fermentation

MDPI

Article

Fermentative L-Lactic Acid Production Using *Bacillus coagulans* from Corn Stalk Deconstructed by an Anaerobic Microbial Community

Xu Yang *, Zhiyuan Shi, Tongyu Wang, Xiangyu Meng, Lili Song, Zhiping Zhang, Jingnan Zhang and Tao Wei *

School of Food and Bioengineering, Zhengzhou University of Light Industry, Zhengzhou 450002, China; 332103040229@email.zzuli.edu.cn (Z.S.); 542003020215@zzuli.edu.cn (T.W.); 2017082@zzuli.edu.cn (X.M.); sll@zzuli.edu.cn (L.S.); zzp@zzuli.edu.cn (Z.Z.); zjn@zzuli.edu.cn (J.Z.)

* Correspondence: yangxu@zzuli.edu.cn (X.Y.); weit8008@zzuli.edu.cn (T.W.); Tel./Fax: +86-371-8660-8661 (T.W.)

Abstract: This study investigated the feasibility of producing L-lactic acid (LA) from dry corn stalk (DCS) that was pretreated by ensiling by an anaerobic microbial community consisting of *Bacillus coagulans*, *Lactobacillus fermentum*, and *Enterococcus durans*. After 28 days of ensiling, the LA and acetic acid content in the microsilage was 2.04 ± 0.08% and 0.38 ± 0.01%, respectively, and the pH was 4.47 ± 0.13. *Enterococcus and Lactobacillus* became the dominant microbiota during the ensiling process. Twenty-eight-day-old microsilage was then subjected to fermentation by *B. coagulans* to produce LA in a simultaneous saccharification and co-fermentation process. The enzymatic hydrolysis yield reached >96%. The maximal concentration of LA reached 18.54 ± 0.52 g/L with a substrate concentration of 5%, where the yield of LA was 0.31 ± 0.01 g/g DCS and the optical purity of the product LA was >97%. Anaerobic ensiling is viable for the pretreatment of biomass for the production of value-added chemicals.

Keywords: dry corn stalk; ensiling; microbial community; high-throughput sequencing; lactic acid

Citation: Yang, X.; Shi, Z.; Wang, T.; Meng, X.; Song, L.; Zhang, Z.; Zhang, J.; Wei, T. Fermentative L-Lactic Acid Production Using *Bacillus coagulans* from Corn Stalk Deconstructed by an Anaerobic Microbial Community. *Fermentation* **2023**, *9*, 611. https://doi.org/10.3390/fermentation9070611

Academic Editors: Yaohua Zhong and Yinbo Qu

Received: 1 June 2023
Revised: 19 June 2023
Accepted: 25 June 2023
Published: 28 June 2023

1. Introduction

Converting lignocellulose biomass (LCB) into sustainable value-added products is an urgent global need. In nature, the degradation and recycling of organic carbon are mainly mediated by anaerobic microorganisms, which work together to degrade straw in a divide-and-conquer manner [1]. However, although many microbial strains have been screened from anaerobic ecosystems, there is not yet an artificial microbial community capable of sustaining the high conversion yields required to degrade lignocellulose for use in the bioenergy and renewable chemicals markets [2]. Bioprocessing of agricultural biomass is also hampered by the need for extensive pretreatment and separation steps to decrease the impact of lignin and to hydrolyze the carbohydrate polymers into fermentable sugars (glucose, xylose, and so on) [3]. Many hydrolysis byproducts have toxic effects on individual microorganisms [4]. Therefore, it is attractive to employ decomposition strategies using dynamic anaerobic communities, in which the microorganisms belong to complementary lignocellulose-degrading groups that distribute decomposition products and metabolites among members and hence mitigate the overall toxicity to the community. Emerging methods in microbial enrichment, sequencing, and bioinformatics offer new opportunities to decipher the functions of anaerobic microbial communities and use them in the sustainable production of bioenergy and chemicals.

Microbial communities can achieve task division and functional complementarity through the interactions between microorganisms, and they have important applications in the fields of bioremediation, high-value-added chemical synthesis, biofuel production, and pollutant degradation [5]. Researchers have added exogenous microorganisms to enhance microsilage production [6]. During the fermentation process, the abundance and

diversity of genes encoding microbial carbohydrate-active enzymes increased significantly, especially those responsible for the degradation of starch, arabinoxylan, and cellulose [7]. The recalcitrance of complex organic polymers such as lignocellulose is one of the main obstacles to the sustainable production of bio-based chemicals from biomass [8]. Researchers developed a model microbial community for lignocellulose degradation, whose specific goal was to reduce the production of formaldehyde toxins during the decomposition of methoxylated aromatic compounds. This community included lignin-degradation agents such as *Pseudomonas putida* and cellulose-degradation agents such as *Cellulomonas* FIMI. The uniqueness of the system lay in the addition of *Methylorubrum extorquens*, which can grow on formaldehyde, to degrade the toxic intermediates [9]. Researchers have reviewed various studies on the "bottom-up" route to construct microbial communities with a limited number of culturable strains for more efficient lignocellulose conversion [10].

Research results show that the anaerobic ensiling of agricultural dry straw can be used as a new treatment method for the directional transformation of bio-based chemicals [11]. However, such research has only focused on whether the dry straw can be successfully ensiled in anaerobic conditions and whether the nutrient composition of the resulting microsilage is suitable for use as feed for cattle and sheep or as raw material for biogas fermentation [12]. The ensiling of straw is a complicated and uncontrollable operation, the product quality is unstable, and the process is not repeatable. Research involving ensiling pretreatment for the conversion of straw to high-value-added bio-based chemicals (such as butanol and lactic acid (LA)) has not received much attention. However, a small number of research results show that the ensiling of straw can decrease the required treatment intensity of the straw to achieve efficient conversion to bio-based chemicals [13].

In this study, the research object was the anaerobic ensiling process of dry corn stalk (DCS). A synthetic community of lactic acid bacteria (LAB), including *Bacillus coagulans* NBRC 12583, *Lactobacillus fermentum* KF5, and *Enterococcus durans* 075, efficiently and selectively degraded the lignocellulose of DCS. The total bacterial community composition and relative abundance of the microbial (bacterial and fungal) taxa in the process of DCS degradation were analyzed by high-throughput sequencing, and changes (succession) in the microbial community structure were clarified. The purpose of this study was to lay a foundation for understanding the synergism among microorganisms in the process of DCS degradation. Furthermore, the feasibility of converting the product microsilage into LA was investigated using simultaneous saccharification and co-fermentation (SSCF) with *B. coagulans* NBRC 12583. We demonstrated that the anaerobic ensiling pretreatment method applied here is viable for use in biomass transformation. Our study provides support for the biological pretreatment of DCS for the production of LA.

2. Materials and Methods

2.1. DCS and Bacterial Strains

DCS was obtained from Henan Tianguan Group Co., Ltd., Henan Province, China. The material used contained 33.95 ± 0.93% glucan, 17.99 ± 0.55% xylan, 27.72 ± 0.79% acid-insoluble lignin (AIL), 2.71 ± 0.03% acid-soluble lignin (ASL), and 2.25 ± 0.01% ash. Commercial cellulase Cellic Ctec2 was provided by Novozyme (Tianjin, China) with a filter paper activity of 120 filter paper units (FPU)/mL. *B. coagulans* NBRC 12583 (ATCC 7050), *L. fermentum* KF5 (ATCC 23271), and *E. durans* 075 (ATCC 19432) were screened from pickled vegetables from Sichuan, China, and identified by 16S rRNA gene sequence analysis. Simplified De Man, Rogosa, and Sharpe (MRS) medium was prepared according to the requirements of *B. coagulans* NBRC 12583, *L. fermentum* KF5, and *E. durans* 075. The ingredients were 20 g/L glucose, 10 g/L yeast extract, 2 g/L diammonium hydrogen citrate, 5 g/L sodium acetate, 0.3 g/L $MgSO_4$, 2 g/L K_2HPO_4, and 0.23 g/L $MnSO_4$.

2.2. Microbial Ensiling

DCS microsilage samples were prepared and collected at the Zhengzhou University of Light Industry. A diagram of the anaerobic fermentation and sampling processes is

shown in Figure 1. Simply, ensiling was carried out using crushed DCS. The microsilage was adjusted to a water content of 60%. The DCS was thoroughly mixed with *B. coagulans* NBRC 12583 (1.0×10^6 cells per gram of DCS), *L. fermentum* KF5 (1.0×10^6 cells per gram of DCS), *E. durans* 075 (1.0×10^6 cells per gram of DCS), and distilled water. In the anaerobic fermentation container (Figure 1) used for the ensiling, 1.0 kg (wet weight) of DCS was packed. The top of the fermentation container was sealed with water to ensure an anaerobic environment for ensiling. The fermentation containers were stored at room temperature for 49 days. Samples were taken every 7 days for determination and were designated as DCS1, DCS2, DCS3, DCS4, DCS5, DCS6, and DCS7, respectively. The prepared samples were stored at −20 °C.

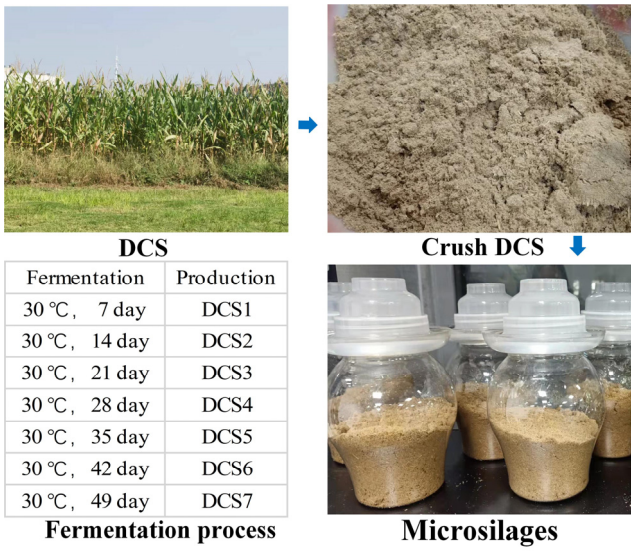

Figure 1. Production diagram for ensiling used in this study with the sampling points indicated. DCS: dry corn stalk; DCS1–DCS7: DCS microsilage for 7, 14, 21, 28, 35, 42, and 49 days, respectively.

2.3. Enzymatic Hydrolysis of Materials

To investigate the effect of the ensiling pretreatment on DCS, enzymatic hydrolysis experiments were carried out on the resulting microsilage. DCS4 samples (autoclaved, 5.0 g dry matter) were mixed with 100 mL of 0.1 mol/L citric acid buffer (pH 4.80) and cellulase (10 FPU g^{-1} cellulose) at 50 °C (in a constant-temperature oscillating water bath, 200 rpm) for 48 h in a 250 mL sealed triangular bottle. The supernatant was sampled periodically (at 8, 16, 24, 32, 40, 48, 56, 64, and 72 h, respectively) during the enzymatic hydrolysis to calculate the hydrolysis yield from Formulas (1) and (2) after determining the concentrations of glucose and xylose. For the enzymatic hydrolysis, triplicate reactions were performed separately, and the results were averaged.

$$\text{Cellulose hydrolysis yield (\%)} = (\text{Total glucose in enzymatic hydrolysate (g)} \times 0.9)/(\text{Initial cellulose content of raw material (g)}) \times 100\% \quad (1)$$

$$\text{Hemicellulose hydrolysis yield (\%)} = (\text{Total xylose in enzymatic hydrolysate (g)} \times 0.88)/(\text{Initial hemicellulose content of raw material (g)}) \times 100\% \quad (2)$$

2.4. LA Batch Fermentations

Fermentation to produce LA was carried out by simultaneous saccharification and co-fermentation (SSCF) with DCS4 (autoclaved) as the substrate; the substrate concentration was 5% during the fermentation process. Cellulase was added at 10 FPU g^{-1} cellulose. SSCF was started by inoculation with 10% (*v*/*v*) *B. coagulans* NBRC 12583. Details of the ability of *B. coagulans* NBRC 12583 to metabolize glucose, xylose, and complex sugars to produce LA are shown in the Supporting Information (Tables S1–S3). SSCF was carried out

in a 5 L fermenter (BIOSTAT B, Sartorius, Germany) at 52 °C and 100 rpm, and 3 mol/L NaOH solution was used to maintain the pH at 6.0. The SSCF was performed for 84 h, and samples were collected every 12 h. The concentration of LA in the system was determined, and the conversion yield of LA was calculated from Formula (3).

$$\text{LA yield (g/g DCS)} = (\text{Total LA in fermentation broth (g)})/(\text{Initial weight of DCS (g)}) \quad (3)$$

2.5. High-Throughput Sequencing Analysis

Samples of DCS and silage were sent to Sangon Biotech (Shanghai, China) Co., Ltd., for high-throughput sequencing analysis of bacteria and fungi. The bacterial amplification region was V3–V4 of the 16S rRNA gene, and the fungal amplification region was ITS1–ITS2. The main processes were as follows: (1) sample pretreatment; (2) extraction of DNA; (3) PCR amplification; (4) purification and recovery of DNA; and (5) sequencing.

The paired-end reads obtained by sequencing were first spliced by overlap, and then the sequence quality was controlled and filtered after distinguishing the samples. Then, operational taxonomic unit (OTU) clustering analysis and species taxonomic analysis were performed. On the basis of the analysis results, a variety of diversity indices of OTUs were analyzed, and the sequencing depth was determined.

2.6. Chemical Composition of DCS Samples

Cellulose, hemicellulose, and lignin were determined by the National Renewable Energy Laboratory (NREL, CO, USA) using a two-step hydrolysis method [14]. Samples were accurately weighed (to give W0 in g, accurate to 0.0001 g), 3 mL of 72% sulfuric acid was added, and the mixture was reacted at 30 °C for 60 min. The hydrolysate was rinsed into a 100 mL screw-cap bottle using 84 mL of distilled water. The bottle was kept at 121 °C for 60 min. The liquid was then filtered after cooling. The concentrations of glucose (C_{glu}), xylose (C_{xyl}), and arabinose (C_{ara}) were determined by high-performance liquid chromatography (HPLC); see below for the analysis conditions. The contents of cellulose and hemicellulose were calculated using Equations (4) and (5). The remaining filtrate was measured at 205 nm using a spectrophotometer (with 4% sulfuric acid as the blank control). The content of ASL was calculated using Equation (8). The filter residue was baked at 105 °C to a constant weight of W_1 (in g), held at 550 °C for 2 h, and then weighed again (to give W_2, in g). The AIL and ash contents were calculated using Equations (6) and (7).

$$\text{Cellulose}(\%) = (C_{glu} \times 87 \times 0.90 \times 10^{-3})/W_0 \times 100\% \quad (4)$$

$$\text{Hemicellulose}(\%) = ((C_{xyl} + C_{ara}) \times 87 \times 0.88 \times 10^{-3})/W_0 \times 100\% \quad (5)$$

$$\text{AIL}(\%) = (W_1 - W_2)/W_0 \times 100\% \quad (6)$$

$$\text{Ash}(\%) = W_2/W_0 \times 100\% \quad (7)$$

$$\text{ASL}(\%) = (D \times A \times L)/(110 \times W_0) \times 100\% \quad (8)$$

For Equation (7), L is the total volume of the reaction system (mL), D is the dilution ratio of filtrate, and A is the absorption coefficient of corn stalk at 205 nm.

2.7. Determination of Free Organic Acids and pH

Sterilized H_2O was added to 2–3 g samples (the ratio of sterilized H_2O to raw material was 10:1). After shaking for 30 min, the supernatant was obtained by centrifugation at 11,167× *g* for 10 min and the pH value was determined. The supernatant was filtered (0.22 μm), and the composition of organic acids was determined by HPLC.

2.8. HPLC Analysis Conditions

Glucose, xylose, arabinose, LA, acetic acid, and propionic acid were determined by HPLC using a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm, 9 µm particle size; Hercules, CA, USA). The column temperature was 65 °C, and the flowrate of the mobile phase (5 mM H_2SO_4) was 0.6 mL/min.

2.9. Optical Purity Calculation of LA

The content of L-LA was measured using an SBA-40C Biosensor Analyzer (Shandong Province Academy of Sciences, China), and the total LA content was measured by HPLC. The formula for calculating the optical purity of L-LA was:

$$\text{Optical purity (\%)} = \text{(L-LA)/(Total LA)} \times 100\% \quad (9)$$

2.10. Characterization of DCS and Microsilage

The morphology of the DCS and microsilage was investigated by scanning electron microscopy (SEM, JSM-6490 LV). The crystal structure of the DCS and microsilage was investigated by X-ray diffraction (XRD; XRD-6100Lab). The XRD analysis conditions were as follows: pipe flow 20 mA, pipe pressure 40 kV, scanning range 5°–80°, and scanning speed 2°/min. The formula for calculating the crystallinity index (CrI) of the sample was:

$$\text{CrI} = (I_{002} - I_{am})/I_{002} \times 100\% \quad (10)$$

where I_{002} is the intensity of the main crystallite at 22.5°, and I_{am} is the minimum intensity corresponding to the peak in the amorphous region around 18.0°.

2.11. Statistical Analysis

All treatments were performed in triplicate unless specified otherwise. Analysis of variance (ANOVA) was used to statistically analyze the data. The Origin Pro 9.1 software (Origin Lab, Northampton, MA, USA) was used to perform the statistical analyses and draw graphs.

3. Results and Discussion

3.1. Characteristics of Microsilage

The pH value, fermentation time, and the content of organic acids (especially LA and acetic acid) in DCS microsilage can be considered important indicators of the success of microbial ensiling [15]. The pH value in the DCS decreased from 6.59 ± 0.02 to 4.47 ± 0.13 after 28 days (Figure 2), at which point the fermentation pattern was controlled to avoid undesirable activities of microbes with acid stress caused by the production of both acetic acid and LA. In the early stage of ensiling, organic acids were produced by the LAB via the use of water-soluble carbohydrates (such as glucose 1.73 ± 0.05% (*w*/*w*) and xylose 2.44 ± 0.09% (*w*/*w*)) in the DCS, which could not be detected in the reaction system after fermentation for 7 days. In DCS4, the contents of LA and acetic acid were 2.04 ± 0.08% (*w*/*w*) and 0.38 ± 0.01% (*w*/*w*), respectively (Figure 2). Butyric acid is a common indicator of insufficient preservation [16]; butyric acid was not detectable in the microsilage, showing efficient preservation. Traces of xylose and arabinose were detected in the microsilage. Both of them are related to the degradation of hemicellulose. Hemicellulose fixes the shape of the plant cell wall; as growing straw matures, the content of hemicellulose gradually increases and enhances the hardness of the plant material [17]. The degradation of hemicellulose showed that the microorganisms in the ensiling mixture could destroy the internal structure of the straw, which was beneficial for the subsequent enzymatic hydrolysis and fermentation of the material.

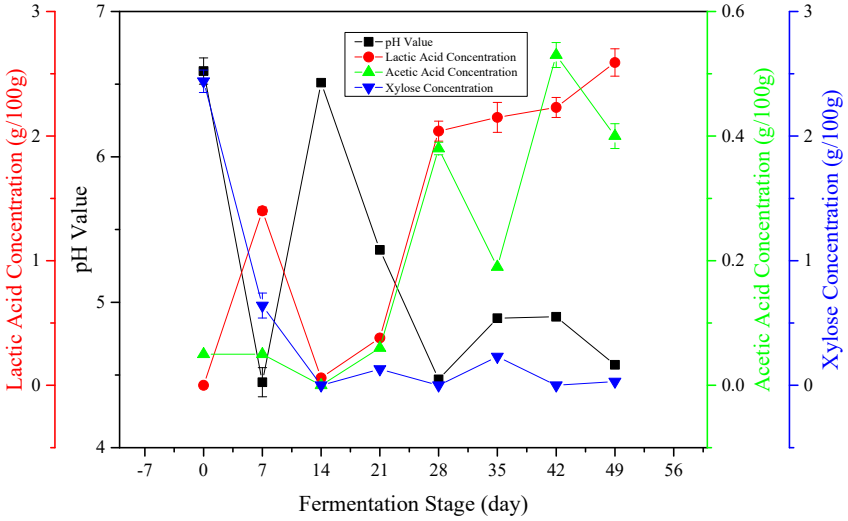

Figure 2. Physicochemical changes in DCS samples. DCS: dry corn stalk; DCS1–DCS7: DCS microsilage for 7, 14, 21, 28, 35, 42, and 49 days, respectively.

3.2. Effect of Ensiling on Carbohydrate Fractions of DCS

According to the traditional theory, lignin plays an important role in limiting the enzymatic conversion of biomass, and thus traditional research usually focuses on how to remove lignin to improve the efficiency of the enzymatic hydrolysis of raw materials. However, some studies have shown that a reduction in lignin content alone cannot fully explain the resistance of lignocellulose to enzymatic hydrolysis. Indeed, >50% delignification has been reported to possibly lead to the collapse of cellulose pores, decreasing the accessibility of the cellulose [18]. The restrictive effect of lignin on enzymatic hydrolysis is mainly due to spatial hindrance from the lignin network structure [19]. A slight change in the lignin content can significantly improve the efficiency of enzymatic hydrolysis [20]. These findings show that changes in the macromolecular microstructure of lignocellulosic materials play an important role in the process of enzymatic hydrolysis, but simply decreasing the content of lignin is not sufficient to facilitate their use in biorefining. This is also the problem for most physicochemical pretreatment technologies for biomass (such as steam explosion at 170–220 °C, dilute acid hydrolysis, or alkaline, organic solvent, or ionic liquid treatment). The use of various physicochemical methods to completely destroy the structure of raw materials can achieve the maximum removal of lignin and can improve the conversion efficiency in enzymatic hydrolysis to some extent, but it cannot clarify the synergistic mechanism of enzymatic hydrolysis from the molecular level.

As can be seen from Table 1, the ensiling of DCS had no significant effect on the cellulose fractions of the DCS, whereas hemicellulose was degraded during the ensiling, indicating that there was little carbohydrate loss during the process. The complete ensiling process thus destroyed the internal structure of the DCS while retaining most of the cellulosic biomass, which would be expected to greatly promote the reuse of the cellulose for subsequent LA fermentation. The low hemicellulose content might have been caused by the combined effect of the enzymatic and acid hydrolysis during ensiling. Hemicellulose is the most susceptible fraction of lignocellulose to enzymatic or acid/alkaline hydrolysis [21]. The partial degradation of raw material components could lead to the destruction of the internal structure of the LCB. In addition, the LCB degradation led to an increase in the AIL content during ensiling, but not to a decrease in bioaccessibility, which was confirmed by the subsequent enzymatic hydrolysis and LA fermentation results.

Table 1. The compositions of dry matter fraction of DCS and DCS microsilage (g/100 g dry matter).

	Cellulose	Hemicellulose	AIL	ASL	Ash
DCS	33.95 ± 0.93	17.99 ± 0.55	27.72 ± 0.79	2.71 ± 0.03	2.25 ± 0.01
DCS1	33.31 ± 0.86	17.57 ± 0.63	27.14 ± 0.85	3.10 ± 0.03	2.23 ± 0.01
DCS2	33.71 ± 0.97	17.39 ± 0.47	28.48 ± 0.91	3.15 ± 0.02	2.56 ± 0.03
DCS3	32.65 ± 0.73	16.98 ± 0.51	28.66 ± 0.73	3.06 ± 0.03	2.45 ± 0.01
DCS4	32.16 ± 0.82	15.89 ± 0.33	27.41 ± 0.56	2.97 ± 0.08	2.32 ± 0.06
DCS5	32.85 ± 0.96	15.65 ± 0.41	28.65 ± 0.81	3.08 ± 0.02	2.46 ± 0.02
DCS6	32.66 ± 0.63	15.14 ± 0.45	27.53 ± 0.63	3.15 ± 0.07	2.48 ± 0.02
DCS7	32.12 ± 0.76	14.85 ± 0.21	26.99 ± 0.58	3.15 ± 0.02	2.45 ± 0.03

The numbers in parentheses are standard deviations (n = 3). DCS: dry corn stalk; DCS1–DCS7: DCS microsilage for 7, 14, 21, 28, 35, 42, and 49 d, respectively; DM: dry matter; AIL: acid-insoluble lignin; ASL: acid-soluble lignin.

3.3. 16S rRNA Gene Sequencing Analysis of the Bacterial Community

In terms of the relative abundance, Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes were the dominant phyla in all the samples (Figure 3A1). Proteobacteria was the most abundant phylum, especially in sample DCS1, where its relative abundance of OTUs was 66.15%. In DCS4 (i.e., after 28 days of ensiling), the highest relative abundance was shown by Firmicutes (57.55%). Contrastingly, Bacteroidetes were much more abundant in DCS (14.40%) than in DCS4 (1.79%).

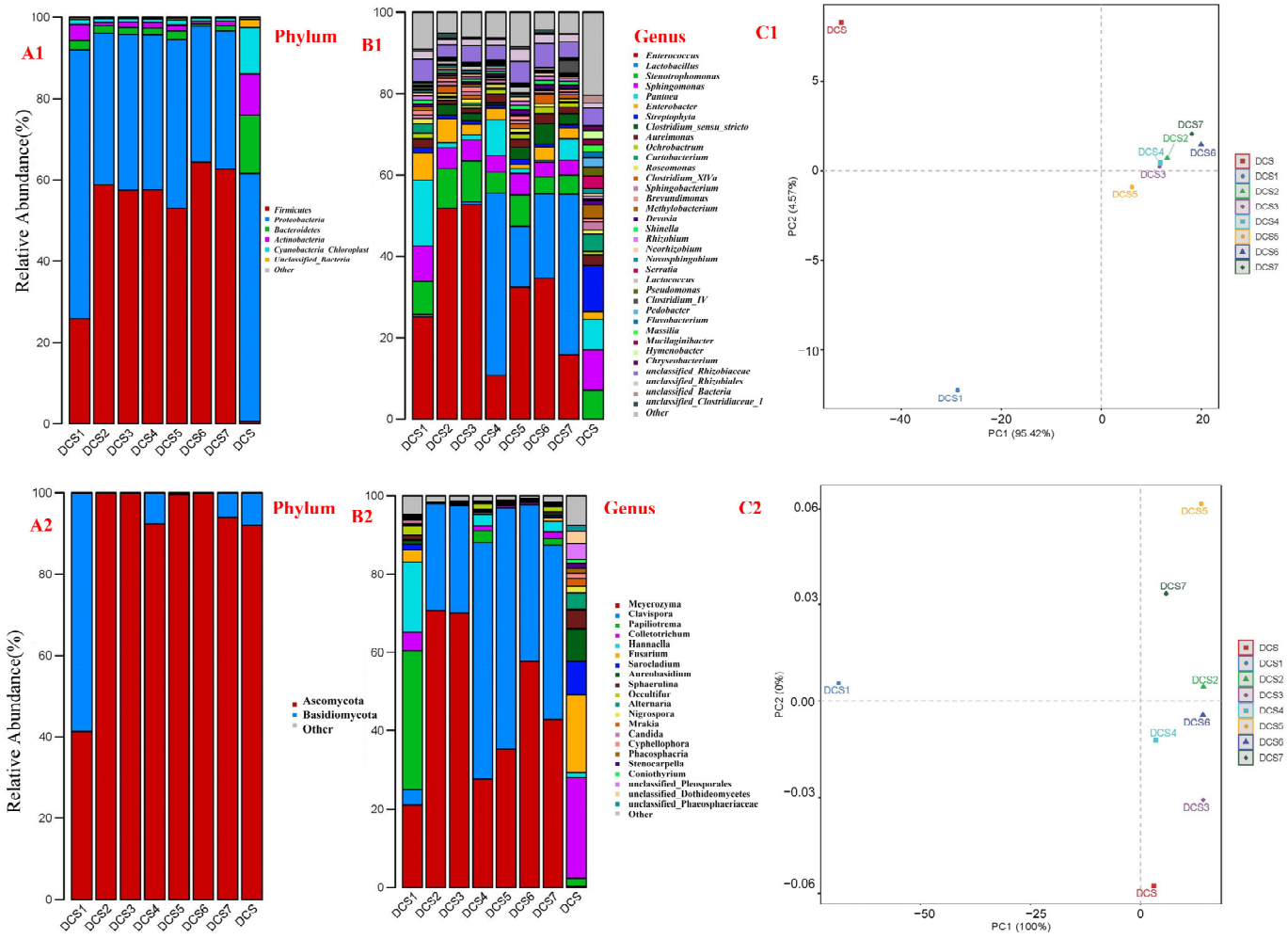

Figure 3. Relative abundance of bacteria and fungi in DCS samples at the phylum level (**A1**,**A2**) and genus level (**B1**,**B2**), and principal component analysis (**C1**,**C2**). DCS1–DCS7: DCS microsilage for 7, 14, 21, 28, 35, 42, and 49 days, respectively.

At the genus level (Figure 3B1), the dominant bacteria in the DCS raw material were *Streptophyta* (11.43%), *Sphingomonas* (9.88%), *Pantoea* (7.56%), *Stenotrophomonas* (7.03%), and *Curtobacterium* (4.16%). There were few *Enterococcus* (0.02%) and *Lactobacillus* (0.00%) units in the raw material. However, after fermentation, which involved the addition of the exogenous bacteria *B. coagulans*, *L. fermentum*, and *E. durans*, *Enterococcus* and *Lactobacillus* became the dominant genera. The inoculation of *Enterococcus* and *Lactobacillus* (1.0×10^6 cells per gram of DCS) could inhibit the rapid proliferation of a small number of epiphytic microorganisms in the DCS, resulting in a niche priority effect [22]. The relative abundance of *Enterococcus* was 0.02% in the DCS raw material, but it was 25.18% in sample DCS2 and 52.17% in DCS3. Potentially, the *Enterococcus* influenced the pH of the microsilage and increased the amount of LA, especially during the early stage of ensiling. At low pH, the undesirable growth of spoilage microorganisms in microsilage is avoided [23]. However, *Enterococcus* has a lower acid-stress-tolerance at a low-pH environment than *Lactobacillus* [24]. Therefore, it might be deduced that *Lactobacillus* was more competitive than *Enterococcus* and dominated in the later stages of the fermentation in the present study. Indeed, the dominant bacteria in sample DCS4 were *Lactobacillus*, with a relative abundance of 44.95%, compared with 0.00% in the DCS raw material. It is reasonable to suppose that the *Lactobacillus* inoculated into the microsilage in this study proliferated. *Streptophyta* (11.43%) and *Sphingomonas* (9.88%) were the dominant genera in the DCS raw material, but they became less abundant in the microsilage samples. The high relative abundance of *Lactobacillus* and *Enterococcus* and the high LA concentration and low pH in the microsilage in the present study were consistent with previous findings [25].

As shown in the bacterial principal component analysis (PCA) diagram (Figure 3C1), at the OTU level, the variance contribution of PC1 was 95.42%. The variance contribution of PC2 was 4.57%. The two PCs thus reflected 99.99% of the total bacterial species information in the samples. The results indicated apparent differences in the bacterial communities between the DCS and the ensiled material. However, the samples obtained at time points after 14 days of ensiling were not clearly separated from each other, which indicated that the bacterial community changed only slowly in the later stage of ensiling [26].

3.4. ITS1–ITS2 Sequencing of the Fungal Community

At the genus level (Figure 3B2), the dominant fungi in the DCS raw material were *Colletotrichum* (25.71%), *Fusarium* (19.90%), *Sarocladium* (8.35%), and *Aureobasidium* (8.29%), which gradually dropped to <1% abundance in the process of ensiling. After the exogenous enhanced fermentation, *Meyerozyma* and *Clavispora* became the dominant fungal taxa. The dominant fungi in sample DCS4 were identified as *Clavispora*, with a relative abundance of 60.43%, compared with 0.03% in the DCS raw material. The relative abundance of *Meyerozyma* was 0.29% in the DCS raw material, but it dramatically increased to 69.92% in sample DCS3 and then fell to 27.58% in DCS4. *Colletotrichum* (25.71%) and *Fusarium* (19.91%) were the main fungal genera in the DCS raw material, but they became less abundant in the microsilage samples. As shown in the fungal PCA diagram (Figure 3C2), at the OTU level, the variance contribution of PC1 was 100%. The distance between samples DCS2, DCS4, and DCS6 was the shortest, indicating that the fungal composition of these samples was the most similar at the OTU level. Natural ensiling is a traditional technology for forage preservation. LAB generally play an important role. In this paper, exogenous LAB and epiphytic bacterial communities were used for the straw microsilage to promote the internal structural degradation of LCB. Because of the anaerobic environment and low pH value of the system, the proportion of fungi was low [27].

Generally speaking, the microbial community diversity decreases and stabilizes during ensiling. The exogenous LAB added in the process here became the dominant microbiota. Our results showed that the microbial pretreatment method applied here potentially enabled the further use of the ensiled straw.

3.5. Cell Wall of DCS Samples

It can be seen (Figure 4, 0 days) that the surface of the DCS raw material was flat and the structure was dense, which would restrict the use of the lignocellulose by microorganisms. The DCS was composed of hemicellulose, lignin, and cellulose, where the lignin layers surrounding hemicelluloses and cellulose in the plant cell walls protected them from deconstruction [28]. However, degradation by microorganisms and their enzymes changed the surface structure of the lignocellulosic materials significantly. Here, a series of regular mesh structures were formed on the straw surface in DCS4, while there were signs of collapse in the center of the mesh (Figure 4, 28 days). The collapse of the pores in the microsilage was mainly due to degradation of the hemicellulose, indicating that some of the hemicellulose was metabolized by microorganisms in the process of ensiling, which was consistent with the results in the detection of hemicellulose components (Table 1). SEM showed that the pretreatment of the DCS with mixed LAB promoted the accessibility of the lignocellulose, which is expected to be beneficial for the degradation efficiency in subsequent enzymatic hydrolysis and LA fermentation [29].

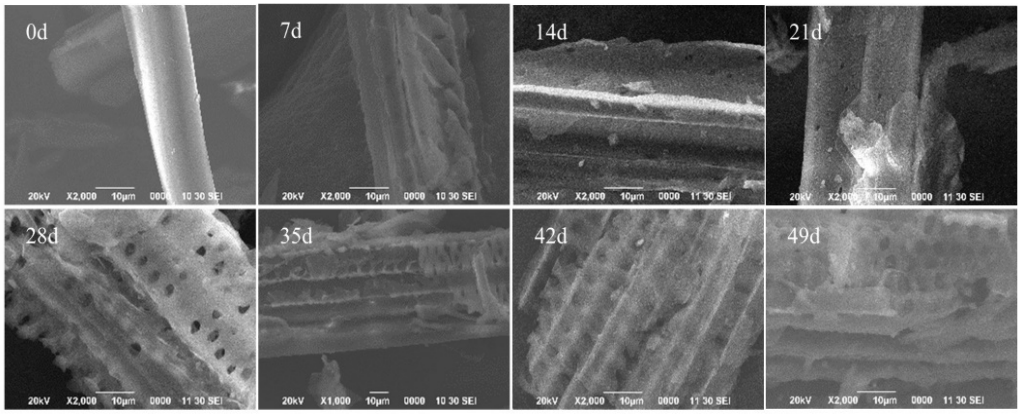

Figure 4. Scanning electron micrographs of DCS samples before and after ensiling. DCS1–DCS7: DCS microsilage for 7, 14, 21, 28, 35, 42, and 49 days, respectively.

Among several influencing factors, crystallinity is considered to significantly affect the enzymatic saccharification of LCB. The amorphous part of straw includes not only amorphous cellulose but also hemicellulose and lignin [30]. Here, XRD was used to measure the crystallinity of the whole material, including the hemicellulose and lignin as well as amorphous cellulose [31]. The XRD patterns of the DCS with different ensiling times showed diffraction peaks for crystalline cellulose at 15.9° and 22.0° and amorphous cellulose at 18.4°. The crystallinities of the cellulose in the samples DCS (the raw material), DCS1, DCS2, DCS3, DCS4, DCS5, DCS6, and DCS7 were 58.57%, 61.39%, 61.65%, 63.54%, 62.60%, 62.10%, 61.37%, and 61.19%, respectively (details are shown in the Supporting Information Figure S1, DTG curves and infrared spectroscopy analysis of corn stalk samples are shown in the Supporting Information Figures S2 and S3). The degradation and modification of the amorphous components hemicellulose and lignin may have decreased the overall amorphous characteristics of the material [32]. The component analysis (Table 1) showed that the content of hemicellulose decreased during the ensiling and that the consumption of hemicellulose was higher than that of cellulose. The crystallinity was higher than that of DCS due to a relative decrease in the amorphous content after ensiling. However, during the ensiling process, the crystallinity of the material first increased and then decreased, which was consistent with the change in the hemicellulose content, indicating that the amorphous components were released in the early stage of fermentation and metabolized in the later part.

3.6. SSCF of Microsilage for LA Production

After 28 s of ensiling, i.e., in DCS4, the contents of LA and acetic acid were 2.04 ± 0.08% (*w*/*w*) and 0.38 ± 0.01% (*w*/*w*), respectively. The pH value decreased from 6.59 ± 0.02 in DCS to 4.47 ± 0.13 in DCS4. An ensiling time of 28 days was required to obtain a stable product from the DCS. The microbiota was relatively stable, and the dominant bacteria in DSC4 were *Lactobacillus* and *Enterococcus*. After the degradation of the DCS for 28 days, a series of regular mesh structures were formed on the straw surface, while there were signs of collapse in the center of the mesh. For these reasons, the substrate used for LA fermentation was DCS treated microbially for 28 days (i.e., DCS4). This material was then fermented by *B. coagulans* NBRC 12583. To decrease the inhibition of hydrolytic enzymes by the accumulation of sugars via metabolism, researchers developed the SSCF process, in which substrate saccharification and fermentation occur in the same reactor [33]. Studies have demonstrated that SSCF can accelerate the rate of hydrolysis and saccharification of substrate, decrease the required volume of the reactor, and improve the yield of LA [34]. A further advantage of using SSCF here is that the optimal temperature of the cellulase is consistent with the fermentation conditions for *B. coagulans* [35]. *B. coagulans* has been reported as a potential industrial LA producer because of its ability to grow and produce LA at high temperatures. Moreover, it can produce optically pure L-LA, and it is suitable for SSCF because of its ability to use glucose and xylose, which enables the simultaneous conversion of cellulose and hemicellulose from LCB [36].

To explore the best conditions for enzymatic hydrolysis and LA fermentation from microbially pretreated DCS, *B. coagulans* NBRC 12583 was added 36 and 48 h after the addition of cellulase, respectively. After 36 h of cellulase hydrolysis, the glucose and xylose concentrations were 15.23 ± 0.62 g/L and 10.93 ± 0.29 g/L, respectively. The highest observed LA concentration was then 12.06 ± 0.21 g/L, 12 h after the addition of *B. coagulans*. After 48 h of cellulase hydrolysis, the glucose and xylose concentrations were 18.12 ± 0.66 g/L and 11.72 ± 0.32 g/L, respectively (Figure 5). The enzymatic hydrolysis yields of cellulose and hemicellulose reached 96.97 ± 0.90% and 97.10 ± 0.33% respectively. The highest observed LA concentration was then 18.54 ± 0.52 g/L, 16 h after of the addition of *B. coagulans*. The optical purity of LA was 97.06 ± 1.29%, and the LA yield was 0.31 ± 0.01 g/g DCS. Thus, the time of adding *B. coagulans* to the fermentation system was a key factor for optimizing the system.

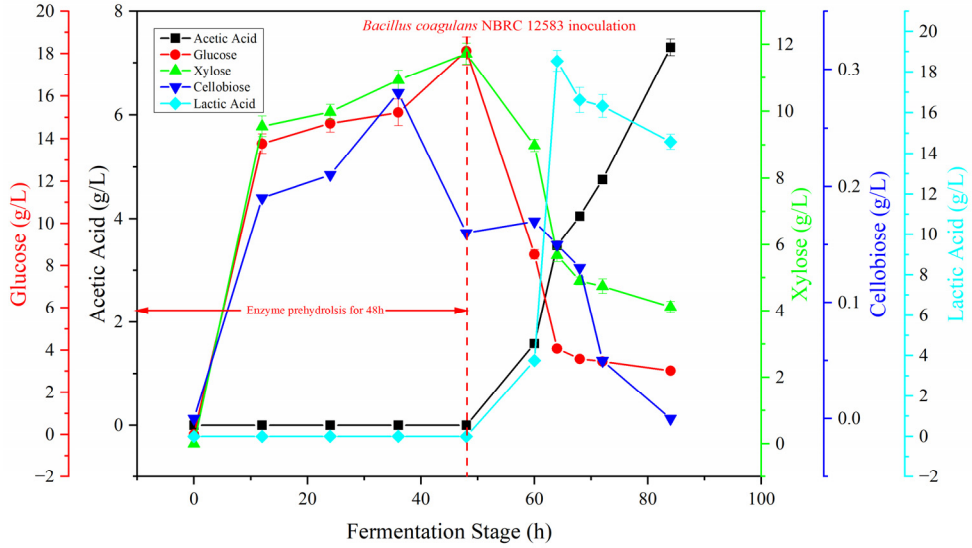

Figure 5. Simultaneous saccharification and co-fermentation (SSCF) performance of DCS4 at 5% substrate loading. DCS4: DCS microsilage after 28 days.

Data on the quantitative conversion of raw materials to products are important for the evaluation of a biochemical process for the production of bio-based chemicals. To

systematically evaluate the conversion process of DCS, statistical analysis of the ensiling pretreatment was performed. As can be seen from Figure 6, the recovery yields of cellulose and hemicellulose in the ensiling pretreatment were 87.81% and 81.88%, respectively, indicating that the sugars were protected in the process of the destruction of the structure of the straw.

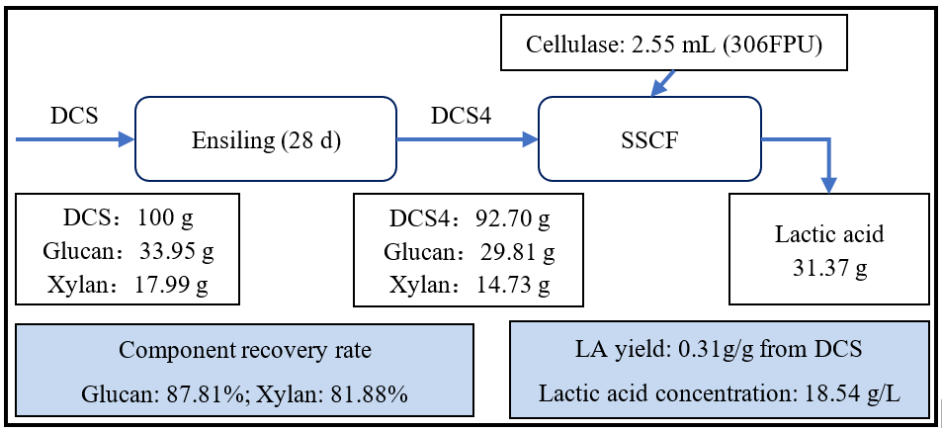

Figure 6. Analysis for ensiling pretreatment of DCS and SSCF of DCS4. SSCF: simultaneous saccharification and co-fermentation. DCS4: DCS microsilage for 28d. DCS: dry corn stover.

In the SSCF of the material obtained by ensiling for 28 days, after 48 h of enzymatic (i.e., cellulase) hydrolysis and 16 h of fermentation by *B. coagulans*, the concentration of LA in the fermentation broth reached 18.54 ± 0.52 g/L, the LA yield was 0.31 ± 0.01 g/g DCS (Figure 6), and the sugar (glucose and xylose)–acid conversion yield was 62.92%. Thus, 31.37 g of LA was obtained from 100 g of DCS (dry matter, DM). On the basis of our analyses, using the processes described here, it would take 3.19 t of DCS (DM) and 82 L of cellulase to produce 1 t of LA. In the work of Ouyang et al. [37], using dilute sulfuric acid pretreatment, *B. coagulans* was successfully applied to produce LA from wheat straw (35.80% cellulose and 20.45% hemicellulose) in a one-pot system, and the LA yield was 0.44 g/g. The LA conversion from wheat straw pretreated with H_2SO_4-steam explosion by *B. coagulans* MA-13 and *B. coagulans* IPE22 was 0.27 g/g and 0.46 g/g, respectively [38]. In the work of Zhang et al. [39], using a process consisting of biomass pretreatment by dilute sulfuric acid and subsequent SSCF, 46.12 g of LA could be produced from 100 g of dry wheat straw with a supplement of 10 g/L corn steep liquid powder at the cellulase loading of 20 FPU/g cellulose with *B. coagulans* IPE22. Here, using corn stalk deconstructed by an anaerobic microbial community as the substrate, the LA yield was 0.31 g/g, similar to that in the above studies. The criteria for evaluating the viability of a biomass pretreatment method are as follows: avoidance of the degradation of cellulose; production of the maximum amount of simple sugars; avoidance of the formation of byproducts harmful to hydrolysis and fermentation; and economic controllability [40]. Compared with other pretreatment methods, anaerobic ensiling pretreatment has the advantages of being a simple process with a high sugar yield, a low production of inhibitors, and a low cost. It represents a new solution for biomass use. Several weeks are generally needed to reach stability in microbial pretreatment. However, biomass plants have a stacked demand during the process of collecting straw, and raw materials will not be processed within a short time. If the microbial pretreatment is conducted concurrently with on-farm storage, the pretreatment time ceases to be a problem.

Emerging methods in microbial enrichment, sequencing, and bioinformatics provide new opportunities to decipher the functions of microbial communities. In the anaerobic ensiling process, the abundance and diversity of genes encoding carbohydrate-active enzymes (CAZymes) need to be resolved, especially the genes responsible for degrading starch, arabino-xylan, and cellulose, in order to improve the quality of silage. In addition, in the process of lactic acid fermentation, it is necessary to adopt a method of high-substrate-

fed batch SSCF to simultaneously and efficiently convert cellulose and hemicellulose into LA.

4. Conclusions

In this study, the research object was the anaerobic ensiling process of DCS. A synthetic community of lactic acid bacteria including *B. coagulans* NBRC 12583, *L. fermentum* KF5, and *E. durans* 075 efficiently and selectively degraded the lignocellulose of DCS. Further, the feasibility of converting the product microsilage into lactic acid was investigated using SSCF with *B. coagulans* NBRC 12583. We demonstrated that the anaerobic ensiling pretreatment method applied here is viable for use in biomass transformation. Our study provides support for the biological pretreatment of DCS for the production of lactic acid. LCB is the most abundant renewable resource on earth, but most of is not used effectively and rationally. If the anaerobic ensiling of straw can be carried out in the field, it can alleviate environmental pollution and also decrease the problems related to straw storage in factories (such as needing a large storage area for the straw, the ease of contamination of stored straw with mildew, and the possibility of spontaneous combustion). Anaerobic ensiling can be accomplished by an operational method that is low-cost and easy. Continuous ensiling equipment is available in the market, which begins the ensiling process while harvesting the straw.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9070611/s1, Table S1: Comparison of the characteristics of the conversion of different concentrations glucose into lactic acid with *Bacillus coagulans* NBRC 12583; Table S2: Comparison of the characteristics of converting different concentrations xylose into lactic acid with *Bacillus coagulans* NBRC 12583; Table S3: Comparison of the characteristics of the conversion of mixed sugars (glucose:xylose) into lactic acid in different proportions with *Bacillus coagulans* NBRC 12583; Figure S1: XRD patterns of DCS samples before and after ensiling pretreatment; Figure S2: DTG curves of corn stalk samples before and after ensiling pretreatment; Figure S3: Infrared spectroscopy analysis of corn stalk samples before and after ensiling pretreatment.

Author Contributions: Conceptualization, methodology, software, investigation, data curation, writing—original draft, funding acquisition, X.Y. Investigation, Z.S. Investigation, T.W. (Tongyu Wang). Methodology, data curation, X.M. writing—review and editing, funding acquisition, L.S. Writing—review and editing, funding acquisition, Z.Z. Writing—review and editing, J.Z. Writing—review and editing, funding acquisition, supervision, T.W. (Tao Wei). All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Key Research Projects of the Science and Technology Department of Henan Province (grant number 232102321144), Key Research Projects of the Science and Technology Department of Henan Province (grant number 232102110150), Key Research Projects of the Science and Technology Department of Henan Province (grant number 212102310077) and Zhongyuan Science and Technology Innovation Leading Talent Project (grant number 224200510017).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data supporting the findings of this study are included in this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Goldford, J.E.; Lu, N.X.; Bajic, D.; Estrela, S.; Tikhonov, M.; Sanchez-Gorostiaga, A.; Segre, D.; Mehta, P.; Sanchez, A. Emergent simplicity in microbial community assembly. *Science* **2018**, *361*, 469–474. [CrossRef] [PubMed]
2. Qian, X.J.; Chen, L.; Sui, Y.; Chen, C.; Zhang, W.M.; Zhou, J.; Dong, W.L.; Jiang, M.; Xin, F.X.; Ochsenreither, K. Biotechnological potential and applications of microbial consortia. *Biotechnol. Adv.* **2020**, *40*, 107500. [CrossRef] [PubMed]
3. Reshmy, R.; Paulose, T.A.P.; Philip, E.; Thomas, D.; Madhavan, A.; Sirohi, R.; Binod, P.; Awasthi, M.K.; Pandey, A.; Sindhu, R. Updates on high value products from cellulosic biorefinery. *Fuel* **2022**, *308*, 122056. [CrossRef]

4. Zhu, J.J.; Jiao, N.X.; Zhang, H.; Xu, G.L.; Xu, Y. Detoxification of lignocellulosic prehydrolyzate by lignin nanoparticles prepared from biorefinery biowaste to improve the ethanol production. *Bioproc. Biosyst. Eng.* **2022**, *45*, 1011–1018. [CrossRef] [PubMed]
5. Sorbara, M.T.; Pamer, E.G. Microbiome-based therapeutics. *Nat. Rev. Microbiol.* **2022**, *20*, 365–380. [CrossRef]
6. Zheng, M.Y.; Zhou, W.; Zou, X.; Wu, S.; Chen, X.Y.; Zhang, Q. Fermentation Quality and Bacterial Diversity of Broussonetia papyrifera Leaves Ensiled with *Lactobacillus plantarum* and Stored at Different Temperatures. *Agronomy* **2022**, *12*, 986. [CrossRef]
7. You, L.J.; Bao, W.C.; Yao, C.Q.; Zhao, F.Y.; Jin, H.; Huang, W.Q.; Li, B.H.; Kwok, L.Y.; Liu, W.J. Changes in chemical composition, structural and functional microbiome during alfalfa (*Medicago sativa*) ensilage with *Lactobacillus plantarum* PS-8. *Anim. Nutr.* **2022**, *9*, 100–109. [CrossRef]
8. Liu, Z.H.; Le, R.K.; Kosa, M.; Yang, B.; Yuan, J.S.; Ragauskas, A.J. Identifying and creating pathways to improve biological lignin valorization. *Renew Sust. Energ. Rev.* **2019**, *105*, 349–362. [CrossRef]
9. Lee, J.S.A.; Baugh, A.C.; Shevalier, N.J.; Strand, B.; Stolyar, S.; Marx, C.J. Cross-Feeding of a Toxic Metabolite in a Synthetic Lignocellulose-Degrading Microbial Community. *Microorganisms* **2021**, *9*, 321. [CrossRef]
10. Lin, L. Bottom-up synthetic ecology study of microbial consortia to enhance lignocellulose bioconversion. *Biotechnol. Biofuels Bioprod.* **2022**, *15*, 14. [CrossRef]
11. Lillington, S.P.; Leggieri, P.A.; Heom, K.A.; O'Malley, M.A. Nature's recyclers: Anaerobic microbial communities drive crude biomass deconstruction. *Curr. Opin. Biotech.* **2020**, *62*, 38–47. [CrossRef] [PubMed]
12. Jomnonkhaow, U.; Sittijunda, S.; Reungsang, A. Assessment of organosolv, hydrothermal, and combined organosolv and hydrothermal with enzymatic pretreatment to increase the production of biogas from Napier grass and Napier silage. *Renew Energ.* **2022**, *181*, 1237–1249. [CrossRef]
13. Peng, X.; Wilken, S.E.; Lankiewicz, T.S.; Gilmore, S.P.; Brown, J.L.; Henske, J.K.; Swift, C.L.; Salamov, A.; Barry, K.; Grigoriev, I.V.; et al. Genomic and functional analyses of fungal and bacterial consortia that enable lignocellulose breakdown in goat gut microbiomes. *Nat. Microbiol.* **2021**, *6*, 499–511. [CrossRef]
14. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Determination of Structural Carbohydrates and Lignin in Biomass*; National Renewable Energy Laboratory: Boulder, CO, USA, 2011.
15. Yang, X.; Li, H.L.; Chang, C.; Chen, J.Y.; Ma, X.J. The integrated process of microbial ensiling and hot-washing pretreatment of dry corn stover for ethanol production. *Waste Biomass Valori.* **2018**, *9*, 2031–2040. [CrossRef]
16. Zheng, Y.; Lee, C.; Yu, C.; Cheng, Y.S.; Simmons, C.W.; Zhang, R.; Jenkins, B.M.; VanderGheynst, J.S. Ensilage and bioconversion of grape pomace into fuel ethanol. *J. Agric. Food Chem.* **2012**, *60*, 11128–11134. [CrossRef] [PubMed]
17. Jensen, M.B.; de Jonge, N.; Dolriis, M.D.; Kragelund, C.; Fischer, C.H.; Eskesen, M.R.; Noer, K.; Moller, H.B.; Ottosen, L.D.M.; Nielsen, J.L.; et al. Cellulolytic and Xylanolytic Microbial Communities Associated with Lignocellulose-Rich Wheat Straw Degradation in Anaerobic Digestion. *Front. Microbiol.* **2021**, *12*, 645174. [CrossRef]
18. Zhu, L.; O'Dwyer, J.P.; Chang, V.S.; Granda, C.B.; Holtzapple, M.T. Structural features affecting biomass enzymatic digestibility. *Bioresour. Technol.* **2008**, *99*, 3817–3828. [CrossRef]
19. Wang, S.; Li, F.; Wu, D.; Zhang, P.; Wang, H.; Tao, X.; Ye, J.; Nabi, M. Enzyme Pretreatment Enhancing Biogas Yield from Corn Stover: Feasibility, Optimization, and Mechanism Analysis. *J. Agric. Food Chem.* **2018**, *66*, 10026–10032. [CrossRef]
20. Wen, J.L.; Sun, S.N.; Yuan, T.Q.; Xu, F.; Sun, R.C. Fractionation of bamboo culms by autohydrolysis, organosolv delignification and extended delignification: Understanding the fundamental chemistry of the lignin during the integrated process. *Bioresour. Technol.* **2013**, *150*, 278–286. [CrossRef]
21. He, L.W.; Lv, H.J.; Wang, C.; Zhou, W.; Pian, R.Q.; Zhang, Q.; Chen, X.Y. Dynamics of fermentation quality, physiochemical property and enzymatic hydrolysis of high-moisture corn stover ensiled with sulfuric acid or sodium hydroxide. *Bioresour. Technol.* **2020**, *298*, 122510. [CrossRef]
22. Ren, H.; Feng, Y.; Pei, J.; Li, J.; Wang, Z.; Fu, S.; Zheng, Y.; Li, Z.; Peng, Z. Effects of *Lactobacillus plantarum* additive and temperature on the ensiling quality and microbial community dynamics of cauliflower leaf silages. *Bioresour. Technol.* **2020**, *307*, 123238. [CrossRef] [PubMed]
23. Liu, H.D.; Zhang, L.Q.; Sun, Y.; Xu, G.B.; Wang, W.D.; Piao, R.Z.; Cui, Z.J.; Zhao, H.Y. Degradation of lignocelluloses in straw using AC-1, a thermophilic composite microbial system. *PeerJ* **2021**, *9*, 12364. [CrossRef] [PubMed]
24. Yang, L.L.; Yuan, X.J.; Li, J.F.; Dong, Z.H.; Shao, T. Dynamics of microbial community and fermentation quality during ensiling of sterile and nonsterile alfalfa with or without *Lactobacillus plantarum* inoculant. *Bioresour. Technol.* **2019**, *275*, 280–287. [CrossRef] [PubMed]
25. Liu, F.; Bai, J.; Huang, W.; Li, F.; Ke, W.; Zhang, Y.; Xie, D.; Zhang, B.; Guo, X. Characterization of a novel beta-cypermethrin-degrading strain of *Lactobacillus pentosus* 3–27 and its effects on bioremediation and the bacterial community of contaminated alfalfa silage. *J. Hazard. Mater.* **2022**, *423*, 127101. [CrossRef] [PubMed]
26. Diaz-Garcia, L.; Huang, S.X.; Sproer, C.; Sierra-Ramirez, R.; Bunk, B.; Overmann, J.; Jimenez, D.J. Dilution-to-Stimulation/Extinction Method: A Combination enrichment strategy to develop a minimal and versatile lignocellulolytic bacterial consortium. *Appl. Environ. Microb.* **2021**, *87*, e02427-20. [CrossRef] [PubMed]
27. Jimenez, D.J.; Korenblum, E.; van Elsas, J.D. Novel multispecies microbial consortia involved in lignocellulose and 5-hydroxymethylfurfural bioconversion. *Appl. Microbiol. Biot.* **2014**, *98*, 2789–2803. [CrossRef]

28. Jard, G.; Dumas, C.; Delgenes, J.P.; Marfaing, H.; Sialve, B.; Steyer, J.P.; Carrere, H. Effect of thermochemical pretreatment on the solubilization and anaerobic biodegradability of the red macroalga Palmaria palmata. *Biochem. Eng. J.* **2013**, *79*, 253–258. [CrossRef]
29. Li, P.P.; He, C.; Li, G.; Ding, P.; Lan, M.M.; Gao, Z.; Jiao, Y.Z. Biological pretreatment of corn straw for enhancing degradation efficiency and biogas production. *Bioengineered* **2020**, *11*, 251–260. [CrossRef]
30. Zhao, X.B.; Wang, L.; Liu, D.H. Peracetic acid pretreatment of sugarcane bagasse for enzymatic hydrolysis: A continued work. *J. Chem. Technol. Biot.* **2008**, *83*, 950–956. [CrossRef]
31. Xu, C.Y.; Ma, F.Y.; Zhang, X.Y.; Chen, S.L. Biological Pretreatment of Corn Stover by Irpex lacteus for Enzymatic Hydrolysis. *J. Agr. Food Chem.* **2010**, *58*, 10893–10898. [CrossRef]
32. Wang, J.F.; Xin, D.L.; Hou, X.C.; Wu, J.Y.; Fan, X.F.; Li, K.N.; Zhang, J.H. Structural properties and hydrolysabilities of Chinese Pennisetum and Hybrid Pennisetum: Effect of aqueous ammonia pretreatment. *Bioresour. Technol.* **2016**, *199*, 211–219. [CrossRef] [PubMed]
33. Zhou, X.D.; Ye, L.D.; Wu, J.C. Efficient production of l-lactic acid by newly isolated thermophilic *Bacillus coagulans* WCP10-4 with high glucose tolerance. *Appl. Microbiol. Biot.* **2013**, *97*, 4309–4314. [CrossRef] [PubMed]
34. Li, J.; Shi, S.A.; Wang, Y.; Jiang, Z.H. Integrated production of optically pure L-lactic acid from paper mill sludge by simultaneous saccharification and co-fermentation (SSCF). *Waste Manag.* **2021**, *129*, 35–46. [CrossRef] [PubMed]
35. Chacon, M.G.; Ibenegbu, C.; Leak, D.J. Simultaneous saccharification and lactic acid fermentation of the cellulosic fraction of municipal solid waste using *Bacillus smithii*. *Biotechnol. Lett.* **2021**, *43*, 667–675. [CrossRef]
36. Zheng, Z.J.; Jiang, T.; Zou, L.H.; Ouyang, S.P.; Zhou, J.; Lin, X.; He, Q.; Wang, L.M.; Yu, B.; Xu, H.J.; et al. Simultaneous consumption of cellobiose and xylose by *Bacillus coagulans* to circumvent glucose repression and identification of its cellobiose-assimilating operons. *Biotechnol. Biofuels* **2018**, *11*, 320. [CrossRef]
37. Ouyang, S.; Zou, L.; Qiao, H.; Shi, J.; Zheng, Z.; Ouyang, J. One-pot process for lactic acid production from wheat straw by an adapted *Bacillus coagulans* and identification of genes related to hydrolysate-tolerance. *Bioresour. Technol.* **2020**, *315*, 123855. [CrossRef]
38. Aulitto, M.; Fusco, S.; Nickel, D.B.; Bartolucci, S.; Contursi, P.; Franzen, C.J. Seed culture pre-adaptation of *Bacillus coagulans* MA-13 improves lactic acid production in simultaneous saccharification and fermentation. *Biotechnol. Biofuels* **2019**, *12*, 45. [CrossRef]
39. Zhang, Y.M.; Chen, X.R.; Luo, J.Q.; Qi, B.K.; Wan, Y.H. An efficient process for lactic acid production from wheat straw by a newly isolated *Bacillus coagulans* strain IPE22. *Bioresour. Technol.* **2014**, *158*, 396–399. [CrossRef]
40. Ashokkumar, V.; Venkatkarthick, R.; Jayashree, S.; Chuetor, S.; Dharmaraj, S.; Kumar, G.; Chen, W.H.; Ngamcharussrivichai, C. Recent advances in lignocellulosic biomass for biofuels and value-added bioproducts—A critical review. *Bioresour. Technol.* **2022**, *344*, 126195. [CrossRef]