offers much fewer calories. Xylitol has sweetness index equal to sucrose, and can replace sucrose on a weight to weight basis. It has a very low viscosity in a solution and a negative heat of solution. With these properties, xylitol has found to have increased applications in food industries.

Yeasts have been shown to induce xylose reductase in the presence of sugars, as this enzyme is needed in the first step for growth on xylose. The
presence of substrate in the medium has been shown to induce activities of xylose reductase and xylitol dehydrogenase. The xylose reductase and xylitol dehydrogenase enzymes were induced in *Pachysolen tannophilus* and *Pichia stipitis* during cell growth in a medium containing xylose, whereas glucose in the medium inhibited induction of the enzymes. The presence of D-xylose and L-arabinose were found to induce aldose reductase and xylitol dehydrogenase activity compared to other pentose and hexose sugars, while the induction by D-xylose was comparatively higher to that of L-arabinose. Enzyme activity in cells grown on D-glucose, D-mannose, and D-galactose were very low (Winkelhausen & Kuzmanova 1988).

Xylitol is produced mainly by chemical processes. This process is a hydrogenation of the five-carbon sugar xylose along with the presence of a nickel catalyst at elevated temperature and pressure. The chemical process has shortcomings such as high cost in process purification. To avoid hostile stages of the chemical processes, biotechnological process were investigated for xylitol production. This alternative production involves bioconversion of xylose to xylitol by microorganisms. To reduce cost and environmental problem, natural biomass from agro-industrial waste can be used as a good source for xylose.

Rao et al. (2004) stated that the interest in xylitol has increased considerably in recent years, due to many commercial applications in diverse industrial sectors like food and pharmaceuticals. As industrial biotechnological production routes to xylitol are expensive they currently represent a small fraction of the market share. Over the past few decades more effort has been devoted to the development of affordable and environmental friendly biotechnological approaches by evaluating cheaper lignocellulosic substrates. Increasing commercial and scientific interest in xylitol has led to a strong demand for such products in the global market, more than 125,000 tons
per annum, with a value that is moderately high (4.5–5.5$/kg for bulk purchase and 20$/kg in daily markets) makes it an attractive proposition for commercialization. Some agricultural residues have relatively high xylose content and low recalcitrance making it a potential source of xylose for the production of xylitol such as sugarcane bagasse and corncob.

1.2 OUTLINE OF PRESENT INVESTIGATION

In this project, low cost, detoxified corncob hydrolyzate medium was prepared for xylitol production using Candida sp. The process will be strengthened to maximize the yield of xylitol by changing the physiological and biotic conditions. The reasons which limits the xylitol availability has been identified as the lack of a reliable, low cost, high quality supplier. The idea behind the concept is to mitigate the sugar dependency in the world and through which serve the mankind globally.

A bioreactor is a device in which a substrate derived from cheap source is utilized by living cells/ enzymes to generate a product of higher value.

All stirred tank reactors deal with heterogeneous systems, i.e. dealing with two or more phases, e.g., liquid, gas and solid. Optimal conditions for fermentation demands efficient transfer of mass, heat and momentum from one phase to other. Chemical engineering principles are engaged for design and operation of bioreactors. But the theoretical explanation usually lags behind technical realization (Stanbury et al. 1995).

A bioreactor should provide the following: (i) agitation; (ii) aeration (for O₂ supply); (iii) regulation of factors like pH, temperature, pressure, inoculum concentration, etc.; (iv) maintenance of sterility; (v) withdrawal of cells/medium (for continuous bioreactors). Modern fermenters
are usually integrated with computers for efficient control over the process and data analysis.

In the present investigation, indigenously designed bioreactor was employed for the production of xylitol with a working volume of 15 litres (~65%). However, the mass transfer limitations were predominant the yeast isolate was tested for xylitol production with the optimized medium and process parameters. The $k_La$ value was used to find out the power requirement for the rotation of agitator shaft.