Bioconversion of Lignocellulosic biomass into Xylitol and it’s application

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Abstract

Xylitol could be a present polyalcohol that has an equivalent sweetness and one third of the caloric content once compare to saccharose. The agro-industrial waste deposited within the setting causes issues in nature which will be solved with the utilization and generation of bio products. Xylitol could be a high price polyalcohol created by the reduction of D-xylose (from hemicellulose fraction of lignocellulose) and is utilized in food and pharmaceutical industries. the massive variety of advantageous properties, like its low-calorie sweetening power and anticariogenicity justifies the high industrial interest for xylitol. As a result of have lower caloric value of two.4 cal/g because it is compared to saccharose that has four cal/g, xylitol is taken into account to be as a secure sweetener by the U.S. FDA. (1) Xylitol might be created from second most lush saccharide, xylan made hemicellulose that on chemical reaction produces xylose (2).

Keyword: Agro industrial waste, Hemicellulose, lignocellulosic biomass, Polyalcohol, Xylitol.

Introduction

Xylitol could be a five-carbon polyalcohol or sugar alcohol (alditol) that has an equivalent degree of sweetness as of sucrose. As a result of have lower caloric value of 2.4 cal/g because it is compared to sucrose that has 4 cal/g, xylitol is taken into account to be as a secure sweetener by the U.S. FDA. (1) Xylitol might be created from second most lush saccharide, xylan made hemicellulose that on chemical reaction produces xylose (2).

Xylitol could be a natural sweetener, utilized in several food and medicine applications like soft drinks, ice cream, chew gum and desserts(3). It are often safely consumed by diabetics therefore it's
metabolism do not need hypoglycaemic agent. Xylitol also can be utilized in the diet of diabetics, as a result of it's slowly absorbed through the internal organ system, its initial metabolic steps area unit freelance of hypoglycaemic agent, and it doesn't cause speedy changes in glucose concentration.

D-Xylitol is a five-carbon polyol (five-carbon sugar alcohol), which has the capacity to form complexes with certain cations, including Cu²⁺, Ca²⁺, and Fe²⁺. It displaces water molecules from these metal ions and the hydration layer of proteins. D-Xylitol has attracted worldwide interest because of its unique properties and huge potential. It has almost the same sweetness as sucrose, but lower energy value than sucrose (2.4 cal/g vs. 4.0 cal/g), thus it has been used as a sugar substitute in dietary foods, especially for insulin-deficiency patients. Due to its anticariogenicity, tooth rehardening and remineralisation properties, D-xylitol has been widely applied in the odontological industry. It could also prevent ear and upper respiratory infections and benefit pregnant and nursing women.

It is mandatory to briefly understand the metabolism of carbohydrate in human body for the better understanding of the oral safely of the xylitol. Xylitol is a natural intermediary product which quite often occurs in the glucose metabolism of human and other animals, and also within the metabolism of several plants and micro-organisms. In human, the normal blood xylitol level ranges between 0.03 and 0.06 mg per 100 ml. As an outcome of the benefit with that it is regenerate within the metabolism, xylitol accommodates a low regular-state attention in human blood. The rate of excretion of xylitol in the urine is approximately 0.3 mg per hour; there is no major difference between healthy and diabetic subjects in this sense.

Xylitol production through chemical processes pathway involves high energy usage and cost. Various technique via microorganism biotransformation and catalyst provide a lot of property and environmental friendly feedstock to be used for xylitol production. Lignocellulosic wastes area unit profusely offered, renewable and area unit cheap energy sources typically found in nature. Lignocelluloses comprise of polysaccharide, hemicelluloses and polymer. Hemicellulose content in lignocellulosic biomass is typically within the range of 20–35%, a number of the lignocellulosic raw materials used for the assembly of xylitol in the main embrace corncobs, wheat straw, corn fodder, wheat bran, miscanthus etc. The depletion of natural energy resources and also the environmental impact of their use have semiconductor diode to a growing interest in renewable bio resources and property development. nice efforts and progress are created within the production of bio-based bulk chemicals from renewable bio resources xylitol is an example.

Bioconversion of saccharide to xylitol is often meted out by microorganisms like microorganism, fungi and yeast. Xylitol production is influenced by varied factors in an exceedingly medium. In recent times, important attention has been drawn to the assembly of xylitol from xylose. Fermentation method because the main stage in xylitol production is controlled by many factors together with substrate concentration, carbon supply, salts and gas compounds, inoculums, aeration rate, temperature and pH.

Fungi play a vital role in decomposition of the organic matter through catalyst depolymerisation of the plant plasma membrane, in order that they capable of changing D-xylose to xylitol by same fungi. The study of thin fungi capable of playingbioconversion of D-xylose to xylitol is even considering the high monosaccharide concentration in lignocellulosic residues that area unit cosmopolitan in nature, during which xylose is that the second most lush sugar, once aldohexose. Fungi plays a
vital role within the decomposition of this organic matter through catalyst depolymerisation of plant plasma membrane and future consumption of the degradable product, among them D-xylose\(^{(15)}\).

The great interest in thin fungi stems from their production of enzymes, like the xylanolytic advanced, that's capable of changing xylan-rich lignocellulosic residues to D-xylose with, future xylitol production by bioconversion performed by equivalent fungi \(^{(18)}\). In healthy humans, D-xylitol is metabolized to glucose-6-phosphate through an insulin-independent pathway within the liver and red blood cells. It's a awfully slow metabolism method from D-xylitol to D-glucose, thus during this approach the glucose and also the hypoglycaemic agent concentration raise gently. In insulin-deficiency conditions, D-xylitol might be used as a sugar substitute. Adding the low energy content and also the smaller thermogenic effects, D-xylitol seems to be a horny various for non-insulin dependent diabetics \(^{(16)}\).

Materials and methods

Isolation of Fungal Strain

Garden Soil was collected and serially diluted. Serially diluted soil sample were plated in mineral salt medium which contain 1% xylan. The plated were incubated at 30\(^{\circ}\)C for 7 days.

Lactophenol Cotton Blue Staining

Clean grease free slide was taken and one drop of lacto phenol cotton blue stain was placed. Using tooth pick fungal colonies were placed in the staining solution. Place a cover slip and visualized under microscope.

Pure Culture Technique

The colonies present in the mineral salt medium were again plated in potato dextrose agar medium to facilitate the pure culture of the selected fungal organism. potato dextrose agar plate were incubated at room temperature for 5-7 days.

Substrate Collection

Among various lignocellulosic biomass paddy straw and corn husk were collected in local area of Coimbatore.

Substrate Pre-treatment

The substrate were first washed with distilled water and dried in hot air oven at 80\(^{\circ}\)C. Then substrates were impregnated in 0.25N Hydrochloride acid for overnight to remove lignin content and to remove any microbial lode adhering to the surface of the substrate. After pre-treatment the substrates were dried in hot air oven at the temperature of 80\(^{\circ}\)C for 10-12 hours. After that the pre-treated substrate were milled into small pieces to get hydrolysate.

Substrate Hydrolysate Preparation

The pretreated milled substrate were added with concentrated Hydrochloride acid at the ratio of 1:6 and heated for 121\(^{\circ}\)C for 1 hour. Then ph of the solution was adjusted to 2 by using diluted hydrochloride acid. This will improve proper hydrolysate production. After one hour heating this hydrolysate were filtered by using whatmann no 1 filter paper to remove the unwanted materials.
Detoxification of Hydrolysate

Now this hydrolysate contain many impurities and unwanted toxic substance this was removed by using 2.5% Activated Charcoal. Activated Charcoal was added directly to the hydrolysate and were filtered by using Whatmann no 1 filter paper. Now pure substrate hydrolysates were collected in a pure sterile conical flask. This hydrolysate was stored in refrigerator under 4°C for future use.

Bial's Test

Bial's test is a chemical test for the presence of pentoses. Xylose is a pentose sugar, for qualitative analysis bial’s test was performed. The components present in the bial’s reagent include orcinol, hydrochloric acid, and ferric chloride. 2ml of each hydrolysate (substrate A- paddy straw and substrate B- corn husk) and 3ml bial’s reagent was added in separate labelled test tube. This tubes were heated at 100°C for 4 minutes and cool. observed for the visual color change.

Fermentation

Fermentation was done at laboratory scale level by using shack flask fermentation method., media composition was,

### Table: 1 Media Composition

<table>
<thead>
<tr>
<th>Composition</th>
<th>Gram/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, Sucrose, Glycerol</td>
<td>25</td>
</tr>
<tr>
<td>Substrate hydrolysate</td>
<td>50</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>4</td>
</tr>
<tr>
<td>Potassium dihydrogen orthophosphate</td>
<td>1</td>
</tr>
<tr>
<td>Dipotassium hydrogen orthophosphate</td>
<td>1</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.05</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.50</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 litre</td>
</tr>
</tbody>
</table>

Glycerol, Glucose, Sucrose were sterilized separately by autoclaving at 121°C for 10 minutes and added to remaining sterilized medium prior to inoculation.

Xylanase Activity

Birch wood xylan in acetate buffer was used in xylanase enzyme activity. About 300 μL of 1% xylan and crude enzyme (culture supernatant) was added together, 400 μL of water was added to make up the volume up to 1000 μL and boiled to 50°C for 30 minutes in water bath. This reaction was terminated by adding 1 mL DNSA reagent and kept in boiling water bath for 10 minutes for colour development. A control was kept with the reagent without adding enzyme and absorbance was measured at 540 nm. One IU of activity was expressed as the amount of enzyme required to release 1 μmol of product/min under assay conditions.

Estimation of Xylose

At first 0.5 g of Phloroglucinol was mixed with 100 ml of glacial acetic acid and 10 ml of concentrated HCl to form a coloured reagent. 200 μl of sample was mixed with 5 ml of coloured reagent and heated at 100°C for 4 minutes and cooled down to room temperature and absorbance was measured at 540 nm.
HPLC Analysis

To quantify the amount of xylitol in the fermentation media, High Performance Liquid Chromatography was done under UV visible range at 218 nm. The result was compared with standard xylitol value.

Recovery of Xylitol

After fermentation the fungal cells and spores were removed by using membrane filter apparatus or vacuum filter contain Whatman filter paper. 100 ml the filtered fermentation broth sample was taken in 250ml conical flask with this 2.5 gram of activated charcoal were added and the ph is adjusted to 6.0 with 1M ammonium hydroxide. The flask was placed in a shaker bath at 80°C for one hour at 100 rpm. The mixture was cooled to room temperature and the activated carbon was vacuum filtered with Whatman filter paper. Filtrate was kept in evaporator at 40°C under reduced pressure. The concentrated mixture was cooled and 0.5 mg xylitol was seeded in the filtrate and placed in refrigerator at 8°C for 1 week.

Antimicrobial Activity

Two set of sterial muller hinton broth was prepare in test tube. One was prepare without xylitol and another with 18% xylitol. Organisms were inoculated in the sterile broth. Organisms are E.coli, Pseudomonas sp, Staphylococcus aureus, Streptococcus mutans ,Candida sp. tubes were kept for incubation at 37°C for 3 days, and every day OD value was taken. Growth was represented as turbidity which measured by OD value. Candida sp tube were incubated at room temperature and kept for 7 day every alternative days OD value was taken and tabulated.

Results

Isolation of Fungal Species

Fungal species were identified in the Potato Dextrose Agar media contain xylan.

Lactopheol Cotton Blue Staining

Lactopenhol cotton blue staining was done under oil emersion microscope two fungal stain was identified they are Aspergillus sp

![Figure 1. Aspergillus sp](image-url)
Pure Culture Technique

*Aspergillus sp* was grown in sterile potato dextrose agar medium after 5-7 days incubation. *Aspergillus sp* appear as white color in initial stage and turn into black in color in later incubation period.

![Aspergillus sp](image)

**Figure 2. Aspergillus sp**

Substrate Hydrolysate Production

By using pre-treatment, detoxification and filtration method substrate hydrolysate were prepared and stored in refrigerator for fermentation and future work.

![substrate hydrolysate](image)

[a] [b]

**Figure 3. [a]Paddy straw hydrolysate [b] Corn husk Hydrolysate**

Bial’s Test

Blue or green coloration was occur in both tubes contain baddy straw and corn hush substrate hydrolysate which is compared with commercial glucose and xylose as negative and positive control respectively.
Xylanase Activity
Xylanase activity was measured and tabulated,

Table: 2 Xylanase Activity

<table>
<thead>
<tr>
<th>Substrate and fungal organism</th>
<th>1st day</th>
<th>3rd day</th>
<th>5th day</th>
<th>7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paddy straw <em>Aspergillus</em> sp</td>
<td>0.359</td>
<td>0.649</td>
<td>0.956</td>
<td>1.004</td>
</tr>
<tr>
<td>Corn husk <em>Aspergillus</em> sp</td>
<td>0.375</td>
<td>0.718</td>
<td>0.942</td>
<td>1.016</td>
</tr>
</tbody>
</table>

Estimation Of Xylose
Xylose content in the fermentation broth was measured in alternative days and OD value was taken.

Table: 3 Estimation Of Xylose

<table>
<thead>
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</table>

HPLC Analysis
HPLC analysis was done for each fermentation broth and the result was observed as,
Xylitol Recovery
Xylitol was recovered from the fermentation broth and observed.

Figure 6. Xylitol Recovery [a] Activated charcoal treatment, [b] Vacuum filter, [c] Evaporator, [d] Concentrated liquid
Antimicrobial Activity

When compared with broth culture without and with xylitol, culture broth with xylitol shows significant amount of reduction in the growth was identified by spectroscopic OD value

Table: 3 Anti bacterial assay:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Without xylitol</th>
<th>With xylitol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h 48h 72h</td>
<td>24h 48h 72h</td>
</tr>
<tr>
<td>E.coli</td>
<td>1.031 1.149 1.205</td>
<td>0.928 0.961 1.036</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>0.584 0.753 0.990</td>
<td>0.394 0.506 0.744</td>
</tr>
<tr>
<td>Pseudomonas sp</td>
<td>0.494 0.651 0.816</td>
<td>0.274 0.485 0.701</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.887 1.235 1.558</td>
<td>0.192 0.387 0.799</td>
</tr>
</tbody>
</table>

Table: 4 Anti fungal assay

<table>
<thead>
<tr>
<th>Organism</th>
<th>without xylitol</th>
<th>With xylitol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd d 5th d 7th d</td>
<td>3rd d 5th d 7th d</td>
</tr>
<tr>
<td>Candida sp</td>
<td>0.415 0.484 0.503</td>
<td>0.290 0.325 0.378</td>
</tr>
</tbody>
</table>

Summary and conclusion

Xylitol (five carbon sugar alcohol), an artificial sweetener, could be produced from second most abundant polysaccharide, xylan rich hemicellulose which on hydrolysis produces xylose. It is a sugar substitute used in dietary, food and pharmaceutical industries due to its properties like low energy content, anticariogenicity, tooth rehardening, preventive against otitis, ear and upper respiratory infections etc.

Xylitol is being used as a highly valued ingredient with some interesting and useful properties in food and pharmaceutical products. It can be produced from xylose-rich (hemicellulose fraction of lignocellulose) resources by microbial bioconversion process. Some Microorganisms have the ability to convert xylose into xylitol. Aspergillus sp was selected for the production of xylitol.

Among various lignocellulosic biomass Paddy straw and corn hush were selected as substrate for fermentation. Using these kind of cheaper carbon source fermentation was carried out by fungal strains. To identify that xylose present in the substrate hydrolysate Bial’s test was performed. In this test blue or green color represent the presence of pentose sugar such as xylose. Fermentation was carried out 7 days, during these days the enzyme which is used to degrade xylose is called xylanase activity also measured by using commercial xylan. Other than this xylose concentration also measured by using Phloroglucinol regent and OD value was taken.

After fermentation xylitol was recovered by using activated charcoal treatment and vacuum filtration method. Then 0.5mg of xylitol were seeded in the recovery unit and kept in refrigerator for one week to facilitate crystallization of xylitol. To screening the concentration of xylitol HPLC was done in UV-visible detector at 218nm.

Xylitol have antimicrobial activity against E.coli, streptococcus mutans, Pseudomonas sp, Staphylococcus aureus. By antimicrobial assay method in this broth without xylitol and with xylitol prepared and Optical Density was measured by using spectrooscope. Significant reduction in the growth
was observed the culture contain xylitol. Xylitol also have anti dehydrating agent in fruits this was represented as dipping the fruits such as strawberry and cucumber in a xylitol concentrating solution and without any dipping control was prepared and wait for visible characteristic change. Xylitol retain the freshness of the fruits compared with control.

Acknowledgement

First let submit myself to god almighty for the bountiful blessings. Foremost, I would like to express my sincere thanks to Principal, Hindusthan College of Arts and Science, Dr. PONNUSAMY and I express my deep sense of gratitude and profound thanks to Mrs. Saraswathi khannaiyan, secretary and Mrs. PRIYA SATHEESH PRABHU, Joint secretary of Hindusthan College of Arts and Science, Coimbatore for their support.

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