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Efficient Degradation of Untreated Complex Cellulosic Substrates by Newly Isolated Aerobic *Paenibacillus* Species

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Abstract: A stable aerobic consortium was enriched to degrade crystalline cellulose (Whatman filter paper 1). The degradation efficiency of the consortium after 7 days of incubation was 91% compared to the control. One bacterial isolate, C7, capable of degrading various cellulosic substrates, was obtained from the consortium under aerobic conditions. The sequencing of 16s rDNA revealed that it was related to *Paenibacillus* sp. It degraded 83% of cotton after 3 days of incubation. The degradation efficiency of *Paenibacillus* sp. C7 for filter paper, cotton, and avicel was 90%, 90%, and 92% after 5 days of incubation compared to the control. It also degraded non-pretreated agricultural residues efficiently by 70% for rice straw and 46% for wheat bran in 10 days. Scanning electron micrographs (SEMs) of degraded filter paper after 2 days of incubation indicated smoother and thinner fabrics in its structure. It is a potential cheaper candidate for the degradation of lignocellulosic biomass without any pretreatment.

Keywords: cellulose degradation; aerobic consortium; *Paenibacillus* sp. C7; rice straw; wheat bran

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1. Introduction

Human society produces large amounts of waste materials. These wastes are produced by different sectors, like industry and agriculture, and municipalities [1]. In the two most populated countries in the world, China and India, where agriculture is of paramount importance to the economy and to sustain the large populations, a large amount of wastage is generated from agro-industries and agricultural activities. Almost 60% of agriculture products, especially crop residue, go to waste, highlighting both a challenge and an opportunity for sustainable management practices.

Cellulose is a major component of these wastes, which is generally non-hazardous, but, after accumulation in water or landfills, may cause various environmental issues, including blockages in sewage systems, increases in biological oxygen demand (BOD), many infectious diseases, respiratory allergic reactions, and the spread of stinking odors in the environment [2,3]. Cellulose materials such as newspapers, cardboard, etc., come under municipal solid waste. Large amounts of cellulose waste are discharged annually with the wastewater from the pulp, paper, board, and textile industries [4,5]. There is no proper waste disposal mechanism; mostly, it is degraded by burning or buried in landfills. This may increase the carbon dioxide level in the atmosphere and pollute surrounding areas [1,2].

Cellulose is a complex polymeric structure of amorphous (disordered) and crystalline (ordered) regions. Its degree of polymerization is several hundreds to tens of thousands; therefore, it is difficult to degrade [6]. Cellulose degradation is the process of breaking

down complex polysaccharide chains into simpler carbohydrates, which can be accomplished through enzymatic hydrolysis. The process of hydrolysis is facilitated by cellulolytic enzymes, including endoglucanases, exoglucanases, and β-glucosidases. These enzymes work together to break down cellulose into individual glucose units. The process of breaking down cellulose by enzymatic degradation is complicated, including numerous stages and a diverse range of enzymes. Endoglucanases break the internal connections in cellulose to destabilize its crystalline structure, exoglucanases remove cellobiose units from the non-reducing ends of the cellulose chains, and β-glucosidases break down cellobiose into glucose through hydrolysis. Various microorganisms, such as bacteria, fungi, and actinomycetes, can synthesize these enzymes, each with distinct benefits for various treatment situations [7]. The predicted half-life of cellulose at neutral pH in the absence of enzymes is several million years [8]. Hofsten and Edberg showed that cotton wool and filter paper fibers were decomposed by about 40% in a month in a sewage treatment plant. It was also shown that cellulose decomposition was rapid in heavily polluted, anaerobic, and nutrient-rich water compared to water with fewer nutrients [9]. Warnock et al. (2009) also showed that the half-life of cotton fibers (70% crystalline) buried in soil is 40.2 days [10].

The biological treatment of wastewater utilizes both aerobic and anaerobic processes. Nevertheless, there are clear disparities between these two methods in relation to effectiveness, secondary outcomes, and operational requirements. Aerobic microbes generally show accelerated growth rates and heightened metabolic activity in comparison to anaerobic organisms, leading to the more expeditious degradation of organic matter, such as cellulose [11]. Aerobic degradation frequently results in the complete conversion of organic compounds into carbon dioxide and water, thus reducing the production of potentially toxic or treatment-requiring intermediate by-products. Aerobic systems are typically easier to operate and maintain compared to anaerobic processes [12]. This is because they do not necessitate the severe conditions required for anaerobic processes, such as maintaining a completely oxygen-free environment and managing the creation of methane gas.

A wide range of microorganisms, particularly fungi and anaerobic bacteria, have the ability to break down and use cellulose as a source of carbon and energy [13–15]. Bacteria are increasingly being utilized for cellulose degradation due to their faster growth rate compared to fungi and their exceptional resilience to environmental stress. But, most of the research is focused on anaerobic bacteria [16–19]. There are only a few reports on aerobic bacteria degrading cellulosic wastes. Cellulolytic aerobic bacteria are at the cutting edge of current studies and applications as they have the ability to degrade cellulosic waste. Aerobic bacteria, in contrast to anaerobic bacteria, flourish in surroundings with oxygen and function at their best under these conditions. This characteristic provides specific benefits in many industrial and environmental situations. The limited number of studies on aerobic cellulose-degrading bacteria highlights the intricate nature of aerobic cellulose degradation processes and the unexplored possibilities for utilizing these organisms in environmentally friendly waste management and bioconversion technologies.

It is essential to make significant efforts to increase our understanding of aerobic cellulose degradation in order to enhance and improve biotechnological methods used to convert cellulose-rich biomass into valuable products including biofuels, biochemicals, and biodegradable materials. Researchers can use the metabolic flexibility and adaptability of aerobic bacteria to develop more efficient and eco-friendly methods for managing cellulose waste. This discovery has the potential to improve our knowledge of how microorganisms break down cellulose and also has important implications for supporting sustainable development practices and improving the bioeconomy worldwide [20].

The major technical and economic bottleneck of lignocellulose degradation is the lignin/hemicellulose association and the crystallinity of cellulose. Various pretreatment methods are available to remove lignin and hemicellulosic content from lignocellulosic material. Acid/alkali hydrolysis and high-temperature degradation are not efficient methods as the resulting sugar yields are very low under these harsh conditions and the energy requirement is also high. Therefore, cost-effective hydrolysis is an important goal. In this

study, we tried to address the issue of degrading recalcitrant cellulosic waste from the environment by utilizing aerobic bacteria in a time-efficient and cost-effective manner.

2. Materials and Methods

Avicel PH101 was purchased from Sigma-Aldrich, Dublin, Ireland. Cotton, rice straw, and wheat bran were collected from agricultural fields in Punjab, India. The Whatman filter paper 1 was purchased from Whatman International Ltd., Maidstone, UK. Prior to usage, all cellulosic materials were autoclaved at a temperature of 121 °C for a duration of 15 min. Thermo Fisher Scientific, Waltham, MA, USA, provided the molecular biology reagents. All remaining chemicals and reagents of analytical quality were procured from HiMedia Laboratories Pvt. Ltd., Delhi, India or Merck Life Science Pvt. Ltd., Delhi, India.

2.1. Sample Collection and Enrichment of Aerobic Consortium

A soil sample was obtained from agricultural dumping sites (CSW) in North Punjab, India, for the isolation of cellulose-degrading bacteria. The consortium was developed using Bushnell Hass Broth (BHB) medium (0.2 g $MgSO_4$, 0.02 g $CaCl_2$, 1 g KH_2PO_4, 1 g K_2HPO_4, 1 g NH_4NO_3, and 0.05 g $FeCl_3$ per liter), supplemented with trace metals (0.1 g/L each of zinc sulfate, manganese sulfate, copper sulfate, cobalt chloride, and sodium molybdate) and vitamin solutions (10 mg/L each of thiamine hydrochloride, riboflavin, nicotinic acid, pyridoxine hydrochloride, cyanocobalamin, folic acid, and biotin) and containing Whatman filter paper 1 (0.5%; *w*/*v*) as the main carbon source by the repeated enrichment technique [17]. Prior to use, the BHB medium was sterilized for 15 min at 121 °C. Trace metals and vitamin solutions were sterilized using a 0.22 µm filter and stored at 4 °C till further use. The control flask had BHB medium, autoclaved soil, and 0.5% (*w*/*v*) Whatman filter paper 1. The developed consortium (CSW) was inoculated into BHB (6%; *v*/*v*) with 0.5% (*w*/*v*) Whatman filter paper 1 and incubated at 37 °C and 150 rpm for 10 days. To acclimate the consortium, this process was repeated for six more weeks at 10-day intervals. Cellulose utilization and growth were measured after 3, 5, 7, and 10 days.

2.2. Screening of Cellulose-Degrading Bacteria

To isolate the cellulose-degrading bacteria, the consortium was serially diluted, and aliquots were spread on BHB agar plates, with (0.5%; *w*/*v*) carboxymethyl cellulose (CMC) as the sole source of carbon, and incubated for 24–48 h at 37 °C. Colonies exhibiting distinct morphologies were selected and re-streaked twice on fresh BHB agar plates containing CMC for purification. Cellulose-degrading bacteria were screened by zone of clearance as a qualitative measure of extracellular cellulase activity [21]. BHB CMC agar plates were immersed in a solution of congo red dye (0.1%; *w*/*v*) for 15 min. Subsequently, they were treated with a solution of 1 M NaCl to remove the dye. Then, the isolated colonies were purified and cultivated on BHB medium, utilizing filter paper (0.5%; *w*/*v*) as the primary carbon source, in order to assess their capacity to break down crystalline cellulose.

2.3. Molecular Identification of Cellulose-Degrading Bacteria

The rapid one-step extraction procedure was used to isolate chromosomal DNA [18]. The 16S rRNA gene was amplified with universal forward primer 8F (AGAGTTTGATC-CTGGCTCAG) and reverse primer 1492R (GGTTACCTTGTTACGACTT), using genomic DNA as a template. The reaction mixture for the PCR contained 1× PCR buffer, each deoxynucleoside triphosphate (dNTP) at a concentration of 200 µM, 2 mM $MgCl_2$, each primer at a concentration of 0.4 µM, and 1 U of Taq DNA polymerase in a final volume of 25 µL. The PCR reactions were performed with the following conditions: preheating at 94 °C for 3 min, 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The 16s rRNA PCR amplified product was subjected to restriction digestion using enzymes Msp 1 and Hha 1 to see the variation in the 16s rRNA gene.

The sequencing was conducted by Amnion Biosciences, Bangalore, India. The obtained sequences were then compared with similar sequences available in the NCBI GenBank database using the blast program. A phylogenetic tree was generated using MEGA 7 software, which utilizes molecular evolutionary genetics analysis. The nucleotide sequences of the bacterial strains analyzed in this study were accurately determined and deposited in the Gen-Bank database under accession number MH128356.

2.4. Growth Estimation and Analysis of Cellulose Degradation

Growth was determined with the help of total protein contents after regular intervals of time (after 3, 5, 7, and 10 days) [22]. The selected culture was inoculated in BHB medium with a concentration of 0.5% (*w*/*v*) of various cellulosic materials (such as filter paper, cotton, avicel PH101, A4 sheets, and wastepaper). The inoculated medium was incubated for 5 days at 37 °C, while the uninoculated medium performed as a control. After regular time intervals, each flask with residual substrate was incubated at 4 °C for half an hour, and an ice-cold sample was acidified by the addition of an equal amount of concentrated H_2SO_4 [23]. The flasks containing acidified solution were incubated at a temperature of 37 °C for a duration of one hour in order to fully digest the substrate. Afterwards, a 100 µL portion of the acid-hydrolyzed sample and 900 µL of water were added to bring the total volume to 1 mL. This mixture was then combined with anthrone reagent (0.2%; *w*/*v*) to obtain a final volume of 4 mL. The tubes were subjected to a 10 min heat treatment in a boiling water bath, followed by quick cooling on ice. The spectrophotometer was used to measure the absorbance at a wavelength of 630 nm.

2.5. Biodegradation of Agricultural Residue

Agricultural residues like wheat bran and rice straw were washed thoroughly with distilled water. The agricultural leftovers, such as wheat bran and rice straw, were meticulously rinsed with distilled water. The rice straw was homogenized using a stone grinder and then sieved to obtain particles with a size range of 0.35–0.5 mm. The obtained particles were subsequently washed 2–3 times with tap water to eliminate any soil particles. Following the process of washing, the substrates were left to dry naturally in the air.

The BHB medium was sterilized and supplemented with either 0.5% (*w*/*v*) rice straw or wheat bran as the only source of carbon. The medium was then inoculated with specific bacteria. The flasks were placed in an incubator set at a temperature of 37 °C and shaken at a speed of 150 RPM. At regular intervals, the entire flask was used to determine the growth and utilization of cellulose, as previously reported.

Residual solid agricultural substrates were assayed as previously described [23]. The precipitate after cultivation was collected by filtration using Whatman filter paper to separate solid from liquid media. The collected precipitate was washed with an acetic–nitric reagent and then with water to remove non-cellulosic materials and other impurities. The washed solid was then dried at 80 °C to achieve a constant weight. The dried residual substrates were then gravimetrically determined, with the uninoculated medium as a control. The weight loss of cellulosic materials was calculated by the following calculation:

$$\text{Degradation rate } (\%) = \frac{M_0 - M_t}{M_0} \times 100 \quad (1)$$

where M_0 is the total weight of the dry cellulosic materials at zero time and M_t is the weight of the dry residual substrate. All the experiments were performed in triplicate. We performed statistical analysis to determine the variation in degradation. Standard deviations were calculated for each set using OriginLab 2021 software. The data were plotted with mean values and error bars representing the standard deviations.

2.6. Scanning Electron Microscope (SEM) Analysis

The morphological features of the filter paper were analyzed using SEM at IIT, Delhi. The samples were made conductive by applying a thin layer of silver coating. The microscope maintained an accelerating voltage within the range of 20 kV.

3. Results

3.1. Development of Cellulose-Degrading Consortium

The enrichment technique is one of the most used methods to develop cellulose-degrading microbial consortia [23–28]. The CSW developed by the enrichment technique was capable of degrading filter paper efficiently within 7 days at 37 °C. The maximum degradation was observed between 3 and 5 days. Specifically, CSW degraded 22% of filter paper after 3 days, which increased to 63% after 5 days compared to the control. This indicates that the most significant degradation activity occurred during this period. The degradation of filter paper reached 91% after 7 days (Figure 1).

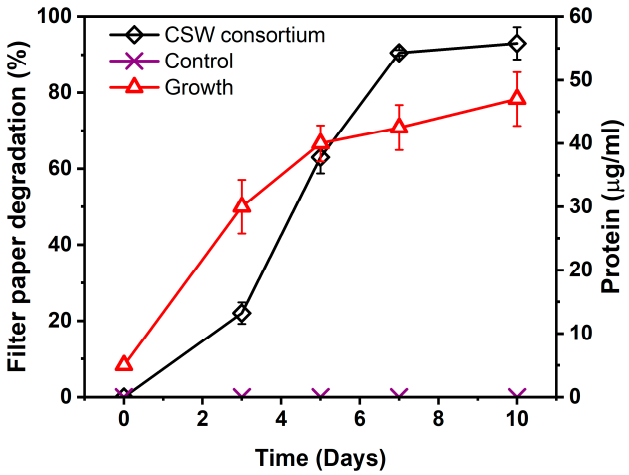

Figure 1. Degradation of Whatman filter paper 1 (0.5% *w*/*v*) as the sole source of carbon by bacterial consortium CSW over the study period and the growth of bacterial consortium also measured in terms of protein concentration.

3.2. Screening and Identification of Cellulose-Degrading Bacteria

A total of 23 bacterial isolates were shown to be positive for CSW on BHA with CMC, based on the zone of clearing observed using congo red dye. Subsequently, all the bacterial isolates were assessed for their capacity to degrade filter paper. Out of the 23 isolates of CSW, 5 isolates had the highest level of growth when exposed to filter paper (Figure S1). After conducting restriction analysis, it was determined that all five isolates of CSW exhibited similarity. The 16S rRNA gene sequence of the highly effective cellulose degrader C7 exhibited a similarity of 94–97% with *Paenibacillus* species.

The phylogeny of *Paenibacillus* sp. C7 was determined by comparing the 16S rRNA gene similarities with previously documented *Paenibacillus* species known for their involvement in cellulose degradation. Figure 2 demonstrates that *Paenibacillus* sp. C7 occupied a unique position in the evolutionary tree compared to other cellulolytic bacteria.

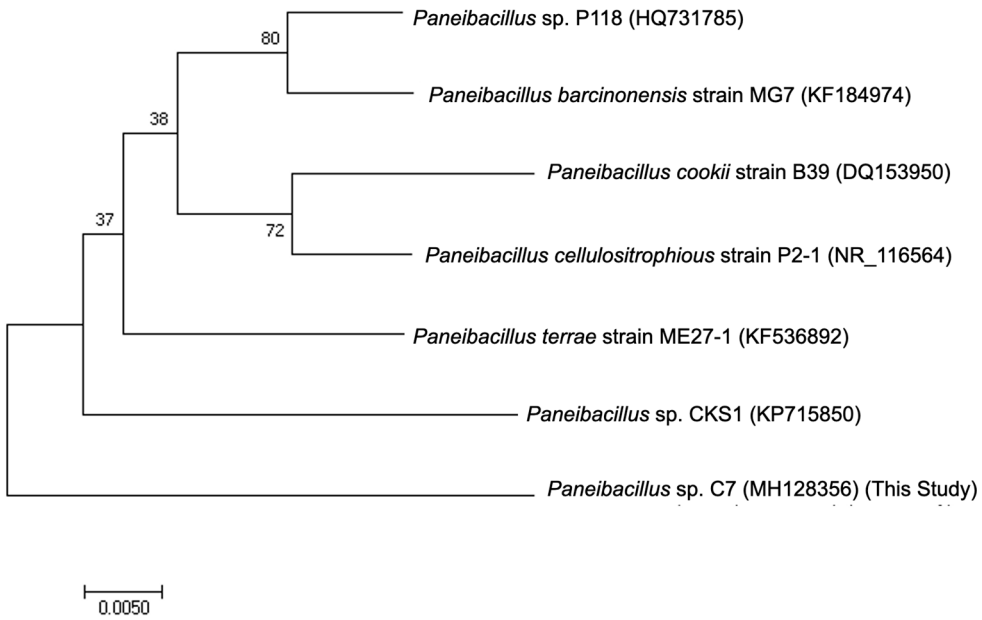

Figure 2. Phylogenetic tree of 16SrRNA gene sequence of *Paenibacillus* sp. C7. Reference sequences from GenBank include the accession number. The scale bar represents substitutions per nucleotide.

3.3. Biodegradation of Cellulosic Substrates by Paenibacillus sp. C7

The pure culture of *Paenibacillus* sp. C7 exhibited greater degradation of filter paper compared to the consortium. This conclusion is based on the results showing that *Paenibacillus* sp. C7 degraded 68% of the filter paper within 3 days of incubation, which further increased to 90% after 5 days. In comparison, the consortium degraded 22% and 63% of the filter paper after 3 and 5 days, respectively. Therefore, further experiments were conducted with a pure culture. This culture showed the efficient degradation of other cellulosic substrates like cotton, avicel PH101, wastepaper, and A4 sheets (Figure 3). It degraded 83% of cotton, which is the purest form of cellulose, after 3 days compared to the control. Avicel was also degraded by up to 92% after 5 days of incubation compared to the control. This culture degraded not only filter paper, cotton, and avicel but also waste newspaper (93%) and A4 sheets (94%) after 10 days of incubation.

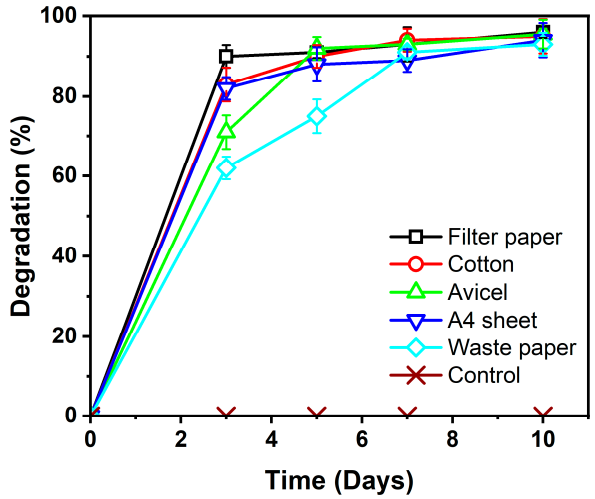

Figure 3. Biodegradation of cellulosic substrates (Whatman filter paper 1, cotton, avicel, A4 sheets, and wastepaper) (0.5%; *w*/*v*) used as the main source of carbon by *Paenibacillus* sp. C7 over the study period.

The morphological alterations that occurred with the degradation of filter paper were examined using SEM (Figure 4). The analysis revealed that the surface of the filter paper was smooth at the beginning (0 h), as shown in Figure 4A. However, after 48 h of incubation, shrinkage of the filter paper became noticeable, as depicted in Figure 4B. Cracks in the filter paper could also be observed after 48 h, indicated by the red arrow.

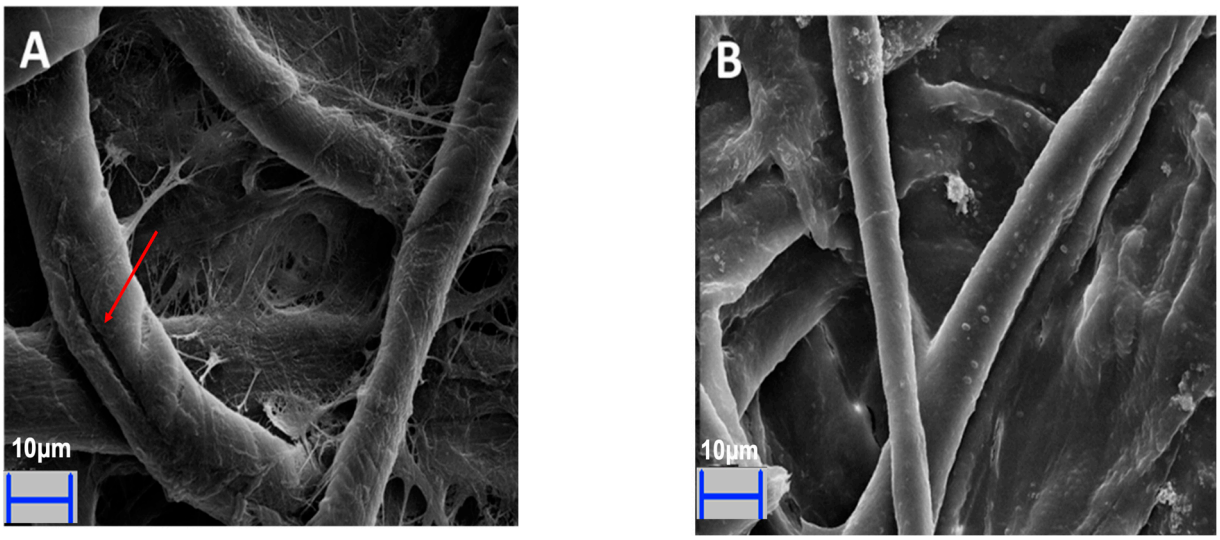

Figure 4. Morphological changes in Whatman filter paper 1 were determined by SEM analysis after 48 h: (**A**) control and (**B**) culture with *Paenibacillus* sp. C7.

3.4. Biodegradation of Agricultural Residues by Paenibacillus sp. C7

The biodegradability of untreated rice straw and wheat bran by *Paenibacillus* sp. C7 was assessed by evaluating the reduction in biomass weight and the amount of total carbohydrate degradation. During the third day of incubation, there was a notable decrease of 52% and 46% in the weight of rice straw and wheat bran, respectively. This reduction further increased to 68% and 62%, respectively, after 10 days of incubation, compared to the control (Figure 5).

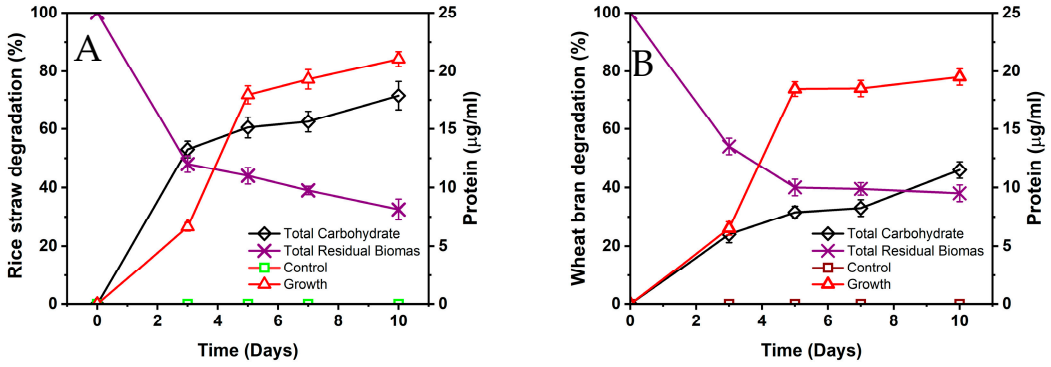

Figure 5. Biodegradation of agricultural residues, i.e., rice straw (**A**) and wheat bran (**B**) (0.5%; *w*/*v*), used as the sole source of carbon by *Paenibacillus* sp. C7 over the study period.

The total carbohydrate content decreased continuously over time and 70% and 46% were consumed after 10 days for rice straw and wheat bran, respectively. The growth of *Paenibacillus* sp. C7 increased simultaneously with the degradation of rice straw and wheat bran (Figure 5). Lower correlation coefficients (R^2) of 0.6892 and 0.7009 (Figure S2) were obtained for wheat bran and rice straw compared to filter paper (0.9909), which shows that lignocellulosic substrates have lignin and hemicellulose in addition to cellulose and, hence,

degradation is slow [23]. The variability in the degradation data is represented by the error bars in the graphs, which show the standard deviations of the measurements.

4. Discussion

After incubation for seven days, our work effectively enriched a stable aerobic consortium that was able to degrade crystalline cellulose with a 91% degradation efficiency. In just three days, a key bacterial isolate identified as *Paenibacillus* sp. C7 was able to degrade 83% of cotton, attributed to its strong cellulolytic activity under aerobic conditions. This isolate successfully broke down untreated agricultural leftovers such as rice straw (70%) and wheat bran (46%) during a ten-day period. It also showed remarkable degradation efficiency for filter paper, cotton, and avicel (90%, 90%, and 92%, respectively) after five days.

There are many reports of anaerobic microbial communities (consortium) that can degrade cellulose efficiently in 3 to 4 days [24,27–30]. However, maintaining a strict anaerobic environment is challenging, whereas aerobic bacteria have the advantage of high cell growth with low maintenance requirements [25]. While it was reported by many researchers that a significant reduction in the weight of cellulosic substrates can occur within 72 h [23,27], our study observed a substantial degradation of all cellulosic substrates after 3 days of incubation, with maximum degradation rates occurring between 3 and 5 days.

Wang et al. (2011) reported a 77% degradation of cotton after 3 days at 50 °C by consortium WCS-6 under aerobic conditions [27]. Avicel was also degraded by up to 92% after 5 days of incubation compared to the control. This culture degraded not only filter paper, cotton, and avicel but also waste newspaper (93%) and A4 sheets (94%) after 10 days of incubation [27]. Haruta et al. (2002) reported 67% and 42% weight loss for newspaper and printing paper, respectively, by an MC1 consortium after 4 days of incubation under anaerobic conditions [31]. For instance, *Paeneibacillus* sp. C7 showed a 68% degradation of filter paper within 3 days, which further increased over time.

Most of the previous research focused on the degradation of pretreated agricultural residues, and some had a lower degradation rate of unpretreated agricultural waste than our current study. Wongwilaiwalin et al. (2010) reported that the degradation rate of unpretreated rice straw (40%) was slow compared to that pretreated (75.3%) by an MC3F consortium after 7 days [25]. Haruta et al. (2002) showed that 60% of rice straw was degraded by a mixture of aerobic and anaerobic microbial communities within 4 days at 50 °C [31]. Hui et al. (2013) showed a 39.71% weight loss of unpretreated rice straw after 12 days by consortium XDC-2 [32]. Kumar et al. (2015) also reported that the maximum degradation of rice straw (49%) was observed within the first three days by *Pseudoxanthomonas* sp. R-28 [23]. Ma et al. (2016) showed a 46.7% reduction in the weight of unpretreated rice straw after 3 days of incubation with *Pantoea ananatis* Sd-1 [33].

Our results are consistent with earlier studies suggesting that other species may be involved in the breakdown of cellulose (Table 1). Previous research has demonstrated the efficacy of several strains in degrading cellulosic materials; however, our work demonstrates a noteworthy efficiency with untreated agricultural wastes, which is not as frequently documented. It shows that *Paenibacillus* sp. C7 may provide an affordable alternative cost-effective and energy-intensive pretreatment procedure for the degradation of biomass.

The fields of biotechnology and environmentally friendly waste management will be greatly impacted by the findings of this investigation. Producing biofuels and other useful bioproducts from lignocellulosic biomass could be more affordable and effective with the help of *Paenibacillus* sp. C7, as it can efficiently degrade untreated agricultural residues, reducing the need for costly pre-treatment processes. Its potential to reduce environmental contamination and promote a circular bioeconomy makes it an attractive choice for managing agricultural waste, especially when it comes to degrading untreated agricultural wastes.

Table 1. Comparison of degradation percentage of filter paper of various microbial consortia.

Consortium	Degradation Percentage of Filter Paper	Number of Days	Temp (°C)	Reference
MC1 (aerobic and anaerobic)	79	4	50	[31]
MC3F (aerobic and anaerobic)	54.7	7	50	[25]
H-C (aerobic and anaerobic)	81	8	40	[26]
Con R (aerobic)	83	15	37	[23]
Bac-3 (aerobic)	95	30	30	[34]
CSW (aerobic)	91	7	37	This study

It is still necessary to assess *Paenibacillus* sp. C7's long-term stability and effectiveness in large-scale applications. Future research ought to focus on improving the culture conditions and investigating whether genetic alterations could be used to increase *Paenibacillus* sp. C7's cellulolytic activity. To comprehend the fundamental mechanisms of *Paenibacillus* sp. C7's cellulose degradation, more investigation is required. Examining the particular enzymes at play and their genetic regulation may shed light on how to make this bacterial isolate more effective. Furthermore, field testing and pilot-scale investigations are necessary to evaluate the feasibility and scalability of employing *Paenibacillus* sp. C7 for waste management and biomass degradation.

5. Conclusions

Considering the reutilization of cellulosic wastes for sugar production through aerobic bacteria in the present investigation seems to be a promising approach as it is difficult to maintain strict anaerobic conditions. The microbial consortium enriched in this study could degrade 91% of filter paper at 37 °C within 7 days. The enriched consortium CSW contained an aerobic organism *Paenibacillus* sp. C7, which was able to degrade all cellulosic substrates efficiently (96%). It could also degrade 70% of untreated rice straw and 46% of wheat bran in 10 days. Utilizing *Paenibacillus* sp. C7 might mitigate the drawbacks associated with pretreating lignocellulosic materials and offer an environmentally sustainable solution. Hence, *Paenibacillus* sp. C7 can effectively address the limitation in the conversion of lignocellulose into biofuels. This finding would have significant implications for the widespread usage of agricultural wastes.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w16131800/s1: Figure S1: Isolates obtained after screening on CMC agar plates were screened by degradation of Whatman filter paper 1 (0.5%; *w*/*v*) as the sole source of carbon after 10 days. Figure S2: Regression fitting model of substrate degradation by *Paenibacillus* sp. C7 for (A) filter paper, (B) rice straw, and (C) wheat bran.

Author Contributions: R.S.Y.: Conceptualization, Methodology, Writing—original draft, Formal analysis. P.: Conceptualization, Methodology. W.H.: Visualization, Investigation, Supervision. C.L.: Software, Validation. J.M.: Validation. Y.F.: Investigation, Supervision. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Bacterial strains reported in this study have been deposited in the Gen-Bank under accession number MH128356.

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Conflicts of 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.

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