Xylitol - Low Calorie Sugar from Indian Brewer’s Spent Grain

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Abstract:
Brewers' spent grain (BSG), the main low-value solid residue, is the major by-product of the brewing industry, constituting around 85% of the total by-products generated. Therefore, this raw material is renewable, cheap, and largely available throughout the year and produced in large quantities. Due to the global intense pressure towards green environmental technology, both academic and industrial researchers are putting more efforts to reduce the amount of such wastes by finding alternative uses apart from the current general use as an animal feed. Different pretreatment methods were evaluated for hydrolysis of BSG to obtain the cellulosic and hemicellulosic sugars to explore possible conversion into value added products such as xylitol; Xylitol, a five-carbon sugar alcohol, has similar sweetness as sucrose. It has unique pharmacological properties such as prevention of tooth decay and ear infection in children. It is used as a sugar substitute for diabetic patients. Xylitol is increasingly being used in chewing gums, candy, soft drinks, ice cream and oral hygiene products.

Xylitol occurs naturally in low levels in fruits and vegetables and hence its extraction from this source is non-feasible. Industrial production of xylitol involves catalytic hydrogenation of xylose. From the composition of processed BSG, it was calculated that 60-70% of the initial complex cellulose and hemicellulose sugars were selectively solubilized in one-step sulfuric acid treatment. Pentose sugars were then selectively fermented using Candida spp to produce xylitol. One ton of dry BSG yields 140 - 160 Kg of xylitol.

Key Words: Brewer’s Spent Grain, Hemicellulose, Hydrolysis, Bio- Xylitol

1.1 Introduction
In today’s era people are becoming more and more calorie conscious as a safety measures for maintaining physical fitness. This trend has shifted the research focus on search for low calorie or zero calorie sweetener. These sweeteners are of two type’s viz artificial and natural sweetener. These sweeteners find applications in food, pharmaceuticals and chemicals.

Artificial sweeteners are sucralose, saccharin, acesulfame K, aspartame, stevioside and neotame [1]. They are good sweetener, but their inability to provide bulk to food products limits their applicability. Moreover, non-nutritive sweeteners confer bitter and metallic aftertaste and incapable to give taste of sucrose.

Chemically, polyols are sugar alcohols or polyhydroxyl group containing alcohols. Unlike high-intensity artificial sweeteners, which are used in very small amounts, polyols are used in the same quantity as sucrose. Some of the commercially available polyols are erythritol, lactitol, maltitol, manifold, sorbitol and xylitol. These sweeteners provide fewer calories and result in a much slower and minor rise in blood sugar level. Hence, these are considered as safe for diabetic patients and therefore the products sweetened with these products may legally be labeled “sugar-free”. These health benefits increases importance of usage of polyols as sugar replacers in a variety of products.

One of such important polyol is xylitol. Xylitol is widely consumed in the food, beverages, pharmaceuticals and confectionery. Xylitol serve as humectants, bulking agents, and freeze point depressants. In foods, xylitol widely used in chewing gums, baked food, jellies, candies and ice cream. In pharma, xylitol is used in
toothpaste, mouthwashes, cough syrup and lozenges. Xylitol has received generally regarded as safe (GRAS) status from the US FDA [2].

1.2 History of Xylitol
In 1890, xylitol was discovered in the laboratories of Fisher and Stahe in Germany and Bétrand in France. In 1943, xylitol was found in nature in some plants. In 1962, xylitol metabolic pathway was found in mammalian tissues. Scientists had classified xylitol as polyol. Engineers and chemists started searching for alternative sweetener due to low sugar supply during Second World War. In 1975, The Finnish Sugar Company started the manufacturing of commercial production of xylitol in southern Finland with a capacity of over 3,000 tons/year [3]. At present, xylitol usage is legally approved for use in foods, pharmaceuticals and health products in more than 50 countries [4]. Xylitol market is growing with CAGR of 6% every year and is estimated to be over US$ 1 billion/year by 2020 and priced at US$ 5–6 per kg [5].

1.3 Natural occurrence of xylitol
Xylitol is naturally available in small quantities in many fruits, berries and vegetables [6]. Reineclaudes or yellow plums have the highest content with 1% on dry solids basis. Some of the xylitol containing plants and vegetables are bananas (*Musa sapientum* L.), raspberries, strawberries, reineclaudes, carrots, fresh cauliflower, white mushrooms etc. [6]. Xylitol is also a normal metabolic intermediate of carbohydrate metabolism in man and animals. The normal xylitol concentration in blood is 0.03-0.06 mg/100 ml of blood [7].

1.4 Physical and Chemical Properties of Xylitol
Since xylitol was discovered in laboratories, natural sources and high importance in various industries, the physical and chemical properties of xylitol have been characterized. Due to better properties over the other sugar alcohols, it is used as a preferred sweetener in foods, pharmaceuticals and cosmetics. Physical and chemical properties of xylitol are shown in Table 1.1. Chemical structure for xylose and xylitol are shown in figure 1.1.

<table>
<thead>
<tr>
<th>Sr no</th>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Empirical formula</td>
<td>C₅H₁₂O₅</td>
</tr>
<tr>
<td>2</td>
<td>Molecular weight</td>
<td>152.1 g/mol</td>
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<tr>
<td>3</td>
<td>Appearance</td>
<td>White crystalline powder</td>
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<tr>
<td>4</td>
<td>Test</td>
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</tr>
<tr>
<td>5</td>
<td>Odour</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>Relative Sweetness</td>
<td>Equal to sucrose</td>
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<tr>
<td>7</td>
<td>Caloric value</td>
<td>2.4 cal/g</td>
</tr>
<tr>
<td>8</td>
<td>Optical rotation</td>
<td>Optically inactive</td>
</tr>
<tr>
<td>9</td>
<td>Melting point</td>
<td>93.4-94.7 °C</td>
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<tr>
<td>10</td>
<td>Boiling point</td>
<td>216 °C</td>
</tr>
<tr>
<td>11</td>
<td>Solubility in water(20 °C)</td>
<td>64.2 g /100 ml</td>
</tr>
<tr>
<td>12</td>
<td>Solubility in ethanol</td>
<td>1.2 g / 100 ml</td>
</tr>
<tr>
<td>13</td>
<td>Solubility in methanol</td>
<td>6.0 g / 100 ml</td>
</tr>
</tbody>
</table>
1.5 Applications
US FDA has approved the use of xylitol for special dietary foods. In 1983, JECFA (a Joint Expert Committee of WHO and FAO) announced xylitol as a safe sweetener for foods [8]. It is used as natural sweetener alone or in combination with other sweeteners in the preparation of a wide variety of low calorie or zero calorie products. Addition of xylitol in yoghurts, jams and deserts improves color, texture and taste of product. Applications of xylitol include chewing gum, candies, toffees, ice cream, chocolates, and caramels in foods and in mouth washes, tooth paste, syrup, chewable tablets, dietetic and diabetic foods in pharmaceuticals [3, 5]. Xylitol is not metabolized by the *Streptococci* (especially *Streptococcus mutans*) normally present in the flora of the mouth. *Streptococci* do not produce caries-promoting acids from xylitol. The clinical and field studies have demonstrated the caries-inhibiting effect of xylitol [7].

1.6 Process for xylitol production
Figure 1.2 shows the summary of various technologies used for xylitol production

1.6.1 Extraction
Xylitol is found naturally in fruits and vegetables (lettuce, cauliflower, yellow plums, raspberry, strawberry, grape, banana), as well as in yeast, lichens, seaweed and mushrooms. Xylitol can be extracted from these sources by solid-liquid extraction, but it’s very less concentration in the raw materials (less than 900 mg/100g) [3, 5]. It is not cost competitive to obtain pure xylitol from plant sources due to their own high cost and relatively low concentrations of xylitol.

1.6.2 Chemical process
Currently industrial production of xylitol from xylose is done by chemical process. Chemical process involves chemical hydrogenation or reduction of pure xylose (99.0 % purity) at high temperature and high pressure in specialized equipment using Raney nickel as catalyst. The potential sources of xylose are xylan containing hard woods or soft woods such as birch wood, sugar cane bagasse, straw and corn cobs. Xylose is extracted from lignocellulosic feedstock by acid hydrolysis, after color removal and purification, xylose treated for chemical hydrogenation at 80–140°C and hydrogen pressures up to 50 atmospheres in the presence of metal catalysts (Raney nickel). The step wise description of industrial chemical process is as follows.
Step 1. Acidic hydrolysis of xylose containing lignocellulosic feedstock
Step 2. Xylose hydrolysate purification till desired purity achieved
Step 3. Pure xylose hydrogenation to xylitol using nickel catalyst
Step 4. Purification of xylitol from mixture of polyols
Step 5. Xylitol crystallization

![Figure 1.1 Chemical structures of xylose and xylitol](image)
Although, chemically produced xylitol is similar in structure and properties to the natural substance, this process has some serious issues. The chemical process produces xylitol with only 50% yield from xylose, and xylitol produced is with other polyols due nonselectivity of the process. This xylitol solution requires further purification by chromatography and then concentration and crystallization of the product to obtain pure xylitol [9]. This chemical process requires specialized equipments to get required temperature and pressure, produces toxic chemicals, and creates environmental threats due to harsh conditions. These issues make current industrial process as costly and environment unfriendly [10, 11].

Currently, Danisco (industry of DuPont Company) is a major supplier of xylitol in world. It manufactures xylitol from xylose by chemical hydrogenation using hardwood sources. Danisco, also developed integrated process to produce xylitol from xylose wastes of the pulp and paper industry.

### 1.6.3 Biological process

Biological process for production of xylitol is a promising alternative for chemical production method. It involves two approaches, one is fermentation based using microorganism and other is enzyme based. Fermentation based approach involves use of fungi, yeast, bacteria and recombinants. Enzyme based approach involves use of xylose reductase (XR). The microbiological process uses bacteria, fungi, yeast, and recombinant strains to produce xylitol from pure xylose or a hemicellulosic hydrolysate. Xylose can be used as pure xylose or xylose hydrolysate with or without detoxification. Enzymatic process is discussed in detail as below.

#### 1.6.3.1 Enzymatic process

The enzymatic production of xylitol from pure xylose is a good alternative for chemical production due to complete stochiometric conversion to xylitol. Kitpreechavanich et al. studied the enzymatic conversion of d-xylose into xylitol using xylose reductase (XR) of *Candida pelliculosa* coupled with the oxidoreductase system of *Methanobacterium* sp. and achieved 90% conversion of xylose to xylitol at 35°C and pH 7.5 in only 24 h time period. This reaction completed with stochiometric conversion of xylose and equivalent consumption of NADPH, with successful regeneration of coenzyme using a membrane reactor [12]. Similarly, Neuhauser et al. also studied the fed batch enzymatic conversion of xylose to xylitol using NADH dependent *C. tenuis* XR coupled with formate dehydrogenase (FDH) from *C. boidinii* at pH-controlled enzyme reactor with recycling of enzyme and 2.8 g/L/h productivity [13].

#### 1.6.3.2 Fermentation process

Biotechnological production of xylitol from lignocellulosic waste

Xylose and other sugar such as mannose, galactose, arabinose and rhamnose are obtained from lignocellulosic biomass and finds tremendous applications in both pharmaceutical and food industry. Xylose is second largest available cheap sugar on the earth. Xylose to xylitol conversion process plays significant role in bioprocess refinery concept. Thus, the process development from xylose to xylitol using biomass feedstock is gaining more attention of scientific community.

Figure 1.3 shows a simplistic flowchart for biological production of xylitol from lignocellulosic. Waste generated in agriculture is tested for xylitol production because all these waste generates xylose after acidic hydrolysis method. A pretreatment or hydrolysis step method on these lignocellulosic feedstock releases pentose sugar more susceptible to biotechnological usage. Examples of such pretreatment methods are phosphoric acid [14] and sulphuric acid [15]. The waste treatment with such acids at temperature of above 100 °C, leads to generation of other toxic inhibitor which inhibits the microorganisms, hence detoxification of xylose hydrolysate becomes top most priority before biological treatment.

The fermentative production of xylitol involves usage of microorganism as biocatalyst using yeast, bacteria and fungi. This process uses xylitol production from commercial pure xylose or hemicellulosic hydrolysate with or without detoxification. The production of xylitol using bacteria and fungi has been studied to a lesser extent compared to that using yeast strains. Bacteria studies are *Enterobacter liquefaciens* [16], *Corynebacterium* sp. [17] and *Glucobacter oxydans*. Very few studies are available for filamentous fungi also [18]. Yeasts are studied extensively as compared to bacteria and fungi in last few decades by several researchers, as yeast are good xylitol producers [11, 19, and 20]. Barbosa et al. studied forty four yeast strains of five genera for xylose to xylitol production and found that *Candida guillermondii* and *C. tropicalis* were
the best xylitol producers [21]. These yeasts produced xylitol titer of 77.2 g/L from from 104 g /L of xylose in high cell density fermentation under under aerobic conditions. da Silva and Afschar optimized fermentation conditions in continuous cultivation of *Candida tropicalis* for xylitol production using *C. tropicalis*. They also produced xylitol with 77-80 % yield from 100 g /L d-xylose [22].

![Fig 1.2 Technologies available for xylitol production (Parajo et al, 1998)](image-url)
This study of yeast for xylitol production by various researchers has confirmed *Candida* sp as best xylitol producers and best candidate for further research. In the fermentation process using yeast, the yield of xylitol obtainable from d-xylose is in a range of 65–85% of the theoretical value [11]. Fermentative xylitol production is dependent on certain factors, such as process parameters, expensive nutrients, type or process and huge water consumption. Thus, Industrial application of fermentation process is challenging and time consuming. Overall process time increases due some process activities like sterilization of media, fermenter, seed culture development with substantial input of energy, labor, and time, leading to decreased process productivity. But, It is having advantages over chemical process due to its overall lower cost, selectivity, use of xylose without any purification, milder reaction condition and no any environmental threat due to use of catalyst [10].

Hence, the fermentative production for xylitol is good viable alternative for chemical process but its viability has some challenges such as optimization of process and culture variables, microbial stability, and nutritional factors related to carbon, nitrogen and micronutrients.

### 2.0 Material and Methods

#### Feedstock

BSG was obtained from a local brewery (Bombay Breweries, Taloja Mumbai, India). The BSG obtained was having a moisture content of about 75 %w/w. It was dried at 50°C to moisture content less than 5 wt %w/w. The feedstock material was then stored in clean dry bags until required for processing or analysis. Solvents of
purity 99.5% w/w was purchased from M/s Hayman and M/s Sigma Chemicals; Sulfuric acid (95-98% w/w purity) was also purchased from M/s Fisher Scientific.

**Experimental:**

**Analytical Methods:**

a) Feedstock Analysis: The particle size of reduced to below 1mm using a hammer mill and was subjected to analysis as per standard NREL procedures (11, 12).

The moisture was determined by oven drying at 105°C to constant weight. The mineral components were determined as ash, after incineration at 550°C. The extractives were determined by ethanol extraction using Soxhlet apparatus [13]. Cellulose, hemicelluloses and lignin were determined by acid hydrolysis of the extractive-free material, followed by chromatographic quantification of the sugars and by products in the hydrolysate stream using HPLC system (Agilent, 1200, 1260 USA), equipped with UV-VIS and RI detectors. Chromatographic separation was achieved on a Pb-based column (Bio-Rad, Richmond, CA, USA) at 85°C, using 0.005 M H2SO4, at a flow rate of 0.6 mL/minute. The acid insoluble fraction was by gravimetrically estimated and reported as acid-insoluble lignin [14].

**Pretreatment:**

Brewery Spent Grain was sun dried and milled using roller mill to achieve desire particle size.

The milled spent grain was mixed with water to achieve slurry containing 20% w/w solids. The slurry pH was adjusted to 1.5 and then it was transferred to a high pressure autoclave. It was cooked at the desired temperature for 30 minutes, after which the solid and liquid streams were separated and independently analyzed for cellulose and hemicellulose sugars. The detailed process flow is depicted in Figure.

**Low pH Pretreatment**

- **BSG:** 100 gram +400 Gram of process water
- pH Adjusted to 1.5 using Sulfuric Acid
- **Reactor:** High Pressure Reactor
  - Temperature: 120°C; 140°C, 160°C, 180°C
  - RPM: 500 Retention Time: 30 Minutes
  - Steam flashing at 120°C from the bottom of High Pressure Reactor
  - Cooling of pretreated slurry at 32°C
  - Solid-Liquid Separation by Filtration after cooling
- **Wet Cake:** Washing with 1:1 Hot Water
- First Liquid / Filtrate
- Final Filtrate
- Analysis of Wet Cake and Final Filtrate by NREL Methods
The final filtrate was concentrated to achieve 5.0 % w/w Xylose under vacuum at 60°C using Rota Vapour. This concentrated slurry was further used for Inoculum preparation and fermentation of xylose to xylitol using adapted strain of Candida tropicalis.

2.1 Microorganism and culture conditions
C. tropicalis was procured from NCIM 3123 and further adapted using xylose rich slurry after pretreatment of BSG to achieve maximum conversion efficiency. C. tropicalis was maintained on YM agar and sub cultured after every 4 weeks.

2.4. Inoculum preparation and fermentation
Inoculum (25 ml) was prepared in 100 ml Erlenmeyer flasks, with the following media components (g/l): Xylose 30; yeast extract 10; peptone 20; K2HPO4 0.5; KH2PO4 0.5; MgSO4Æ7H2O 0.5 and ammonium sulphate 2. pH 5.0 and incubated for 24 h on a rotary shaker (250 rpm) at 30°C. After 24 h the cells were recovered by centrifugation.

2.5. Preparation of adapted inoculum for xylitol production
The inoculum was grown on BSG hydrolysate with supplementation of nutrients (g/l): yeast extract 5; peptone 10; K2HPO4 0.5; KH2PO4 0.5; MgSO4Æ7H2O 0.5 and ammonium sulphate 2. The cells were recovered by centrifugation and resuspended in fresh hydrolysate.

2.6. Fermentation medium
Fermentation medium (100 ml) was prepared in 250 ml Erlenmeyer flasks with diluted (until about 50 g/l xylose was obtained) BSG hydrolysate with supplementation of nutrients (g/l): yeast extract 5; peptone 10; K2HPO4 0.5; KH2PO4 0.5; MgSO4Æ7H2O 0.5 and ammonium sulphate 2. Adapted cells (5%, 1.0 OD) were used for inoculation and incubated for 48 h at 30°C on a rotary shaker at 250 rpm.

3.0 Results and Discussion
The mild acid pretreatment at pH and 120 Deg C yields 65 % xylose in filtrate stream with lesser byproducts. The filtrate was neutralized using caustic and further concentrated to achieve 5 % w/w Xylose sugars. In the present investigation BSG hydrolysate is prepared using mild acid pretreatment at lower temperature which contains lesser byproducts and fermentation inhibitors. The yeast strain adapted to this hydrolysate further used for production of Xylitol which yielded 0.7 g/ g of Xylose in 48 hours reaction time.
4.0 Conclusion

It has been demonstrated that Xylitol can be made using Brewery Spent Grain Hydrolysate prepared using very mild pH and temperature conditions. The 0.7 g/g of Xylitol : Xylose yield achieved in 48 hour reaction time with adapted C tropicalis strain. Brewery spent grain explored for Xylitol making which is cost effective and abundantly available feedstock.

References:


