Production of an environmentally friendly, pressed composite material from straw using biological binders developed from modified residual beer yeast and spent microbial culture media containing the polysaccharide levan

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Abstract
This study shows the possibility of producing compressed biocomposite materials made from pressed straw without the use of phenol-formaldehyde resins. We used a biological binder consisting of modified residual beer yeast and spent culture media containing a microbial levan polysaccharide, which was obtained from an Azotobacter vinelandii culture. The physical and mechanical properties of the pressed straw depended on the type of biobinder used as well as on the conditions surrounding the preparation of the straw and the biocomposite material pressing. Removal of the fat-wax shell from the straw improved the characteristics of the material. Pressed materials obtained using the spent culture media as a biological binder were more water-resistant than materials obtained using modified residual beer yeast. Swelling, as measured by thickness, was 38% and 62% using the former and latter biobinders, respectively. The ultimate bending strengths of the pressed materials prepared using the modified residual beer yeast and spent culture media were 13.7 and 13.9 MPa, respectively. The addition of paraffin to the biological binder mixtures prior to pressing improved the water resistance of the pressed materials but reduced the ultimate bending strength. The addition of glutaraldehyde to the modified residual beer yeast improved the water resistance of the resulting biocomposite materials and did not affect its ultimate bending strength. All samples did not emit methanol or phenol, but there was emission of ammonia and formaldehyde at concentrations that did not exceed the threshold limit value. Obtained biocomposites could be used as the materials with heat-insulating properties.

1. Introduction
In many countries, the manufacture of particle boards is a traditional use for wood. However, the negative impact of mass deforestation has caused producers from these countries to search for alternate raw materials, such as lignocellulose-containing materials from agricultural wastes such as straw. The quantity of lignocellulose-containing raw materials produced worldwide amounts to millions of tons each year [1; 2]. The chemical composition of straw is similar to wood. In cereal straw, the lignin content is 12-20% by weight, which is considered high compared to deciduous tree wood, which has a lignin content of 14-25% [3; 4; 5].

The average market price of straw is several times less than wood, and considerably less money is required to crush and dry straw. In recent years, cereal straw has become the main raw material used for the manufacture of pressed biocomposite materials with heat-insulating properties, which can be used for constructing ecologically safe houses. For example, in the USA, cereal straw is considered the second most suitable agricultural fibre after bagasse for the manufacture of wood composites [6]. There are works which are devoted to studying of sound absorption coefficient and other characteristics of a composite material based on fibers of rice straw and polypropylene [7], influence of the fiber content of straw of rice and wheat on the sound
absorption, thermal conductivity and compressive strength of rigid polyurethane foams [8], and also developments and estimation of efficiency of new heat-insulating material from rice straw with use of high-frequency hot pressing [9]. However, biocomposite materials made from straw have very low durability and collapse at high humidity. The use of phenol-formaldehyde and other synthetic resins are traditionally used as binding agents, which is undesirable since they emit toxic phenol and formaldehyde into the environment [10; 11; 12; 13]. This problem can be solved by the complete replacement of toxic binding agents with natural ones [15] or by decreasing the amount of synthetic binding agents used by means of the addition of other agents [14]. The use of natural protein and polysaccharide-based binding agents is limited due to their high cost.

A number of studies have shown that some pressed materials can be generated from wood waste (sawdust, wood chips, and shavings) using nontoxic protein- and polysaccharide-containing binding agents [16; 17; 18; 19]. However, the use of straw as a raw material for the production of chipboards is restricted by the presence of wax, which has a complex chemical composition. Wax is not distributed evenly in straw, as it is in wood, and is almost completely localized in the surface layers of the straw stalks. The presence of this anti-adhesion layer on the surface of straw particles interferes with the surface wetting of particles and impairs gluing. The use of straw particles in the production of chipboards is still possible but will require additional scientific investigation.

Therefore, the objective of the present research was to optimize the pressing conditions and modify straw particles for use in the manufacture of biocomposite materials using nontoxic bio-binding adhesives.

2. Material and Method

2.1 Materials

Rye grass straw (Secále cereálé) was used as the main raw material for the manufacture of pressed biocomposites. Straw was bought from the research farm "Yalga" at the Mordovian Research Institute of Agriculture. Residual beer yeast was obtained from JSC SUN INBEV (Saransk, the Republic of Mordovia, Russia). Nutrients used to make the cultivation media for the A. vinelandii D-08 strain were obtained from the Romodanovsky Sugar Plant, LLC, (Republic of Mordovia, Russia); the Kemlyansky Distillery, LLC, (Republic of Mordovia, Russia); and the PJSC Saransky Dairy (Saransk, Republic of Mordovia, Russia). The A. vinelandii D-08 strain was acquired from the Russian National Collection of Industrial Microorganisms, Moscow, Russia (http://www.genetika.ru/vkpm/) (VKPM) and has the following registration number: VKPM B-5787.

2.2 Generation of biological binders

A biological binder was prepared from residual beer yeast and the spent culture media containing a levan polysaccharide. Residual beer yeast was subjected to treatment with a sodium hydroxide solution at a ratio of 1:1 for 30 minutes. After treatment, the yeast cells were pelleted by centrifugation at 6000 rpm for 15 minutes and the supernatant was discarded [20; 21]. The residual beer yeast was then used as a base for the biobinder, to which glycerol and boric acid were added as a plasticizer and antiseptic, respectively.

The spent culture media containing a levan polysaccharide was generated by cultivating the A. vinelandii D-08 strain in a medium containing food industry waste products, including molasses, distillery dregs and lactoserum. The medium was inoculated with a 10% inoculum. Bacterial growth was carried out using a temperature controlled shaker incubator (ES-20/60; BioSan, Riga, Latvia) in conical flasks for 72 hours at 28°C and 250 rpm. A total of 1% (w/w) of boric acid was added as an antiseptic to the resulting spent culture media.

2.3 Estimation of the physical and chemical properties of the biological binders

The yield of the levan polysaccharide was determined by precipitation of the polysaccharide from the spent culture media with 96% ethanol at a ratio of 1:2, with subsequent drying of the pellet at a temperature of 105±2.5°C until a constant weight was reached [22]. The biomass was determined by measuring the dry cell weight. The protein concentration was determined by the Bradford method [23]. The viscosity of the biobinder was determined with a portable rotor viscometer (Viscotester VT-04F; RION, Tokyo, Japan). Identification of the levan polysaccharide was carried out by Fourier transform infrared spectroscopy (IR Prestige-21; Shimadzu Corporation, Tokyo, Japan) and processed by means of the IR solution programme.
2.4 Biocomposite preparation

To prepare the pressed biocomposite materials, the straw was ground on a planetary ball grinder (Retsch PM 400; RETSCH, Haan, Germany) at 380 rpm for 40 seconds to obtain particles with a size of 5-10x2.4x0.5-1.0 cm. The mixture used for pressing contained 100 g of ground straw with a moisture content of 4-5% mixed with 50 ml of spent culture media or 70 ml of modified residual beer yeast. The biobinder was evenly distributed on the surface of the straw by atomization (SM-BH; LLC PromTehSnab (ILTEK-prom), Moscow, Russia). Exsiccation of the mixture used for pressing was carried out in a drying chamber at a temperature of 70°C until a moisture content of 6-8% was reached. To increase the water resistance of the resulting biocomposite materials prepared with the modified residual beer yeast, glutaraldehyde and paraffin were added to the mixture for before pressing. The moisture content of the straw that was added to the mixture was measured by a thermogravimetric moisture analyser (MS-70; A.N.D., Tokyo, Japan).

The resulting mixture was loaded into a compression mould (5×15 cm). After a cold prepressing (mixture was pressed at room temperature at the pressure of 0.5 MPa for 1 min). The mixture was subjected to a hot pressing for 10 minutes on a hydraulic press (Gotech-7014-A50; Gotech Testing Machines Inc., Taipei, Taiwan) at 140-160°C with a pressure of 26.1-39.2 MPa (pressure from 2 to 5 MPa per 1 mm of thickness of composite material).

2.5 Determination of the physical-mechanical properties of the bio composites

The samples of pressed biocomposite materials were tested to determine their physical-mechanical properties and were compared to GOST 10632-2014 requirements. The ultimate bending strength was determined according to the GOST 10635-88 using a universal testing machine (Gotech AI-7000 M; Gotech Testing Machines Inc., Taipei, Taiwan). The swelling of the pressed biocomposite materials and their water absorption was determined according to GOST 10634-88. The density of the materials was measured on an automatic high-resolution densitometer (H-300S; Hildebrand, Taiwan-Germany). The distribution of straw and the binding of the biocomposite materials were investigated using a scanning electron microscope (Hitachi Tabletop SEM TM, 300; Hitachi, Tokyo, Japan). The emission of phenol and formaldehyde by the pressed biocomposite materials was determined using a gas-chromatograph (Shimadzu GC-2010 Plus; Shimadzu Co, Japan). The emission of ammonia and methanol was determined by spectrophotometry (Shimadzu UV-3600; Shimadzu Co, Japan) [24].

3. Results and discussion

Our investigation showed that the physical, chemical and adhesive properties of the spent culture media and modified residual beer yeast biobinder depended on the conditions surrounding their preparation. The yield of the levan polysaccharide and the spent culture media viscosity from the A. vinelandii culture strongly depended on the ratio of the components used in the medium (molasses, distillery dregs and lactoserum). Incubation of A. vinelandii in a medium containing molasses, distillery dregs and lactoserum at a 5:2:3 ratio (by mass, respectively) for 48 hours resulted in the accumulation of 14.5 g/l of levan. The viscosity of the spent culture media was 0.4 DPa*sec. The protein content and the amount of biomass in the spent culture media was 2.80 mg/ml and 13.54 g/l, respectively.

A. vinelandii strains are known to produce two polysaccharides, levan and alginate. According to the Russian National Collection of Industrial Microorganisms, the A. vinelandii D-08 strain produces levan when cultured in the standard reference medium. However, in our work, we used a medium consisting of molasses, distillery dregs and lactoserum, which resulted in the synthesis of a levan polysaccharide as determined by infrared spectroscopy, which is one of the most efficient methods for substance identification. We carried out a comparative analysis of the infrared spectra of the levan polysaccharide and commercial alginate, the results of which are presented in Figure 1. The infrared spectra of alginate and the levan polysaccharide had absorption bands that are typical of all polysaccharides. The alginate infrared spectrum (Figure 1, Curve 1) had absorption bands at 1616 cm⁻¹ and 1419 cm⁻¹ that are caused by presence of deprotonated carboxyl groups. It is known that alginate consists of D-mannuronic and guluronic acid residues covalently linked in different sequences or blocks. The infrared spectrum of the levan polysaccharide did not have bands typical for carboxyl groups, but...
there were absorption bands at 923 cm\(^{-1}\) and 830 cm\(^{-1}\) that are caused by the vibrations of \(\nu(\text{CO})\) groups, demonstrating the presence of furanose rings typical of levan [25; 26].

![Figure 1: Fourier transform infrared spectroscopy (FTIR-spectra) of a commercial polysaccharide alginate (1) and the polysaccharide levan (2) produced by \(A.\ vinelandii\ D-08\)](image)

Thus, a comparative analysis of the infrared spectra of the levan polysaccharide and alginate showed that the cultivation of \(A.\ vinelandii\) of D-08 in medium containing food industry waste resulted in the synthesis of a levan polysaccharide.

The adhesive properties and viscosity of the modified residual beer yeast depended on the sodium hydroxide treatment conditions used. The best adhesive properties and highest viscosity resulted from using a 4% sodium hydroxide treatment. Under these conditions, the viscosity of the residual beer yeast was 40-120 DPa*sec, the strength of the adhesive joint of the paper was 421.8 N/m, and the tensile strength of the adhesive joint of wood was 11.7 MPa.

Our previous studies have shown that the yeast biomass at an appropriate chemical modification acquires adhesive properties and this modified yeast biomass can be used for bonding paper, fabrics and wood [20; 27]. But glue based on the only modified yeast biomass has low water resistance. To increase water resistance of the glue prepared from modified yeast biomass glutaraldehyde were added to glue composition. Glutaraldehyde has two functional groups that can react with amino groups forming azomethine bonds [28]. Our research has shown that addition of the glutaraldehyde can improve the moisture resistance of the adhesive joint, water resistance and reduces the time of the bonding due to the cross-linking of proteins.

In our previous papers, we showed that biobinders with such characteristics could be used for preparing pressed materials made from wood waste using the following conditions: a temperature of 140-160°C, a 5-10 minutes pressing time and a pressure of 30 tons (5 MPa per 1 mm of material thickness). To prepare pressed biocomposite materials made from straw, 70 ml of the modified residual beer yeast suspension (having a viscosity of 1.5 DPa*sec) and 50 ml of spent culture media per 100 g of straw was used. The spent culture media contained 14.5 g/l of levan and had a viscosity of 0.4 DPa*sec [15; 17; 18; 19; 26; 29; 30]. Using of the combined culture medium consisting of molasses, distillery dreg and lactoserum can be explained with the fact that proteins of distillery dreg and lactoserum as a part of culture liquid were capable to act as adhesive and to connect wood particles [20]. The physical-mechanical properties and images of the resulting biocomposites are presented in Table 1 and Figure 2.

Our investigation showed that materials generated from pressed straw prepared without biobinder had very low durability. Even small deformations of the pressed material caused garneting and the destruction of the
material (Figure 2a). When such materials were placed in water they were completely destroyed; thus, it was impossible to determine the swelling by the thickness of these materials (Table 1). The addition of a biobinder resulted in an increase in adhesion between the straw particles and in the durability and density of the pressed materials, but the straw particles could still be easily separated from each other (Figure 2b, 2c). Visual observations and crushing of the pressed materials showed that the adhesion between the straw particles was still weak and, essentially, there were no changes in the straw structure. Increasing the pressure, time and temperature during pressing resulted in an increase in durability and density of the pressed materials. The best results were generated using a pressure of 39.2 MPa and a temperature of 160°C. When the modified residual beer yeast or spent culture media were used as biobinders, the durabilities of the pressed materials were 10.7 MPa and 9.9 MPa, respectively. These values were less than those obtained for the pressed materials prepared from wood waste using the same biobinders [16; 18; 19; 31; 32; 33; 34].

Our investigations and those of other groups showed that hot pressing wood waste in the presence of a biobinder causes changes in the infrared spectra of these materials that indicate the formation of new bonds between the binder and degradation products, such as lignin and hemicellulose, due to polycondensation and polymerization reactions [16; 18; 19]. The chemical compositions of cereal straw and wood do not differ significantly [35]. Therefore, we assumed that the biobinder would interact with the straw particles if the same physical and chemical processes occurred.

However, it is known that cereal straw is unlike wood in that it is covered with a moistened wax shell [20]. Data suggests that even the partial replacement of wood with straw in pressed materials sharply decreases the physical-mechanical properties of the pressed materials. Apparently, even at high temperatures, the wax pushes away water-based biobinders, thereby significantly reducing the adhesion bond between wood particles and components with the biobinder [35]. Increasing the temperature above 180°C was also ineffective due to carbonization and darkening of the pressed materials. Therefore, before mixing the straw with the biobinder, we carried out a physical and chemical pretreatment of the straw to remove the wax shell [10]. The straw was boiled in water for 30-40 minutes to remove the fat-wax shell and then was mixed with the biobinder. The mixture used for pressing was prepared in the same way that the untreated straw mixture was prepared.

![Figure 2](image-url)
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Samples of materials from pressed straw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without biobinder</td>
</tr>
<tr>
<td></td>
<td>Pressure 26.1 MPa</td>
</tr>
<tr>
<td></td>
<td>140°С</td>
</tr>
<tr>
<td>Ultimate bending strength, MPa</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>Density, kg/m³</td>
<td>710±25</td>
</tr>
<tr>
<td>Swelling in water by thickness, %</td>
<td>*-</td>
</tr>
</tbody>
</table>
Examination of the received materials was shown that appearance (Figure 2d, 2e) and physical-mechanical properties of pressed biocomposite materials from straw were significantly improved (Table 1). Bending strength of the best samples of pressed materials prepared from the pretreated straw and modified residual beer yeast or culture liquid containing a polysaccharide levan at pressure of 39.2 MPa and temperature 160°C was 13.7 and 13.9 MPa, respectively. At the same time density of pressed biocomposite materials from straw received with using of culture liquid containing a polysaccharide levan was higher and swelling on thickness was lower as compared with pressed biocomposite materials from straw received with using of modified residual beer yeast. As it was stated above the basis of biobinder from modified residual beer yeast consisted of water-soluble proteins whereas basis of biobinder from culture liquid was presented with a polysaccharide levan.

According to the literature and research results of Montana Polysaccharide Corp., levan consists of fructose residues packed into a spherical structure that does not swell well in water and is capable of forming a crystal structure [35]. It is possible that the increased moisture resistance of the materials generated using spent culture media can be explained by the fact that at high pressure and temperature there was a condensation reaction between levan and the lignin and cellulose components of the straw. The pressing conditions resulted in the formation of levan microcrystals that penetrated into micropores of the cellulose, thereby reducing the water absorption [18; 25; 26; 37; 38]. High swelling values, as determined by measuring thickness (water absorption), were observed when modified residual beer yeast that was used as a biobinder was bound to degraded glue bonds of the hydrophilic biobinder during the course of swelling of the wood particles in wood-particle boards. This phenomenon is called "breakup". According to the literature, paraffin and other components can be added to the mixture used for pressing to increase the moisture resistance of the resulting wood-particle board [39]. Therefore, to decrease the water absorption and swelling of the resulting material, the mixture used for pressing was hydrophobized by adding paraffin to the mixture at a ratio of 1:10 and by the addition of a 5% solution of a glutaraldehyde.

Our investigation showed that the addition of glutaraldehyde before pressing did not greatly affect the strength characteristics of the resulting pressed biocomposite materials but did sharply decrease the swelling on thickness of the material (Table 2).

Table 2: Impact of hydrophobic additives, such as paraffin and glutaraldehyde, on the physical-mechanical properties of the biocomposite materials made from straw pressed with a pressure of 39.2 MPa, at 160°C for 10 minutes.

<table>
<thead>
<tr>
<th>Type of pressed materials from straw</th>
<th>Density, kg/m³</th>
<th>Ultimate bending strength, MPa</th>
<th>Swelling in water by thickness, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material from straw pressed with biobinder from modified residual beer yeast and without additives</td>
<td>1154±32</td>
<td>13.7±0.3</td>
<td>62±2</td>
</tr>
<tr>
<td>Material from straw pressed with biobinder from modified residual beer yeast and with paraffin</td>
<td>954±22</td>
<td>9.7±0.3</td>
<td>32±2</td>
</tr>
<tr>
<td>Material from straw pressed with biobinder from modified residual beer yeast and with glutaraldehyde</td>
<td>1104±38</td>
<td>14.1±0.3</td>
<td>22±2</td>
</tr>
<tr>
<td>Material from straw pressed with biobinder from spent microbial culture media and without additives</td>
<td>1296±41</td>
<td>13.9±1.8</td>
<td>38±2</td>
</tr>
<tr>
<td>Material from straw pressed with biobinder from spent microbial culture media and with paraffin</td>
<td>1096±47</td>
<td>10.9±0.8</td>
<td>23±2</td>
</tr>
</tbody>
</table>
The addition of paraffin slightly increased water resistance of the pressed biocomposite material, but, at the same time, the bending strength was decreased when modified residual beer yeast and spent culture media were used as biobinders (Figure 2). It is possible it was bound to the paraffin as the hydrophobic substance reduced the adhesion and cohesive covalent and physical interactions between the hydrophilic biobinder and the straw particles. An analysis of the amount of the toxic substances emitted showed that all the pressed biocomposite material from straw samples did not emit any detectable amounts of phenol or methanol, but formaldehyde and ammonia were emitted and were found at concentrations that did not exceed the threshold limit values (Table 3).

Table 3: Emission of formaldehyde and ammonia from biocomposite materials made from straw pressed with a pressure of 39.2 MPa, at 160°C for 10 minutes.

<table>
<thead>
<tr>
<th>Type of pressed materials from straw</th>
<th>Formaldehyde emission, mg/m³, TLV-0.035</th>
<th>Ammonia emission, mg/m³, TLV-0.200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material from straw pressed without biobinder and without additives</td>
<td>0.005±0.001</td>
<td>0.037±0.005</td>
</tr>
<tr>
<td>Material from straw pressed with biobinder from modified residual beer yeast and without additives</td>
<td>0.009±0.002</td>
<td>0.115±0.005</td>
</tr>
<tr>
<td>Material from straw pressed with biobinder from modified residual beer yeast and with paraffin</td>
<td>0.009±0.002</td>
<td>0.109±0.009</td>
</tr>
<tr>
<td>Material from straw pressed with biobinder from modified residual beer yeast and with glutaraldehyde</td>
<td>0.017±0.003</td>
<td>0.134±0.011</td>
</tr>
<tr>
<td>Material from straw pressed with biobinder from spent microbial culture media and without additives</td>
<td>0.011±0.004</td>
<td>0.065±0.007</td>
</tr>
<tr>
<td>Material from straw pressed with biobinder from spent microbial culture media and with paraffin</td>
<td>0.012±0.002</td>
<td>0.059±0.007</td>
</tr>
</tbody>
</table>

For the comparative morphological analysis and the establishment of processes for the agglomeration of straw particles with the biological adhesive, scanning electron microscopy was used. Figure 3a shows the samples of the pressed biocomposite materials made from straw that were generated without addition of a biobinder and without pretreatment of the straw by boiling in water. Adhesion of the straw particles was almost undetectable. Only small amounts of agglomeration of the straw particles was observed as a result of the high temperature and pressure used, and the partial degradation of lignocellulose from the straw during pressing process was observed. Numerous microcracks and empty spaces were observed, practically over the entire surface of the material. Figure 3b shows that the biobinder poorly mixed with the raw straw and during the pressing process was partially squeezed out onto the surface of the pressed material. In the materials generated using the treated straw, the biobinder was distributed evenly throughout the pressed materials (Figure 3c). In the presence of paraffin, the microstructure of the bioplastics was similar to the microstructure of the materials received from the raw straw. However, the paraffin was poorly mixed with the biobinder, and it was unevenly distributed, resulting in the formation of separate lumps (Figure 3d).

Thus, cultivation of A. vinelandii D-08 to produce a levan polysaccharide in media containing food industry waste and modification of residual beer yeast allowed for the generation of a biobinder that can be used for the production of ecologically safe pressed materials made from straw. The materials have sufficient durability, and they can be used for the manufacture of furniture and materials with heat- and sound-insulating properties for use in enclosed areas with low humidity.
Conclusions

The conditions used to produce materials made from pressed cereal straw using a biological binder from modified residual beer yeast- and levan-containing culture media, without the use of toxic phenol-formaldehyde resins, were optimized. Physical-mechanical properties of the resulting materials made from the pressed straw depended on the preparation conditions of the mixture components and pressing conditions. Preliminary removal of the hydrophobic shell from the surface of straw increased the durability of the received materials up to 13.9 MPa, which is comparable to standard materials. The addition of paraffin or a glutaraldehyde increased the moisture resistance of the pressed materials depending on the type of biobinder used. No samples emitted methanol or phenol, but the emission of ammonia and formaldehyde at concentrations that did not exceed the threshold limit value was observed. However, density and water absorption of the resulting pressed materials did not correspond to standards. The resulting pressed materials made from straw are ecologically safe and are low-cost, as they are obtained from food industry waste products. They may find applications as cheap heat- and sound-insulating pressed materials for use in enclosed spaces with low humidity.

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