Release of Fermentable Sugars from Corn Silage – The Effect of Biological Pretreatment

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Lignocellulose biomass, as a renewable and biodegradable carbon source, provides a wide range of valuable bioproducts. Their utilization requires an efficient conversion process to break down the complex and variable chemical structure of lignocellulose. In this work, a solid-state fermentation-based pretreatment method for the release of fermentable sugars from corn silage was investigated. The optimal process conditions for water-soluble sugar extraction were initially explored by response surface methodology with the aim to achieve the maximum sugar concentration in the extracts. The optimal extraction conditions were determined: t = 30 min; the liquid-solid ratio L/S = 25 mL g^{-1}; n = 170 rpm; and T = 30 °C. The changes in the content of water-soluble sugars (glucose, fructose, sucrose, maltose, maltotriose) were analyzed during seven days of fungal treatment.

Keywords
solid-state fermentation, corn silage, *Trametes versicolor*, fermentable sugars, response surface methodology

Introduction

Plant biomass is a lignocellulosic material consisting of a complex matrix, containing both polymers (cellulose, hemicellulose, and lignin) and valuable extractable biochemicals (e.g., simple sugars, amino acids, polyphenols, pigments, etc.). The vast amount of residual plant biomass (global annual production is on the order of 140 Gt) that is considered "waste" can potentially be converted into a wide range of value-added products and bioenergy.[1,2] Lignocellulosic biomass consists of an amorphous matrix of hemicellulose and lignin in which cellulose microfibrils are dispersed. Cellulose forms about one-third to one-half of plant tissue. It is comprised of D-glucose subunits linked by β-1,4-glycosidic bonds. Hemicellulose is a heteropolysaccharide and therefore contains various sugar monomers (glucose, mannose, galactose, xylose, and ribose) and carbohydrate derivatives (e.g., galacturonic acid). The cellulose microfibrils are interconnected by intra- and intermolecular hydrogen bonds and surrounded by hemicellulose. The cellulose-hemicellulose matrices are surrounded by lignin, an amorphous, water-insoluble polymer consisting of a complex network of aromatic alcohols. As a result, these complex networks are strongly interconnected and difficult to dissolute, which impedes the microbial attack on internal cellulosic structures.[3]

Any approach intended to use cellulosic material for feed or bioethanol must include the ability to efficiently convert the polysaccharide components (cellulose and hemicellulose) into simple sugars. Therefore, it is essential to apply pretreatment methods to make cutbacks in the recalcitrant nature of lignocellulose and facilitate the conversion of lignocellulosic material into sugars or other value-added products.[4]

Different methods of lignocellulose pretreatments have been developed, including chemical, physical, mechanical, biological, and a combination of different pretreatments. The physical and chemical pretreatments apply acid, alkali, microwave, steam explosion, ionizing radiation, or combined processes that are accompanied by the production of inhibitors and require special equipment and extensive energy consumption.[1,5–7] In contrast to the aforementioned methods, biological pretreatment processes require mild process conditions without excessive energy consumption, as these methods simulate natural conditions for microbial well-being, like composting and ensiling.[8] The microorganisms most often used in biological pretreatments are brown rot, soft rot, and white rot fungi.[9] The literature suggests that biological pretreatment of lignocellulosic biomass by white rot fungi can be an economic and environmentally friendly alternative to

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physical, chemical, or physicochemical pretreatment methods to facilitate enzymatic hydrolysis.[4,10] For that purpose, white-rot fungi are usually cultivated under solid-state fermentation (SSF) conditions in the absence of free-flowing water where solid material can act as a substrate and/or support.[11]

The wide range of solid materials used in SSF can be divided into two main categories: inert materials, which serve only as attachment sites for the microorganism, and non-inert materials, which not only have a function as attachment sites but also provide nutrients to the microorganism. These materials are usually agricultural products or starch- or lignocellulosic-based wastes.[12] The production of chemicals, enzymes, value-added products, secondary metabolites, and organic acids through SSF is a promising and preferred technology in recent years.[13] Accordingly, the SSF process should be integrated into the lignocellulosic biorefinery and waste management system as an environmentally friendly conversion of recyclable and biodegradable waste.[14]

The ability of white-rot fungi to degrade the lignocellulosic structure is the result of their complex hydrolytic enzymes (such as cellulases and hemicellulases) and ligninolytic enzymes (lignin peroxidase, manganese peroxidase, and laccase).[15–18] Biological pretreatment of lignocellulosic biomass as a sustainable green resource leads to physicochemical and ultrastructural changes in the substrate, ultimately resulting in improved saccharification of the biomass and its use for the production of biofuels such as biomethane.[19–23]

Considering that fungi have the capacity to degrade renewable biomass, there is growing interest in the sustainable development for the eco-friendly production of biofuels and high-value chemicals under SSF conditions. One major problem in the production of bioethanol from lignocellulose is that the most commonly used microorganism, the yeast *Saccharomyces cerevisiae*, can only ferment mono- and disaccharides (such as glucose, fructose, maltose, and sucrose) into ethanol. Moreover, the presence of inhibitors, like phenolic compounds, in lignocellulosic liquors reduces the growth and productivity of the yeast and other potential biocatalysts, resulting in decreased ethanol yield due to inhibition of their enzymatic and physiological activities.[24] Laccases have shown potential for improving the efficiencies of enzymatic hydrolysis and fermentation of monosaccharides into ethanol by selectively removing the phenolic compounds.[5,25] The results of our team's experiments indicate that the cultivation of *Trametes versicolor* TV6 under SFF conditions had a significant effect on phenolic compounds recovery (most of the tested polyphenols decreased during SSF), whereas glucose concentration increased.[26] Based on these results, it is possible to develop an experimental one-pot platform to test the ability of *T. versicolor* to remove polyphenols with laccase and release sugars using a hydrolytic enzyme system.

The objective of the biological pretreatment in this study was to break down the lignocellulosic matrix of corn silage in an environmentally friendly manner. Corn silage is heterogeneous, consisting of a mixture of leaves, stalks, and corn cobs, and the complex mechanism of fungal growth make SSF a difficult bioprocess to monitor. It is necessary to optimize the numerous parameters, such as inoculation, moisture content, temperature, aeration, particle size, and supplements, as detailed in our previous work.[27,28] Determination of carbon source consumption and utilization of water-soluble sugars by white-rot fungi during the bioconversion process of lignocellulose is particularly important but rarely studied. In this study, the optimal process conditions for sugar extraction from whole plant corn silage consisting of a mixture of leaves, stalks, and corn cobs were determined. Optimization was performed using Response Surface Methodology (RSM), a well-known mathematical and statistical tool.[29] Biological pretreatment of corn silage was then conducted under solid-state conditions for 7 days with *T. versicolor*, a highly effective lignin-degrading white rot fungus, to investigate the ability to release simple sugars from corn silage. Solid-liquid extraction was used under the previously optimized process conditions to isolate water-soluble sugars from corn silage during biological treatment. To our knowledge, this is the first attempt to measure fermentable sugars during cultivation of *T. versicolor* on corn silage.

Materials and methods

Substrate and microorganism

Corn silage was obtained from Osatina grupa d.o.o., Viškovci, Croatia, and stored at -20 °C before use in biological pretreatment by *T. versicolor*.

Trametes versicolor TV-6 (MZKI, Ljubljana, Slovenia) was cultivated on a potato dextrose agar (PDA) medium for seven days at 27 °C in an incubator with the air fan set to 20 % and an absence of light (KB 115, BINDER GmbH, Germany).

Chemicals

PDA was purchased from Biolife (Biolife Italiana Sr. L. Viale Monza, Milan, Italy). HPLC grade acetonitrile was obtained from Fisher Chemical (Loughborough, UK). D(+)-glucose was obtained from Gram Mol Ltd. (Zagreb, Croatia). D(−)-fruc-

tose, D(+)-sucrose, D(+)-maltose monohydrate, and maltotriose were obtained from Acros Organics (Geel, Belgium).

Experimental design

Experimental design for sugars extraction evaluation

Before commencing the biological treatment of corn silage, the extraction of water-soluble sugars was optimized. The corn silage samples were thawed, dried to a moisture content of 92.85 - 94.46 % on a dry basis (referred to as "db"), and milled to a particle size of ≤ 1 mm. To eliminate the possible interference of naturally occurring microbes from the substrate and the released sugar during extraction, the samples were sterilized by autoclaving at 121 °C for 15 min.

A three-level four-factor Box-Behnken experimental design with three replications in the central points was employed to determine the optimum level of investigated extraction conditions, including extraction time (t), liquid-solid ratio (L/S), shaking speed (n), and extraction temperature (T) (Table 1). Twenty-seven extraction runs, according to the Box-Behnken design, were performed. The experimental data was assayed by RSM analysis using the Design-Expert software version 12 (Stat Ease Inc., Minneapolis, USA). According to the ANOVA (criteria $p < 0.05$), a second-order polynomial model (Eq.1) was used for fitting the experimental values as previously described:[30]

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{j<1}^{k} \beta_{ij} X_i X_j \quad (1)$$

where Y is the predicted response (the content of glucose and fructose); β_0, β_i, β_{ii}, β_{ij} are the regression coefficients for intercept, linear, quadratic, and interaction terms, respectively; X_i, X_j are the independent variables; k is the number of variables.

Sugar extractions from the biologically treated corn silage were performed under optimal extraction conditions (t = 30 min; liquid-solid ratio L/S = 25 mL g^{-1}; n = 170 rpm; and T = 30 °C) determined by RSM based on the Box-Behnken experimental design. In all experiments (using untreated or treated sterilized corn silage), one gram of milled substrate (particle size 1 mm) was extracted with distilled water in sealed flasks placed in a shaking water bath (Julabo, SW-23, Seelbach, Germany). After extraction, the extracts were centrifuged (Multifuge 3L-R Centrifuge, Heraeus, Germany) at 10 000 *g* for 10 min. The obtained supernatants were used for the determination of sugar content. All experiments were conducted in duplicate.

Table 1 – *Uncoded and coded levels of independent variables used in Box-Behnken design for the extraction process*

Independent variables (Factors)	Variable levels		
	–1	0	+1
X_1 – extraction time (t, min)	30	60	90
X_2 – liquid-solid ratio (L/S, mL g^{-1})	15	20	25
X_3 – shaking speed (n, rpm)	100	150	200
X_4 – extraction temperature (T, °C)	27	37	47

Biological pretreatment of corn silage

A seven-day biological pretreatment of corn silage using *T. versicolor* was conducted in laboratory jars according to a previously published procedure.[27] The initial process conditions were as follows: $m_{substrate}$ = 50 g, inoculum = 5 mycelial discs (1 cm in diameter), initial moisture content (w) = 75 %, T = 27 °C. Samples for sugar extraction and ultra-high liquid performance chromatography (UHPLC) analysis were collected before, during, and after the biological treatment. A total of 28 laboratory jars were inoculated. Twice a day, at 8 am and 6 pm, samples were collected from two jars. These four jars were mixed together, sterilized at 121 °C for 15 minutes, and then used for additional experiments. The extractions were conducted in duplicate, resulting in a total of eight samples analyzed by UHPLC each day. All results are reported as an average value of repetition (n = 8) ± standard deviation, and expressed per dry matter of substrate (mg g$^{-1}_{db}$).

Analytics

Determination of sugar content by ultra-high performance liquid chromatography

The content of individual sugars was determined by UHPLC (Nexera XR UHPLC, Shimadzu, Japan) using a refractive index detector (RID-20A). Prior to analysis, corn silage extracts were filtered through a 0.45 μm syringe PTFE filter into 2-mL vials. The samples were placed in a thermally controlled autosampler at 4 °C and injected into the system using the Shimadzu auto-injector (SIL-20ACXR). The chromatographic column InertSustain NH_2 and isocratic elution for 20 minutes was used for the separation of sugars with a flow rate of 1.0 mL min^{-1}. The mobile phase consisted of acetonitrile and water (75:25, v/v). Column oven (CTO-20AC) temperature was maintained at 40 °C. Data were collected and analyzed by the Lab-Solutions program (version 5.71 SP2). Chromatogram peaks were identified by comparing retention times of separated sugars against known standards, and by spiking samples with standards. Standard stock solutions of glucose, fructose, sucrose, maltose, and

maltotriose were prepared in redistilled water (γ = 10 mg mL^{-1}). Calibration curves (peak area *vs*. standard concentration) of the standards were created by diluting stock standard solutions in ultra-pure water to range from 0.1 to 1.0 mg mL^{-1}. Concentrations of sugars in the extracts were calculated from calibration curves, and the results expressed as the mass of individual sugar per dry basis of corn silage (mg g$^{-1}_{db}$). The dry matter content of the substrate was determined using the fast moisture analyzer (HR-73, Mettler Toledo, Zürich, Switzerland) by drying the sample to constant mass.

Results and discussion

In this work, the process conditions for sugar extraction were optimized with the aim of obtaining the extracts from corn silage with maximum sugar concentration. After optimization of the extraction, the corn silage was biologically treated by culturing *T. versicolor* under SSF conditions for seven days. Subsequently, the sugar extraction was performed from the biologically treated samples to study the effects of biological treatment on the release of simple sugars.

Determination of optimal extraction conditions

As already mentioned, the aim of this experiment was to find the optimal extraction conditions for individual sugar extraction (glucose, fructose, maltose, and sucrose) using RSM based on the Box-Behnken experimental design. According to the obtained results given in Table 2, it is evident that the content of glucose, fructose, maltose, and sucrose in the extracts obtained under the investigated experimental conditions, fell within the range 6.86 – 9.37 mg g$^{-1}_{db}$, 9.21 – 15.47 mg g$^{-1}_{db}$, 0.06 – 0.87 mg g$^{-1}_{db}$, and 0.33 – 3.82 mg g$^{-1}_{db}$, respectively.

The concentrations of maltose and sucrose were too low compared to the concentrations of fructose and glucose for mathematical modeling using the RSM method. However, when modeling the glucose content using the second-order model, a linear dependence of glucose content on temperature and shaking speed were observed (Fig. 1a). Furthermore, for fructose, as the dominant fermentable sugar with high coefficient correlation ($R > 0.91$) and insignificant lack of fit ($p > 0.05$), the significant regression model (intercept term, $p < 0.05$) indicated a reasonable fit of the data to the quadratic model (Fig. 1b). Temperature had the most significant effect on both sugar extractions confirmed by the significant linear term for glucose extraction and the combined effect of linear and quadratic terms for fructose extraction. Besides temperature, both sugar extractions were affected by shaking speed, as confirmed by the significant linear term for glucose and significant quadratic term, as well as an interaction term for shaking speed and liquid-solid ratio for fructose (Table 3).

Moreover, the extraction parameters for fructose and glucose were not identical. However, the RMS method allows for the combination of the

Table 2 – *Content range of quantified sugars: glucose, fructose, maltose, and sucrose in corn silage extracts obtained under 27 experiments of Box-Behnken design*

run	t (min)	L/S (mL g^{-1})	n (rpm)	T (°C)	$C_{glucose}$	$C_{fructose}$	$C_{maltose}$	$C_{sucrose}$
					(mg g$^{-1}_{db}$)			
1	60	15	200	37	8.94	10.66	0.41	1.84
2	90	15	150	37	8.33	14.62	0.32	2.71
3	60	25	100	37	8.38	10.75	0.57	0.65
4	30	20	200	37	8.93	9.87	0.51	1.94
5	60	15	100	37	6.99	12.15	0.44	1.35
6	60	20	150	37	8.28	14.45	0.55	2.66
7	30	20	150	27	8.33	14.39	0.22	2.72
8	30	20	100	37	8.56	10.70	0.63	3.20
9	60	20	200	47	7.61	9.81	0.13	3.82
10	60	20	200	27	7.84	9.67	0.44	3.59
11	90	25	150	37	7.82	13.97	0.08	2.40
12	30	20	150	47	6.99	12.27	0.46	1.31
13	60	15	150	47	6.87	12.65	0.54	2.18
14	30	25	150	37	7.99	14.14	0.09	2.38
15	60	20	150	37	7.95	13.80	0.28	2.50
16	60	20	100	27	7.75	9.45	0.31	3.64
17	60	25	150	27	8.80	14.89	0.32	2.53
18	30	15	150	37	8.29	14.69	0.06	2.30
19	90	20	150	27	8.22	14.43	0.37	2.41
20	90	20	100	37	7.41	12.34	0.31	1.33
21	60	25	200	37	8.98	14.74	0.30	1.99
22	60	20	150	37	7.97	14.05	0.20	2.53
23	90	20	150	47	6.86	12.23	0.12	0.33
24	60	20	100	47	7.70	9.21	0.12	3.62
25	60	15	150	27	8.23	14.49	0.40	2.63
26	60	25	150	47	7.27	11.84	0.09	1.27
27	90	20	200	37	9.37	15.47	0.87	2.28

t – extraction time (min), L/S – liquid-solid ratio (mL g^{-1}), n – shaking speed (rpm), T – extraction temperature (°C)

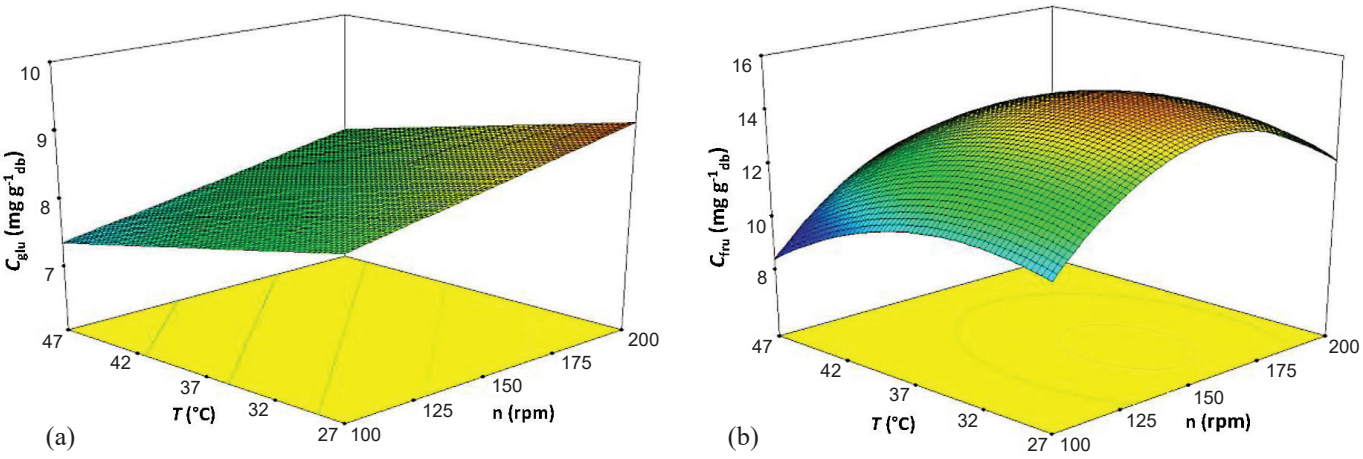

Fig. 1 – *Response surface 3D plot obtained through numerical optimization as a function of statistically significant interaction variables (temperature (T) and shaking speed (n)) for extraction of (a) glucose, and (b) fructose at a fixed time (30 min) and liquid-solid ratio (25 mL g^{-1})*

Table 3 – *Regression coefficients of significant terms (p values < 0.05), coefficient of correlation (R), and lack of fit values of the predicted second-order polynomial models for the response variable, glucose, and fructose in corn silage extracts*

Coefficients	Glucose	Fructose
β_0	8.02	14.10
β_1 – time	–	–
β_2 – liquid-solid ratio	–	–
β_3 – shaking speed	0.41	–
β_4 – temperature	–0.49	–0.78
$\beta_1\beta_2$	–	–
$\beta_1\beta_3$	–	–
$\beta_1\beta_4$	–	–
$\beta_2\beta_3$	–	1.37
$\beta_2\beta_4$	–	–
$\beta_3\beta_4$	–	–
β_1^2	–	–
β_2^2	–	–
β_3^2	–	–2.68
β_4^2	–	–1.36
R	0.66	0.91
p – value of lack of fit	0.098	0.056

studied variables to maximize concentration of the studied sugars in the extract. Therefore, optimal extraction conditions were determined through numerical optimization based on desirability functions, resulting in the following conditions: t = 30 min; liquid-solid ratio L/S = 25 mL g^{-1}; n = 170 rpm; and T = 30 °C. Criteria for optimization were maximum value of dependent variables with independent variables set within range.

Validation of the model for predicting the optimum response values was conducted using the recommended optimum extraction conditions for extraction sugars from corn silage. The experimental values agreed with the predicted ones (Table 4).

Biological pretreatment and sugar analysis of the biologically pretreated corn silage

Sugar extraction from biologically pretreated corn silage was performed under determined optimal extraction conditions. The sugar content in the obtained extracts of biologically pretreated corn silage during seven days of *T. versicolor* cultivation in SSF is presented in Fig. 2 and Fig. 3. All results are reported as the average value of repetition (n = 8) ± standard deviation, and finally expressed as per dry matter of substrate (mg g$^{-1}_{db}$). The initial corn silage samples (representing Day 0) were adjusted to the desired moisture level and sterilized in the same manner as they were prepared for inoculation. The initial sugar content in the corn silage extracts were in the range of 12.09 ± 0.14 mg g$^{-1}_{db}$ for

Table 4 – *Experimental and predicted values of response (glucose and fructose content) at optimal extraction conditions*

Experimental values		Predicted values	
Glucose	Fructose	Glucose	Fructose
C_{glu} = 8.28 mg g$^{-1}_{db}$	C_{fru} = 12.09 mg g$^{-1}_{db}$	C_{glu} = 8.75 mg g$^{-1}_{db}$	C_{fru} = 14.38 mg g$^{-1}_{db}$

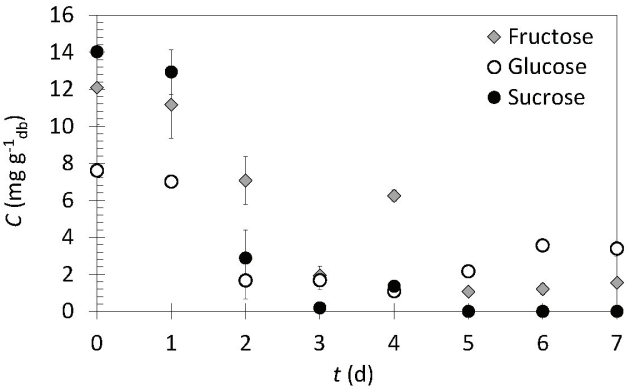

Fig. 2 – *Concentration of fructose, glucose, and sucrose in the extract of pretreated corn silage by T. versicolor for 7 days*

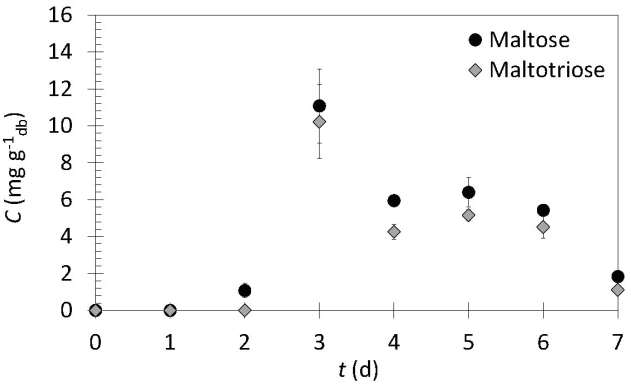

Fig. 3 – *Concentration of maltose and maltotriose in the extract of pretreated corn silage by T. versicolor for 7 days*

fructose, 7.61 ± 0.22 mg g^{-1}_{db} for glucose, and 14.03 ± 2.55 mg g^{-1}_{db} for sucrose. Notably, no maltose was detected in the obtained extracts, in contrast to the samples used for extraction optimization where maltose was identified, with a content ranging from 0.06 to 0.87 mg g^{-1}_{db}.

The changes in the concentration of individual sugars during the SSF process are directly related to the action of the complex enzyme system of the microorganism at work.[3] In the initial growth phase, the microorganism utilizes simple carbohydrates for its growth and metabolic activity. As development progresses, the mycelium penetrates the interior of the lignocellulosic material, causing the pores of the material to expand. This mycelium penetration, coupled with the action of the lignolytic enzymes, leads to the breakdown of bonds between the complex polymer lignin and the complex carbohydrates. This makes the carbohydrates more sensitive to the action of the hydrolytic enzymes. The mechanism of action of lignolytic and hydrolytic enzymes on the complex structure of lignocellulosic biomass is explained in our previously published review article.[3]

According to the results obtained (Fig. 2), during the first three days of treatment, the content of fructose and glucose decreased, and were in the range of 1.93 ± 0.26 mg g^{-1}_{db} (fructose), and 1.68 ± 0.37 mg g^{-1}_{db} (glucose). This decline is the consequence of *T. versicolor* proliferating over the substrate and utilizing available sugars for metabolic activity. The content of disaccharide sucrose had decreased to 0.19 ± 0.06 mg g^{-1}_{db} after Day 3, indicating that invertase catalyzes the hydrolysis of sucrose into its subunits (glucose and fructose). An increase in fructose content was observed on Day 4 (6.24 ± 1.26 mg g^{-1}_{db}), after which, on the Day 5, the fructose content decreased and remained almost constant until the end of the SSF process. The glucose content increased after Day 5 and reached the value of 3.39 ± 0.52 mg g^{-1}_{db} by the end of the SSF process.

Disaccharide maltose started to be released after Day 2, and reach its maximum after Day 3 (11.08 ± 2.12 mg g^{-1}_{db}), with oligosaccharide maltotriose also being released (10.22 ± 2.13 mg g^{-1}_{db}) (Fig. 3). After Day 3, both sugar contents started to be metabolized by *T. versicolor* and their concentrations in the extracts slowly decreased with time.

The process of sugar utilization can be explained as follows: at the beginning of the SSF process, when the inoculum from the Petri dishes is placed on the outer surface of the corn silage, the fungus consumes the available simple sugars for its growth. Later, as the fungus multiplies and penetrates into the macro- and micropores of the solid, it starts to utilize polysaccharides as an energy source.[31] There are limited data on sugar utilization during solid-state fungal fermentation-based (pre)-treatment methods. Isikhuemhen *et al.* studied the release of sugars from canola plant biomass under SSF conditions using six different strains of white-rot fungi.[32] All organisms released sugars during the three-week incubation period. In our previous work, where we investigated *T. versicolor* growth in submerged cultivation using different carbon sources (glucose, fructose, and sucrose), we observed that glucose uptake stopped before fructose consumption due to the higher affinity of *T. versicolor* for glucose as a growth substrate, and that *T. versicolor* started to utilize sucrose even before it started to grow on glucose and fructose.[33] In a study by Šelo *et al.,* the effect of biological treatment of grape pomace through cultivation of *T. versicolor* was investigated. During the first five days of the SSF process, due to the action of hydrolytic enzymes, there was a decrease in the mass fraction of sucrose and an increase in the mass fraction of glucose, fructose, arabinose, and xylose in the sugar extracts.[26] Zeko-Pivač *et al.* also cultivated the white rot fungus *T. versicolor* using brewer's spent grain as a lignocellulosic substrate. During the SSF process, they observed the production of hydrolytic

enzymes (xylanases, β-glucosidases, and cellulases) and lignolytic enzymes (laccases and manganese peroxidases), a decrease in the content of hemicellulose, cellulose, and lignin, and an increase in the mass fraction of reducing sugars.[34] In an earlier study, our group performed the SSF procedure to grow *T. versicolor* on corn silage in a tray bioreactor. The initial percentage of hemicellulose and lignin in the corn silage was 10.00 $\%_{db}$ and 9.79 $\%_{db}$, respectively. After Day 7 of the SSF process, the mass fraction of hemicellulose and lignin had reduced by 44.3 % and 47.1 %, and the activities of laccase (260 U dm^{-3}) and manganese peroxidase (76 U dm^{-3}) were detected.[27]

Even though the concentrations of sugars were lower after the SSF process compared to untreated corn silage, the presented research provides valuable information about individual sugar profiles during fermentation. Additionally, the obtained results expand the opportunities for monitoring depletion of carbon sources. Furthermore, it is evident that *T. versicolor* possesses the ability to hydrolyze sucrose as well as polysaccharides, which implies that the SSF process should be optimized to enhance the production of hydrolytic enzyme cocktails. This produced hydrolytic enzyme cocktail could be used for saccharification of the lignocellulosic biomass to improve sugar liberation. *T. versicolor* was used in an eco-friendly manner for efficient delignification and saccharification of hardwood, resulting in glucose yields and improved lignin extractability, which supports the aforementioned hypothesis.[35] Recently, it has been demonstrated that *T. versicolor* produces plant cell wall degrading enzymes (cellulolytic, hemicellulolytic, pectinolytic, esterase, and auxiliary activity) that enable efficient conversion of cellulose and hemicellulose into monosaccharides.[36] Moreover, saccharification of lignocellulosic biomass with unpurified enzyme cocktails contributes to reducing the cost of the bioconversion process.[37]

Conclusion

The optimal extraction conditions for water-soluble sugar extractions were determined as follows: t = 30 min; ratio L/S = 25 mL g^{-1}; n = 170 rpm, and T = 30 °C. The initial content of water-soluble sugars in the corn silage extracts obtained before fungal treatment were in the range of 12.09 ± 0.14 mg g^{-1}_{db} for fructose, 7.61 ± 0.22 mg g^{-1}_{db} for glucose, and 14.03 ± 2.55 mg g^{-1}_{db} for sucrose. During the treatment, *T. versicolor* utilized available glucose, fructose, and sucrose for its growth, while the release of maltose and maltotriose occurred with time. The results demonstrate that the hydrolytic enzyme system of *T. versicolor* has the ability to convert complex lignocellulosic biomass into valuable sugar units. In addition, to improve the saccharification of biomass and its conversion into bioethanol, the production of a hydrolytic enzymes cocktail should be optimized, and research in this direction will continue in the future. Optimization of the process for saccharification of lignocellulosic biomass using crude and purified cocktails of hydrolytic enzymes will be the focus of future research.

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