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(54) **ENZYME-TRIGGERED SELF-CULTURED
BACTERIA RETTING FOR NATURAL FIBER**

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(57) **ABSTRACT**

A process for retting biomass includes contacting an enzyme solution with the biomass, degrading at least a portion of the pectin in the biomass using the enzyme to release monomers, proliferating bacteria using the monomers as a feed-stock, and separating the cellulosic fibers after degrading at least the portion of the pectin to produce natural fibers. The enzyme solution comprises an enzyme, and the biomass comprises pectin and cellulosic fibers.

FIG. 1A

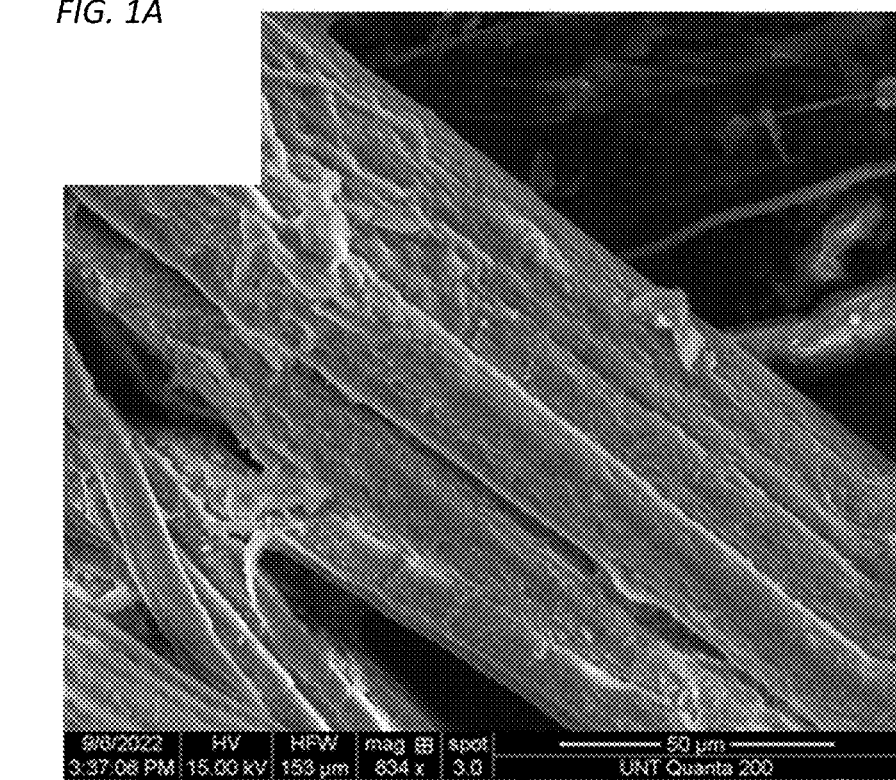


FIG. 1B

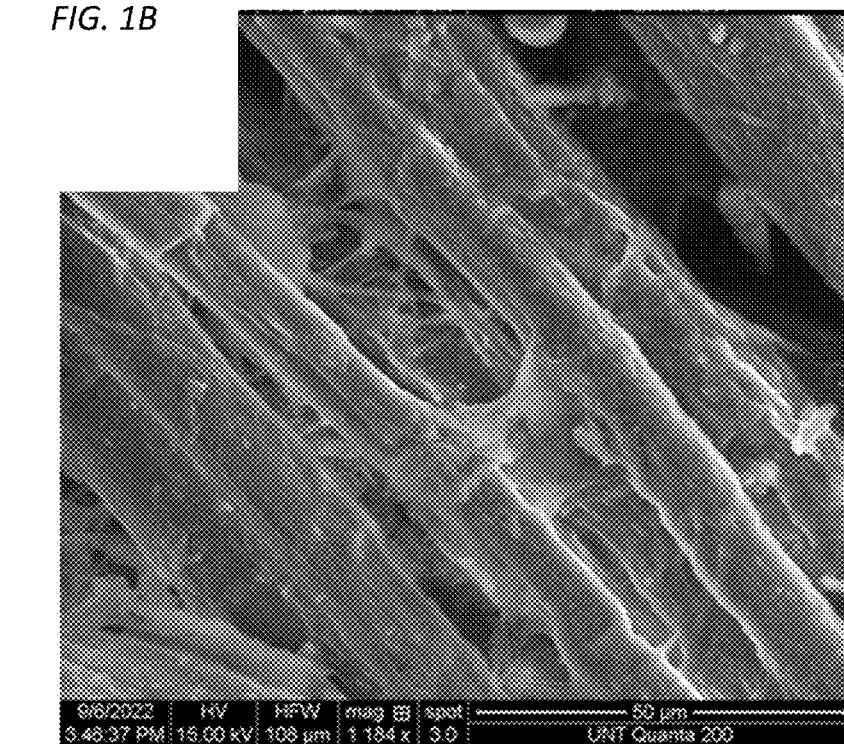


FIG. 1C

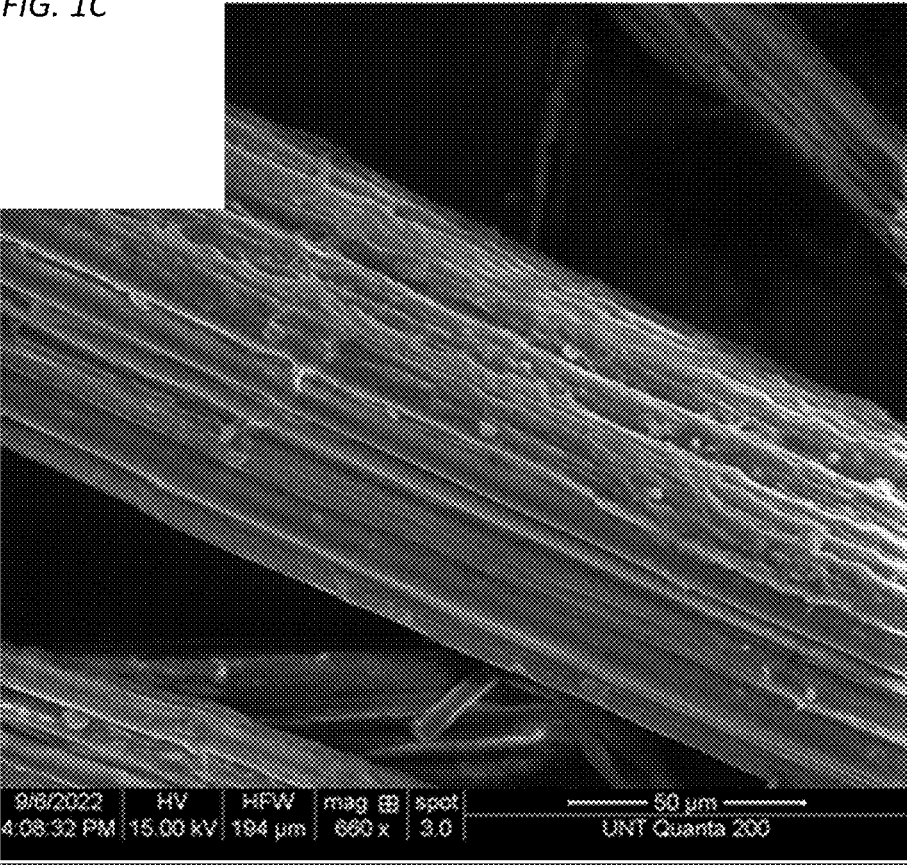
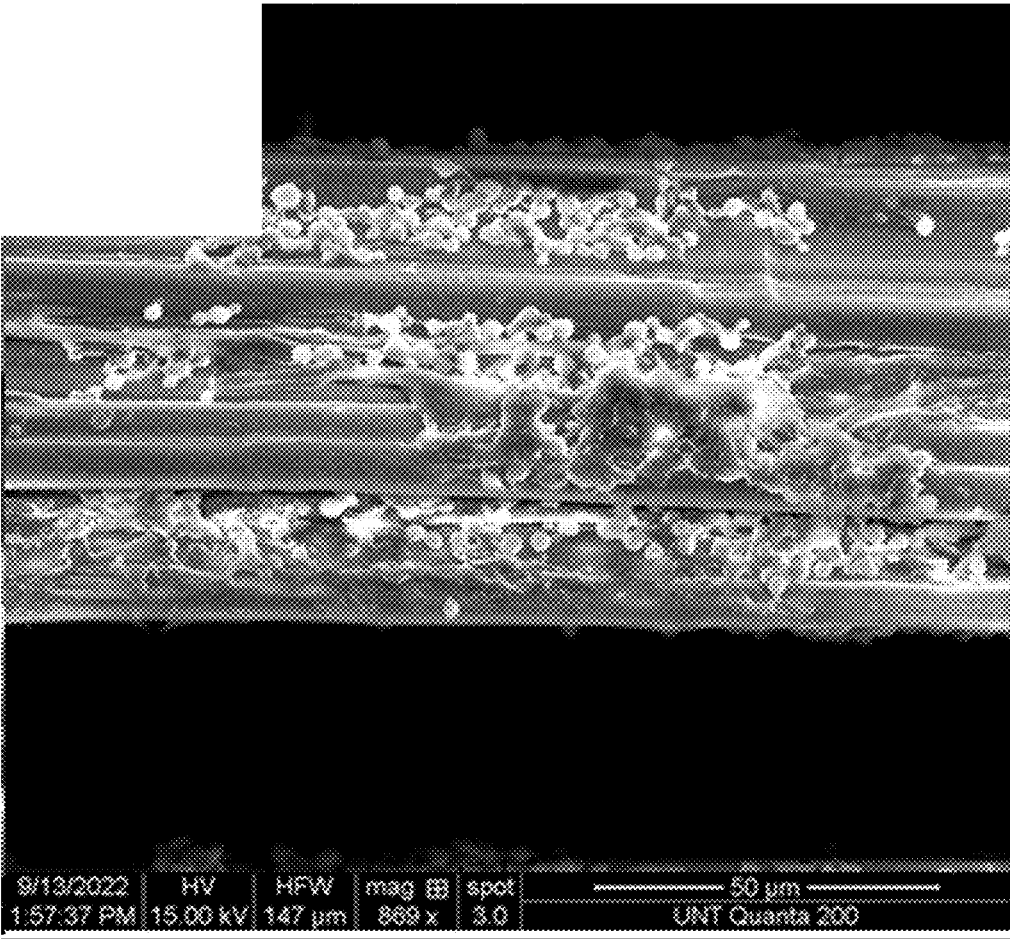


FIG. 1D



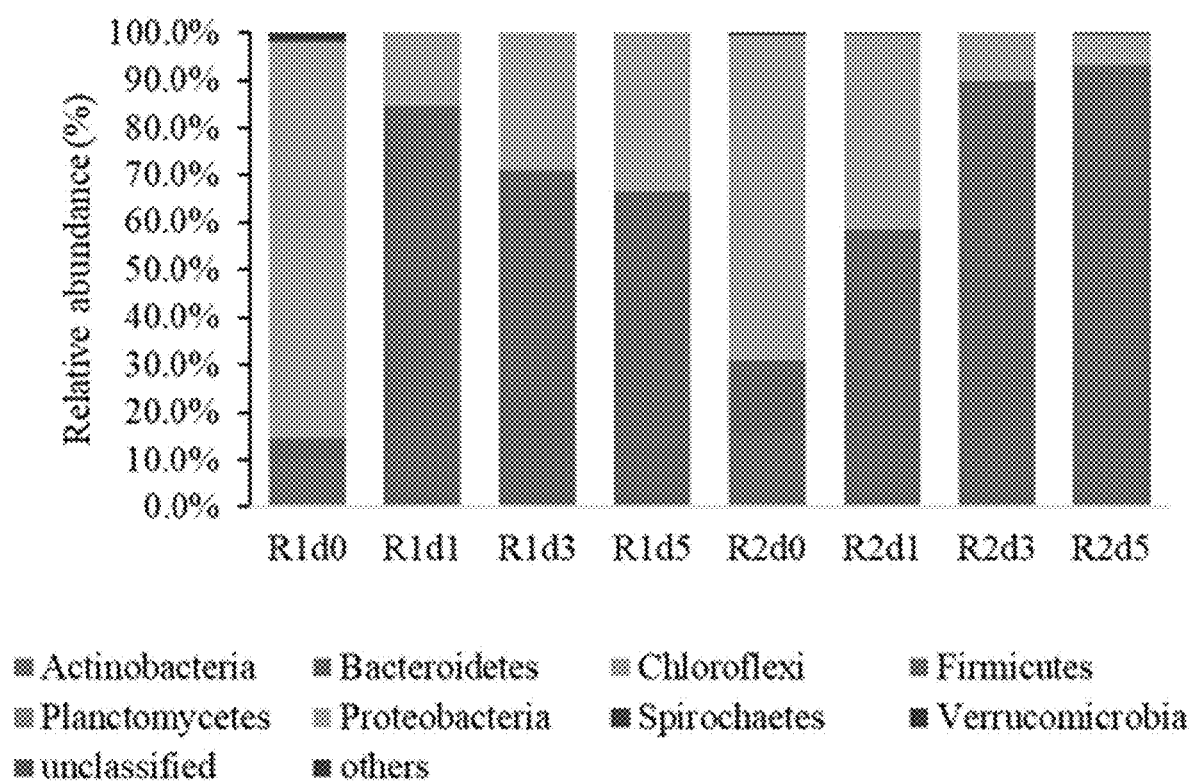


FIG. 2A

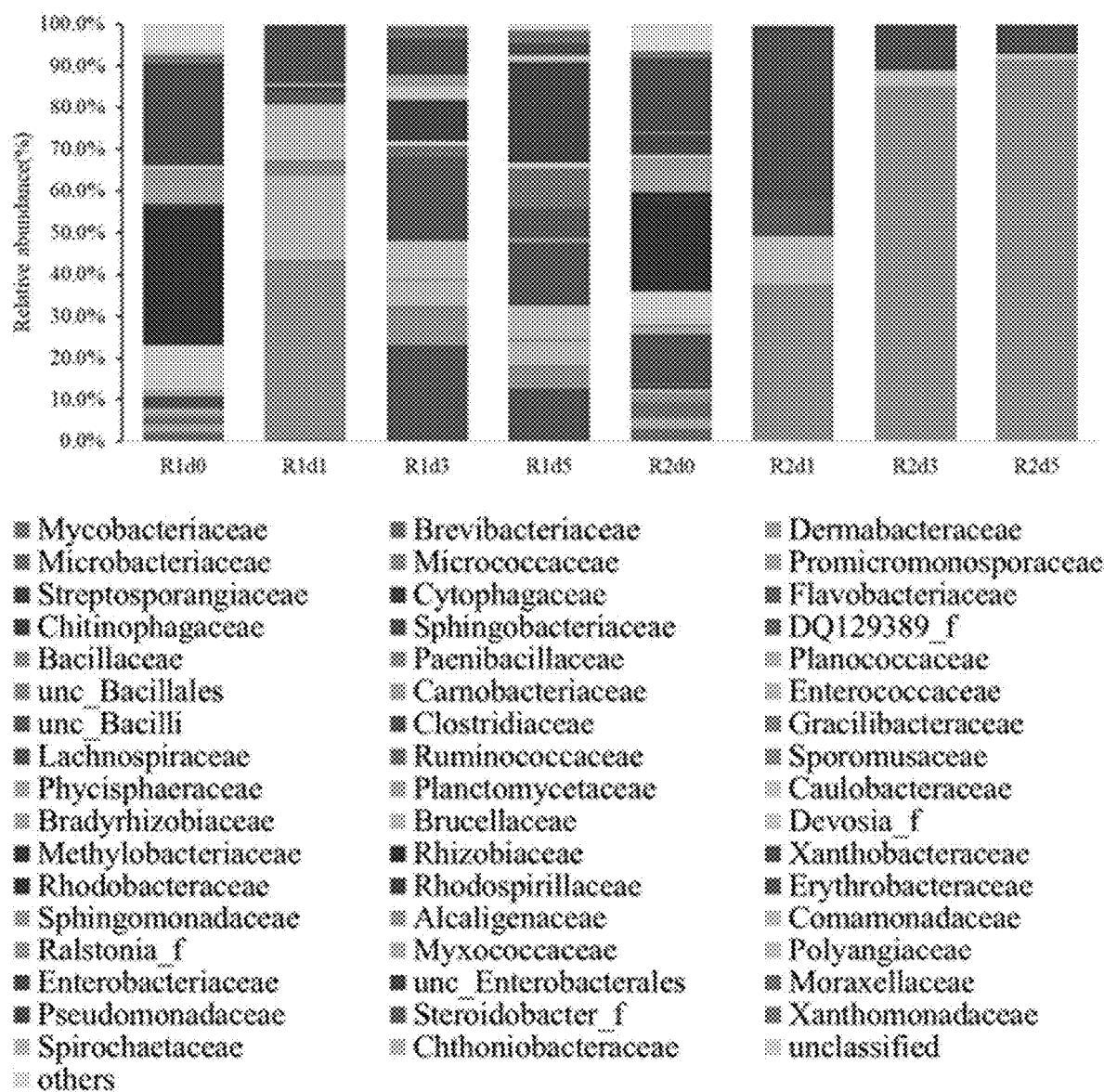


FIG. 2B

ENZYME-TRIGGERED SELF-CULTURED BACTERIA RETTING FOR NATURAL FIBER

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/525,899 filed on Jul. 10, 2023 and entitled "ENZYME-TRIGGERED SELF-CULTURED BACTERIA RETTING FOR NATURAL FIBER," the entire disclosure of which is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Grant DE-SC0022736 awarded by the Department of Energy. The government has certain rights in this invention.

BACKGROUND

[0003] Natural fiber has a growing demand in the composite industry because of its sustainability and unique properties. The global natural fibers market size is expected to grow to \$85.25 billion in 2027 at a compound annual growth rate (CAGR) of 5.0% as a result of increasing demand for sustainable, eco-friendly products from a range of industries such as textiles, construction, and automotive manufacturing.

[0004] Natural fibers, which are renewable and sustainable, have become potential alternatives to petroleum-based fibers in the field of automotive components and composites. Interest in using industrial hemp (*Cannabis sativa* L.) bast fiber is increasing in the U.S. as the 2018 Farm Bill legalized the commercial production of hemp. The USDA National Agricultural Statistics Service released its first National Hemp Report in 2022 (USDA, 2022) indicating that hemp has been explored as a potential cash crop in many states of the United States. While the cultivation of industrial hemp has increased, the potential of hemp fiber in the development of a green biomass-based economy has not been widely explored due to decades of prohibition.

SUMMARY

[0005] In some embodiments, a process for retting biomass comprises contacting an enzyme solution with the biomass, degrading at least a portion of the pectin in the biomass using the enzyme to release monomers, proliferating bacteria using the monomers as a feedstock, and separating the cellulosic fibers after degrading at least a portion of the pectin to produce natural fibers. The enzyme solution comprises an enzyme, and the biomass comprises pectin and cellulosic fibers.

[0006] In some embodiments, a process for retting biomass comprises contacting biomass with a bacterial solution, expressing an enzyme from the bacteria, degrading at least a portion of the pectin in the biomass using the enzyme to release monomers, proliferating the bacteria using the monomers as a feedstock, and separating the cellulosic fibers after degrading at least a portion of the pectin to produce natural fibers. The biomass comprises pectin and cellulosic fibers, and the bacterial solution comprises at least one culture of bacteria.

[0007] These and other features will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] For a more complete understanding of the present disclosure and the advantages thereof, reference is now made to the following brief description, taken in connection with the accompanying drawings and detailed description, wherein like reference numerals represent like parts.

[0009] FIGS. 1A-1D illustrate SEM micrographs of hemp fiber sampled from day 1 (FIG. 1A, FIG. 1C) and day 5 (FIG. 1B, FIG. 1D) of water retting and enzyme-triggered self-cultured bacteria retting, where a and b from water retting, c and d from the bacterial retting. Fibers were wrapped by gummy substance (FIG. 1A) and water retting did not remove the gummy substance (FIG. 1B). The gummy substance was removed partially (FIG. 1C) at the beginning of the bacterial retting. Bacteria colonized and adhered on the fiber surface after 5 days of the bacterial retting (FIG. 1D).

[0010] FIGS. 2A-2B illustrates the relative abundance of the phylum-level (FIG. 2A) and family-level (FIG. 2B) bacterial community composition during retting. Only bacterial phyla with relative abundance >1% and bacterial family with relative abundance >5% are shown.

DETAILED DESCRIPTION

[0011] It should be understood at the outset that although an illustrative implementation of one or more embodiments are provided below, the disclosed systems and/or methods may be implemented using any number of techniques, whether currently known or in existence. The disclosure should in no way be limited to the illustrative implementations, drawings, and techniques below, including the exemplary designs and implementations illustrated and described herein, but may be modified within the scope of the appended claims along with their full scope of equivalents.

[0012] The conversion process from biomass to natural fiber is called retting. Retting is an important process for fiber extraction. The fiber quality is highly related to the retting process used. The main components of hemp bast are cellulose (53-91%), hemicellulose (4-18%), lignin (1-21%), and pectin (1-17%). The retting process loosens the cellulosic fiber and fiber bundles from pectin and other cementing compounds such as hemicellulose and lignin, thus separating the fibers from the non-cellulosic material. It has been a challenge to efficiently produce long and high-quality fibers.

[0013] Obtaining natural fibers from biomass usually requires using chemicals and/or high heat. Retting methods can include chemical retting, thermal mechanical retting, and water retting. Chemical retting uses various chemicals in water to loosen the fibers, which generates a significant amount of wastewater and is not environmentally friendly. Thermal mechanical retting uses high heat combined with mechanical processes, which consumes a significant amount of energy. For the water retting method, traditionally, it is time-consuming (usually several weeks) and often leads to inconsistent fiber quality.

[0014] Biological retting methods include enzymatic retting, bacterial retting, and fungal retting. Enzymatic retting of flax fibers with the multi-enzyme Viscozyme L. and chelator ethylenediaminetetraacetic acid (EDTA) can be

performed. The flax fibers can be efficiently retted without destruction of the cellulosic fiber. Fungal retting (e.g., using white rot fungi from the group Basidiomycotina) in sealed plastic bags can also remove non-cellulosic compounds efficiently within 2 weeks. The retting process can be shortened to 3-4 days by culturing selected pectinolytic bacterial strains *Clostridium* sp. L1/6 and *Bacillus* sp. ROO40B and inoculating these strains into the water retting tank. Over the past few decades, research on microbial communities involved in retting has led to an increasing interest in the more environmentally friendly bacterial retting process.

[0015] The contribution of bacteria in the retting process can also be explored. The bacteria *Bacillus cereus* HDYM-02 can significantly change bacterial successions during flax retting and accelerated the process compared to natural retting. The cellulase-free crude enzyme produced by *Bacillus cereus* HDYM-02 contained high pectinase and mannanase activity, which act synergistically in the retting of flax. The pectinolytic bacteria can reduce the jute retting period and improved fiber quality. Studies have been conducted on the isolation of bacteria from retting solutions and the inoculation of specific bacteria into the retting process. Several bacterial species such as *Bacillus* spp., *Cellulomonas* spp., *Pseudomonas fluorescens*, and *Microbacterium* sp. can accelerate the natural fiber retting process.

[0016] Studies have been conducted on the isolation of bacteria from retting solutions and the inoculation of specific bacteria into the retting process. It has been found that *Bacillus macerans*, the most common bacterium, took 4-10 days to ret jute at 37° C. Another bacterium, *Pseudomonas aeruginosa*, took 4-8 days for retting. *Pseudomonas* species were found to be particularly important in the pectin degradation of plant fiber. It has also been found that the anaerobic *Clostridium felsineum* bacterial strains reduced the duration of the water retting process from 12 to 6 days at a temperature of 20° C. Samples in water containing the bacterial strain also had higher fiber modulus than those retted in plain water. The bacteria retting process is greatly influenced by the temperature, and it has been concluded that the temperature for bacterial growth can be between 25 to 35° C. for most bacteria species, which also helps to maintain fiber quality. Inoculation with both aerobic and anaerobic pectinolytic bacteria can shortened the retting time. An aerobic pectinolytic bacteria strain (*Bacillus* sp. ROO40B) and an anaerobic pectinolytic bacteria strain (*Clostridium* sp. L1/6) can be introduced into the water-retting process of hemp stems. It was found that ROO40B did not show cellulolytic activity. This was an important implication for strain selection since the cellulolytic activity was found in all anaerobic pectinolytic strains so far. At a temperature of 35° C., the inoculum sped up the retting process, with the best fiber quality obtained after 3-4 days. The fiber quality, for example as measured by easy scutching, bright color, retting degree, cleanliness, homogeneity, fitness, etc., can be significantly improved as well. It was also found that excessive bacterial proliferation and/or excessive retting time can alter and weaken the structure of the fiber wall. Therefore, a proper retting time is an important variable in the process of achieving good quality fiber by bacteria retting.

[0017] During bacterial retting, the relative abundance of different bacterial populations changes. Several studies of microbial succession during the retting process demon-

strated that microbial communities change depending on the retting conditions. The bacterial succession of water retting begins with the proliferation of aerobic bacteria (e.g., often *Bacillus* spp.), and as oxygen is gradually consumed, anaerobic bacteria (e.g., *Clostridium* spp.) begin to multiply. Incubation of additional pectinolytic bacteria such as *Bacillus cereus* HDYM-02 also changed the bacterial succession during retting. Higher enzyme activity in the bacteria-inoculated retting process can be observed, as evidenced by the increase in galacturonic acid and reducing sugars in the bacterial retting liquid and a decrease in fiber-associated gum compared with regular water retting. It has also been shown that the addition of specific enzymes can accelerate the release of fiber and can shorten the retting time to 24 hours. Some enzymes such as pectinase can degrade the non-cellulosic polymer pectin in hemp fibers. Hemp fiber tensile strength after 2, 5, 24, and 48 hours of pectinase retting at 25° C. and 50° C. has been compared, and the maximum tensile strength of hemp fiber was obtained after 48 hours of retting at 25° C. with a 4% pectinase water solution.

[0018] Isolation and purification of enzymes is an expensive process; therefore, the cost of enzymatic retting alone is high. It is not economically friendly to apply enzymes to each batch of retting, especially for large-scale industrial applications. Natural fibers inevitably carry bacteria from the natural environment during the planting and harvesting process. Bacteria can use those carbon-based polymers as their food source. Additionally, retting conditions suitable for enzymatic action are sufficient for bacteria to proliferate. However, little is known about bacterial succession after a small amount of enzyme application during retting.

[0019] Disclosed herein is a process for retting that uses enzymes along with bacterial retting. Specifically, bacterial communities associated with the retting of natural fibers (e.g., hemp bast fibers, flax fibers, etc.) can be used along with the addition of pectinase enzyme. In some aspects, the addition of residue from long-term pectinase retted natural fiber core can also be used. By performing microbial community analysis and bioinformatics analysis, a significant effect of using small amounts of pectinase on bacterial community succession under retting conditions can be observed. This leads to the possibility of enriching a portion of the bacteria with a small amount of enzymes (e.g., pectinase) and the potential that these in situ generated bacteria could be used to continue retting fibers.

[0020] Disclosed herein in some aspects is a method involving the use of enzymes to trigger the aggregation of bacteria in biomass. The aggregated bacteria can be used for the retting of the biomass to obtain the lignocellulosic natural fiber.

[0021] In some aspects, a disclosed bacteria retting method for separating natural fibers from biomass can comprise a number of steps. Initially, the biomass can comprise any suitable biomass that provides fibers. In general, the biomass can contain the fibers along with other organic components such as pectin, hemicellulose, and lignin. Various types of biomass containing natural fibers can include, but are not limited to, wood, bamboo, flax, jute, hemp, ramie, or any combination thereof.

[0022] The biomass can be optionally pre-processed to aid in the retting process. For example, the biomass can be dried, milled, chopped, or otherwise processed prior to using the biomass in the retting process. In some aspects, the

biomass preparation process can comprise cutting or chopping the biomass into a suitable size for the expected fibers. Since the fibers tend to run parallel to the length of the biomass stalks or bast, cutting the biomass can determine the maximum fiber size while also making the resulting fibers relatively uniform in length. The length of the biomass used in the process can be based on the final fiber use.

[0023] In a first step, the biomass can be contacted with an enzyme solution. The enzyme solution can comprise an enzyme, and the biomass can comprise pectin and cellulose fibers. The enzyme solution can result in the pectin being broken down using various mechanisms to trigger the bacterial aggregation on the fibers. The enzyme can be pectinase that breaks pectin through different mechanism such as protopectinase, polygalacturonase, pectin lyase, and pectin-esterase. The duration of this period can vary depending on the temperature and enzymes. The purpose of this processing is to release part of small monomers through the enzyme catalyzed reaction while proliferating bacteria that can use these small monomers as carbon sources.

[0024] Within this initial step, the enzyme solution can be in a suitable carrier such as an aqueous fluid. The concentration of the enzyme(s) in the solution can be between about 0.05% w/v to about 2% w/v, or alternatively between about 0.1% w/v to about 1% w/v. In some aspects, the enzyme can comprise pectinase produced by any suitable biological agent. For example, pectinase can be produced and obtained (e.g., expressed, etc.) from a suitable fungus such as *Aspergillus niger*. The enzymatic process can be carried out with a temperature at about room temperature or at elevated temperatures (e.g., from about 20° C. to about 55° C.) using the enzyme solution. The temperature of the process can be based on the allowable temperature for the enzyme while balancing costs associated with heating the biomass in the solution.

[0025] The enzymatic step can be carried out between about 0.1 to about 10 days, between about 1 to about 5 days, or between about 2 to about 4 days. The enzyme solution can degrade the pectin of natural fibers as a trigger agent for bacterial proliferation. In some aspects, the enzyme can degrade at least a portion of the biomass to release monomers that the bacteria can use. As an example, a laboratory-scale bacterial aggregation can proliferate Bacillaceae to a relative abundance greater than 80% with 200 mL of 1% pectinase (e.g., from *Aspergillus niger*) solution and 5 g of hemp bast fiber within 3 days at 40° C.

[0026] In a second step, bacterial fiber retting with bacteria stock solution can be carried out. In some aspects, the bacterial fiber retting can use bacteria stock solution. In some aspects, the bacterial retting can be carried out under controlled pH and temperature conditions to ensure consistent degradation of the pectin. The retting conditions including pH, temperature, and oxygen level, can be controlled to maintain the activity of proliferated bacteria. This procedure uses enzymes secreted by bacteria proliferated in first step to break down the pectin of the fiber bundle. The retting time can be varied from 2-4 days until the fiber bundles are loose and easy to be separated by hand. When a bacterial stock solution is used, it can be added to the biomass after the initial enzymatic step.

[0027] In some aspects, the stock solution with bacteria can be reused by proper control such as maintaining acidity using a proper buffer. For example, the bacteria solution can be reused up to four times in the pH range of 4-5 with the

pectinase as a trigger. The indicator of reusability of bacterial solutions can be pH, relative abundance of certain bacteria, or enzyme activity. When a buffer is used, any suitable buffer can be added to the solution. Any suitable bacteria can be used. In some aspects, the bacteria comprises Bacillaceae, and in some aspects, can be *Bacillus cereus* HDYM-02.

[0028] The use of the enzymatic step can affect the bacterial growth progression during the retting process. Due to the use of natural fibers in the biomass, a variety of bacteria can be present in the biomass at the start of the process. Various bacteria can be present initially such as Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes. The relative abundance of some of the initial bacteria such as Actinobacteria can diminish while others such as Firmicutes and Bacillaceae can have the greatest relative abundance at the later stages of retting when the enzyme step is performed.

[0029] While described as occurring in separate steps, in some aspects, the steps can overlap. For example, the enzyme can be added to the biomass and the bacterial solution can be introduced prior to a breakdown of the biomass with the enzymes. The bacteria may produce or express enzymes that can further break down the biomass. In some aspects, the enzymes produced by the bacteria can be the same as the enzyme(s) used in the first step. In this aspect, the initial introduction of the enzyme(s) can aid in starting the bacterial growth process.

[0030] In some aspects, the enzymatic step (e.g., the initial step) may be omitted and the use of a proper bacterial strain or strains can be used to allow the bacteria to express the enzymes in-situ. This process can allow the same or a different bacterial strain to proliferate to perform bacterial retting of the fibers in the biomass.

[0031] In a third step, separation of the retted fiber through mechanical separation processes such as beating, tapping, or stirring can be carried out. Any other separation means can also be used. This process can be completed at room temperature. The purpose of this procedure is to further separate fibers suspended in the aqueous solution.

[0032] The method provides several advantages over traditional methods of retting. The use of enzymes allows for the efficient aggregation of bacteria, which can further degrade pectin in biomass. The bacterial stock solution can also be reused because the bacteria produced can further secrete enzymes to ensure consistent and efficient degradation of pectin. In addition, the method is environmentally friendly and cost effective, and it does not require the use of toxic chemicals.

Examples

[0033] The disclosure having been generally described, the following examples are given as particular embodiments of the disclosure and to demonstrate the practice and advantages thereof. It is understood that the examples are given by way of illustration and are not intended to limit the specification or the claims in any manner.

[0034] Industrial hemp (*Cannabis sativa* L.) from Canada was obtained through EcoEnvision, LLC (Frisco, Texas, USA). Pectinase (contains pectinase from *Aspergillus niger* and maltodextrin as the carrier) was purchased from LD Carlson. This product is used in commercial winemaking with the advantages of low prices and easy accessibility.

[0035] Hemp fiber was cut into ¼-inch (0.635 cm) lengths and retted in a 40° C. water bath using four different retting schedules (R1-R4) as shown in Table 1. Pectinases from *Aspergillus niger* have activity over a wide range of temperatures (from 20° C.-70° C.) (Sandri & Silveira, 2018).

TABLE 1

Retting schedules		Sample labeling			
Sched- ule	Description of retting process	Day 0	Day 1	Day 3	Day 5
R1	Hemp fiber (5 g) was retted with tap water (200 mL) only	R1d0	R1d1	R1d3	R1d5
R2	Hemp bast fiber (5 g) was retted with 1% pectinase in tap water (200 mL)	R2d0	R2d1	R2d3	R2d5
R3	Hemp bast fiber (5 g) was retted with 1% pectinase in tap water (200 mL) and 1 g solid residues from hemp core retting (with cultured bacteria)	R3d0	R3d1	R3d3	R3d5
R4	Hemp bast fiber (5 g) was retted with tap water (200 mL) and 1 g of solid residues from hemp core retting (with cultured bacteria)	R4d0	R4d1	R4d3	R4d5

[0036] The optimum pectinase activity is at 40° C. (Ajayi et al., 2021) or 50° C. (Jalil et al., 2021). The reason for choosing 40° C. here was to preserve the greater activity of pectinase while saving energy. Solid residues (with bacteria cultured) from hemp core retting were prepared by retting hemp core with 1% pectinase enzyme at ambient laboratory temperature for two months. The retting solution was collected at beginning of each retting schedule (day 0) and on the 1st, 3rd, and 5th day during retting and stored at -20° C. for bacterial sequencing analysis (R1d0-R4d5, Table 1). The pH in the retting liquid was monitored using a Mettler Toledo pH meter (SevenCompact™ S210). Micromorphological fiber analysis was conducted on fibers collected randomly from day 1 and day 5 of four retting schedules. The samples were dried and mounted to the aluminum stub using conductive adhesive and observed under FEI Quanta 200 Environmental Scanning Electron Microscope (ESEM) with spot size of 3.0 and accelerating voltage of 15 kV.

Microbial Community Analysis by Illumina MiSeq Sequencing

[0037] Microbial cells from 3 mL of the retting solution were harvested by centrifugation for 5 min at 10,000×g. Genomic DNA was recovered from the pellet using the DNeasy PowerSoil Pro Kit and the automated QIAcube Connect robot (Qiagen, Carlsbad CA). 16S rRNA gene was amplified using universal bacterial primers targeting the V4 hypervariable region (Apprill et al., 2015, Parada et al., 2016). The PCR reaction contained: DNA template (~10-100 ng), 0.5 µl of each primer (10 µM), 2.5 µl 10× AccuPrime PCR Buffer II, 2.5 µL BSA (1.6 mg/µL), 1.5 µl MgCl (50 mM), 0.1 µl AccuPrime Taq High Fidelity (5 U/µl), and PCR grade water to a final volume of 25 µl. PCR amplification was carried out as follows: denaturization at 94° C. for 2 min, 25 cycles of 94° C. for 30 s, 52° C. for 30 s, 68° C. for 40 s, then 68° C. for 5 min and held at 4° C. PCR products were viewed using gel electrophoresis (1.5% agarose). Successful PCR amplification products were

cleaned using AMPure XP (Beckman Coulter, Chaska, MN) magnetic bead-based purification. After cleanup, the PCR products were indexed using Illumina Nextera XT Index Kit v2 (Illumina, San Diego, CA) following the manufacturer's instructions and purified again using AMPure XP magnetic beads. Each 50 µl index PCR reaction contained: 5 µl 10× AccuPrime PCR Buffer II, 5 µl Nextera XT indexing primers 1, 5 µl Nextera XT indexing primers 2, 0.2 µl AccuPrime Taq High Fidelity (5 U/µl), 5 µl purified DNA, and PCR grade water. The PCR recipe was as follows: denaturation at 94° C. for 3 min, 8 cycles of 94° C. for 30 s, 55° C. for 30 s, 68° C. for 30 s, then 68° C. for 5 min and held at 4° C. PCR products were quantified using the Qubit dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA), and pooled in equimolar amounts. The pooled sample was then denatured, diluted, loaded, and sequenced using MiSeq Reagent V2 (500 cycles) kit (Illumina Part #15044223, Rev B.).

Bioinformatics Analysis

[0038] Sequences generated from the MiSeq were processed through the Mothur v.1.36.1 pipeline (Schloss et al., 2009). Paired-end sequences were assembled, primers and barcodes were removed, and short sequences (<100 bp) and low-quality sequences (homopolymers >8) were excluded from the dataset. Sequence alignments were performed using reference sequences from the SILVA database (Glockner et al., 2017). The sequences that could not be aligned were removed and the aligned sequences were further filtered to remove gaps. Redundant sequences were reduced using the Unique.seqs command and a precluster (diffs=2) algorithm, and chimeras were removed after identification using UCHIME (Edgar et al., 2011). Taxonomic classification was conducted using the Ribosomal Database Project (RDP) classifier with a minimum confidence of 80% (Wang et al., 2007), and the up-to-date curated EzBiocloud database as a reference (Yoon et al., 2017). Sequences classified as mitochondria, chloroplast, archaea, and eukaryote, as well as unknown sequences, were removed from the dataset. Principle coordinate analysis (PCoA) based on UniFrac distances was used to investigate differences in the microbial community among different retting schedules (Lozupone & Knight, 2005).

Results

[0039] In water retting (schedule R1; see Table 1), the fibers were covered with gummy substances at the beginning of the retting (FIG. 1a) and on the fifth day of the retting (FIG. 1b). The gummy substance such as pectin were not completely broken down or degraded in the short period of time under water retting condition. By contrast, the fibers were relatively clean under retting schedule R2 (FIGS. 1c and 1d) and R3. Both schedules included added pectinase, which degrades pectin around the fiber bundles; however, the gummy substances were still visible on part of the fiber bundle surface. After five days of retting, bacteria accumulated on the surface of fiber bundles. Accumulated bacteria started to degrade the remaining gummy substances. At the beginning of the retting schedule R4, the fiber bundles were still surrounded by gummy substances, because pectinase was not added. Bacterial proliferation on the fiber surface was not observed under ESEM.

[0040] A total of 2,010,228 sequencing reads were generated (125,639±6,270 average per sample). Bacterial relative

abundance changes were observed at the phylum level (FIG. 2a). A total of 17 bacterial phyla were detected. Five bacterial phyla (Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Spirochaetes) were able to be classified to family level (greater than 1% in the samples). Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes were the top 4 bacterial phyla in all retting conditions, among which Proteobacteria and Firmicutes accounted for $93.13\% \pm 0.093\%$ of bacteria. Actinobacteria were present in hemp and all the initial stages of hemp retting methods; however, their abundance diminished during the retting process. Pectinase altered the succession of bacterial communities. In the pectinase-treated retting process, the relative abundance of Firmicutes in schedule R2 increased at later stages of retting (day 3 and 5) from 2.5% to 89.8% and 93.1%. Difference between pectinase-treated retting process and non-pectinase-treated retting process was more obvious at the family level, where Rhodospirillaceae (23.7%), Clostridiaceae (14.7%), Sphingobacteriaceae (12.5%), and Ruminococcaceae (9.6%) were the most abundance families present in the late stage of non-pectinase-treated retting R1d5, and Bacillaceae (91.4%) were the most abundance family that appeared in the late stage of pectinase-treated retting R2d5.

[0041] Ribeiro et al. (2015) previously studied the microbial diversity changes during hemp field retting. It was observed that Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes were abundant at the phylum level, where Proteobacteria was the most abundant phylum. Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes have also appeared in the phyla level of the dataset during simulated six weeks of field hemp retting in the research completed by Law et al. (2020). Results of their research also confirmed that microorganisms were already “on-site” within or on the hemp prior to retting and continued to be present throughout the field retting process, which did not show large shifts in population over retting time. This example identified the “on-site” bacterial phyla on hemp before retting. However, the bacterial communities were altered markedly in the presence of pectinase. Proteobacteria were present at relatively high abundance (greater than 50%) at the beginning of the retting, but their relative abundance tended to decrease during the R2, R3, and R4 retting processes. This was not the case for the water retting R1. The relative abundance of Firmicutes increased with retting time in the R2 and R3 retting schedules with added pectinase. The Bacteroidetes phylum was present at the beginning of each retting method. This bacterial phylum tended to proliferate from the third day of water retting (R1d3) and had a significant abundance on the fifth day of retting schedule R4 (R4d5). Bacteroidetes are known to be anaerobic (Wexler, 2007), and anaerobic bacteria are associated with a foul odor during water retting (Bajpai, 2014). A significant abundance (greater than 5%) of Actinobacteria in hemp and the initial stages of all retting processes (R1d0, R2d0, R3d0, R4d0) was also detected. However, their relative abundance dropped to less than 0.2% during the rest of the retting process, possibly due to the shift towards anoxic conditions. Evaluation of the hemp microbiome done by Barnett, et al. (2020) confirmed that Actinobacteria were in higher relative abundance in the soil, rhizosphere, and root tissue of hemp. These bacterial phyla (Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes) have similarly been found in a variety of natural fiber retting conditions including flax water retting (Djemiel, et al., 2017; Djemiel, et al., 2020),

Kenaf water retting (Visi, et al., 2013), and jute retting (Munshi, et al., 2008; Das, et al., 2014).

[0042] The relative abundance of family-level bacterial communities during retting (FIG. 2b) revealed more detailed bacterial compositions. It was noted that the relative abundance of Bacillaceae during retting with pectinase (schedule R2) increased with the duration of retting, from 0.3% at the beginning of the retting process increasing to 84.9% and 91.4% on the third and fifth days, respectively. Bacillaceae were not able to be further classified to the genus level. The value of members of the Bacillaceae family in the field of ecological function include involvement in the degradation of soil organic matter, nitrogen cycle, and phosphate cycle (Mandic-Mulec et al., 2016). Research completed by Zhao, et al. (2016) showed that flax retting with the addition of Bacillaceae member *Bacillus cereus* HDYM-02 as inoculum altered bacterial succession significantly and effectively accelerated the retting process. They also speculated that the presence of Paenibacillaceae in flax retting inoculated with *B. cereus* HDYM-02 further contributed to degradation of the gummy pectic substances during the retting process. Paenibacillaceae were the dominant microorganisms in the retting residues of hemp cores. The relative abundance of Paenibacillaceae in the retted hemp core was 68.9%, possibly explaining why Paenibacillaceae dominated the bacterial populations in the retting schedule R4, which contained the bacterial inoculum from retted hemp core. Paenibacillaceae can be classified into the genera *Brevibacillus*, *Paenibacillus*, and *Cohnella*. *Brevibacillus* was identified as the dominant genus in R4. Members of *Brevibacillus* have been shown to produce pectin lyase (Demir, et al., 2014), which suggests these bacteria may be contributing pectinase enzymes to the retting system.

[0043] Retting typically begins as an aerobic process. Aerobes or facultative anaerobes such as *Bacillus* and *Paenibacillus* predominated the initial bacterial communities (Tamburini, et al., 2003). Firmicutes, particularly members of the genus *Clostridium*, was reported to be the dominant phylum during the water retting of flax fibers, while the aerobic environment of dew-retting of flax fibers was low in *Clostridium* (Djemiel, et al., 2017). The more anaerobic environment of water-retting than dew-retting was thought to be the primary reason for the difference in *Clostridium* abundance (Donaghy, et al., 1990; Tamburini, et al., 2003), as *Clostridium* species are obligately anaerobic (Bowman, 2011). Clostridiaceae (including the genus *Clostridium*) was also found in the water retting (FIG. 2b, R1d3 and R1d5). On the third and fifth days of retting schedules R2 and R3, the most abundant bacterial communities belonged to the family Bacillaceae. Bacillaceae include facultative anaerobes that can produce acetate from organic matter through anaerobic digestion (Park, et al., 2015).

[0044] This example explored the succession of bacteria in the presence of a small amount of pectinase during the hemp retting process and the possibility of using retting residues of hemp core as a bacterial starter inoculum. The results indicated that a small amount of pectinase had a significant effect on the abundance of Bacillaceae. The addition of pectinase resulted in an acidic retting environment that likely enriched for Bacillaceae during the retting process within three days. After certain pectinase-producing bacteria dominated the communities in the retting system, it may be possible to recycle the retting solution and accelerate the treatment of subsequent batches of bast fibers.

[0045] Having described various compositions, systems, and processes for retting, certain aspect can include, but are not limited to:

[0046] In a first aspect, a process for retting biomass comprises: contacting an enzyme solution with the biomass, wherein the enzyme solution comprises an enzyme, and wherein the biomass comprises pectin and cellulosic fibers; degrading at least a portion of the pectin in the biomass using the enzyme to release monomers; proliferating bacteria using the monomers as a feedstock; and separating the cellulosic fibers after degrading at least the portion of the pectin to produce natural fibers.

[0047] A second aspect can include the process of the first aspect, wherein the enzyme in the enzyme solution has a concentration between 0.05-2% w/v.

[0048] A third aspect can include the process of the first or second aspect, wherein the enzyme comprises pectinase.

[0049] A fourth aspect can include the process of any one of the first to third aspects, wherein the enzyme breaks down the pectin through a mechanism including at least one of protopectinase, polygalacturonase, pectin lyase, and pectinesterase, or any combination thereof.

[0050] A fifth aspect can include the process of any one of the first to fourth aspects, wherein the biomass comprises wood, bamboo, flax, jute, hemp, ramie, or any combination thereof.

[0051] A sixth aspect can include the process of any one of the first to fifth aspects, wherein the bacteria comprises Bacillaceae.

[0052] A seventh aspect can include the process of any one of the first to sixth aspects, further comprising: expressing the enzyme from *Aspergillus niger*.

[0053] An eighth aspect can include the process of any one of the first to seventh aspects, further comprising: expressing a second enzyme from the bacteria; and breaking down the biomass using the second enzyme.

[0054] A ninth aspect can include the process of the eighth aspect, wherein the enzyme and the second enzyme are the same.

[0055] A tenth aspect can include the process of any one of the first to ninth aspects, wherein proliferating the bacteria occurs at a pH between 4-5.

[0056] An eleventh aspect can include the process of any one of the first to tenth aspects, wherein separating the cellulosic fibers comprises mechanically separating the cellulosic fibers.

[0057] A twelfth aspect can include the process of any one of the first to eleventh aspects, further comprising: collecting a retting solution after proliferating the bacteria; and reusing the retting solution in a subsequent retting process.

[0058] In a thirteenth aspect, a process for retting biomass comprises: contacting biomass with a bacterial solution, wherein the biomass comprises pectin and cellulosic fibers, wherein the bacterial solution comprises at least one culture of bacteria; expressing an enzyme from the bacteria; degrading at least a portion of the pectin in the biomass using the enzyme to release monomers; proliferating the bacteria using the monomers as a feedstock; and separating the cellulosic fibers after degrading at least the portion of the pectin to produce natural fibers.

[0059] A fourteenth aspect can include the process of the thirteenth aspect, wherein the enzyme comprises pectinase.

[0060] A fifteenth aspect can include the process of the thirteenth or fourteenth aspect, wherein the enzyme breaks

down the pectin through a mechanism including at least one of protopectinase, polygalacturonase, pectin lyase, and pectinesterase, or any combination thereof.

[0061] A sixteenth aspect can include the process of any one of the thirteenth to fifteenth aspects, wherein the biomass comprises wood, bamboo, flax, jute, hemp, ramie, or any combination thereof.

[0062] A seventeenth aspect can include the process of any one of the thirteenth to sixteenth aspects, wherein the bacteria comprises Bacillaceae.

[0063] An eighteenth aspect can include the process of any one of the thirteenth to seventeenth aspects, wherein proliferating the bacteria occurs at a pH between 4-5.

[0064] A nineteenth aspect can include the process of any one of the thirteenth to eighteenth aspects, wherein separating the cellulosic fibers comprises mechanically separating the cellulosic fibers.

[0065] A twentieth aspect can include the process of any one of the thirteenth to nineteenth aspects, further comprising: collecting a retting solution after proliferating the bacteria; and reusing the retting solution in a subsequent retting process.

[0066] For purposes of the disclosure herein, the term “comprising” includes “consisting” or “consisting essentially of.” Further, for purposes of the disclosure herein, the term “including” includes “comprising,” “consisting,” or “consisting essentially of.”

[0067] Accordingly, the scope of protection is not limited by the description set out above but is only limited by the claims which follow, that scope including all equivalents of the subject matter of the claims. Each and every claim is incorporated into the specification as an embodiment of the present invention. Thus, the claims are a further description and are an addition to the embodiments of the present invention. The discussion of a reference in the Description of Related Art is not an admission that it is prior art to the present invention, especially any reference that may have a publication date after the priority date of this application. The disclosures of all patents, patent applications, and publications cited herein are hereby incorporated by reference, to the extent that they provide exemplary, procedural or other details supplementary to those set forth herein.

[0068] While embodiments of the invention have been shown and described, modifications thereof can be made by one skilled in the art without departing from the spirit and teachings of the invention. The embodiments described herein are exemplary only, and are not intended to be limiting. Many variations and modifications of the invention disclosed herein are possible and are within the scope of the invention. Where numerical ranges or limitations are expressly stated, such express ranges or limitations should be understood to include iterative ranges or limitations of like magnitude falling within the expressly stated ranges or limitations (e.g., from about 1 to about 10 includes, 2, 3, 4, etc.; greater than 0.10 includes 0.11, 0.12, 0.13, etc.). For example, whenever a numerical range with a lower limit, R_L , and an upper limit, R_U , is disclosed, any number falling within the range is specifically disclosed. In particular, the following numbers within the range are specifically disclosed: $R = R_L + k * (R_U - R_L)$, wherein k is a variable ranging from 1 percent to 100 percent with a 1 percent increment, i.e., k is 1 percent, 2 percent, 3 percent, 4 percent, 5 percent, . . . , 50 percent, 51 percent, 52 percent, . . . , 95 percent, 96 percent, 97 percent, 98 percent, 99 percent, or 100 percent.

Moreover, any numerical range defined by two R numbers as defined in the above is also specifically disclosed. Use of the term “optionally” with respect to any element of a claim is intended to mean that the subject element is required, or alternatively, is not required. Both alternatives are intended to be within the scope of the claim. As used herein, the term “and/or” can mean one, some, or all elements depicted in a list. As an example, “A and/or B” can mean A, B, or a combination of A and B. Use of broader terms such as comprises, includes, having, etc. should be understood to provide support for narrower terms such as consisting of, consisting essentially of, comprised substantially of, etc.

What is claimed is:

1. A process for retting biomass, the process comprising: contacting an enzyme solution with the biomass, wherein the enzyme solution comprises an enzyme, and wherein the biomass comprises pectin and cellulosic fibers; degrading at least a portion of the pectin in the biomass using the enzyme to release monomers; proliferating bacteria using the monomers as a feedstock; and separating the cellulosic fibers after degrading at least the portion of the pectin to produce natural fibers.
2. The process of claim 1, wherein the enzyme in the enzyme solution has a concentration between 0.05-2% w/v.
3. The process of claim 1, wherein the enzyme comprises pectinase.
4. The process of claim 1, wherein the enzyme breaks down the pectin through a mechanism including at least one of protopectinase, polygalacturonase, pectin lyase, and pectinesterase, or any combination thereof.
5. The process of claim 1, wherein the biomass comprises wood, bamboo, flax, jute, hemp, ramie, or any combination thereof.
6. The process of claim 1, wherein the bacteria comprises Bacillaceae.
7. The process of claim 1, further comprising: expressing the enzyme from *Aspergillus niger*.
8. The process of claim 1, further comprising: expressing a second enzyme from the bacteria; and breaking down the biomass using the second enzyme.
9. The process of claim 8, wherein the enzyme and the second enzyme are the same.

10. The process of claim 1, wherein proliferating the bacteria occurs at a pH between 4-5.

11. The process of claim 1, wherein separating the cellulosic fibers comprises mechanically separating the cellulosic fibers.

12. The process of claim 1, further comprising: collecting a retting solution after proliferating the bacteria; and reusing the retting solution in a subsequent retting process.

13. A process for retting biomass, the process comprising: contacting biomass with a bacterial solution, wherein the biomass comprises pectin and cellulosic fibers, wherein the bacterial solution comprises at least one culture of bacteria;

expressing an enzyme from the bacteria; degrading at least a portion of the pectin in the biomass using the enzyme to release monomers; proliferating the bacteria using the monomers as a feedstock; and separating the cellulosic fibers after degrading at least a portion of the pectin to produce natural fibers.

14. The process of claim 13, wherein the enzyme comprises pectinase.

15. The process of claim 13, wherein the enzyme breaks down the pectin through a mechanism including at least one of protopectinase, polygalacturonase, pectin lyase, and pectinesterase, or any combination thereof.

16. The process of claim 13, wherein the biomass comprises wood, bamboo, flax, jute, hemp, ramie, or any combination thereof.

17. The process of claim 13, wherein the bacteria comprises Bacillaceae.

18. The process of claim 13, wherein proliferating the bacteria occurs at a pH between 4-5.

19. The process of claim 13, wherein separating the cellulosic fibers comprises mechanically separating the cellulosic fibers.

20. The process of claim 13, further comprising: collecting a retting solution after proliferating the bacteria; and reusing the retting solution in a subsequent retting process.

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