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HIGHLIGHTS

- *Bacillus* strains were able to grow and degrade wheat bran releasing soluble sugars.
- Members of bacterial consortia were selected based on cluster analysis.
- Consortium of bacterial strains increased significantly cellulolytic activities.
- Efficacy of the consortium in wheat bran pretreatment was increased significantly.
- *Bacillus* consortia showed an increase in cellulose bioconversion than pure cells.

GRAPHICAL ABSTRACT
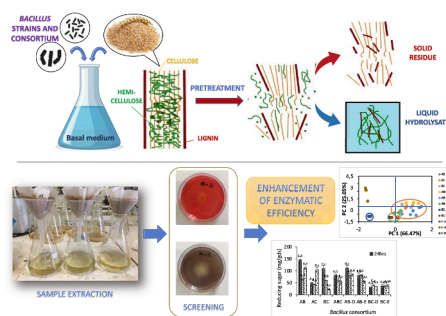
ARTICLE INFO

ABSTRACT

In the downstream process, the bioconversion of lignocellulosic biomass can be improved by applying a biological pretreatment procedure using microorganisms to produce hydrolytic enzymes to modify the recalcitrant structure of lignocellulose. In this study, various *Bacillus* strains (*B. subtilis* B.01162 and B.01212, *B. coagulans* B.01123 and B.01139, *B. cereus* B.00076 and B.01718, *B. licheniformis* B.01223 and B.01231) were evaluated for the degrading capacity of wheat bran in the submerged medium using enzymatic activities, reducing sugars and weight loss as indicators. The obtained results revealed that the *B. subtilis* B.01162, *B. coagulans* B.01123 and *B. cereus* B.00076 could be promising degraders for the wheat bran pretreatment. Besides, the application of their consortium (the combination of 2–3 *Bacillus* species) showed the positive effects on cellulose bioconversion compared with monocultures. Among them, the mixture of *B. subtilis* B.01162 and *B. coagulans* B.01123 increased significantly the cellulase, *endo*-glucanase, and xylanase enzyme activity resulting in accelerating the lignocellulose degradation. Our results served a very good base for the development of microbial consortium for biological pretreatment of lignocellulosic raw materials.

1. Introduction

Lignocellulosic biomass (LB) is one of the most potent sources for replacing the fossil carbon from fuel production due to its great availability and renewability. Wheat bran (WB) is an agricultural residue of the rolled milled wheat grain; its main layers include pericarp, aleurone and testa tissue. In chemical composition, WB is rich in cellulose, hemicellulose and lignin a complex polymer matrix trapping cellulose and hemicellulose inside its interspace (Farkas et al., 2019; Hell et al., 2014; Stevenson et al., 2012). Because this recalcitrant lignocellulosic structure often prevents the enzymatic access in saccharification steps of bioconversion processes, the pretreatment of LB is necessary to break down the lignin seal and open the bundle form of cellulose. Among various approaches, the biological pretreatment using the enzymatic hydrolysis from microorganisms is attractive. Therefore, it has gained great attention since their mild working conditions do not require high energy input and chemical additions.

For the lignocellulose biodegradation, several types of enzymes can be applied such as ligninolytic enzymes, cellulolytic and hemicellulolytic enzymes (de Gonzalo et al., 2016; Gupta et al., 2016; Vasco-Correa et al., 2016). However, the lignocellulolytic bioconversion efficiency by commercial enzymes is often lower than that of microorganisms (Poszytek et al., 2016). Mono-strains or microbial consortia can secrete hydrolytic enzymes during their metabolism, disintegrate the cellulose, hemicellulose and lignin, so that the biopolymers are converted into smaller fragments (Ferdeş et al., 2020; Jiménez et al., 2017). Naturally, a large variety of organisms have been found to degrade the lignocellulosic biomass successfully. Gram-positive *Bacillus* is well-known for its degradation capacity of the wide sole of substrates incorporate from agricultural residues, producing monetary creation of compounds. The certain high impacts of *Bacillus* species such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus coagulans* and *Bacillus cereus* were reported to produce the array of cellulolytic enzymes such as cellobiase-rich cellulase, *endo*-glucanase using agriculture residuals as sole substrate (Aulitto et al., 2017; Bano et al., 2013; Chantarasiri, 2015; Shu-bin et al., 2012; van Dyk et al., 2009). Previous studies showed that the activity of the bacteria mixtures (or consortia) in lignocellulose degradation was higher than that of each component since their communities can perform complex tasks which individual strain must struggle to overcome (Kato et al., 2004; Haruta et al., 2002). Several publications claim that stable complex microbial communities enhance the rates of bioconversion of non-sterilized and sterilized potential biorefinery cellulose substrates to valuable products (Cortes-Tolalpa et al., 2016; Wongwilaiwalin et al., 2010; Haruta et al., 2002). Furthermore, the synergistic action of thousands of microorganisms has been recently applied in most organic wastes, such as agricultural residues, sludge wastewater in pulp or paper mills (Boopathy et al., 2015; Cortes-Tolalpa et al., 2017). Recently, Deng and Wang (2016) reported a higher amount of degraded lignocellulose in 15 of 27 mixed cultures in comparison with single culture under similar physical conditions. Despite various *Bacillus* species are well-known to produce cellulase enzymes which account for the breakdown of recalcitrant lignocellulosic biomass, the research focused on their applications in this field still has been limited. Moreover, the potential for using the mixtures of cellulose degrading microbes inspired us to construct *Bacillus* consortium with improved cellulase activities for higher bioconversion efficiency.

To achieve the effective bacterial consortium for lignocellulosic degradation, whether prevailed synergism or antagonism between species in consortium must be clarified, driven by the complementary mechanisms, the competition for nutrients or antagonistic interaction. In this study, the efficacy of individual *Bacillus* strains, and their consortium on biological pretreatment of wheat bran as model lignocellulosic biomass, was focused.

2. Materials and methods

2.1. Materials

A total of eight *Bacillus* strains including *Bacillus subtilis* (B.01162; B.01212); *B. licheniformis* (B.01223; B.01231); *B. coagulans* (B.01123; B.01139); *B. cereus* (B.00076; B.01718) were received from the National Collection of Agricultural and Industrial Microorganisms (NCAIM, Hungary). These strains were grown for 24 h in the nutrient medium (NCAIM 0025) containing 1 g/L yeast extract, 2 g/L meat extract, 5 g/L peptone, 5 g/L sodium chloride, then being stored at 4 °C until use.

Wheat (*Triticum aestivum*) bran was purchased from Denes-Natural Kft. (Pecs, Hungary). The label chemical components of wheat bran were about 3.4% (w/v) fat (0.8% (w/v) saturated fatty acids), 56.2% (w/v) carbohydrates (5.0% (w/v) sugar), 16.3% (w/w) protein, 0.03% (w/w) salt.

2.2. Screening for cellulase producer

The substrate-agar plate assay was used to screen the high cellulase producer from various strains of *Bacillus* bacteria. Carboxymethyl cellulose (CMC): agar plates contain (g/L): CMC, 5.0; $NaNO_3$, 2.5; yeast extract, 1.0; KH_2PO_4, 1.0; NaCl, 1.0; $MgSO_4.7H_2O$, 0.6; $CaCl_2$, 0.1; $FeCl_3$, 0.1; agar, 15.0. Medium was adjusted to pH 7.0 by NaOH 1 M solution.

In brief, 0.1 ml of the growing culture of *Bacillus* strain was streaked on the surface of CMC agar plates, then incubated at 30 °C for 24 h in an incubator (C85G V LB Standard, Helkama Forste Kft., Hungary) for *endo*-glucanase enzyme release. At the end of the incubation, the agar plates were stained by 0.5% congo red, dyed for 10 min before being de-stained by NaOH 1 M solution and rinsed by 0.5% acetic acid solution and cold water at the last step. The formation of a clear zone around the colony in CMC agar plates indicated the hydrolysis of CMC by enzyme *endo*-glucanase.

2.3. Biological pretreatment of wheat bran

The monoculture and microbial consortium constructed from the effective strains were conducted in a basal medium containing wheat bran 2% (w/v). Basal medium was prepared with nutrient components (gL^{-1}) such as lactose, 5.0; NH_4NO_3, 5.0; KH_2PO_4, 1.0; NaCl, 1.0; $MgSO_4.7H_2O$, 0.6; $CaCl_2$, 0.1; $FeCl_3$, 0.01. The pH 7.0 of the medium was obtained by using NaOH 1 M solution before autoclaving. After cooling down the flasks, the equivalent inoculum was added to 250 ml flask containing 150 mL of medium to obtain 10^5 CFU/mL, then incubated at 30 ± 2 °C for 7 days, 140 rpm agitation speed. Every 24 h of incubation, the culture broth sample was centrifuged at 17.968 (x g) of relative centrifugal force for 10 min at room temperature to remove the cell and supernatant. All the samples were kept at −20 °C for further analysis.

The microbial consortium was cultivated followed similar procedures of the cultivation of individual species. Various microbial communities were established by combining individual strains which were freshly incubated for 24 h in separated flasks. Two-member and three-member consortia are constructed by adding 10^5 cells/mL of each strain into testing flasks at the ratio of 1:1 or 1:1:1, respectively.

2.4. Evaluation the degradation rates

After 7 days of pretreatment by *Bacillus* strains and their consortia, the residue remaining was quantitatively determined by gravimetric methods (Qiu and Chen, 2012). Briefly, after each biotreatment, the solid compounds were suspended in water to remove adherent bacteria, separated using Whatman filter paper (MN 612, Macherey-Nagel, Germany) and dried at 105 °C for 24 h. Then, the samples were stored in the desiccator for 48 h to reach the saturated moisture and weighted to determine the total weight loss.

2.5. Determination of reducing sugar yield

Reducing sugar concentration was determined by the Somogyi-Nelson method (Dénes et al., 2013), using the spectrophotometer (Helios Gamma, Unicam, United Kingdom) at 540 nm. The blue color developed by reduction of arsenomolybdic acid to molybdenum and cuprous oxide which was formed by the reaction of reducing sugar with alkaline copper tartrate at high temperature was determined. A series of different concentrations of glucose solution were prepared and used to establish the calibration curve. The working solution was diluted properly before measurement.

2.6. Assays of enzyme activities

The crude enzyme solution was harvested at 24 h interval up to 7 days, by centrifugation at 14000 rpm in 10 min at ambient temperature. The extracted supernatants were used to measure enzyme activities including total cellulase, *endo*-glucanase, xylanase and β-glucosidase activities. Filter paper, carboxymethyl cellulose (CMC), birchwood xylan substrates were used for determination of total cellulase, *endo*-glucanase and xylanase activities, respectively. Total cellulase activity was assayed by reacting 0.5 mL crude enzyme solution with one strip of filter paper weight 50 mg (equivalent dimension of 1×6 cm), using 1 mL of 0.05 M sodium citrate buffer, pH 7.0 and incubated at 50 °C for 1 h (Yu et al., 2016). Endo-glucanase activity was measured at pH 7.0 at 50 °C for 15 min of reaction, containing 1% (w/w) CMC in the 0.05 M citrate buffer. Total cellulase activity and *endo*-glucanase activity (U/mL) was calculated using the following formula (Shareef et al., 2015):

$$FPase(or\ CMCase)activity\ (U/ml) = \frac{Abs \times d \times V_r}{V_s \times t \times M_G \times s}$$

where Abs is the absorbance, d is the dilution factor, V_r is the volume of the reaction medium, V_s is the volume of the sample, t is the incubation time; s: slope of the calibration curve of glucose, M_G is the molecular mass of glucose.

In the case of xylanase, the reaction mixture contains enzyme preparation with 1 mL of 1% xylan substrate (w/w) and 1 mL of buffer solution (50 mM citrate at pH 7.0), the enzyme activity was determined after 10 min of reaction at 50 °C in water bath. Xylanase activity was measured using the formular below:

$$Xylanase\ activity\ (U/ml) = \frac{Abs \times d \times V_r}{Vs \times t \times M_X \times s}$$

where Abs is the absorbance, d is the dilution factor, V_r is the volume of the reaction medium, V_s is the volume of the sample, t is the incubation time; s: slope of the calibration curve of xylose, M_X is the molecular mass of xylose.

Following the incubation step, hemicellulase assay was conducted by placing the tube with reactive mixtures in boiling water for 15 min to stop the reaction. The mixture was then allowed to cool down at room temperature before reading the optical density at 540 nm in accordance with the Somogyi-Nelson method (Farkas et al., 2019). One enzyme unit is defined as the amount of enzyme used to release 1 μmol of reducing sugar per minute under room conditions.

The β-glucosidase activity was assayed by determining the amount of p-nitrophenol liberated from the reaction between p-nitrophenol-β-D-glucopyranoside (PNPG) and enzyme solution. Adding 1 mL of PNPG (10 mM solution in 50 mM citrate buffer, pH 4.8) to 1 mL of the supernatant, then the mixture was incubated at 50 °C in the water bath for 10 min. The reaction was stopped by the addition of 1 mL of 1 M Na_2CO_3, then centrifuged to remove the insoluble substances. The absorbance of released p-nitrophenol was measured spectrophotometrically at 410 nm. One unit of β-D-glucosidase activity is defined as the amount of 1 μmol of p-nitrophenol released per minute under conditions of the assay.

2.7. Statistical analysis

All experimental sets were prepared in three replications with several treatments by utilization of individual strains and their consortia. The data were processed in Microsoft Excel spreadsheet and expressed as the mean ± SE of different independent replicates. One-way analysis of variables (one-way ANOVA) followed by TUKEY post hoc multiple comparison tests was conducted using SPSS software (version 20.0) to test the differences between the variances. Data were considered significant at $p < 0.05$.

Multivariate methods including cluster analysis and principal component analysis were conducted to describe diversity patterns between strains and consortia's hydrolysis capacity, by focusing on the significant differences between groups or clusters. In addition, enzyme production capacity of 8 individual strains was obtained in clustering results using Euclidean shortest distance method.

Using the Principal Component Analysis (PCA) method, samples pretreated by microbial consortium were compared based on the data's average value, including enzyme activities, reducing sugars yields, using the Principal Component Analysis (PCA) method. The correlation matrix between variables is calculated to transform orthogonal, creating new axes (eigenvectors) installed as the original variables' linear combination. The percentage variations of two principal components in investigated variable were obtained in the PCA diagram using SPSS 20.0. The contribution rates of each variable to PC1, PC2 and their interrelations were also performed.

3. Results and discussion

3.1. Screening of Bacillus strains for pretreatment of wheat bran

Screening of the cellulolytic bacteria was conducted using CMC agar plates with congo red straining method (Hendricks et al., 1995). In this study, clear zones appeared on the surface of CMC agar of all samples, indicating the cellulase enzymes, specifically *endo*-glucanases. Some previous studies also claimed the cellulase activity of *Bacillus* strains using congo red (Chantarasiri, 2015; Kim et al., 2012; Shareef et al., 2015). On another aspect, dry weight loss and the reducing sugar concentration could be used as indicators for the estimation the bioconversion efficiency of microorganism. According to Fig. 1A, the control has a weight loss of approximately 30% due to the autohydrolysis process during the sterilization using autoclaving. For *Bacillus* pretreatment samples, the weight losses of substrate are relatively high ranging from 54% to 60% except samples from *B. licheniformis* B.01223 and B.01231 strains. These mass loss data indicated the advantages of biological pretreatment using *Bacillus* species for wheat bran as substrate, which gained higher efficiency than physical pretreatment or utilization of commercial enzymes according to the previous work of Hell and coworkers (2015). Additionally, Zhang et al. (2016) claimed around 22% of biomass lost after 6 days under submerged cultivation of *Bacillus* strain in fresh medium with 1% (w/v) rice straw as substrate.

Based on the Fig. 2A, the weight losses seemed to be in a correlation with the released reducing sugars produced after the biological pretreatment (except with *B. subtilis* B.01212 strain). The released reducing sugars were well-associated with the enzymatic activities of the microorganisms, which catalyzed the glycosidic linkages of celluloses or hemicellulose to produce glucose, xylose or oligosaccharides with reducing ends and thus, increasing in concentration of soluble sugars. Among investigated species, *B. cereus* and *B. coagulans* produced significantly higher amount of reducing sugars than other strains ($p < 0.05$). On the contrary, *B. subtilis* B.01212 and *B. licheniformis* B.01223 showed the lowest yield of reducing sugars (28 mg/gds and 18 mg/gds at 24 h, respectively), indicating the lower efficiency of hydrolysis. In all samples, the reducing sugar concentration increased with time of incubation
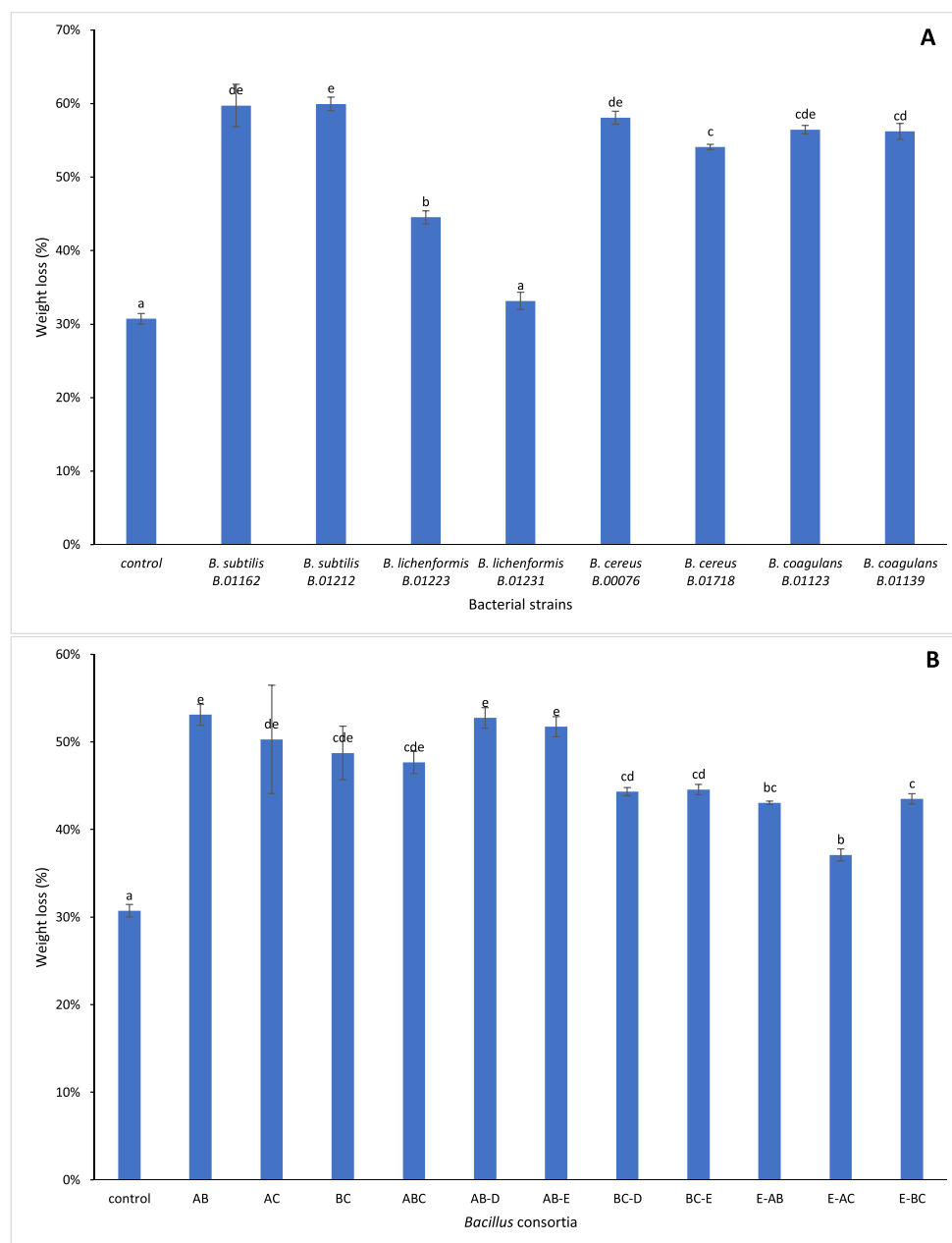
Fig. 1. Dried weight loss of wheat bran after 7-day of cultivation of *Bacillus* strains (A) and *Bacillus* consortia (B) (members of *Bacillus* consortia shown in Table 1). Data were reported as the mean ± SD (standard deviation). Mean values with different letters above the bars differ according to Tukey's test at P < 0.05.

within the first 72 h, then significantly dropped on the day after. It might be explained by the negative effect on microbial metabolism caused by concentrated sugar in culture medium (Reischke et al., 2014). Thus, the longer treatment lead to the loss in biomass from bacterial metabolism, which was also reported in with previous works (Guo et al., 2018; Wan and Li, 2010).

To extensively elaborate the mechanism of cellulose degradation, the enzyme activities were assayed at 72 h of harvesting and the results were summarized in Table 2. Firstly, the total cellulase activities ranged from 0.038 U/mL to 0.336 U/mL. Among these strains, strains of *B. cereus* and *B. coagulans* achieved the highest cellulase with no significant difference from each other, reached above 0.300 U/mL. *B. subtilis* B.01212 and *B. licheniformis* B.01223 showed low total cellulase activities of 0.038 U/ mL and 0.045 U/mL, respectively. Other species (0.115 U/mL by *B. licheniformis* B.01231 and 0.242 U/mL by *B. subtilis* B.01162) posed moderate total cellulase activity in quantification. Similarly, high

endoglucanase enzyme activities were also observed in strains that distinguished as the best total cellulase producers, including *B. cereus* B.00076, *B. cereus* B.01718, *B. coagulans* B.01123, *B. coagulans* B.01139 and *B. subtilis* B.01162 strains, with activities of 1.327 U/mL, 1.303 U/ mL, 1.374 U/mL, 1.325 U/mL and 1.241 U/mL, respectively. Studying the cellulose degradation activities of 11 strains isolated from water and soil samples of hot springs in India, it was shown that strains of *Bacillus* genus presented similar enzymatic profiles of total cellulase activities and endo-glucanase activities (Singh et al., 2019). They reported that the maximum cellulase activities and endo-glucanase were found after 72 h by the cultivations of *B. subtilis* BHUJPV-H12, *B. subtilis* BHUJPV-H19, *B. subtilis* BHUJPV-H23 and *B. stratosphericus* BHUJPV-H5 strains (Singh et al., 2019). Ladeira et al. (2015a) reported that *Bacillus sp.* was able to produce 0.290 U/mL endo-glucanase activity at 50 °C incubation. In the present study, other strains produced enzymes at a much lower activity rate with 0.169 U/mL by *B. licheniformis* B.01223 and
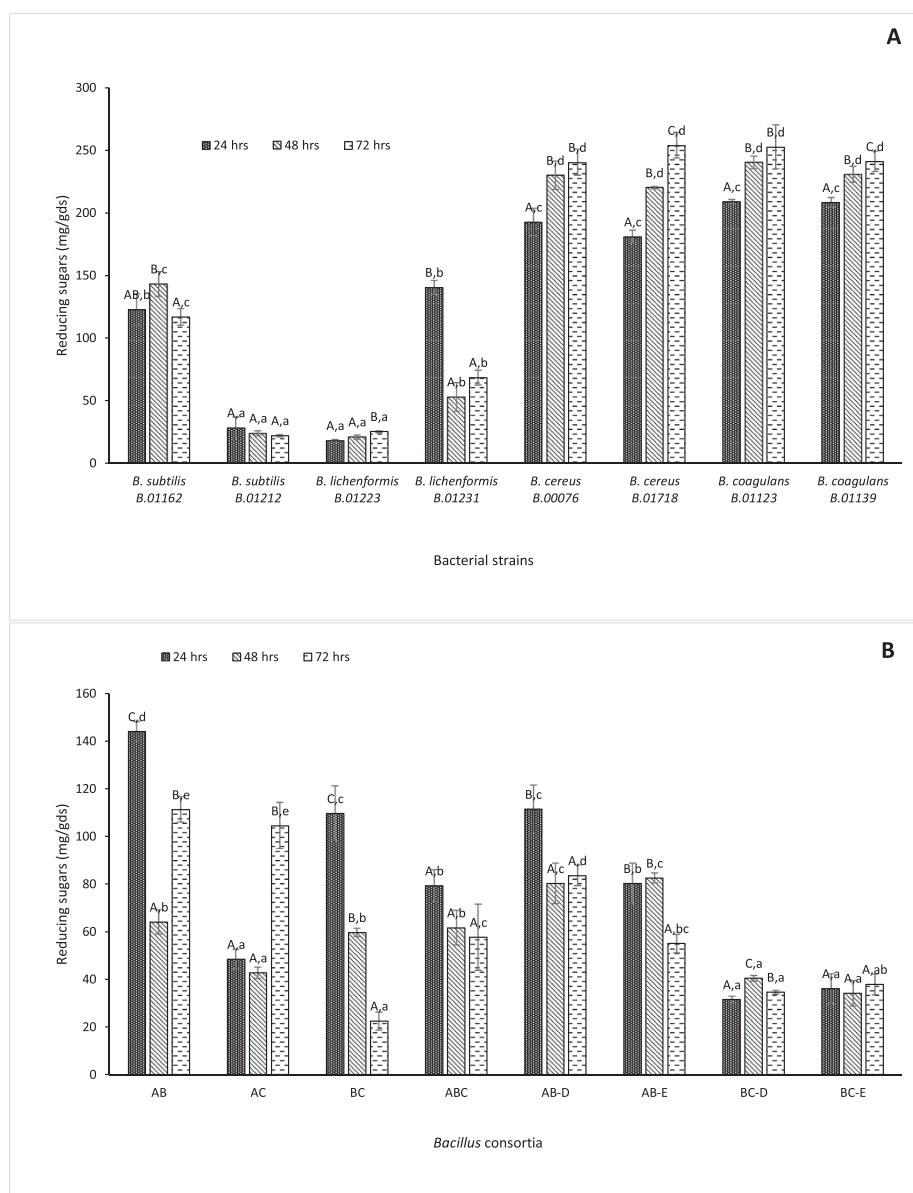
Fig. 2. Reducing sugar concentration of *Bacillus* strains (A) and *Bacillus* consortia (B) after 24, 48 and 72 h of cultivation. Data are reported as the mean ± SD (standard deviation). Capital letter (A,B,C) indicate the difference by treatment time and lower-case letters (a, b, c, d, e) demonstrated difference by strains. Mean values with different letters above the bars differ according to Tukey's test at P < 0.05.

0.174 U/mL by *B. licheniformis* B.01212 at 30 °C of incubation. Maximum activity of β-glucosidase was obtained by *B. cereus* B.01718, value at 0.129 U/mL. *B. coagulans* B.01123, *B. coagulans* B.01139 strains showed the β-glucosidase activities of 0.118 U/mL, while a comparable result of 0.108 U/mL was obtained by *B. cereus* B.00076 and *B. subtilis* B.01162 strains. Although possessing the low total cellulase and endoglucanase but the high content of β-glucosidase produced by *B. subtilis* B.01212 strain in shake flask fermentation may attribute to the degradation of wheat bran. Agrawal et al. (2014) also claimed that the overall rate of bioreaction could be enhanced by high β-glucosidase activities which contributed to reducing of end-product inhibition.

Hemicellulose removal by bacteria is one of the most promising approaches to enhance the digestibility of lignocellulolytic biomass. As one of the main heteropolymers of the hemicellulose, xylan with its hydrogen and covalent bonds forms a sheathing layer to cellulose, accounts for the structural integrity of lignocellulose. Therefore, the cleavage of xylan by xylanase enzymes can significantly increase cellulose accessibility (Hu et al., 2013; Moreira and Filho, 2016; Sindhu

et al., 2016). Numerous previous studies found the high xylanase activity using wheat bran as sole substrate by shake flask or solid-state cultivation of *B. coagulans* (Choudhury et al., 2006; Heck et al., 2005). Roy & Habib (2009) discovered neutral xylanase secretion of *Bacillus cereus* using oat spelt xylan, birchwood xylan and beechwood xylan as sole substrate, and the maximum activities were reported at 40 °C and pH 6.0. In our study, *B. subtilis* B.01162, *B. cereus* B.00076, *B. cereus* B.01718, *B. coagulans* B.01123, *B. coagulans* B.01139 strains showed considerable xylanase activities in the cell-free culture supernatant. The maximum value was reached by the cultivation of *B. subtilis* B.01162 strain (2.081 U/mL) for 72 h of shaking at 140 ppm. The other strains *B. subtilis* B.01212, *B. licheniformis* B.01223 and *B. licheniformis* B.01231, however, produced lower xylanase activities as well as cellulase.

In the fermentation using microorganisms, the pH value is also a common indicator for their growth. Fig. 3 illustrates the correlation of pH value with the reducing sugars during single strain cultivation. It was found that pH of samples *B. licheniformis* B.01231, *B. cereus* B.00076 and *B. coagulans* B.01123 and B.01139 strains tended to stay constantly at a
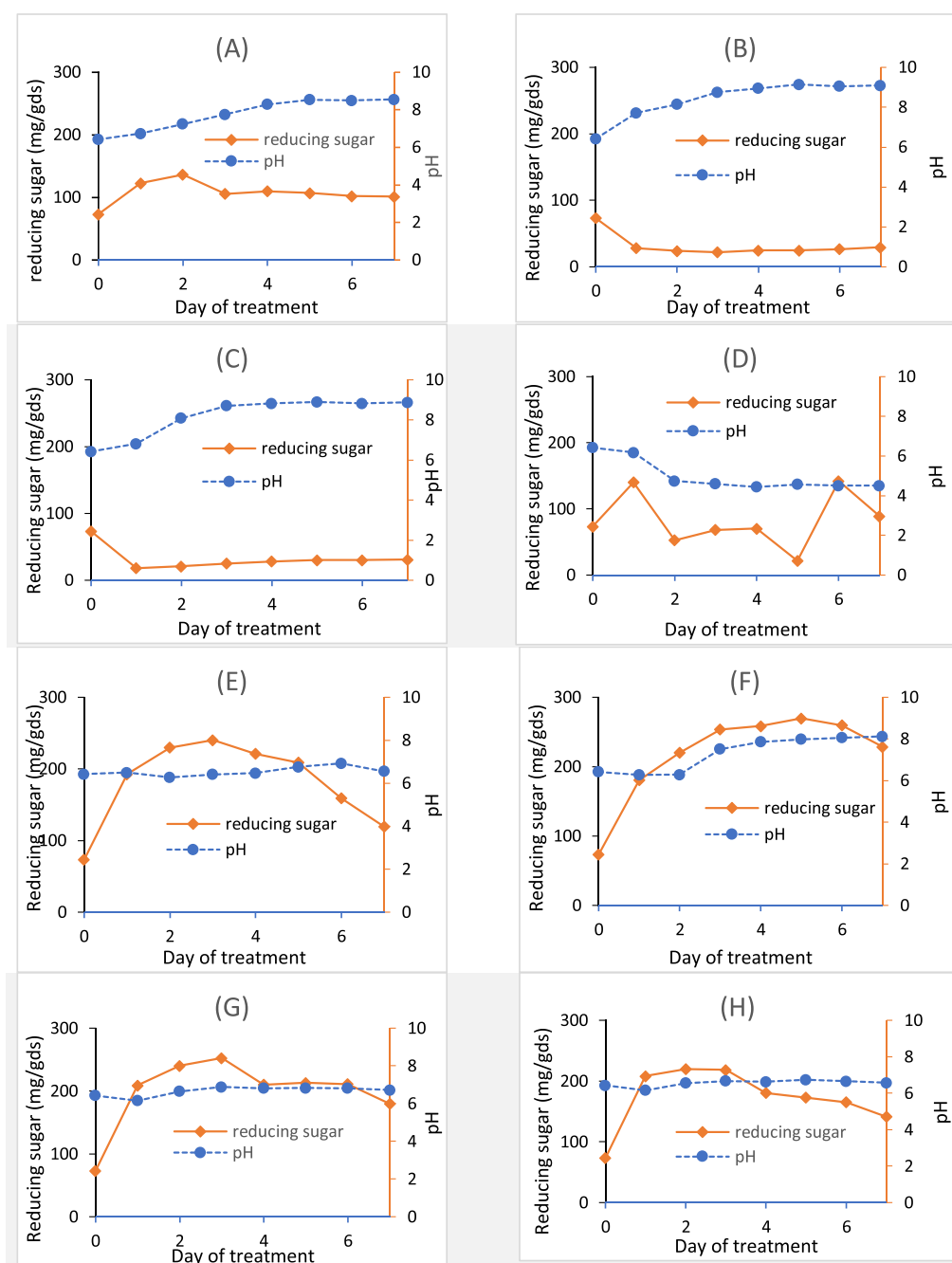
Fig. 3. Relevance of pH value with reducing sugar yield produced in pretreatment by individual *Bacillus* strains. *B. subtilis* B.01162 (A), *B. subtilis* B.01212 (B), *B. licheniformis* B.01223 (C), *B. licheniformis* B.01231 (D), *B. cereus* B.00076 (E), *B. cereus* B.01718 (F), *B. coagulans* B.01123 (G), *B. coagulans* B.01139 (H).

value of pH 7.0 or decreased due to the presence of organic acid during microbial metabolism. By contrast, pH value of the liquid extract from *B. subtilis* B.01162, B.01212, *B. licheniformis* B.01223, and *B. cereus* B.01718 strains increased by the cultivation time. The amino acid consumption of cells could explain this trend in the absence of glucose as the sole nutrient. As a consequence, ammonia could release and attract protons to form ammonium in an aqueous solution and increase pH value. In a previous study, Haruta et al. (2002) utilized microbial consortium for the hydrolysis of rice straw and observed the pH values pH 6.0 within 4 days, before increasing to pH 8.0 – 9.0. Additionally, the pH profile during cultivations of *Bacillus* sp. SMIA-2 strain on sugarcane bagasse, carried by Ladeira et al. (2015b), revealed the increase of pH value at the end of the fermentation process to 8.5 from the initial value of pH 7.1.

3.2. Selection of members for organization of microbial consortium

Cluster analysis technique using Cluster with Ward's method was used to group the strains of *Bacillus* aerobic bacteria based on the enzyme activity (total cellulase enzymes, endo-glucanase, β-glucosidase, xylanase enzymes) and the reducing sugar contents (Fig. 2B). The total of 24 variances was classified into 3 groups A, B and C. Cluster A (includes *Bacillus cereus* and *Bacillus coagulans* species) showed the highest enzyme production. These strains also performed the promising ligno-cellulolytic biomass degradation capacity based on the high produced reducing sugar. *Bacillus subtilis* B.01162 strain was grouped in the cluster B, presenting the moderate capacity for hydrolytic enzyme production. The strains of *B. licheniformis* and *B. subtilis* B.0112 were clustered together to the cluster C, which gave insufficient effect on the bio-

hydrolysis process of wheat bran. These results were in line with previous works, which reported that *B. coagulans* and *B. cereus* could secrete a high level of cellulolytic activities towards lignocellulosic materials (Aulitto et al., 2017; Chantarasiri, 2015; Kovács et al., 2010). For remaining cluster with the presence of *Bacillus licheniformis*, the decrease in secreted cellulases could be explained by the presence of proteases. Schallmey, Singh & Ward (2004) discovered the use of *B. licheniformis* for thermal-tolerant protease production. Protease was claimed to have the negatively effect on hydrolysis efficiency (Wang and Hsu, 2006). Haab and co-workers (1990) explained that protease tightly binds to cellulase proteins, thus modifying homogeneously cellulase proteolytically. The low concentration of protease accounted for the extremely rapid loss of cellulase and xylanase activity (Whelan and Pembroke, 1989). Characteristics of each group were analyzed and presented in Fig. 4. In the first group (cluster A), bacterial strains showed superior cellulase enzymes and reducing sugar yield as compared to xylanase activity. On the other hand, xylanase activity in cluster B varied less and remained at a high level but there was no significant effect on the lignocellulolytic degradation, which proved the moderate reducing sugar yield. The last one (cluster C) including two strains of *B. licheniformis* and *B. subtilis* B.01212 strain, which produced the lowest enzymatic activities as well as reducing sugar yields. Overall, the results of cluster analysis appear to be useful for the classification of numerous strains and selection of effective strains based on their bio-information. Regarding these analyses, different combinations of strains of *B. subtilis*

B.01162, *B. coagulans* B.01123, *B. cereus* B.00076, *B. licheniformis* B.01223 and *B. licheniformis* B.01231, were constructed to evaluate their interaction as well as degradation capacity in co– and multi-cultures.

3.3. Performance of microbial consortium on biological pretreatment

According to the screening experiment, five strains of different *Bacillus* species were selected for the construction of eleven different consortia (Table 1). The consortium's lignocellulolytic activities were investigated by determining the biomass degradation during 3 days of incubation period. The variety number of members of the microbial communities and the methods of incubation were claimed to have a significant effect on pretreatment of lignocellulose substrate (p < 0.05). The consortium of *B. subtilis* B.01162 and *B. coagulans* B.01123 strains regarding 2-member mixture, and 3-member mixture including 2 above strains and *B. licheniformis* B.01223 strain added at 24 h later after the first inoculation, gave the highest weight losses ranged from 51.74 to 53.1% (Fig. 1B). Zhang et al. (2016) reported that XDC-2 consortium gave the best weight loss of corn straw of 40% with high xylanase production capacity. Meanwhile, individual strain of *Bacillus*, *Clostridium* and *Bacteroides* cultivation accounted for the lower percentage of solid cleavage, <85% of those of XDC-2 consortium (Table 2).

The increasing pH from neutral to alkaline was observed in the first 3 days in hydrolysate of some consortia with high degradation rate. This fact was in agreement with previous work conducted by Shruti and
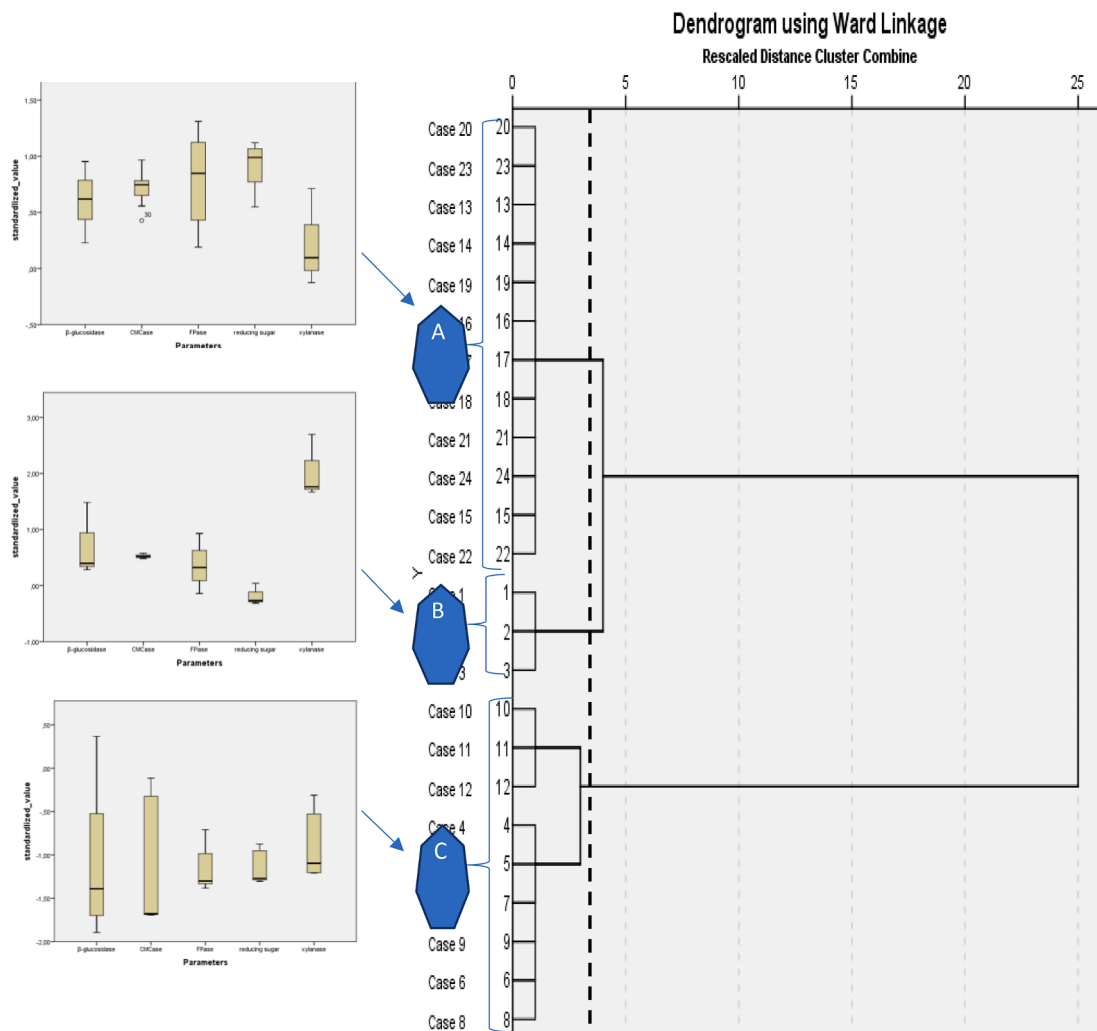

Fig. 4. Cluster analysis and its characteristic using Ward's minimum variance, based on hydrolytic enzyme and reducing sugar in pretreatment by *Bacillus* strains.

Table 1

Description of the cellulolytic consortia. All synthetic microbial communities were at least 3 replicates.

Type of Mix	Synthetic community	*Bacillus* strains			
5 selected strains		**I**	**II**	**III**	**IV/V**
2-member consortia	AB	*B. subtilis* B.01162	*B. coagulans* B.01123		
	AC	*B. subtilis* B.01162		*B. cereus* B.00076	
	BC		*B. coagulans* B.01123	*B. cereus* B.00076	
3-member consortia	ABC	*B. subtilis* B.01162	*B. coagulans* B.01123	*B. cereus* B.00076	
	AB-D	*B. subtilis* B.01162	*B. coagulans* B.01123		*B. licheniformis* B.01223
	AB-E	*B. subtilis* B.01162	*B. coagulans* B.01123		*B. licheniformis* B.01231
	BC-D		*B. coagulans* B.01123	*B. cereus* B.00076	*B. licheniformis* B.01223
	BC-E		*B. coagulans* B.01123	*B. cereus* B.00076	*B. licheniformis* B.01231
	E-AB	*B. subtilis* B.01162	*B. coagulans* B.01123		*B. licheniformis* B.01231
	E-AC	*B. subtilis* B.01162		*B. cereus* B.00076	*B. licheniformis* B.01231
	E-BC		*B. coagulans* B.01123	*B. cereus* B.00076	*B. licheniformis* B.01231

Table 2

Enzymatic production of various enzymes by individual *Bacillus* strains in the pretreatment hydrolysate at 72 h of cultivation.

STRAIN	Enzyme activities (U/mL)			
	FPase	CMCase	β-glucosidase	Xylanase
B. subtilis B.01162	0.242[c]	1.241[c]	0.109[bc]	2.081[f]
B. subtilis B.01212	0.034[a]	0.167[a]	0.076[bc]	0.339[ab]
B. lichenformis B.01223	0.045[a]	0.169[a]	0.060[ab]	0.264[a]
B. lichenformis B.01231	0.115[b]	0.914[b]	0.030[a]	0.714[bc]
B. cereus B.00076	0.336[d]	1.327[cd]	0.108[bc]	1.157[de]
B. cereus B.01718	0.315[d]	1.303[cd]	0.129[c]	1.438[e]
B. coagulans B.01123	0.311[d]	1.374[d]	0.118[c]	0.989[cd]
B. coagulans B.01139	0.303[d]	1.325[cd]	0.118[c]	0.927[cd]

Kumar (2015) using microbial consortium isolated from wood rot and rice field soil sample for the degradation of rice straws, in which lignocellulose degradation increased with increasing pH value. However, it was also found that pH probably did not interfere the degradation rate by individual species. For instance, 60% of solid residue loss under 7 days of incubation by some pure *Bacillus* strains was found in different pH culture medium which range from pH 6.4 – 8.7. The competitive interactions among bacteria in nature are ubiquitous, even in the synergistic consortium. The relative decrease in reducing sugar yield was found in microbial consortia compared to individual strains. When wheat bran was used as substrate, species form consortium synergistically acted to modify on lignocellulose structure. Deng & Wang (2016) proved that bacteria in synergistic mixed cultures had higher metabolic activity than those in pure culture, showing their competition in nutrient consumption, thus led to the drop of fermented sugar yield during co-culture incubation.

Additionally, two-step cultivation approach was also applied to determine the interaction between strains. The *B. licheniformis B.01223* strain (D) cooperated with AB consortium than other microbial communities when added 24 h later. 1-day-grown AB consortium took the dominant role in degrading consortium performance with strain D, resulting in a high degradation rate. In contrast, initially inoculated *B. licheniformis* B.01223 strain before the cultivation of *B. subtilis* B.01162 and *B. coagulans* B.01123 strains did not reach the expectation for high degradation efficiency. Under those cultivation methods, it was observed that dominant strains which initially cultivated determined the degradation performance of applied microbial consortium. A minority population could became the most metabolically active strain, upon which the survival of the entire consortium depends (Brenner et al., 2008). Thus, a suitable cultivation strategy plays the important role in achieving the high degradation efficiency in pretreatment of lignocellulosic biomass using *Bacillus* consortium.

In another notice, the positive effect of complex microbial communities to enhance the enzymatic production in the bioconversion of lignocellulose to valuable products which also claim in several works (Jiménez et al., 2014; Wongwilaiwalin et al., 2010; Haruta et al., 2002).

Positive bacterial interaction promoted the production of total cellulase, endo-glucanase and xylanase compared to those by individual strain incubation. The enzymatic activities of selected species and their high impact consortia at 72 h of cultivation can be noted in the Table 3.

The maximal filter paper enzyme activity and xylanase activity secreted in consortia of AB, BC and ABC were almost 2-fold higher and over 3-fold higher than that of monocultures, respectively. The endo-glucanase activities of the listed consortia were found to be slightly higher than single cell cultivation, from 20 to 60%. The other mixtures of microbial communities did not show any improvement in cellulase production or even observing the negligible role of enzymatic activities in degradation process. It was also observed the repression of β-glucosidase activity in pretreatment by microbial consortia. Dabhi et al. (2014) used bacterial consortium comprising of *Pseudomonas* and *Bacillus* strains for the degradation of banana waste by solid state fermentation. As the result, the highest of 0.178 U/mL total cellulase activity, 1.716 U/mL of endo-glucanase activity were both observed after 20 days and 0.602 U/mL of β-glucosidase activity after 25 days. Singh et al. (2019) developed thermophilic cellulose degrading consortia which produced mostly higher total cellulase activity than the individual strains, maximum value as 0.300 U/mL at 72 h of incubation.

Table 3

Enzymatic production of various enzymes by monoculture and co-culture in the pretreatment hydrolysate at 72 h of cultivation.

STRAIN	Denoted by	Enzyme activities (U/mL)			
		FPase	CMCase	β-glucosidase	Xylanase
B. subtilis B.01162	A	0.242[b]	1.241[b]	0.109[a]	2.081[c]
B. coagulans B.01123	B	0.311[d]	1.374[b]	0.118[a]	0.989[ab]
B. cereus B.00076	C	0.336[de]	1.327[b]	0.108[a]	1.157[b]
B. licheniformis B.01223	D	0.045[a]	0.169[a]	0.060[b]	0.264[a]
B. subtilis B.01162 - *B. coagulans* B.01123	AB	0.370[ef]	2.153[c]	0.030[b]	2.692[c]
B. subtilis B.01162 - *B. cereus* B.00076	AC	0.262[bc]	1.513[b]	0.026[b]	2.140[c]
B. coagulans B.01123 - *B. cereus* B.00076	BC	0.385[f]	1.483[b]	0.030[b]	2.440[c]
B. subtilis B.01162 -*B. coagulans* B.01123 - *B. cereus* B.00076	ABC	0.518 [g]	1.564[b]	0.033[b]	2.428[c]
B. subtilis B.01162 –*B. coagulans* B.01123 -*B. licheniformis* B.01223	AB-D	0.300[cd]	1.437[b]	_	2.514[c]

However, these consortia failed to promote the production of endo-glucanase activity and β-glucosidase activity, which were found very low compared to pure cell treatment. The range of enzyme activities were found of 0.012 U/mL – 0.196 U/mL and 0.1622 – 0.400 U/mL for FPase and endo-glucanase, respectively, and they are in similar to the work done by Gupta, Samant, & Sahu (2012).

To elaborate more the degradation mechanism of these microbial consortia, principal component analysis (PCA) was employed to analyze the contributions of different cellulase enzymes in the first 3 days (including total cellulase, endo-glucanase, β-glucosidase and xylanase) to the hydrolysis process and the results were depicted in Fig. 5. FPase, CMCase, xylanase were positively explained by the PC1 (66.47%) while PC2 (25.05%) loaded with β-glucosidase activity. The 2-member consortium including AB, AC, CD and 3-member consortium denoted as AB-C, AB-D located at the right quadrant with high scores of cellulolytic

enzyme activities, while the remaining 6 consortia took place in other quadrants with the lowest intensities of hydrolytic enzymes. It appeared that the combination of high potential strains as *B. subtilis* B.01162, *B. coagulans* B.01123 and *B. cereus* B.00076 strains promoted the production of hydrolytic enzyme activities of FPase and xylanase than their individual cultivation. The role of β-glucosidase in degradation by the consortium was negligible, causing less efficiency in the removal of cellobiose, which may inhibit the hydrolytic actions of other cellulase enzymes. Enzymatic production capacities of different *Bacillus* consortia were presented in Fig. 5. The crowd cluster with orange circle illustrated microbial flora that produced most of cellulase enzymes classified in PC1 (66.47%) as FPase, CMCase and xylanase.

Fig. 2B demonstrated the reducing sugar yield measured after different time periods (at 24, 48 and 72 h of incubation). It could be predicted the drop of sugar yields under cultivation of mixed of bacteria
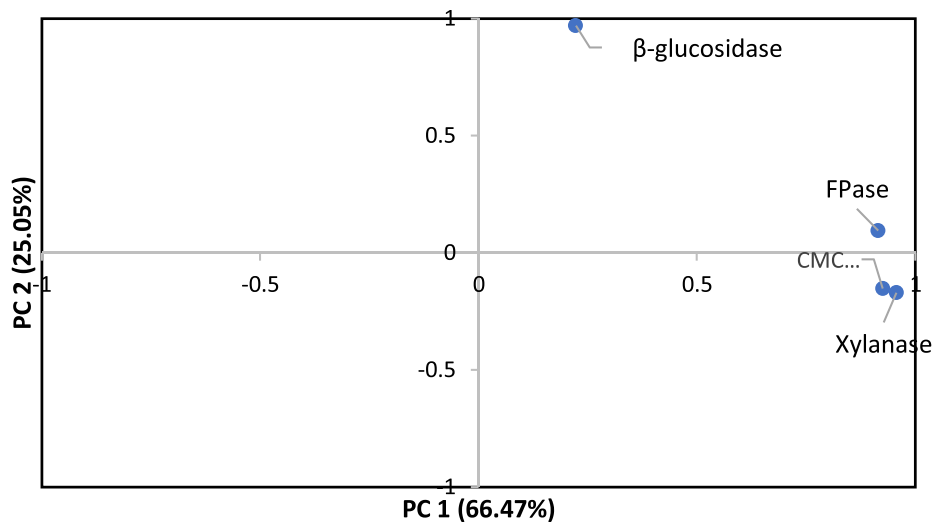
(A)
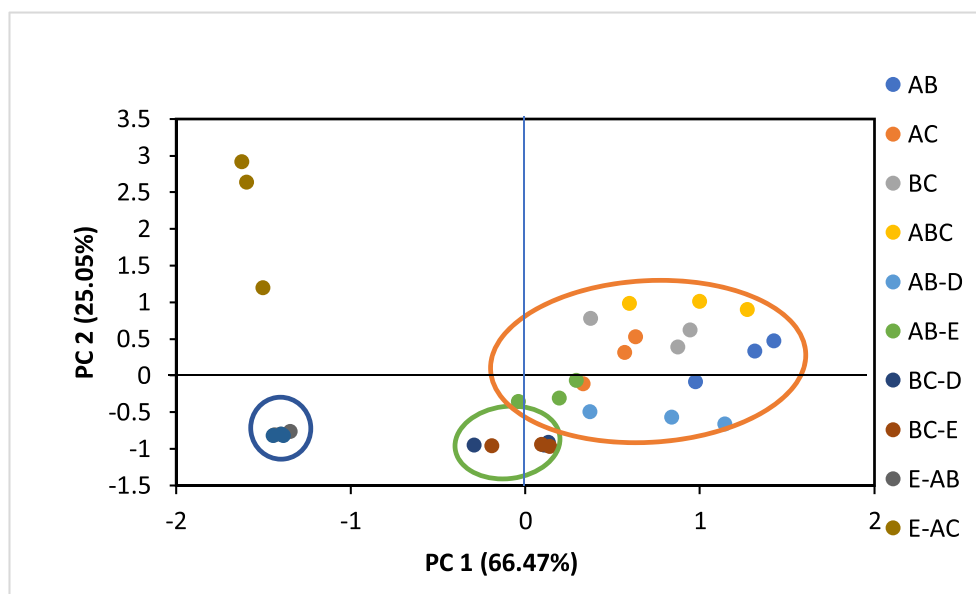
(B)

Fig. 5. Principal component analysis (PCA) plot (A: component plot in rotated space; B: plot of regression factor on the first and second axes from PCA of 11 *Bacillus* consortia).

instead of individual species. Among of them, those caused the large loss in substrate's weight also produced higher fermented sugars than others. Interestingly, they graded with high scores for PC1 component during incubation. From these results, the enhancement in production of FPase, CMCase and xylanase by certain consortia in lignocellulolytic degradation was observed. The low activities of β-glucosidase enzyme could lead to inefficient cleavage of cellobiose, thus limiting hydrolysis by cellulase enzymes secreted by co-cultures, which contributes to the decrease in reducing sugar yields. Despite the drawbacks caused by low endoglucanase and β-glucosidase activities, the enhancement of important degradative enzymes such as xylanase and total cellulase activities secreted by the consortium demonstrated the promising role of cellulolytic strains in the biological pretreatment of lignocellulosic biomass. Among the genus *Bacillus*, some strains such as *B. subtilis, B. cereus, B. coagulans* possess high cellulolytic activity (Chantarasiri, 2015; Choudhury et al., 2006; Roy and Habib, 2009; Safitri et al., 2015). The most important role of *Bacillus* species was their hydrolytic capacity through the synergistic action of enzyme array to form important products such as DP3, DP2 carbohydrate fraction, glucose and xylose (data are not shown). The total yield of reducing sugars produced by the consortium was lower than in the monoculture, and it decreased with the increase in the number of microorganisms in the communities. Generally, the simple sugars such as glucose, maltose are easierly and preferly ultilised by microbes, thus this phenomena can be explained by the quality of sugars released in the medium as well as the competitive consumption of nutrients by members in the same ecological system. More studies are needed to understand the interaction between members of consortium, fermentation dynamics such as the growth of cells, consumption rate of sugars etc., and to control the quality of pretreated biomass. These experiments are in progress in our laboraty.

4. Conclusions

This study provides an insight in the potential of biological pretreatment of wheat bran by culturing different *Bacillus* strains and their consortium. The 3-member consortium comprising strains of *Bacillus subtilis* B.01162, *Bacillus coagulans* B.01123 and *Bacillus cereus* B.00076 were found to be the most efficient degraders with high cellulases activities and efficient digestibility of solid substrates. The pretreatment of wheat bran substrate in submerged medium showed a significant increase in cellulolytic enzyme production compared to monocultures, except β-glucosidase. Function-sorting of different consortium enable us to identify the key organisms involved in the efficient degradation process of lignocellulosic biomass.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT Author Statement

The manuscript was written with input from all authors; V.V., C.F., O.R., A.K. and G.S conducted the experiments and collected data as well as wrote the draft of manuscript; Q.D.N., E.B., M.S., Z.U. and V.K.G. conceptualized, supervised the project, and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

References

Agrawal, A., Kaushik, N., Biswas, S., 2014. Derivatives and applications of lignin – An insight. Scitech J. 01, 30–36.

Aulitto, M., Fusco, S., Bartolucci, S., Franzén, C.J., Contursi, P., 2017. *Bacillus coagulans* MA-13: A promising thermophilic and cellulolytic strain for the production of lactic acid from lignocellulosic hydrolysate. Biotechnol. Biofuels 10, 1–15.

Cortes-Tolalpa, L., Salles, J.F., Elsas, J.D.V., 2017. Bacterial synergism in lignocellulose biomass degradation – complementary roles of degraders s influenced by complexity of the carbon source. Front. Microbiol. 8, 1628.

Dabhi, B.K., Vyas, R.V., Shelat, H.N., 2014. Use of banana waste for the production of cellulolytic enzymes under solid substrate fermentation using bacterial consortium. Int. J. Curr. Microbiol. App. Sci. 3, 337–346.

Bano, S., Ali, S., Qader, U., Aman, A., Noman, M., Durrani, K., 2013. High production of cellulose degrading endo-1,4-β-d-glucanase using bagasse as a substrate from *Bacillus subtilis* KIBGE HAS. Carbohydr. Polym. 91, 300–304.

Boopathy, R., Kern, C., Corbin, A., 2015. Use of *Bacillus* consortium in waste digestion and pathogen control in shrimp aquaculture. Int. Biodeterior. Biodegrad. 102, 159–164.

Brenner, K., You, L., Arnold, F.H., 2008. Engineering microbial consortia : a new frontier in synthetic biology. Trends Biotechnol. 26, 483–489.

Chantarasiri, A., 2015. Aquatic *Bacillus cereus* JD0404 isolated from the muddy sediments of mangrove swamps in Thailand and characterization of its cellulolytic activity. Egypt. J. Aquat. Res. 41 (3), 257–264.

Choudhury, B., Chauhan, S., Singh, S.N., Ghosh, P., 2006. Production of xylanase of *Bacillus coagulans* and its bleaching potential. W. J. Microbiol. Biotechnol. 22 (3), 283–288.

Cortes-Tolalpa, L., Jiménez, D.J., de Lima Brossi, M.J., Salles, J.F., van Elsas, J.D., 2016. Different inocula produce distinctive microbial consortia with similar lignocellulose degradation capacity. Appl. Microbiol. Biotechnol. 100 (17), 7713–7725.

de Gonzalo, G., Colpa, D.I., Habib, M.H.M., Fraaije, M.W., 2016. Bacterial enzymes involved in lignin degradation. J. Biotechnol. 236, 110–119.

Dénes, K., Farkas, C., Hoschke, Á., Rezessy-Szabó, J., Nguyen, Q., 2013. Bioethanol fermentation of Jerusalem artichoke using mixed culture of *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*. Acta Aliment. 42 (Supplement 1), 10–18.

Deng, Y.-J., Wang, S.Y., 2016. Synergistic growth in bacteria depends on substrate complexity. J. Microbiol. 54 (1), 23–30.

Guo, H., Hong, C., Zheng, B., Jiang, D., Qin, W., 2018. Improving enzymatic digestibility of wheat straw pretreated by a cellulase-free xylanase-secreting *Pseudomonas boreopolis* G22 with simultaneous production of bioflocculants. Biotechnol. Biofuels 11, 1–10.

Gupta, P., Samant, K., Sahu, A., 2012. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. Int. J. Microbiol. 2012.

Gupta, V.K., Kubicek, C.P., Berrin, J.-G., Wilson, D.W., Couturier, M., Berlin, A., Filho, E. X.F., Ezeji, T., 2016. Fungal enzymes for bio-products from sustainable and waste biomass. Trends Biochem. Sci. 41 (7), 633–645.

Haab, D., Hagspiel, K., Szakmary, K., Kubicek, C.P., 1990. Formation of the extracellular proteases from *Trichoderma reesei* QM 9414 involved in cellulase degradation. J. Biotechnol. 16 (3-4), 187–198.

Haruta, S., Cui, Z., Huang, Z., Li, M., Chakraborty, I., Igarashi, Y., 2002. Construction of a stable microbial community with high cellulose-degradation ability. Appl. Microbiol. Biotechnol. 59, 529–534.

Heck, J.X., Flôres, S.H., Hertz, P.F., Ayub, M.A.Z., 2005. Optimization of cellulase-free xylanase activity produced by *Bacillus coagulans* BL69 in solid-state cultivation. Process Biochem. 40 (1), 107–112.

Hell, J., Donaldson, L., Michlmayr, H., Kraler, M., Kneifel, W., Potthast, A., Rosenau, T., Böhmdorfer, S., 2015. Effect of pretreatment on arabinoxylan distribution in wheat bran. Carbohydr. Polym. 121, 18–26.

Hell, J., Kneifel, W., Rosenau, T., Böhmdorfer, S., 2014. Analytical techniques for the elucidation of wheat bran constituents and their structural features with emphasis on dietary fiber - A review. Trends Food Sci. Technol. 35, 102–113.

Hendricks, C.W., Doyle, J.D., Hugley, B., 1995. A new solid medium for enumerating cellulose-utilizing bacteria in soil. Appl. Environ. Microbiol. 61 (5), 2016–2019.

Hu, J., Arantes, V., Pribowo, A., Saddler, J.N., 2013. The synergistic action of accessory enzymes enhances the hydrolytic potential of a "cellulase mixture" but is highly substrate specific. Biotechnol. Biofuels 6, 112.

Jiménez, D.J., Korenblum, E., van Elsas, J.D., 2014. Novel multispecies microbial consortia involved in lignocellulose and 5-hydroxymethylfurfural bioconversion. Appl. Microbiol. Biotechnol. 98 (6), 2789–2803.

Kato, S., Haruta, S., Cui, Z.J., Ishii, M., Igarashi, Y., 2004. Effective cellulose degradation by a mixed-culture system composed of a cellulolytic *Clostridium* and aerobic non-cellulolytic bacteria. FEMS Microbiol. Ecol. 51, 133–142.

Kim, Y.-K., Lee, S.-C., Cho, Y.-Y., Oh, H.-J., Ko, Y.H., 2012. Isolation of cellulolytic *Bacillus subtilis* strains from agricultural environments. ISRN Microbiol. 2012, 1–9.

Kovács, Á.T., van Hartskamp, M., Kuipers, O.P., van Kranenburg, R., 2010. Genetic tool development for a new host for biotechnology, the thermotolerant bacterium *Bacillus coagulans*. Appl. Environ. Microbiol. 76 (12), 4085–4088.

Ladeira, S.A., Cruz, E., Delatorre, A.B., Barbosa, J.B., Lelis, M., Martins, L., 2015a. Cellulase production by thermophilic Bacillus sp. SMIA-2 and its detergent compatibility. EJBT 18, 110–115.

Ladeira, S.A., Cruz, E., Delatorre, A.B., Barbosa, J.B., Martins, M.L.L., 2015b. Cellulase production by thermophilic *Bacillus* sp. SMIA-2 and its detergent compatibility. Electron. J. Biotechnol. 18 (2), 110–115.

Farkas, C., Rezessy-Szabo, J.M., Gupta, K.V., Truong, H.D., Friedrich, L., Felfoldi, J., Nguyen, D.Q., 2019. Microbial saccharification of wheat bran for bioethanol fermentation. J. Cleaner Prod. 240, 118269 https://doi.org/10.1016/j.jclepro.2019.118269.

Ferdeş, M., Dincă, M.N., Moiceanu, G., Zăbavă, B.Ş., Paraschiv, G., 2020. Microorganisms and enzymes used in the biological pretreatment of the substrate to enhance biogas production: A Review. Sustain 12 (17), 7205. https://doi.org/10.3390/su12177205.

Moreira, L.R.S., Filho, E.X.F., 2016. Insights into the mechanism of enzymatic hydrolysis of xylan. Appl. Microbiol. Biotechnol. 100 (12), 5205–5214.

Poszytek, K., Ciezkowska, M., Sklodowska, A., 2016. Microbial consortium with high cellulolytic activity (MCHCA) for enhanced biogas production. Front. Microbiol. 7, 1–11.

Qiu, W., Chen, H., 2012. Enhanced the enzymatic hydrolysis efficiency of wheat straw after combined steam explosion and laccase pretreatment. Bioresour. Technol. 118, 8–12.

Reischke, S., Rousk, J., Bååth, E., 2014. The effects of glucose loading rates on bacterial and fungal growth insoil. Soil Biol. Biochem. 70, 88–95.

Roy, N., Habib, M.R., 2009. Isolation and characterization of xylanase producing strain of *Bacillus cereus* from soil. Iran. J. Microbiol. 1, 49–53.

Safitri, R., Priadie, B., Miranti, M., Astuti, A.W., 2015. Ability of baterial consortium: *Bacillus coagulans, Bacilus licheniformis, Bacillus pumilus, Bacillus subtilis, Nitrosomonas* sp. and *Pseudomonas putida* in bioredemediation of waste water in cisirung waste water treatment plant. AgroLife Sci. J. 4, 146–152.

Schallmey, M., Singh, A., Ward, O.P., 2004. Developments in the use of *Bacillus* species for industrial production. Can. J. Microbiol. 50 (1), 1–17.

Shareef, I., Gopinath.S.M, Satheesh, M., Christopher, S.X., 2015. Isolation and identification of cellulose degrading microbes. Int. J. Innov. Res. Sci. Eng. Technol. 4, 6788–6793.

Shruti, S.A., Kumar, M.D., 2015. Lignocellulose Biomass Degradation by microbial consortium Isolated from harvested rice field. Int. J. Curr. Microbiol. App. Sci 4, 274–280.

Shu-bin, L., Ren-chao, Z., Xia, L., Chu-yi, C., Ai-lin, Y., 2012. Solid-state fermentation with okara for production of cellobiase-rich cellulases preparation by a selected *Bacillus subtilis* Pa5. African J. Biotechnol. 11, 2720–2730.

Sindhu, R., Binod, P., Pandey, A., 2016. Biological pretreatment of lignocellulosic biomass – An overview. Biores. Technol. 199, 76–82.

Singh, S., Jaiswal, D.K., Sivakumar, N., 2019. Developing efficient thermophilic Cellulose degrading consortium for glucose production from different isolation of bacterial strains. Front, Energy Res, p. 7.

Stevenson, L., Phillips, F., O'sullivan, K., Walton, J., 2012. Wheat bran: Its composition and benefits to health, a European perspective. Int. J. Food Sci. Nutr. 63 (8), 1001–1013.

Jiménez, D.J., Dini-Andreote, F., DeAngelis, K.M., Singer, S.W., Salles, J.F., van Elsas, J. D., 2017. Ecological insights into the dynamics of plant biomass-degrading microbial consortia. Trends Microbiol. 25 (10), 788–796. https://doi.org/10.1016/j.tim.2017.05.012.

van Dyk, J.S., Sakka, M., Sakka, K., Pletschke, B.I., 2009. The cellulolytic and hemi-cellulolytic system of *Bacillus licheniformis* SVD1 and the evidence for production of a large multi-enzyme complex. Enzyme Microb. Technol. 45 (5), 372–378.

Vasco-Correa, J., Ge, X., Li, Y., 2016. In: Biomass Fractionation Technologies for a Lignocellulosic Feedstock Based Biorefinery. Elsevier, pp. 561–585. https://doi.org/10.1016/B978-0-12-802323-5.00024-4.

Wan, C., Li, Y., 2010. Microbial delignification of corn stover by *Ceriporiopsis* for improving cellulose digestibility. Enzyme Microb. Technol. 47, 31–36.

Wang, H.T., Hsu, J.T., 2006. Usage of enzyme substrate to protect the activities of cellulase, protease and α-amylase in simulations of monogastric animal and avian sequential total tract digestion. Asian-Australasian J. Anim. Sci. 19, 1164–1173.

Whelan, H.A., Pembroke, J.T., 1989. The effect of protease on stability of cellulase and xylanase from *Cellulomonas flavigena*. Biotechnol. Lett. 11, 891–894. https://doi.org/10.1007/BF01026847.

Wongwilaiwalin, S., Rattanachomsri, U., Laothanachareon, T., Eurwilaichitr, L., Igarashi, Y., Champreda, V., 2010. Analysis of a thermophilic lignocellulose degrading microbial consortium and multi-species lignocellulolytic enzyme system. Enzyme Microb. Technol. 47 (6), 283–290.

Yu, X., Liu, Y., Cui, Y., Cheng, Q., Zhang, Z., Lu, J.H., Meng, Q., Teng, L., Ren, X., 2016. Measurement of filter paper activities of cellulase with microplate-based assay. Saudi J. Biol. Sci. 23 (1), S93–S98.

Zhang, D., Wang, Y., Zheng, D., Guo, P., Cheng, W., Cui, Z., 2016. New combination of xylanolytic bacteria isolated from the lignocellulose degradation microbial consortium XDC-2 with enhanced xylanase activity. Biores. Technol. 221, 686–690.