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Effective bio-pretreatment of sawdust waste with a novel microbial consortium for enhanced biomethanation

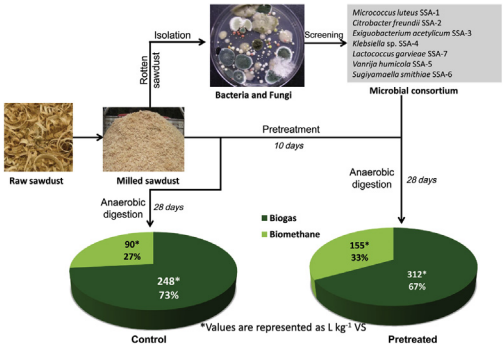
Sameh S. Ali [a,c,1], Abd El-Fatah Abomohra [b,c,1], Jianzhong Sun [a,*]

[a] Biofuels Institute, School of the Environment and Safety Engineering, Jiangsu University, 212013 Zhenjiang, China
[b] School of Energy and Power Engineering, Jiangsu University, 212013 Zhenjiang, China
[c] Botany Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt

HIGHLIGHTS

- Novel microbial consortium isolated from rotten sawdust was isolated.
- Biological pretreatment showed effective biodegradation.
- The pretreatment enhanced biogas production by 25.6% over the control.
- Biomethane production was enhanced by 72.6% over the control.
- The pretreatment enhanced the biomethanation route during anaerobic digestion.

GRAPHICAL ABSTRACT



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ABSTRACT

Anaerobic digestion (AD) is considered an efficient cost-effective technology for sustainable biogas production from lignocellulosic wastes. A novel lignocellulosic degradation microbial consortium (LCDC) was isolated from rotten sawdust, and further used for sawdust pretreatment prior to AD. Results showed that pretreatment of sawdust for 10 days led to significant reduction in cellulose, hemicelluloses, and lignin contents by 37.5%, 39.6%, and 56.7%, respectively, with respect to the control. In addition, the pretreatment enhanced cumulative biogas yield, which reached its maximum value of 312.0 L kg^{-1} VS after 28 days of AD (25.6% higher than the corresponding control). Moreover, the maximum significant cumulative methane yield was recorded after 28 days of AD of the pretreated sawdust (155.2 L kg^{-1} VS), which represented 72.6% higher than the corresponding control. Significantly higher biomethane yield from sawdust pretreated with LCDC confirms that this process is more economical than the previous reports.

1. Introduction

Continuous growth of human population led to increasing energy demands all over the world. The current consumption of petroleum is about 105 times faster than its natural creation (Netravali and Chabba, 2003). If this development continues, the worldwide fossil oil reserves will be exhausted in shorter than 30 years, threatened the world with an energy crisis (Abomohra et al., 2016). In order to realize stable energy alternatives that will meet world demand while mitigating climate change through emissions reduction, different biomass-derived fuels are receiving superior consideration (Wang et al., 2016; Abomohra et al., 2017;

* Corresponding author.
E-mail address: jzsun1002@ujs.edu.cn (J. Sun).
[1] Authors contributed equally to this study.

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Patinvoh et al., 2017). Using anaerobic digestion (AD) for renewable energy production has increased in popularity over the last decades. The technology is eco-friendly, economical, and can produce renewable energy and biofertilizers as a byproduct from a wide range of organic resources (Kimming et al., 2011; Lizasoain et al., 2016). Nevertheless, AD of some substrates can compete directly with human food resources. In order to mitigate this conflict, sustainable strategies are being developed to include agro-industrial and municipal wastes. Although sawdust is a type of bioenergy wastes largely disposable in several countries, it is so far less exploited as a substrate for biogas production (Castoldi et al., 2017). However, the rate-limiting step in the bioconversion of sawdust into biogas during AD process is the degradation of lignocellulose, which consists mainly of cellulose, hemicellulose, and lignin. The later is hardly metabolized by most microbial species during AD leading to high resistance of lignocellulose to enzymatic attack because of the tight association between lignin, cellulose, and hemicellulose (Alexandropoulou et al., 2016).

Pretreatments, including solubilization and biodegradation of lignin and cellulosic components of the substrates, are essential to enhance biogas production by overcoming hydrolysis limitations (Nizami et al., 2010). Different pretreatments including physical, chemical, and biological have been proposed for the enhancement of lignocellulosic feedstocks digestibility (Hendriks and Zeeman, 2008; Carrere et al., 2016). The aim of an efficient pretreatment is delignification, sugar solubilization and reduction of cellulose crystallization in the used lignocellulosic feedstock (Alexandropoulou et al., 2016). Among the different pretreatment strategies, biological and alkaline pretreatment methods are considered most promising to enhance the AD of lignocellulosics, since both methods are quite effective in the breakdown of lignin (Carrere et al., 2016). Alkaline hydrolysis with NaOH and NH_4OH has been successfully applied to treat lignocellulosic materials such as straw or hardwood (Ali and Sun, 2015). However, enhancement of bio-digestibility of lignocellulosic wastes by biological processes is a promising strategy due to its simplicity, low capital cost, and low consumption of energy and chemicals. Many selective lignin-degrading microorganisms have been used individually to reduce the recalcitrance of lignocellulosic biomass and to enhance biogas production (e.g. Lalak et al., 2016; Sindhu et al., 2016). However, degradation of lignocellulosic biomass in nature involves synergistic and cooperative actions of microbes producing a variety of cellulolytic, hemicellulolytic, and lignin degrading enzymes (Kumar et al., 2008). Therefore, the capability of lignin degradation by a single bacterium was reported to be much weaker than using lignin degradation consortium. Alexandropoulou et al. (2016) concluded that in comparison with the traditional chemical pretreatment, lignin degradation consortium would be a good choice in the pretreatment of lignocellulosic wastes. However, using of identified microbial consortium might be more useful to understand the synergism between different bacteria for enhanced biodegradability and biomethanation. To the best of the authors' knowledge, there have been no reports that explored the pretreatment using a consortium of lignocellulolytic yeast (*Sugiyamaella smithiae* and *Vanrija humicola*) and bacteria (*Citrobacter freundii*, *Klebsiella* sp., *Exiguobacterium acetylicum*, *Lactococcus garvieae*, and *Micrococcus luteus*) isolated from rotten sawdust. The objective of the present study was to investigate the effects of a novel lignocellulosic degradation microbial consortium (LCDC), composed of bacteria and yeasts, for efficient biodegradation and enhanced biomethanation. The consortium was isolated from rotten sawdust. Changes in the main chemical compositions after pretreatment were studied. In addition, the impact of biological pretreatment on AD of sawdust and biogas & biomethane production was estimated.

2. Materials and methods

2.1. Raw materials

Sawdust containing a mixture of several lignocellulosic materials, mainly Casuarina and Pine, was collected in February 2015 from softwood industry. It was transported to the laboratory in clean polyethylene bags. It was subsequently shredded to reduce its size to less than 3 cm. The shredded sawdust was dried and milled with a lab grinder. The final powder was collected after passing through 0.8 mm pore size sieve. Finally, it was sterilized in autoclave at 121 °C for 30 min and vacuum-stored at 4 °C for further experiments. Two weeks old inoculum from a running digester was used as a starter as previously described by Ali and Sun (2015).

2.2. Isolation and molecular identification

The milled unsterilized sawdust was kept at room temperature under humid conditions for 15 days. A weight of 30 mg was used to establish 5 ml of enrichment cultures in M9 minimal salts containing (g L^{-1}); 12.8 Na_2HPO_4, 3.0 KH_2PO_4, 0.5 NaCl, 1.0 NH_4Cl, and enriched with 2 g L^{-1} of milled sawdust, then incubated at 30 ± 1 °C for 21 days. Microbial isolation and purification were performed using serial dilution plating technique followed by several subculturing.

Genomic DNA of the promising candidates was extracted using Dr. GenTLE® (TaKara Clontech, USA) according to manufacturer's instructions. For molecular identification of bacteria, 16S rRNA genes were amplified by PCR using16S rRNA gene specific 27F and 1513 universal primers. The 18S rRNA of yeasts was determined as described by White et al. (1990). The amplified product was purified by QIAquick PCR purification kit (Qiagen, USA) according to the manufacturer's instructions. Sequences were aligned in the international GenBank database (http://www.ncbi.nlm.nih.gov/) using BLAST. Evolutionary analyses were conducted using MEGA 7.0. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site.

2.3. Screening of cellulolytic and ligninolytic activity

2.3.1. Cellulolytic assay

For quantitative measurement of cellulolytic activity, the carboxymethyl cellulose (CMC) agar (10.0 g CMC, 2.0 g $NaNO_3$, 0.5 g KCl, 1.0 g K_2HPO_4, 0.5 g $MgSO_4$, 20 μM $FeSO_4$, and 15.0 g agar in 1.0 L dist. water at pH 7.6) was inoculated with the tested isolates. The inoculated CMC-agar plates were incubated at 30 ± 1 °C for 3 days. The clearance zone displaying CMCase activity was detected by staining the CMC-agar plates with 0.1% Congo red solution for 15 min, and then destained with 1 M NaCl for 20 min (Teather and Wood, 1982). The extracellular enzymes produced by the positive cellulolytic strains on CMC-agar medium were concentrated by precipitation using 60% ammonium sulfate (Santhi et al., 2014). The extracellular endo-glucanase activity of the concentrated enzyme solution was determined by agar plug well assay using 10 μL enzyme solution supplemented with 1.0% CMC (Dantur et al., 2015).

2.3.2. Lignin degradation assay

After cultivation of the isolated species in axenic cultures as previously described in Section 2.2, each plate was sprayed with a solution of nitrated lignin as previously described by Taylor et al. (2012) and incubated at 30 °C for 3 days. Yellow colonies confirmed nitrated lignin degradation. Nitrated lignin UV–Visible

assay was carried out for the positive lignin-degrading isolates using the procedures that previously described by Ahmad et al. (2010). Briefly, a stock solution of nitrated milled wood lignin from sawdust was prepared according to the modified protocol of Taylor et al. (2012). Milled sawdust (5 mg) was treated in 1 ml of acetic acid and 0.2 ml of concentrated nitric acid. After neutralization to pH 7, the solution was diluted 25-fold in sterile H_2O. In order to measure lignin degradation activity, 60 μl of the microbial supernatant, 320 μl of nitrated lignin, 20 μl of 2 mM H_2O_2 were mixed. After incubation for 20 min, the change in absorbance was recorded at 430 nm. Each assay was carried out in triplicates against blank in which nitrated lignin and bacterial culture supernatant were replaced by 50 mM NaCl with pH 7.4.

2.4. Experimental setup

Microbial cells of the promising candidates grown in liquid media were harvested by centrifugation at 5000*g* for 15 min, washed 2 times using saline solution and homogenized, in equal ratios of different isolates (*w*/*w*), in 200 mL of sterilized saline solution to form lignocellulose degradation consortium (LCDC).

For biological pre-treatment, 600 g of sterilized air-dried sawdust was weighted into 5 L Erlenmeyer flasks and inoculated with 300 mL of microbial consortium (corresponding to 12 mg dry weight/g sawdust). As a control, 200 mL of sterilized saline solution without inocula was added to the substrate. The flasks were closed with cotton stoppers and incubated at 30 ± 1 °C for 10 days. For AD, two 5 L batch digesters were used for each treatment, each containing the pretreated substrate and with 250 g of start inoculum. Digesters were anaerobically incubated for 35 days at 30 ± 1.3 °C with continuous mixing. Digestate and biogas samples were taken at respective times for further analysis.

2.5. Analytical methods

Total carbon (TC), total solids (TS), volatile solids (VS), and total nitrogen (TN) were measured according to the APHA standard methods (APHA, 1998). Organic matter (OM) was calculated as the percentage of VS/TS. Total dissolved solids (TDS) were measured according to Peters et al. (2003). Cellulose, hemicellulose and lignin contents were determined by the extraction unit for determining raw fiber content according to the procedures proposed by Soest et al. (1991). The volume of the generated biogas was monitored daily via water displacement method (Yan et al., 2015). The composition of biogas was analyzed using 1 ml injection volume by GC (Agilent HP5972, Wilmington, DE, USA) fitted with 10 m capillary column with an internal diameter of 0.32 mm. Oven, injector, and detector temperatures were adjusted at 40 °C for 8 min, 120 °C splitless mode, and 100 °C, respectively (Mamimin et al., 2012). Argon was used as a carrier gas at a flow rate of 14 ml min^{-1}. The detection was performed with an Agilent 5973 mass spectrometer (Agilent Technologies) at electron ionization mode of 70 eV. Daily biogas/methane production rate and cumulative yield were expressed as L kg^{-1} VS d^{-1} and L kg^{-1} VS, respectively.

2.6. Statistical analysis

Results are presented as the mean ± standard deviation (SD). The statistical analyzes were carried out using SPSS (IBM, v20). Data obtained were analyzed statistically to determine the degree of significance using one-way analysis of variance (ANOVA) at probability level (P) ≤ 0.05.

3. Results and discussion

3.1. Isolation and identification

Out of 17 isolates, there were 7 isolates that highly displayed stable color reaction during the first step of screening for lingocellulolytic activity (Table S1, Supplementary data). Three of them showed a stable and significant reaction for lignin degradation activity. Lytic activity and phylogenetic identification of the 7 positive isolates; namely SD-1, SD-5, SD-6, SD-7, SD-11, SD-13, and SD-15; are shown in Table 1. The phylogenetic analysis of lignocellulolytic bacterial (Fig. 1A) isolates SD-1 and SD-5 revealed their belonging to the phylum Proteobacteria and denoted as *Citrobacter freundii* SSA-2 and *Klebsiella* sp. SSA-4, respectively. In addition, bacterial isolates SD-6 and SD-7 revealed their belonging to the phylum Firmicutes and denoted as *Exiguobacterium acetylicum* SSA-3 and *Lactococcus garvieae* SSA-7, respectively (Fig. 1A). The bacterial isolate SD-15 revealed its belongings to the phylum Actinobacteria and denoted as *Micrococcus luteus* SSA-1 (Fig. 1A). Moreover, the phylogenetic analysis of lignocellulolytic yeast isolates SD-13 and SD-11 revealed their belongings to the phylum Basidiomycota and Ascomycota and denoted as *Vanrija humicola* SSA-5 and *Sugiyamaella smithiae* SSA-6, respectively (Fig. 1B). Overall, the nucleotide sequences of bacterial and yeast strains displayed higher than 99% sequence identity with their closest relatives. *Micrococcus luteus* strain SSA-1 showed the highest lignolytic activity, followed by *Citrobacter freundii* strain SSA-2 and *Klebsiella* sp. strain SSA-4 (Table 1). In that context, Mmango-Kaseke et al. (2016) reported that *Micrococcus* sp. SAMRC-UFH3 isolated from sawdust appears to be a potentially important candidate for lignocellulosic waste degradation for the use in bioethanol

Table 1
Phylogenetic identification of isolates showed positive extracellular endo-glucanase (Ext-EGase) and lignolytic activity.

Code	Strain name	Group	Accession No.	Closest relative	Similarity (%)	Lignocellulytic activity (U ml^{-1})	
						Ext-EGase	Lignolytic
SD-1	*Citrobacter freundii* SSA-2	Bacteria	KY486009	*Citrobacter freundii* [BDCP018810]	99%	2.5 ± 0.19^{a}	1.2 ± 0.11^{a}
SD-5	*Klebsiella* sp. SSA-4	Bacteria	KY486011	*Klebsiella* sp. Kb [KT716257]	99%	2.0 ± 0.15^{b}	0.9 ± 0.12^{a}
SD-6	*Exiguobacterium acetylicum* SSA-3	Bacteria	KY486010	*Exiguobacterium acetylicum* Lmb007 [KT986080]	99%	3.2 ± 0.21^{c}	<0.2
SD-7	*Lactococcus garvieae* SSA-7	Bacteria	KY486014	*Lactococcus garvieae* FJ6 [KX671996]	100%	3.7 ± 0.19^{def}	<0.2
SD-11	*Sugiyamaella smithiae* SSA-6	Yeast	KY486013	*Stephanoascus smithiae* CBS 5657 [AJ606463]	99%	4.8 ± 0.24^{d}	<0.2
SD-13	*Vanrija humicola* SSA-5	Yeast	KY486012	*Vanrija humicola* NRRL Y-12944 isolate Lee755 [FJ153213]	99%	4.0 ± 0.26^{e}	<0.2
SD-15	*Micrococcus luteus* SSA-1	Bacteria	KY486008	*Micrococcus luteus* KL3 [KT901825]	99%	3.6 ± 0.17^{f}	1.5 ± 0.18^{b}

Values of lytic activity are the mean of three measurements ± SD.
Means with the same letter in the same column showed insignificant difference (at $P \leq 0.05$).

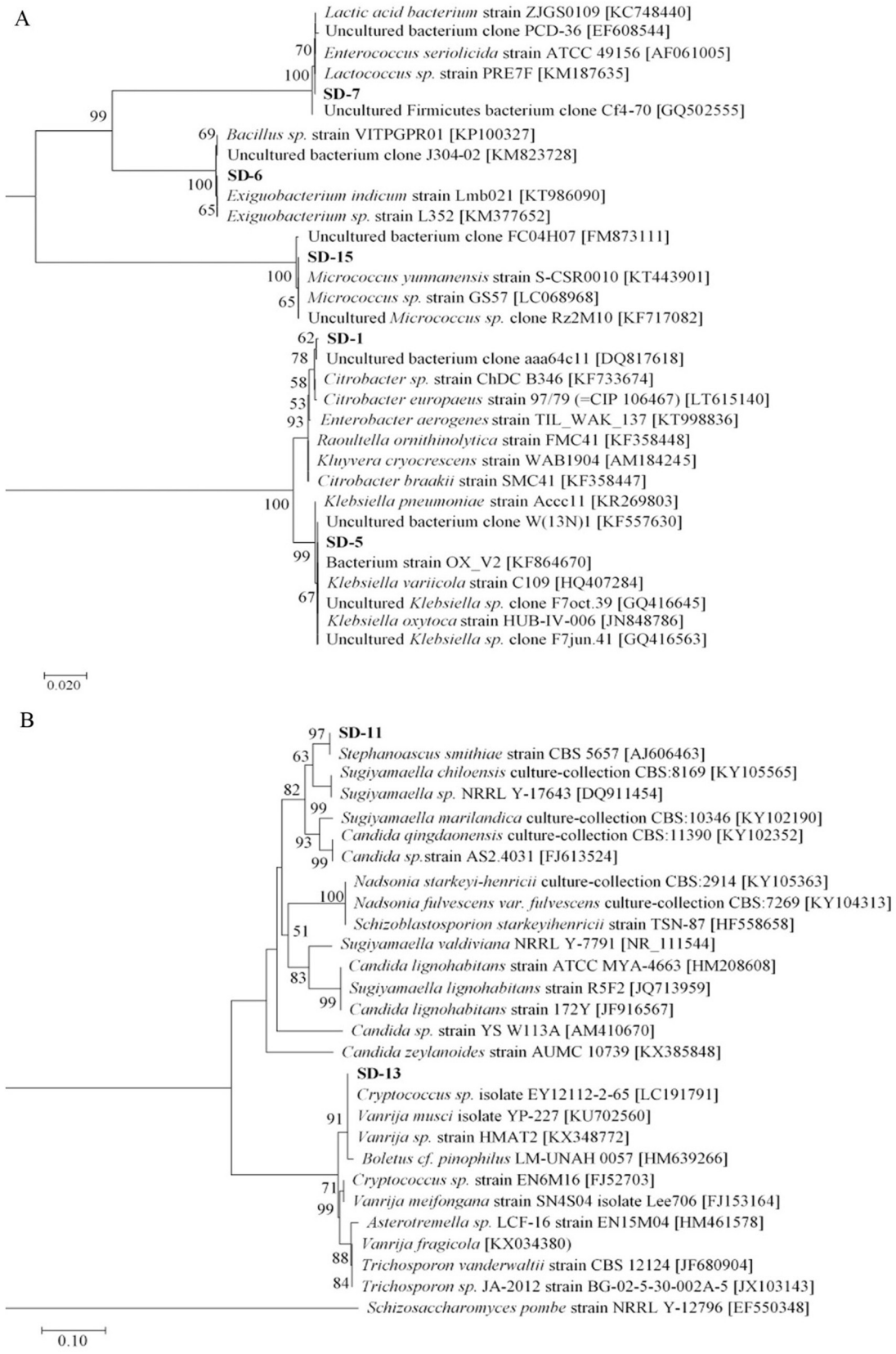

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences of bacterial strains SD-1, SD-5, SD-6, SD-7 and SD-15 (A), and that based on 18S rRNA gene sequences of yeast strains SD-11 and SD-13 (B).

production. In addition, the ability of cellulolytic *Klebsiella* sp. strain BRL6-2 to degrade lignin for industrial lignocellulose degradation and consolidated bioprocessing of biofuels was reported by Woo et al. (2014). Moreover, cellulose and lignin degrading-*Citrobacter freundii* was used by Abhishek et al. (2015) for efficient pretreatment of lignocellulosic biomasses for biofuel production. It has been reported that three strains of *Exiguobacterium acetylicum* showed high cellulolytic and hemicellulolytic activities (Zainudin et al., 2013). Furthermore, *Vanrija humicola* was isolated by Thongekkaew et al. (2012) from lignocellulosic biomass in pulp and paper mill for the production of biofuels.

3.2. Effect of biological pretreatment

Table 2 shows the main characteristics of milled sawdust and inoculum used in the present study. Total solids of sawdust represented 91.9% of the dried biomass, with organic matter of 90.3% per dry weight. Cellulose was the main component of sawdust, representing 31.5% of the dried biomass; while hemicelluloses and lignin represented 26.1% and 24.9%, respectively. Cellulose and hemicellulose (holocellulose) are the main carbon sources in AD process. The holocellulose content of the studied sawdust is in accordance with other research studies on sawdust (Jurado et al., 2013; Alexandropoulou et al., 2016), while lignin content showed relatively higher value.

Pretreatment induces desired physical and chemical changes in the lignocellulosic biomass, enhancing the biodegradability process which leads to enhanced biogas production. The pretreatment aims mainly to break lignocelluloses into three major polymeric constituents, lignin, cellulose, and hemicellulose. It breaks the lignin seal and hemicellulose sheathing over cellulose, and then disrupts the crystalline structure of cellulose (Jaffar et al., 2016). Table 3 summarizes the effect of LCDC pretreatment for 10 days on sawdust biodegradation. TS and VS of the pretreated substrate were reduced by 83.4 and 56.9%, respectively, with respect to the control. In addition, pretreatment significantly reduced cellulose, hemicelluloses, and lignin by 37.5, 39.6, and 56.7%, respectively, with respect to the control. This confirms the efficiency of the used LCDC for lignocellulosic biodegradation of sawdust. However, the concentration of dissolved carbohydrates was significantly enhanced by 70.6% over the control as a result of pretreatment; which confirms the higher solubilization of dry biomass to dissolved sugars, a fact which also justifies the recorded biomass loss. The rate of biomass loss observed in the present study is higher than that reported using individual microorganisms, such as *Leiotrametes menziesii*, *Pleurotus ostreatus, Trametes versicolor, Phanerochaete chrysosporium, Ceriporiopsis subvermispora, Polyporus tricholoma*, *Coprinopsis cinerea*, and *Abortiporus biennis* which were also used for biodegradation of lignocellulosic biomass (Sasaki et al., 2011; Castoldi et al., 2014; Nuchdang et al., 2015; Alexandropoulou et al., 2016). The higher biomass loss rate might be attributed to the synergism among the complex population of bacteria and yeast that exist within LCDC.

3.3. Effect of pretreatment on biogas and methane production

Fig. 2 shows the daily biogas production rate and biogas yield of the pretreated and untreated sawdust. The maximum daily biogas production for the untreated samples (15.7 L kg^{-1} VS d^{-1}) was recorded after 19 days of AD with a lag phase of 4 days. However, pretreatment enhanced the biogas production spontaneously, which reached its maximum (15.9 L kg^{-1} VS d^{-1}) after 13 days of AD (Fig. 2A). As a result of enhancement of biogas production rate, the cumulative biogas yield increased continuously during the digestion period. The maximum significant cumulative biogas yield for the pretreated samples was 312.0 L kg^{-1} VS after 28 days of AD, which represented 25.6% higher than the corresponding control (Fig. 2B). The recorded cumulative biogas yield is comparatively better than that obtained by Córdoba et al. (2016), who used spent sawdust after *Gymnopilus pampeanus* cultivation, and obtained maximum biogas yield of 147.8 mL g^{-1} VS after 105 days of AD.

The untreated sawdust showed a maximum daily methane production of 7.4 L kg^{-1} VS d^{-1} after 20 days of AD. However, pretreatment enhanced methane production rate, showing its maximum of 9.8 L kg^{-1} VS d^{-1} after 18 days of AD (Fig. 3A). As a result, the cumulative methane yield was continuously increased during the digestion period. The maximum significant cumulative methane yield was recorded after 28 days of pretreated sawdust digestion (155.2 L kg^{-1} VS), which represented 72.6% higher than the corresponding control. Noteworthy, the recorded increase in methane yield as a result of the pretreatment is not only due to enhancement of biogas production, but also because of increased methane ratio in the produced biogas (Fig. 3B). Liu et al. (2014) studied the effect of *P. chrysosporium* on methanation of corn stover and corn stover silage. They reported that the methane yield of corn stover silage was improved up to 21.2% through fungal pretreatment, while the methane yield of corn stover was not significantly improved. Recently, Alexandropoulou et al. (2016) reported that biological pretreatment with *A. biennis* for 30 days results in an increase in biomethane production by 43%, which is 68.8% lower than that reported in the present study using LCDC.

3.4. Effect of pretreatment on the digestate

Table 4 shows the chemical analysis of the digestate residue collected after 28 days of AD. The digestate residue showed the relatively low content of dry matter of around 6% (*w/v*). The pretreated digestate showed a significant reduction in carbon content by 30.7% with respect to the control. However, TDS and ammonia significantly increased by 25.9% and 54.2%, respectively, over the control. Lower carbon content and higher TDS in the digestate of the pretreated sawdust confirm the better biodegradability. Since anaerobic biodegradability of organic matter is

Table 2
The main characteristics of sawdust and inoculum used in the present study.

Constituents	Milled sawdust	Inoculum
Total solids (TS,% dry basis)	91.9 ± 2.3	9.33 ± 0.71
Volatile solids (%, dry basis)	83.3 ± 1.8	5.68 ± 0.65
Organic matter (OM,% dry basis)	90.3 ± 2.2	60.87 ± 0.67
Carbon (% dry basis)	51.26 ± 1.1	29.65 ± 0.84
Cellulose (%, dry basis)	31.5 ± 1.3	ND
Hemicellulose (%, dry basis)	26.1 ± 2.1	ND
Lignin (%, dry basis)	24.9 ± 1.7	ND
TDS (mg L^{-1})	NA	910 ± 27
pH	NA	7.01 ± 0.12

Values are the mean of three measurements ±SD.
ND not detectable; NA not applied.

Table 3
Changes in the main constituents of sawdust (%, dry basis) after 10 days of pretreatment.

Constituents	Control	Pretreated
TS[a]	92.4 ± 2.1	15.3 ± 0.8*
VS	74.9 ± 1.6	32.3 ± 1.1*
Cellulose	31.2 ± 1.9	19.5 ± 1.5*
Hemicellulose	25.5 ± 1.9	15.4 ± 0.9*
Dissolved carbohydrates	1.7 ± 0.09	2.9 ± 0.23*
Lignin	25.2 ± 1.2	10.9 ± 0.6*

Values are the mean of three replicates ±SD.
[a] It was calculated in relation to dry mass of the pretreated sawdust.
* Showed significant difference with the corresponding control (at $P \leq 0.05$).

related to its composition, alkalinity is an ideal parameter to monitor AD process. Jaffar et al. (2016) concluded that for biomethane production with ideal degradation of digestate residue into methane, the digestate residue should be in neutral pH range of around 6.5–7.4. In the present study, the untreated digestate showed higher acidity (pH 5.38) than the pretreated one, which showed neutral pH of 6.86 (Table 4). Therefore, the alkalinity profile of the digestate samples suggests that the digestate from the pretreated sawdust is in the desired pH range for biomethanation.

Delignification of lignocellulosic substrate is likely to be the major process of the cost for AD. Conventional physico-chemical methods for lignin degradation require large inputs of energy and also cause environmental pollution. Therefore, biological pretreatment of lignocellulosic biomass is considered as an efficient, eco-friendly and cheap alternative. Significant higher biomethane yield from sawdust pretreated with LCDC confirms that it is more economical in comparison with the previous reports. However, experience in the operation of biological delignification processes

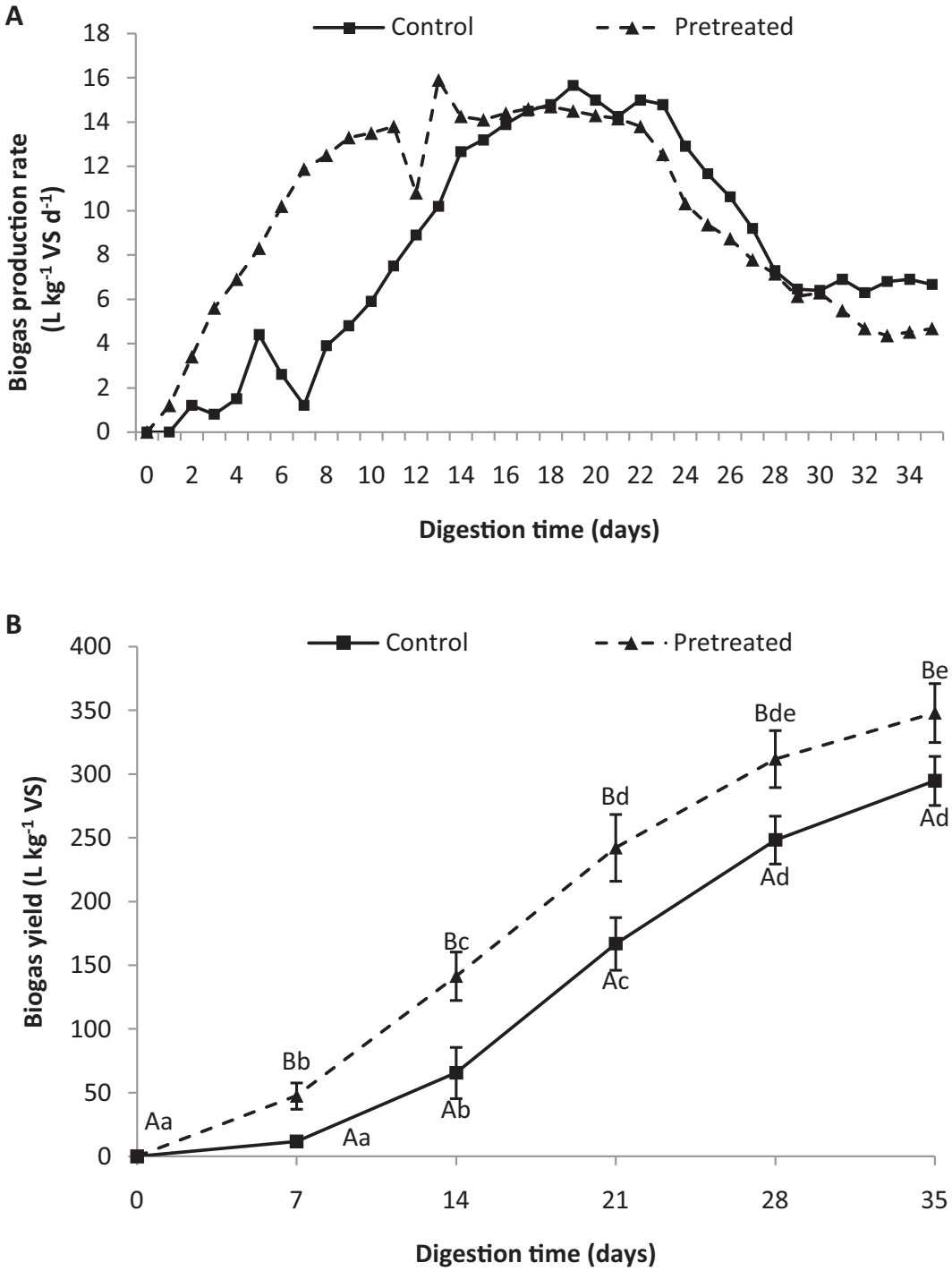

Fig. 2. Daily biogas production rate (A) and cumulative biogas yield (B) during anaerobic digestion of sawdust for 35 days. Error bars show the SD of two replicates with 3 measurements of each. Means with the same small letter in the same series showed insignificant difference (at $P \leq 0.05$), and means with the same capital letter at the same digestion time showed insignificant difference (at $P \leq 0.05$).

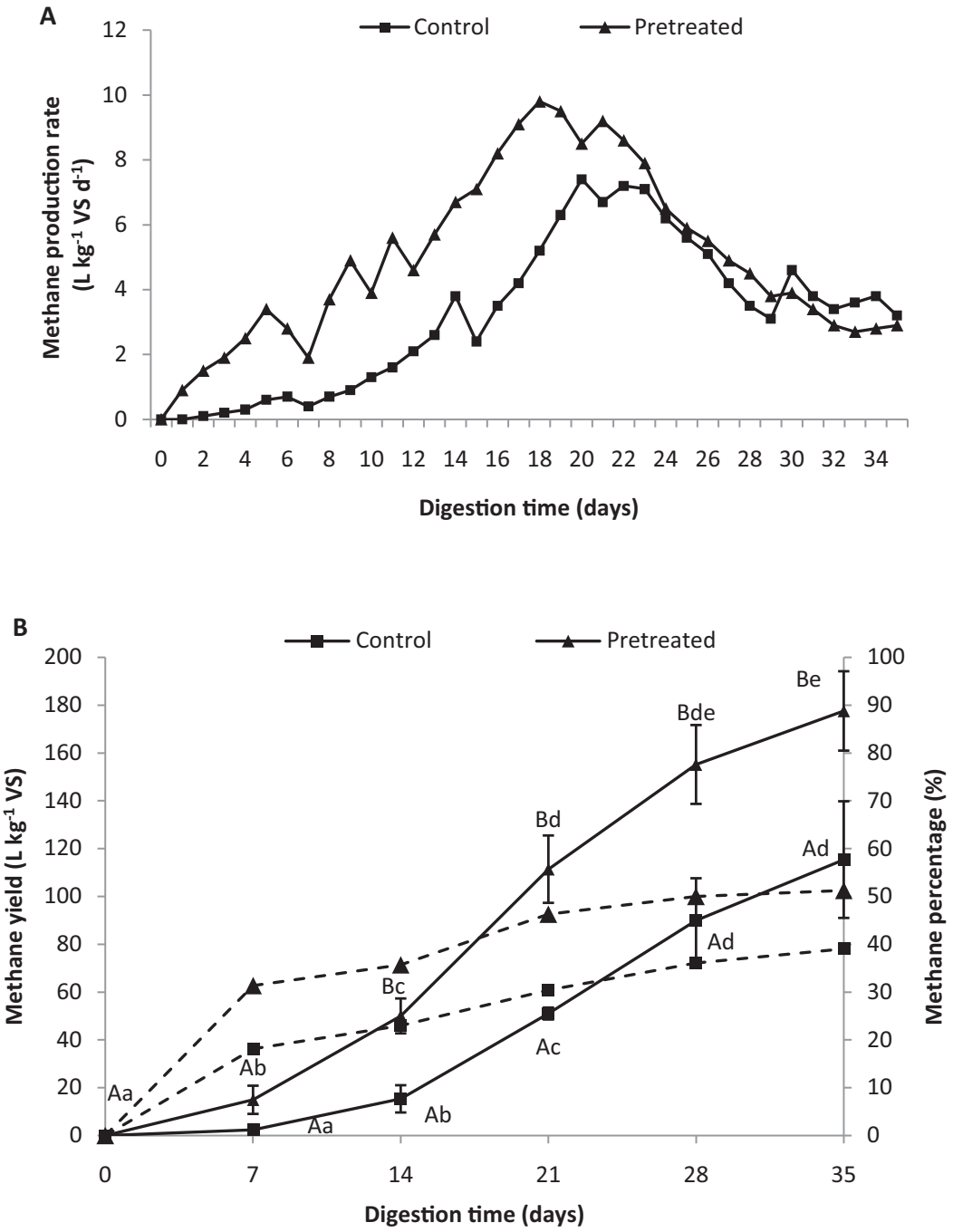

Fig. 3. Daily methane production rate (A) and cumulative methane yield (B) during anaerobic digestion of sawdust for 35 days. Dashed lines show the percentage of methane in the biogas. Error bars show the SD of two replicates with 3 measurements of each. Means with the same small letter in the same series showed insignificant difference (at $P \leq 0.05$), and means with the same capital letter at the same digestion time showed insignificant difference (at $P \leq 0.05$).

Table 4
Changes in the main composition of sawdust after 28 days of anaerobic digestion.

Constituents	Control	Pretreated
Carbon (% DW)	50.49 ± 3.45	35.01 ± 1.87*
TKN (mg L^{-1})	324 ± 57	351 ± 65ns
Nitrogen (% DW)	2.22 ± 0.19	2.13 ± 0.17ns
Ammonia (% DW)	313.7 ± 19	483.6 ± 25*
TDS (mg L^{-1})	1183 ± 112	1489 ± 104*
TP (mg L^{-1})	5.19 ± 7.35	5.92 ± 9.36ns
pH	5.38 ± 0.15	6.86 ± 0.19*

Values are the mean of two replicates with 3 measurements of each ±SD.
* Showed significant difference with the corresponding control (at $P \leq 0.05$).
ns Showed insignificant difference with the corresponding control (at $P \leq 0.05$).

at pilot plant or industrial scale is needed to establish realistic process costs.

4. Conclusion

The results of this study showed that pretreatment with the innovative LCDC composed of 5 bacteria and 2 yeasts for 10 days prior to AD could significantly enhance the hydrolysis and biomethanation of sawdust. The pretreatment significantly affected cellulose, hemicellulose, lignin, and dissolved carbohydrates contents of the studied sawdust. Compared to the untreated controls, pretreated sawdust yielded 72.6% more biomethane. The

increase in methane yield was attributed to the improved biodegradability which resulted in enhanced biogas production by 24.5%, and changes in its chemical composition towards biomethanation, demonstrating the positive effect of the isolated microbial consortium.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.03.187.

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