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Original scientific paper

Effective valorisation of barley bran for simultaneous cellulase and *β*-amylase production by *Paenibacillus chitinolyticus* CKS1: Statistical optimization and enzymes application

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Abstract: The agricultural raw industry generates large amounts of annually by--products that create disposal problems. Hitherto, there have been no reported papers about the simultaneous production of cellulase and *β*-amylase from these raw materials using *Paenibacillus* sp. that would reduce the costs. Thus, in this paper simultaneous cellulase (CMC-ase and avicelase) and *β*-amylase production using barley bran and the application of the natural isolate *Paenibacillus chitinolyticus* CKS1 and potential enzymes in the hydrolysis process was studied. Response surface methodology was used to obtain the maximum enzyme activity (CMC-ase 0.405 U mL^{-1}, avicelase 0.433 U mL^{-1} and *β*-amylase 1.594U mL^{-1}). Scanning electron microscopy showed degradation of the lignocellulosic–starch structure of barley bran after fermentation. The CKS1 bacterial supernatant, which contains cellulases and *β*-amylase, could hydrolyze cotton fibres and barley bran, respectively. The main products after enzymatic hydrolysis of cotton fibres and barley bran, glucose (0.117 g g_{mat}^{-1}) and maltose (0.347 g g_{mat}^{-1}), were quantified by high performance liquid chromatography (HPLC). The produced enzymes could be used for hydrolysis of cotton fabric and barley bran to glucose and maltose, respectively. Application of simultaneous enzymes production using an agricultural by-product is economically and environmentally accepted and moreover, valuable biotechnological products, such as glucose and maltose, were obtained in this investigation.

Keywords: agricultural by-product; *P. chitinolyticus* CKS1; enzymes; process optimization; degradation; hydrolysis products.

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INTRODUCTION

Industrial processing of raw materials from agriculture generates large amounts of by-products annually. These agricultural by-products are mainly composed of sugars, fibres, proteins, and minerals, which makes it very interesting from the economic and environmental point of view, as it is suitable for obtaining low-cost value-added products.[1] Cellulose is the major component of these by-products. The biomass production of cellulose is estimated at about1.5 trillion tons per year, making it an essentially inexhaustible source of raw material for environmentally friendly and biocompatible products.[2] Due to its complex structure, lignocellulosic biomass (mainly composed of lignin, cellulose, and hemicelluloses) is often very recalcitrant for complete microbial degradation.[3–6] However, beside lignocellulosic structure some agro-industrial residues contain polysaccharide starch. These kinds of residues, containing both cellulose and starch, can be valorised as an inexpensive raw material for the production of bio-based products or enzymes – cellulases and amylases.

Cellulases are regarded as one of the key enzymes for bioconversion of lignocellulosic biomass into fermentable sugars, which could then be converted in bioethanol.[7] The conversion of soluble sugars from cellulose requires the combined action of three enzymes in cellulase systems, which include endoglucanase, exoglucanase or cellobiohydrolase, and β-glucosidase. All these enzymes act synergistically to release glucose as the end product.[7,8] Amylases are also important industrial enzymes which hydrolyze starch molecules to dextrin and smaller polymers composed of glucose[9,10] units. β-Amylases is an exo-hydrolase that specifically cleaves α-1,4 linkages to produce a maltose.[11,12]

Barley bran is a complex material that is the major by-product of the barley milling process. Due to its lignocellulosic–starch structure, it is mainly used as a substrate for microorganisms fermentations and for the production of the enzymes laccase, xylanase and amylasem,[13–15] while barley stalk is mainly used for cellulase production[16] or barley bran in a combination with other waste substrate.[17]

Although in recent years, the enzymes of the genus *Paenibacillus* have gained ever more attention because of their potential industrial application,[18–21] but the simultaneous production of two or more enzymes using lignocellulosic–starch materials has not hitherto been reported. According to above mentioned, the aim of this study was to investigate the simultaneous cellulase (endoglucanase – CMC-ase and exoglucanase – avicelase) and β-amylase production from *Paenibacillus* sp. using barley bran waste material by the strain *P. chitinolyticus* CKS1. Optimization of cellulase and β-amylase production was performed using a statistical model under response surface methodology (RSM) based on the central composite design (CCD). Scanning electron microscopy (SEM) was used to detect changes in morphology of the barley bran after fermentation by *P. chitin-*

lyticus CKS1. Application of *P. chitinolyticus* CKS1 cellulase in the hydrolysis of cotton fabric was examined, as well as the hydrolysis of barley bran using amylase, respectively. High performance liquid chromatography (HPLC) was performed in order to determine the end-products of enzymatic hydrolysis of cotton fabric and barley bran by the strain CKS1. The cost of the simultaneously production of two enzymes on agro-industrial residues could be significantly reduced by using the proposed procedure.

EXPERIMENTAL

Microorganism and inoculum preparation

The *P. chitinolyticus* CKS1 was a natural isolate from a soil sample (GenBank accession No. KP715850).[18]The inoculum was prepared by growing the microorganism in 300 mL Erlenmeyer flask with 30 mL of International Streptomyces Project 1 (ISP1) broth containing 3 g L^{-1}yeast extract and 5 g L^{-1}casein hydrolysate. The medium was inoculated at 30 °C for 20 h in a rotary shaker at 150 rpm. This inoculum was used for the fermentation process.

Raw material – barley bran and cotton fibre

Barley bran (Factory Klas, Sarajevo, Bosnia and Herzegovina; particle size 0.8–2 mm; composition 4.5 % cellulose, 35.5 % starch, 17 % proteins and 4 % fats) was used as the substrate for microorganism growth and for enzymatic hydrolysis.

Desized and bleached cotton fabric (165 g m^{-2}) was used as the substrate for the enzymeatic hydrolysis.

Enzyme assay for cellulase and amylase

Cellulase (CMC-ase and avicelase) activity was measured by reduction of 3,5-dinitrosalicylic acid in the presence of glucose released during the enzymatic hydrolysis of cellulose according to the method of Müller,[22] as described in a previous study.[18]

CMC-ase (endoglucanase) activity was determined using the following procedure: 0.500 mL of enzyme solution was mixed with 0.500 mL of 1 % carboxymethyl cellulose (CMC) solution in 0.1 M acetate buffer (pH 4.80) and incubated in a rotary shaker at 150 rpm, at 50 °C for 15 min. Subsequently, 1 mL of DNS reagent was added, the reaction mixture boiled for 15 min, cooled to room temperature and mixed with 5 mL of distilled water. The absorbance was read on the UV/Vis spectrophotometer (Ultrospec 3300 pro, Amersham Bioscience, Sweden) at 540 nm (25 °C) against a blank (non-incubated enzyme). One unit of CMC-ase activity was defined as the amount of enzyme that released 1 μmol of glucose equivalents per min.

Avicelase (exoglucanase) activity was determined at 80 °C following the same procedure as for CMC-ase activity determination with the exception that 1 % avicel in place of 1 % CMC solution in 0.1 M acetate buffer (pH 4.80) was used as the substrate. One unit of avicelase activity was defined as the amount of enzyme that released 1 μmol of glucose equivalents per min.

The activity of β-amylase was measured by a modified Bernfeld method,[23] as described in a previous study.[19]

Fermentation processes

All experiments were performed in 300 mL Erlenmeyer flasks with a working volume of 30 mL. Casein hydrolysate (30 mL) was used as the liquid medium for the fermentations. In each flask with casein hydrolysate (5 g L^{-1}), different concentrations of yeast extract and barley bran were added. After sterilization at 121 °C for 20 min, an overnight bacterial culture at

a concentration of 10 % was inoculated into fresh medium on a rotary shaker with a mixing speed of 150 rpm at 30 °C for 3 days. The culture medium was centrifuged at 6000 *g* for 15 min to remove the cells. The crude cell-free supernatant was analyzed for cellulase and *β*-amylase activity.

Scanning electron microscopy (SEM)

For the microscopic study of the changes in the morphological characteristics of barley during the fermentations, samples were soaked in 3.5 % glutaraldehyde for 2–4 h, and dried by treatment with 50, 70, 90, 95 and 100 % ethanol, followed by storing overnight in a desiccator for the removal of moisture. Scanning electron micrographs of the samples were obtained after treatment with gold for 15 min. Morphological changes were captured using an electron microscope (TESCAN Mira3 XMU at 10 kV).

Enzymatic hydrolysis of the cotton fibre and barley bran

Crude bacterial supernatant obtained from the barley bran fermentation by the strain CKS1 was used for the enzymatic hydrolysis of cotton fibre and barley bran, respectively. Hydrolysis of cotton fibre was performed in 300 ml Erlenmeyer flasks at 50 °C with 1.33 % of cotton fibre, 37.5 mL of crude bacterial supernatant obtained after fermentation and with 37.5 ml of 0.1 macetate buffer pH 4.8 with 150 rpm agitation.

Hydrolysis of barley bran was performed in 300 mL Erlenmeyer flasks at 50 °C with 1 % barley bran, 37.5 ml of crude bacterial supernatant obtained after fermentation and with 37.5 ml of 0.1 M acetate buffer pH 4.8 with 150 rpm agitation.

After the reaction, the samples were centrifuged (6000*g*, 10 min) to remove unhydrolysed residue and the total reducing sugars in the supernatant, calculated as glucose equivalents, was estimated by the DNS method.[22]

HPLC analyses of enzymatic hydrolysis

The barley bran hydrolysis product was analyzed by high performance liquid chromatography (HPLC). After enzymatic hydrolysis, the samples were centrifuged (6000*g*, 10 min) to remove unhydrolysed residue. The sample was then filtered through a 0.22 μm membrane filter.

For analysis of obtained sample, a Dionex Ultimate 3000 Thermo Scientific (Waltham, USA) HPLC system was used. A reverse phase column (Hypersil gold C18, 150 mm×4.6 mm, 5 μm) at 40 °C was employed. The run time was 10 min. Water (HPLC grade, JT Baker (USA)) was used as the sole mobile phase at an elution rate 0.4 mL min^{-1}. All data acquisition and processing was realized using Chromeleon Software. The separated hydrolysis products were identified by comparison with standard glucose and maltose.

RESULTS

Fitting the process variables

The experimental design (design matrix) and corresponding responses are presented in Table I.

The relationship between the two independent variables (barley bran concentration and yeast extract concentration) and three responses (CMC-ase, avicelase and *β*-amylase activity) fitted well with the quadratic model.

The relationship between responses and the tested variables are given by the following equations:

$$Y_1 = -0.085 + 0.073A + 0.030B - 7.58\times10^{-3}AB + 0.013A^2 - 2.77\times10^{-3}B^2 \quad (1)$$

$$Y_2 = -0.032 + 0.043A + 0.0125B - 2.583\times10^{-3}AB + 0.018\mathrm{A}^2 - 1.488\times10^{-3}\mathrm{B}^2 \quad (2)$$

$$Y_3 = -0.649 + 0.402A + 0.249B - 0.025AB + 0.028A^2 - 0.028B^2 \quad (3)$$

where Y_1, Y_2 and Y_3 are the responses, and the A and B are the independent variables. The analyses of variance (ANOVA) for the quadratic models are given in Table II.

TABLE I. The design matrix and the corresponding responses; A – barley bran, B – yeast extract, Y_1 – CMC-ase activity, Y_2 – avicelase activity, Y_3 – β-amylase activity

Run	Independent variables		Responses		
	A / %	B / g L^{-1}	Y_1 / U mL^{-1}	Y_2 / U mL^{-1}	Y_3 / U mL^{-1}
1	4.0	2.0	0.389	0.421	1.643
2	4.0	6.0	0.297	0.381	1.328
3	2.5	6.0	0.164	0.182	0.648
4	1.0	6.0	0.028	0.027	0.114
5	2.5	1.6	0.208	0.211	0.723
6	2.5	6.4	0.121	0.170	0.521
7	0.7	4.0	0.032	0.041	0.149
8	4.3	4.0	0.424	0.487	1.638
9	0.7	4.0	0.028	0.023	0.122
10	2.5	4.0	0.156	0.174	0.84
11	4.3	4.0	0.420	0.475	1.724
12	2.5	4.0	0.183	0.191	0.857
13	1.0	2.0	0.029	0.036	0.129
14	2.5	1.6	0.184	0.195	0.754

TABLE II. The analysis of variance (ANOVA) for the fitted models; A – barley bran concentration; B – yeast extract concentration; Y_1 – CMCase activity; Y_2 – avicelase activity and Y_3 – β-amylase activity

Source	Y_1		Y_2		Y_3	
	F-value	P-value $Prob > F$	F-value	P-value $Prob > F$	F-value	P-value $Prob > F$
Model	159.45	< 0.0001[a]	358.55	< 0.0001[a]	367.04	< 0.0001[a]
A	755.47	< 0.0001[a]	1730.18	< 0.0001[a]	1753.62	< 0.0001[a]
B	15.03	0.0047[a]	6.79	0.0313[a]	20.73	0.0019[a]
AB	6.20	0.0376[a]	1.23	0.2997[b]	9.29	0.0159[a]
A^2	10.13	0.0129[a]	35.24	0.0003[a]	6.60	0.0332[a]
B^2	1.47	0.2594[b]	0.73	0.4185[b]	20.56	0.0019[a]
Lack of fit	1.13	0.4208[b]	2.84	0.1454[b]	0.87	0.1569[b]
R^2	0.9901		0.9956		0.9957	
Adjusted R^2	0.9839		0.9928		0.9929	
Predicted R^2	0.9685		0.9843		0.9869	
$C.V.$ / %	9.61		6.49		6.16	
Adequate precision	32.134		48.630		49.422	

[a]Significant coefficient ($P < 0.05$); [b]non-significant coefficient

In respect to the three responses, the models are significant and the lack of fit is not significant ($P > 0.05$: 1.13, 2.84 and 0.87), which confirms that the quadratic models are valid for obtaining the maximum enzyme activity. The determination coefficient (R^2, adjusted R^2 and predicted R^2) for the three models are almost 1 (0.9901, 0.9956 and 0.9957), which indicates good correlation between the experimental and predicted values (Fig. 1A–C).

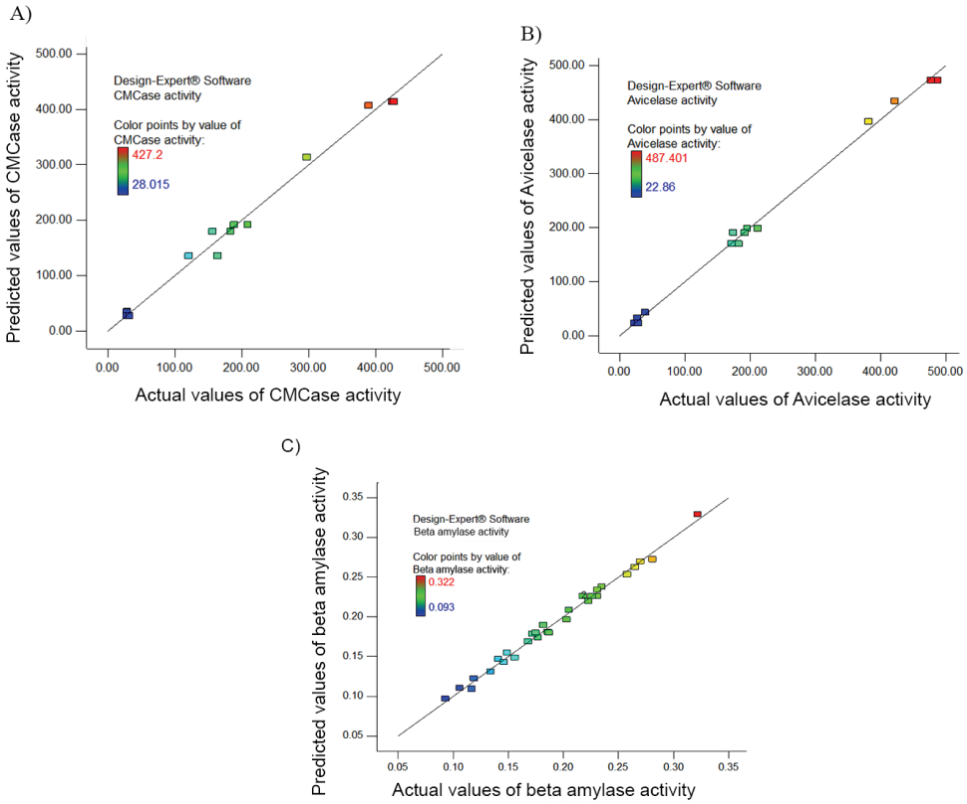

Fig. 1. Plots of the measured and model-predicted values of the response variable: A) CMC-ase production, B) avicelase and C) *β*-amylase production.

The adequate precision ratio for each response is greater than 4 (32.134, 48.630 and 49.422), which indicates that the signal was adequate. The low value of the coefficient of variation (*C.V.*) for the tested models indicates high precision of the obtained experimental results (Table II).

Influence of the fermentations parameters on CMC-ase, avicelase and β*-amylase activity*

The influence of two independent variables on the *β*-amylase production was in following order: barley bran concentration (*A*) > yeast extract concentration

(*B*). The concentration of barley bran, which is the most significant factor in cellulase production, varied from 0.7–4.3 % (Table I) and the maximum CMC--ase and avicelase production was obtained using 4.3 % barley bran (Table I, Run 8). Under the optimal conditions, the maximum CMC-ase and avicelase production was 0.405 and 0.433 U mL^{-1}, respectively, using 4.0 % barley bran and 2.0 g L^{-1} yeast extract (Fig. 2A and B).

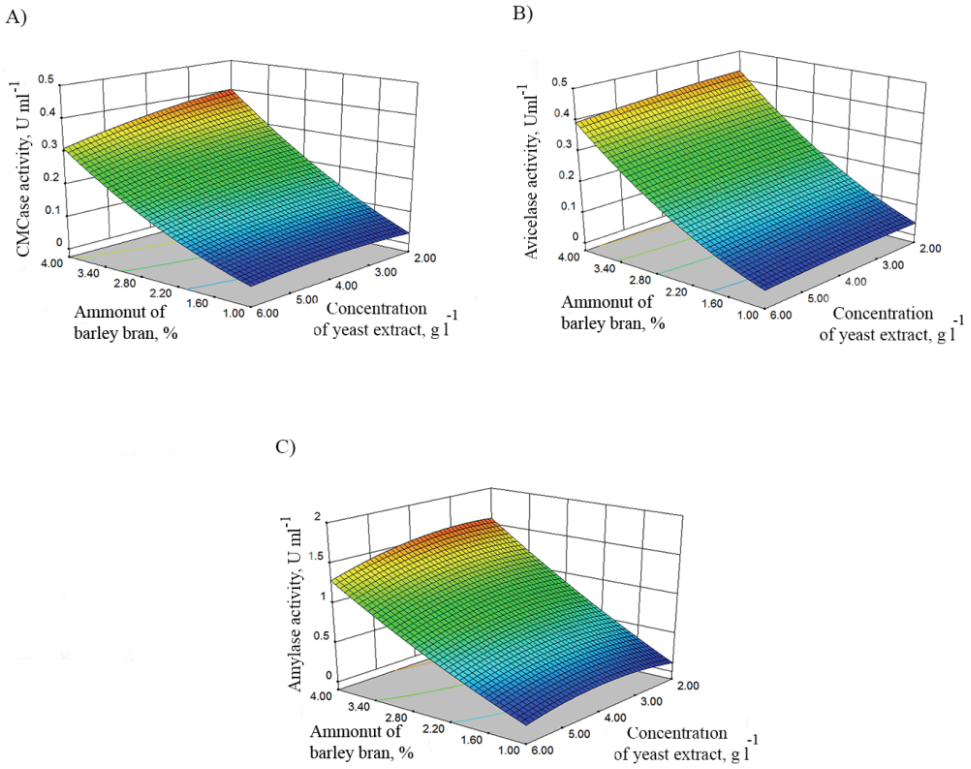

Fig. 2. Surface plot of the interactive effects of barley bran and yeast extract on: A) CMC-ase, B) avicelase and C) *β*-amylase production.

While producing cellulase, the strain CKS1 is able to produce *β*-amylase with maximum activity of 1.724 U mL^{-1} using 4.3 % barley bran and 4.0 g L^{-1} yeast extract (Table I, Run 11). Under the optimal conditions, maximum *β*-amylase activity was 1.594 U mL^{-1} using 4.0 % barley bran and 2.0 g L^{-1}yeast extract (Fig. 2C). The yeast extract is the second independent variable with positive influence on the CMC-ase, avicelase and *β*-amylase activity. In the performed experiments, the concentration of yeast extract was varied from 1.6 to 6.4 g L^{-1} and the maximum activity of CMC-ase was 0.424 U mL^{-1}, of avicelase 0.484 U $mL^{-1}$and of *β*-amylase 1.724 U mL^{-1}, obtained with 4.0 g L^{-1} yeast extract

(Table I, Run 8 for CMC-ase and avicelase activity and Run 11 for β-amylase activity).

SEM analysis of barley bran sample (Fig. 3) showed that before fermentation (Fig. 3A and B), the surface was smooth, without cracks or major pores and with starch granules, while after fermentation these surfaces were perforated with gaps (holes) and were without starch particles (Fig. 3C and D).

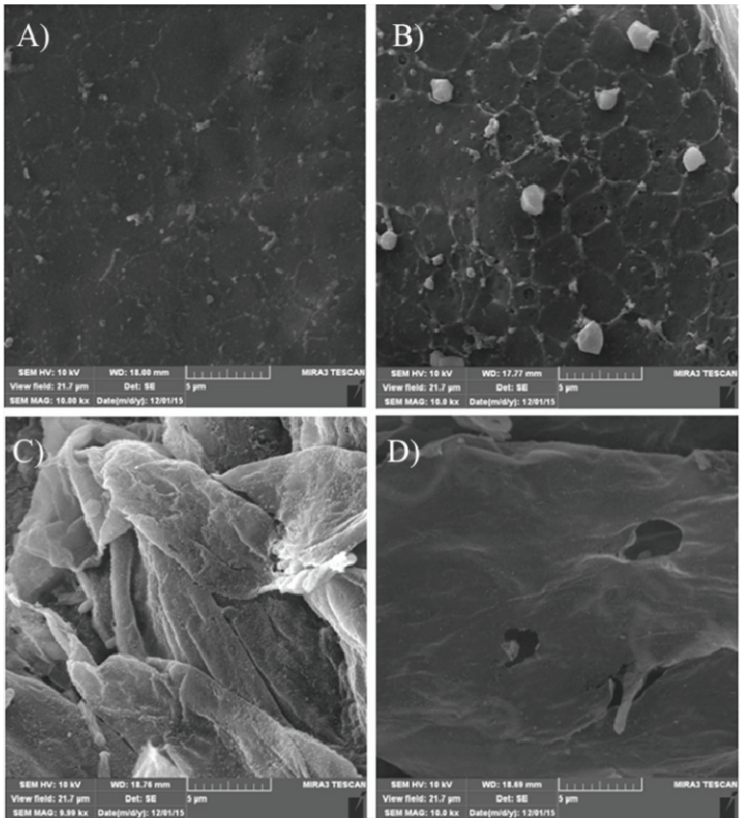

Fig. 3. SEM analysis of the morphological changes in barley bran before (A, B) and after the fermentation process (C, D) by *P. chitinolyticus* CKS1.

Validation of the models

The validation of the optimization of the models was performed for one point selected from the numerical optimization results. The optimal conditions that were obtained from the desirability function approach were: barley bran concentration 4.0 % and yeast extract concentration 2.0 g L^{-1}. In order to evaluate the validity of the models, additional experiments were performed under the optimal conditions (Table III).

TABLE III. Validation of the model; *P* – predicted, *V* – validated

Working volume, mL	Barley bran, %	Yeast extract, g L^{-1}	CMCase activity, U mL^{-1}		Avicelase activity, U mL^{-1}		*β*-amylase, activity U mL^{-1}	
			P	*V*	*P*	*V*	*P*	*V*
30	4	2	0.405	0.413	0.433	0.438	1.594	1.463
100	4	2	0.405	0.447	0.433	0.457	1.594	1.731

The experiment was performed using two working volumes, 30 and 100 mL in Erlenmeyer flasks of 300 mL and in 1000 mL "flying saucer" shake flasks for aerobic cultivation, respectively.

The predicted values for the three outcomes were the following: CMC-ase activity 0.405 U mL^{-1}, 95 % prediction interval (*PI*) 0.350–0.460 U mL^{-1}, avicelase activity 0.433U mL^{-1}, 95 % prediction interval (*PI*) 0.400–0.470 U mL^{-1} and *β*-amylase activity 1.594 U mL^{-1}, 95 % prediction interval (*PI*) 1.450–1.740 U mL^{-1}. The measured values for the three outcomes (CMC-ase, avicelase and *β*-amylase activity) fitted within the 95 % *PI* ranges and were very close to the most probable predicted values, *i.e.*, a CMC-ase activity of 0.413±0.011 U mL^{-1} was obtained for the working volume of 30 mL and 0.447±0.012 U mL^{-1} for the working volume of 100 mL, an avicelase activity of 0.438±0.012U mL^{-1} for the working volume of 30 mL and 0.457±0.13 U mL^{-1} for the working volume of 100 mL, a *β*-amylase activity of 1.463±0.009 U mL^{-1} was obtained for the working volume of 30 mL and 1.731±0.007U mL^{-1} for the working volume of 100 mL, showing that the models were reliable.

Hydrolysis of cotton fibre and barley bran

To examine the cellulolytic and amylolytic potential of the strain CKS1, the crude bacterial supernatant was used for the hydrolysis of the cotton fabric and the barley bran. During cotton hydrolysis, the maximum concentration of released sugar, measured by the DNS method (results not shown), was reached after 72 h. HPLC analysis (Supplementary material to this paper, Fig. S-1A) of the sample after 72 h of hydrolysis showed the presence of glucose as the major hydrolysis product in a concentration of 0.117 g g_{mat}^{-1} (g glucose per g of cotton material). Considering that the bacterial supernatant, which produced the strain CKS1, contains amylase besides cellulase, the possibility of enzymatic hydrolysis of lignocellulosic–starch raw materials or barley bran was also investigated. Similarly to the hydrolysis of cotton material, the released sugar concentration was measured by the DNS method (results not shown) and maximum was reached after 24 h. HPLC analysis (Supplementary material, Fig. S-1B) of the sugar sample after 24 h of hydrolysis showed the presence of maltose as the major hydrolysis product in a concentration of 0.347 g g_{mat}^{-1}.

DISCUSSION

In previous research, *P. chitinolyticus* showed individual cellulase[18] and *β*-amylase production[19,20] on different substrates. In this study, for the first time, the simultaneous production of these enzymes by the strain CKS1 using one substrate was shown, which would reduce the cost of their production.

Barley bran is produced in the milling process of barley and is mainly composed of cellulose, hemicellulose and lignin.[24] During the milling process, besides the shell, inner parts of the kernel (rich in starch), are also milled thus in addition to a lignocellulosic structure, barley bran may also contain starch.[25] Due to the high content of lignin and hemicellulose, barley is mainly used in fermentation processes for the production of the enzyme laccase,[13] while the starch content in barley bran enables growth of microorganisms for amylase production.[15]

There is no literature data concerning cellulase production and process optimization using barley bran and *Paenibacillus*. With regards to cellulase production by microorganisms using barley bran or barley stalks as a substrate, only few reports are available. Assareh and co-workers[16] used untreated barley stalks to produce cellulase using a new bacterial isolates *Geobacillus* sp. A combination of barley bran and corn stalks was used for the production of CMC-ase in *Aspergillus fumigatus*, during the selection of fungus cellulase producers.[17]

In this work, barley bran was used as a substrate in a liquid medium for *P. chitinolyticus* CKS1 growth and the production of enzymes. Cellulases are inducible enzymes and their production is affected by the nature of the carbohydrate used during fermentation.[26] According to the literature, different waste materials that contains cellulose, used in the fermentation process, could also be cellulase inducers.[27] The strain CKS1 could produce both cellulase, CMCase and avicelase during its growth in a medium with barley bran. Other studies reported higher CMC-ase activity than that found in the present study. For example, during its growth on barley stalks, *Geobacillus* sp. produced CMC-ases with maximum activity 143.5 U mL^{-1}.[16] Several studies in the literature indicated that the source of carbon used in the fermentation process is one of the most important factors affecting the yield of cellulase.[28] For example, barley stalk is a good inductor of CMCase with maximum activity 143.5 U mL^{-1} *Geobacillus* sp.,[16] sugarcane bagasse was used for avicelase production (1.06 U mL^{-1}) by *Geobacillus stearothermophiles*,[29] while barley was used as an avicelase (0.10 U mL^{-1}) inductor in the fungus *Scytalidium thermophilum*.[28] However, the main point of this work was the simultaneous production of cellulase (CMC-ase and avicelase) and *β*-amylase in order to obtain their synergistic effect on enzyme-catalyzed processes.

During growth in a medium with barley bran, *P. chitinolyticus* CKS1 produced two types of cellulase, CMC-ase and avicelase, with different activities. The activity of the produced avicelase was higher than that of the produced

CMC-ase suggesting that the crude bacterial supernatant from the strain CKS1 predominantly contained higher exoglucanase levels. Ladeira and co-workers[30] reported that *Bacillus* sp. SMIA-2 could produce both CMC-ase and avicelase with higher avicelase activity than CMC-ase (0.29 *vs*. 0.83 U mL^{-1}). Moreover, according to Kostylev and Wilson,[31] the mechanisms by which different types of cellulases enhance each others activities are complex and not completely understood, and the published data is often inconsistent.

When a microorganism is presented with two substrates for carbon and an energy source, it first consumes only that substrate which supports a faster growth rate.[32] Since barley bran consists of cellulose and starch, the strain CKS1 first consumes the starch and then begins to synthesize the enzymes necessary for the utilization of the second substrate – cellulose.

It is well known that amylases are inducible enzymes and that the type of carbon source is a very important factor in amylases production. The maximum *β*-amylase activity obtained in this study using barley bran as the substrate was lower than the *β*-amylase activity produced by other microorganisms using different substrates.[33,34] However, the lower activity could be some characteristic of *P. chitinolyticus* sp. that are described as non-amylolytic in Bergey's manual.[35]

Yeast extract, as an additional supplement, rich in nitrogen, amino acids and vitamins, affects the growth of microorganism and thus enzymatic activity. In this study, a further increase in yeast extract concentration to above 4.0 g L^{-1} caused a slight decrease in the production of the enzymes. A high concentration of nitrogenous compounds could alter the hydrophobicity of the cell wall, which would decrease the production of cellulase.[36]

The strain CKS1 showed the ability to degrade cellulosic components and starch particles (Fig. 3). The changes in the morphological structure of the barley bran after fermentation indicate that *P. chitinolyticus* CKS1 could use both cellulose and starch for growth and simultaneous cellulase and *β*-amylase production.

According to the literature, no studies are available concerning the simultaneous production of cellulase and amylase by *Paenibacillus* sp. using the RSM approach. Thus, the strain CKS1 is the first reported *Paenibacillus* in which cellulase and *β*-amylase production was optimized through statistical design using barley bran. Although the levels of the produced cellulase and *β*-amylase, using barley bran as the fermentation substrate are low in comparison with values given in the literature, this method could be highly applicable in cellulase and *β*-amylase production using other agricultural by-products or low-cost substrates.

In order to evaluate the effectiveness of waste cotton decomposition by CKS1, waste cotton material from the textile industry was used as a hydrolysis substrate. Cotton fabrics are predominantly composed of cellulose (99 %) and therefore, it is possible to enzymatically hydrolyze cotton to obtain soluble sugars

such as glucose, and other oligosaccharides as the final products.[37,38] Cellulases that hydrolyze the β-1,4-glycosidic bonds in cellulose molecules are suitable for the enzymatic hydrolysis of cotton fabrics.[37] Although cellulase production using *Paenibacillus* sp. was reported earlier in the literature,[18,39–41] their potential application in the processes of enzymatic hydrolysis of cellulosic and lignocellulosic material has not been determined. In the literature, results concerning enzymatic hydrolysis are expressed using total reducing sugars. For example, total reducing sugars concentration of 50–80 mg g_{mat}^{-1} was reported in the work of Vallinachiyar.[42] They used different concentrations of cotton fibres (5–50 %) for hydrolyses with the cellulases produced by the fungus *Aspergillus nidulans*. The glucose concentration obtained by hydrolysis of cotton using the cellulase produced by CKS1 was almost double in comparison to literature data of total reducing sugars.

As it was previously stated in this study for cellulase application, the potential application of amylolytic enzymes produced by the genus *Paenibacillus* in starch hydrolysis is not specified. Generally, microorganisms mainly produce α-amylase, while a relatively small number of microorganisms have the ability to produce β-amylase. In this study, maltose was detected as the main product after enzymatic hydrolysis.

CONCLUSIONS

Recent trends in enzymatic production include valorisation of inexpensive raw materials, especially agricultural by-products, to valuable biotechnological products. Considering that the production of the enzyme is expensive, preference is given to microorganisms that produce simultaneously two or more enzymes using cheap substrates. This study represents a novelty in using barley bran as an agricultural by-product for simultaneous cellulase (CMC-ase and avicelase) and β-amylase production using the genus *Paenibacillus.* The produced enzymes could hydrolyze cotton fibres and barley bran, respectively, to form glucose and maltose as the end products of hydrolysis. These results suggest that CKS1 is a promising candidate for utilization in many industrial processes, especially ones that include saccharification of lignocellulosic biomasses. Moreover, this study provides several possibilities for further investigations of the enzymatic potential of *P. chitinolyticus* CKS1, which includes saccharification of waste material and biomass for bioethanol production.

SUPPLEMENTARY MATERIAL

HPLC analysis is available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

ЕФИКАСНО ИСКОРИШЋЕЊЕ ЈЕЧМЕНИХ МЕКИЊА У ПОСТУПКУ СИМУЛТАНЕ ПРОИЗВОДЊЕ ЦЕЛУЛАЗА И *β*-АМИЛАЗА ПОМОЋУ *Paenibacillus chitinolyticus* CKS1: СТАТИСТИЧКА ОПТИМИЗАЦИЈА И ПРИМЕНА ДОБИЈЕНИХ ЕНЗИМА

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Индустрија прераде пољопривредних сировина ствара велике количине споредних производа – агроиндустријског отпада који се акумулира током целе године и чије одлагање представља велики еколошки проблем. Литературни подаци показују да до сада нема забележених радова о искоришћењу оваквих отпадних сировина у процесима симултане производње ензима целулаза и *β*-амилаза помоћу бактеријског соја *Paenibacillus chitinolyticus* CKS1 а у циљу смањења трошкова производње ензима. У овом раду је приказана симултана производња ензима целулаза (ендоглуканаза и егзоглуканаза) и *β*-амилаза на отпадном супстрату – јечменим мекињама природног бактеријског изолата CKS1 као и потенцијална примена добијених ензима у процесу хидролизе. Применом методе статистички план и раног експеримента (метода одзивних површина), под оптималним условима, максимум производње ендоглуканаза износио је 0,405 U mL^{-1}, егзоглуканаза 0,433 U mL^{-1} и *β*-амилаза 1,594 U mL^{-1}. Деградација лигноцелулозно-скробног отпадног супстрата – јечмених мекиња, након ферментације сојем CKS1 је потврђена применом скенирајуће електронске микроскопије (SEM). Сирови бактеријски супернатант соја CKS1, који садржи целулазе и *β*-амилазе је коришћен за хидролизу памучног материјала, као и јечмених мекиња. Главни производи ензимске хидролизе памучног материјала и јечмених мекиња, глукоза (0,117 g g_{mat}^{-1}) и малтоза (0,347 g g_{mat}^{-1}) детектовани су течном хроматографијом високих перформанси (HPLC). Произведени ензими су коришћени у поступцима хидролизе памучног материјала и јечмених мекиња, као извори целулозе и скроба. Симултана производња ензима целулаза и амилаза на отпадном сусптрату – јечменим мекињама, као и примена датих ензима у процесима хидролизе а у циљу добијања биотехнолошки важних производа – глукозе и малтозе, са економског и еколошког аспекта може бити врло користан и занимљив технолошки процес.

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