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(54) **Title**: NOVEL COMPOSITION FOR PREPARING POLYSACCHARIDE FIBERS

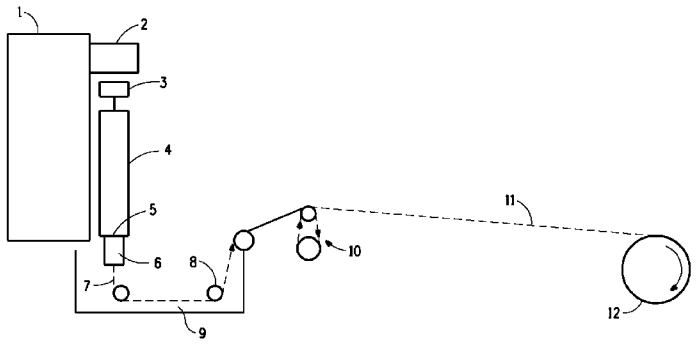

FIG. 1

(57) **Abstract**: This invention pertains to a novel process for preparing fibers from poly(α(1→3) glucan). The fibers prepared according to the invention, have"cotton-like" properties, are useful in textile applications, and can be produced as continuous filaments on a year-round basis. The process comprises solution spinning from a novel solution of poly(α(1→3) glucan) in a mixture of water and N-methylmorpholine-N-oxide followed by coagulation in a liquid coagulant that comprises a liquid that is not water..

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Novel Composition for Preparing Polysaccharide Fibers

This application is a PCT application and claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application Serial Number 61/543,423, filed on October 5, 2011, and U.S. Provisional Patent Application Serial Number 61/543,428, filed October 5, 2011. The disclosures of the foregoing applications are incorporated by reference in their entirety.

Field Of The Invention

The present invention is directed to a process for solution spinning poly(α(1→3) glucan) from a solution thereof in a mixture of water and N-methylmorpholine-N-oxide, and to the solution itself. The poly(α(1→3) glucan) employed was synthesized by the action of an enzyme.

Background Of The Invention

Polysaccharides have been known since the dawn of civilization, primarily in the form of cellulose, a polymer formed from glucose by natural processes via β(1→4) glycoside linkages; see, for example, *Applied Fibre Science*, F. Happey, Ed., Chapter 8, E. Atkins, Academic Press, New York, 1979. Numerous other polysaccharide polymers are also disclosed therein.

Only cellulose among the many known polysaccharides has achieved commercial prominence as a fiber. In particular, cotton, a highly pure form of naturally occurring cellulose, is well-known for its beneficial attributes in textile applications.

It is further known that cellulose exhibits sufficient chain extension and backbone rigidity in solution to form liquid crystalline solutions; see, for example O'Brien, U.S. Pat. No. 4,501,886. The teachings of the art suggest that sufficient polysaccharide chain extension could be achieved only in β(1→4) linked polysaccharides and that any significant deviation from that backbone geometry would lower the molecular aspect ratio below that required for the formation of an ordered phase.

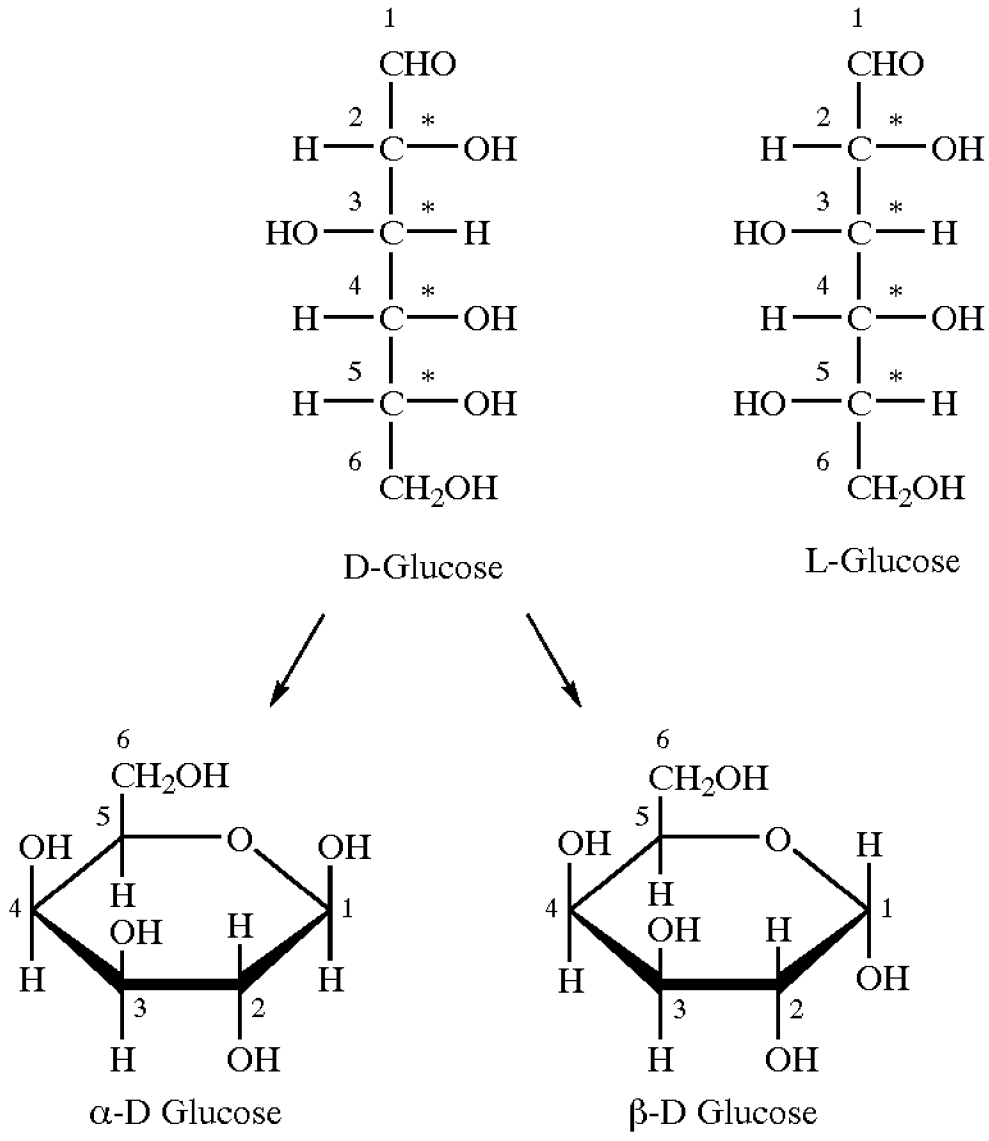
More recently, glucan polymer, characterized by α (1→3) glycoside linkages, has been isolated by contacting an aqueous solution of sucrose with GtfJ glucosyltransferase isolated from *Streptococcus salivarius*, Simpson et al., Microbiology, vol 141, pp. 1451-1460 (1995). Highly crystalline, highly oriented, low molecular weight films of α(1→3)-D-glucan have been fabricated for the purposes of x-ray diffraction analysis, Ogawa et al., Fiber Diffraction Methods, 47, pp. 353-362 (1980). In Ogawa, the insoluble glucan polymer is acetylated, the acetylated glucan dissolved to form a 5% solution in chloroform and the solution cast into a film. The film is then subjected to stretching in glycerine at 150° C. which orients the film and stretches it to a length 6.5 times the original length of the solution cast film. After stretching, the film is deacetylated and crystallized by annealing in superheated water at 140° C. in a pressure vessel. It is well-known in the art that exposure of polysaccharides to such a hot aqueous environment results in chain cleavage and loss of molecular weight, with concomitant degradation of mechanical properties.

Polysaccharides based on glucose and glucose itself are particularly important because of their prominent role in photosynthesis and metabolic processes. Cellulose and starch, both based on molecular chains of polyanhydroglucose are the most abundant polymers on earth and are of great commercial importance. Such polymers offer materials that are environmentally benign throughout their entire life cycle and are constructed from renewable energy and raw materials sources.

The term “glucan” is a term of art that refers to a polysaccharide comprising beta-D-glucose monomer units that are linked in eight possible ways, Cellulose is a glucan.

Within a glucan polymer, the repeating monomeric units can be linked in a variety of configurations following an enchainment pattern. The nature of the enchainment pattern depends, in part, on how the ring closes when an aldohexose ring closes to form a hemiacetal. The open chain form of glucose (an aldohexose) has four asymmetric centers (see below). Hence there are 2^4 or 16 possible open chain forms of which D and L glucose are two. When the ring is

closed, a new asymmetric center is created at C1 thus making 5 asymmetric carbons. Depending on how the ring closes, for glucose, α(1→4)-linked polymer, e.g. starch, or β(1→4)-linked polymer, e.g. cellulose, can be formed upon further condensation to polymer. The configuration at C1 in the polymer determines whether it is an alpha or beta linked polymer, and the numbers in parenthesis following alpha or beta refer to the carbon atoms through which enchainment takes place.



The properties exhibited by a glucan polymer are determined by the enchainment pattern. For example, the very different properties of cellulose and starch are determined by the respective nature of their enchainment patterns. Starch or amylose consists of α(1→4) linked glucose and does not form fibers among other things because it is swollen or dissolved by water. On the other hand, cellulose consists of β(1→4) linked glucose, and makes an excellent structural material being both crystalline and hydrophobic, and is commonly used for textile applications as cotton fiber, as well as for structures in the form of wood.

Like other natural fibers, cotton has evolved under constraints wherein the polysaccharide structure and physical properties have not been optimized for textile uses. In particular, cotton fiber is of short fiber length, limited variation in cross section and fiber fineness and is produced in a highly labor and land intensive process.

O'Brien, U.S. Patent No. 7,000,000 discloses a process for preparing fiber from liquid crystalline solutions of acetylated poly(α(1→3) glucan). Thus thus prepared fiber was then de-acetylated resulting in a fiber of poly(α(1→3) glucan).

Summary Of The Invention

Considerable benefit accrues to the process hereof that provides a highly oriented and crystalline poly (α(1→3) glucan) fiber without sacrifice of molecular weight by the solution spinning of fiber from the novel solution hereof.

In one aspect the present invention is direct to a solution comprising N-methylmorpholine-N-oxide (NMMO), water, and poly(α(1→3) glucan) (PAG) wherein the concentration of poly(α(1→3) glucan) is in the range of 5 – 20 % by weight with respect to the total weight of the solution; and, wherein the weight ratio of NMMO to water is in the range of 12 to 1.6.

In one embodiment, the solution is isotropic.

In another aspect, the present invention is directed to a process for preparing a poly(alpha(1→3) glucan) fiber, comprising the steps of: dissolving in a mixture of N-methylmorpholine-N-oxide (NMMO) and water, 5 to 20 % by

weight of the total weight of the resulting solution of poly(alpha(1→3) glucan) (PAG) characterized by a number average molecular weight (M_n) of at least 10,000 Da, to form a solution, wherein the weight ratio of NMMO to water in said solution is in the range of 12 to 1.6; causing said solution to flow through a spinneret, forming a fiber thereby, using a liquid coagulant to extract the NMMO from the thus formed fiber.

In one embodiment, the solution is isotropic.

Brief Description Of The Drawing

FIG. 1 is a schematic diagram of an apparatus suitable for air gap or wet spinning of liquid crystalline solutions of hexose polymer to form polysaccharide fibers.

Detailed Description

When a range of values is provided herein, it is intended to encompass the end-points of the range unless specifically stated otherwise. Numerical values used herein have the precision of the number of significant figures provided, following the standard protocol in chemistry for significant figures as outlined in ASTM E29-08 Section 6. For example, the number 40 encompasses a range from 35.0 to 44.9, whereas the number 40.0 encompasses a range from 39.50 to 40.49.

The term “solids content” is a term of art. It is used herein to refer to the percentage by weight of poly(α(1→3) glucan) in the NMMO/water solution hereof. It is calculated from the formula

$$SC = \frac{Wt(G)}{Wt(G) + Wt(NMMO) + Wt(Water)}$$

where *SC* represents “solids content,” and *Wt(G), Wt(NMMO) and Wt(water)* are the respective weights of the poly(α(1→3) glucan), the NMMO, and the water. The term “solids content” is synonymous with the concentration by weight of poly(α(1→3) glucan) with respect to the total weight of solution.

Percent by weight is represented by the term “wt-%.”

While the term "glucan" refers to a polymer, it also encompasses oligomers and low molecular weight polymers that are unsuitable for fiber formation. For the purposes of the present invention, the polymer suitable for the practice thereof shall be referred to as "poly(α(1→3) glucan)."

A polymer, including glucan, and poly(α(1→3) glucan) in particular, is made up of a plurality of so-called repeat units covalently linked to one another. The repeat units in a polymer chain are diradicals, the radical form providing the chemical bonding between repeat units. For the purposes of the present invention the term "glucose repeat units" shall refer to the diradical form of glucose that is linked to other diradicals in the polymer chain, thereby forming said polymer chain.

In one aspect, the present invention provides a solution comprising N-methylmorpholine-N-oxide (NMMO), water, and poly(α(1→3) glucan) (PAG) wherein the concentration of poly(α(1→3) glucan) is in the range of 5 – 20 % by weight with respect to the total weight of the solution; and, wherein the weight ratio of NMMO to water is in the range of 12 to 1.6.

In one embodiment, the solution is isotropic.

For the purposes of the present invention, the term "isotropic solution" refers to a solution exhibiting a disordered morphology. Isotropic solutions stand in contrast with the morphology of liquid crystalline solutions that exhibit ordered regions as described in U.S. Patent 7,000,000. It has surprisingly been found that the embodiment of the solution hereof that is isotropic is useful for the preparation of fibers using common solution spinning methods such as are known in the art.

The poly(α(1→3) glucan) (PAG) suitable for use in the present invention is a glucan characterized by M_n of at least 10,000 Da wherein at least 90 mol-% of the repeat units in the polymer are glucose repeat units and at least 50% of the linkages between glucose repeat units are α(1→3) glycoside linkages. Preferably at least 95 mol-%, most preferably 100 mol-%, of the repeat units are glucose repeat units. Preferably at least 90 %, most preferably 100 %, of the linkages between glucose units are α(1→3) glycoside linkages.

The isolation and purification of various polysaccharides is described in, for example, *The Polysaccharides*, G. O. Aspinall, Vol. 1, Chap. 2, Academic Press, New York, 1983. Any means for producing the α(1→3) polysachharide suitable for the invention in satisfactory yield and 90 % purity is suitable. In one such method, disclosed in U.S. Patent 7,000,000, poly(α(1→3)-D-glucose) is formed by contacting an aqueous solution of sucrose with gtfJ glucosyltransferase isolated from *Streptococcus salivarius* according to the methods taught in the art. In an alternative such method, the gtfJ is generated by genetically modified E. Coli, as described in detail, *infra.* .

The poly(α(1→3) glucan) suitable for use in the present invention can further comprise repeat units linked by a glycoside linkage other than α(1→3), including α(1→ 4), α(1→6), β(1→2), β(1→ 3), β(1→4) or β(1→6) or any combination thereof. According to the present invention, at least 50% of the glycoside linkages in the polymer are α(1→3) glycoside linkages. Preferably at least 90 %, most preferably 100 %, of the linkages between glucose units are α(1→3) glycoside linkages.

According to the present invention, the ratio of NMMO to water on a weight basis in the solution hereof is in the range of 12 to 1.6, as determined from the formula:

$$\text{ratio} = (\text{Wt. NMMO})/\text{Wt. } H_2O)$$

The solution hereof is prepared by combining NMMO, H_2O, and poly(α(1→3) glucan), agitating to obtain thorough mixing. The amount of poly(α(1→3) glucan) in the solution ranges from 5 to 20 % by weight with respect to the total weight of the solution. At concentrations of poly(α(1→3) glucan) below 5 % by weight, the fiber-forming capability of the solution is greatly degraded. Solution concentrations above 16 % by weight are increasingly problematical to form. In the range of 16 to 20 % by weight, increasingly refined solution forming techniques are often required.

In one embodiment, the concentration of poly(α(1→3) glucan) is in the range of 10 to 15 % by weight.

In any given embodiment, the solubility limit of poly(α(1→3) glucan) is a function of the molecular weight, the NMMO/water ratio, the duration of mixing, the viscosity of the solution as it is being formed, the shear forces to which the solution is subjected, and the temperature at which mixing takes place. In general, lower molecular weight poly(α(1→3) glucan) will be more soluble than higher molecular weight, other things being equal. Generally, higher shear mixing, longer mixing time, and higher temperature will be associated with higher solubility. The maximum temperature for mixing is limited by the boiling point and stability of the solvents. The optimum NMMO/water ratio may change depending upon the other parameters in the mixing process.

In another aspect, the present invention is directed to a process for preparing a poly(alpha(1→3) glucan) fiber, comprising the steps of: dissolving in a mixture of N-methylmorpholine-N-oxide (NMMO) and water, 5 to 20 % by weight of the total weight of the resulting solution of poly(alpha(1→3) glucan) (PAG) characterized by a number average molecular weight (M_n) of at least 10,000 Da, to form a spinning solution, wherein the weight ratio of NMMO to water in said solution is in the range of 12 to 1.6; causing said solution to flow through a spinneret, forming a fiber thereby; and, using a liquid coagulant to extract the NMMO from the thus formed fiber. In one embodiment, the spinning solution is isotropic.

While it is not strictly required in the practice of the invention, it is highly desirable to combine the water and the NMMO before the addition of the glucan polymer. The addition of water to NMMO lowers the melting point of the NMMO to the point where it can be used safely without explosive decomposition.

In a further embodiment, the isotropic spinning solution further comprises a poly(α(1→3) glucan) wherein 100 % of the repeat units therein are glucose, and 100 % of the linkages between glucose repeat units are α(1→3) glycoside linkages.

The minimum solids content of poly(α(1→3) glucan) required in the spinning solution in order to achieve achieve stable fiber formation varies according to the specific molecular morphology and the molecular weight of the

poly(α(1→3) glucan), as well as the NMMO/water ratio. It is found in the practice of the invention that a 5% solids content is an approximate lower limit to the concentration needed for stable fiber formation. A solution having a solids content of at least 10% is preferred. A solids content ranging from about 10% to about 15% is more preferred . Preferred is a poly(alpha (1→3) glucan) characterized by a number average molecular weight of ca. 50,000 to 70,000 Daltons. Optimum spinning performance for this particular polymer is achieved at about 10 to about 12% solids content in a NMMO/water mixture wherein the weight ratio of NMMO to water is in the range of 12 to 1.6.

Spinning from the NMMO/water solution can be accomplished by means known in the art, and as described in O'Brien, op. cit. The viscous spinning solution can be forced by means such as the push of a piston or the action of a pump through a single or multi-holed spinneret or other form of die. The spinneret holes can be of any cross-sectional shape, including round, flat, multi-lobal, and the like, as are known in the art. The extruded strand can then be passed by ordinary means into a coagulation bath wherein is contained a liquid coagulant which dissolves NMMO but not the polymer, thus causing the highly oriented polymer to coagulate into a fiber according to the present invention.

Suitable liquid coagulants include but are not limited to glacial acetic acid, or NMMO/water mixtures characterized by a water concentration of at least 75 % by weight. In one embodiment, the liquid coagulant is maintained at a temperature in the range of 20 – 100 °C

In one embodiment, the coagulation bath comprises acetic acid. It is found in the practice of the invention that satisfactory results are achieved by employing as the coagulant liquid an excess of glacial acetic acid. During the course of spinning, the glacial acetic acid absorbs both NMMO and water as the as spun fiber passes through the coagulant bath.

Under some circumstances, a superior result is achieved when the extruded strand first passes through an inert, noncoagulating layer, usually an air gap, prior to introduction into the coagulation bath. When the inert layer is an air gap, the spinning process is known as air-gap spinning. Under other

circumstances, extrusion directly into the coagulation bath is preferred, known as wet-spinning.

Figure 1 is a schematic diagram of an apparatus suitable for use in the fiber spinning process hereof. The worm gear drive, **1**, drives a ram, **2,** at a controlled rate onto a piston, **3,** fitted into a spinning cell, **4**. The spinning cell, **4**, may contain filter assemblies, **5**. A suitable filter assembly includes 100 and 325 mesh stainless steel screens. Another suitable filter assembly includes a Dynalloy X5, 10 micron sintered metal filters, (Pall Corporation, Deland, FL). A spin pack, **6,** contains the spinneret and optionally stainless steel screens as prefilters for the spinneret. The extruded filament, **7**, produced therefrom is optionally directed through an inert non coagulating layer (typically an air gap) and into a liquid coagulating bath, **9**. The extrudate can be, but need not be, directed back and forth through the bath between guides, **8** , which are normally fabricated of Teflon® PTFE. Only one pass through the bath is shown in Figure 1. On exiting the coagulation bath, **9**, the thus quenched filament, **11**, can optionally be directed through a drawing zone using independently driven rolls, **10,** around which the thus quenched filament is wound. The thus prepared filament is then collected on plastic or stainless steel bobbins using a wind up, **12**, preferably provided with a traversing mechanism to evenly distribute the fiber on the bobbin. In one embodiment, the process comprises a plurality of independently driven rolls.

In one embodiment, a plurality of filaments is extruded through a multi-hole spinneret, and the filaments so produced are converged to form a yarn. In a further embodiment, the process further comprises a plurality of multi-hole spinnerets so that a plurality of yarns can be prepared simultaneously.

EXAMPLES

Materials

MATERIAL	Description	Vendor
Dialysis tubing	Spectrapor 25225-226, 12000 molecular weight cut-off	VWR (Radnor, PA).
Sucrose	15 wt-% solids aqueous solution (#BDH8029)	VWR.
Dextran	T-10 (#D9260)	Sigma Aldrich.
Ethanol	Undenatured (#459844)	Sigma Aldrich
Antifoam	Suppressor 7153	Cognis Corporation (Cincinnati, OH).
N-methylmorpholine N Oxide	NMMO	Sigma Aldrich

All other chemicals were obtained from commonly used suppliers of such chemicals.

Molecular Weights

Molecular weights were determined by size exclusion chromatography (SEC) with a GPCV/LS 2000™ (Waters Corporation, Milford, MA) chromatograph equipped with two Zorbax PSM Bimodal-s silica columns (Agilent, Wilmington, DE), using DMAc from J.T Baker, Phillipsburg, NJ with 3.0% LiCl (Aldrich, Milwaukee, WI) as the mobile phase. Samples were dissolved in DMAc with 5.0% LiCl. The degree of polymerization shown in Table 2 is based upon number average molecular weight.

Preparation of glucosyltransferase (gtfJ) enzyme

Seed medium

The seed medium, used to grow the starter cultures for the fermenters, contained: yeast extract (Amberex 695, 5.0 grams per liter, g/L), K_2HPO_4 (10.0 g/L), KH_2PO_4 (7.0 g/L), sodium citrate dihydrate (1.0 g/L), $(NH_4)_2SO_4$ (4.0 g/L), $MgSO_4$ heptahydrate (1.0 g/L) and ferric ammonium citrate (0.10 g/L). The pH of

the medium was adjusted to 6.8 using either 5N NaOH or H_2SO_4 and the medium was sterilized in the flask. Post sterilization additions included glucose (20 mL/L of a 50% w/w solution) and ampicillin (4 mL/L of a 25 mg/mL stock solution).

Fermenter medium

The growth medium used in the fermenter contained: KH_2PO_4 (3.50 g/L), $FeSO_4$ heptahydrate (0.05 g/L), $MgSO_4$ heptahydrate (2.0 g/L), sodium citrate dihydrate (1.90 g/L), yeast extract (Amberex 695, 5.0 g/L), Suppressor 7153 antifoam (0.25 milliliters per liter, mL/L), NaCl (1.0 g/L), $CaCl_2$ dihydrate (10 g/L), and NIT trace elements solution (10 mL/L). The NIT trace elements solution contained citric acid monohydrate (10 g/L), $MnSO_4$ hydrate (2 g/L), NaCl (2 g/L), $FeSO_4$ heptahydrate (0.5 g/L), $ZnSO_4$ heptahydrate (0.2 g/L), $CuSO_4$ pentahydrate (0.02 g/L) and $NaMoO_4$ dihydrate (0.02 g/L). Post sterilization additions included glucose (12.5 g/L of a 50% w/w solution) and ampicillin (4 mL/L of a 25 mg/mL stock solution).

Construction of glucosyltransferase (gtfJ) enzyme expression strain

A gene encoding the mature glucosyltransferase enzyme (gtfJ; EC 2.4.1.5; GENBANK® AAA26896.1, SEQ ID NO: 3) from *Streptococcus salivarius* (ATCC 25975) was synthesized using codons optimized for expression in *E. coli* (DNA 2.0, Menlo Park CA). The nucleic acid product (SEQ ID NO: 1) was subcloned into pJexpress404® (DNA 2.0, Menlo Park CA) to generate the plasmid identified as pMP52 (SEQ ID NO: 2). The plasmid pMP52 was used to transform *E. coli* MG1655 (ATCC 47076™) to generate the strain identified as MG1655/pMP52. All procedures used for construction of the glucosyltransferase enzyme expression strain are well known in the art and can be performed by individuals skilled in the relevant art without undue experimentation.

Production of recombinant gtfJ in fermentation

Production of the recombinant gtfJ enzyme in a fermenter was initiated by preparing a pre-seed culture of the *E. coli* strain MG1655/pMP52, expressing the

gtfJ enzyme, constructed as described *infra*. A 10 mL aliquot of the seed medium was added into a 125 mL disposable baffled flask and was inoculated with a 1.0 mL culture of *E. coli* MG1655/pMP52 in 20% glycerol. This culture was allowed to grow at 37 °C while shaking at 300 revolutions per minute (rpm) for 3 hours.

A seed culture, for starting the fermenter, was prepared by charging a 2 L shake flask with 0.5 L of the seed medium. 1.0 mL of the pre-seed culture was aseptically transferred into 0.5 L seed medium in the flask and cultivated at 37 °C and 300 rpm for 5 hours. The seed culture was transferred at optical density 550 nm (OD_{550}) >2 to a 14 L fermenter (Braun, Perth Amboy, NJ) containing 8 L of the fermenter medium described above at 37 °C.

Cells of *E. coli* MG1655/pMP52 were allowed to grow in the fermenter and glucose feed (50% w/w glucose solution containing 1% w/w $MgSO_4 \cdot 7H_2O$) was initiated when glucose concentration in the medium decreased to 0.5 g/L. The feed was started at 0.36 grams feed per minute (g feed/min) and increased progressively each hour to 0.42, 0.49, 0.57, 0.66, 0.77, 0.90, 1.04, 1.21, 1.41 1.63, 1.92, 2.2 g feed/min respectively. The rate was held constant afterwards by decreasing or temporarily stopping the glucose feed when glucose concentration exceeded 0.1 g/L. Glucose concentration in the medium was monitored using a YSI glucose analyzer (YSI, Yellow Springs, Ohio).

Induction of glucosyltransferase enzyme activity was initiated, when cells reached an OD_{550} of 70, with the addition of 9 mL of 0.5 M IPTG (isopropyl β-D-1-thiogalacto- pyranoside). The dissolved oxygen (DO) concentration was controlled at 25% of air saturation. The DO was controlled first by impeller agitation rate (400 to 1200 rpm) and later by aeration rate (2 to 10 standard liters per minute, slpm). The pH was controlled at 6.8. NH_4OH (14.5% weight/volume, w/v) and H_2SO_4 (20% w/v) were used for pH control. The back pressure was maintained at 0.5 bars. At various intervals (20, 25 and 30 hours), 5 mL of Suppressor 7153 antifoam was added into the fermenter to suppress foaming. Cells were harvested by centrifugation 8 hours post IPTG addition and were stored at -80 ^{o}C as a cell paste.

Preparation of gtfJ crude enzyme extract from cell paste

The cell paste obtained above was suspended at 150 g/L in 50 mM potassium phosphate buffer pH 7.2 to prepare a slurry. The slurry was homogenized at 12,000 psi (Rannie-type machine, APV-1000 or APV 16.56) and the homogenate chilled to 4 °C. With moderately vigorous stirring, 50 g of a floc solution (Aldrich no. 409138, 5% in 50 mM sodium phosphate buffer pH 7.0) was added per liter of cell homogenate. Agitation was reduced to light stirring for 15 minutes. The cell homogenate was then clarified by centrifugation at 4500 rpm for 3 hours at 5-10 °C. Supernatant, containing crude gtfJ enzyme extract, was concentrated (approximately 5X) with a 30 kilo Dalton (kDa) cut-off membrane. The concentration of protein in the gftJ enzyme solution was determined by the bicinchoninic acid (BCA) protein assay (Sigma Aldrich) to be 4-8 g/L.

EXAMPLES 1 – 3 AND COMPARATIVE EXAMPLES A - D

Examples 1 - 3

Polymer P1:

Twenty liters of an aqueous solution was prepared by combining 3000 g of sucrose (in the form of an aqueous solution of 15 wt-%), 60 g of Dextran T-10 , 2 L of undenatured ethanol, and 1 L of 1M KH_2PO_4., The pH was adjusted to pH 6.8 – 7.0 by addition of 10 % KOH. De-ionized water was then added to bring the volume up to 20 L. The buffer concentration in the thus prepared solution was 50 mM.

The thus prepared pH-adjusted solution was then charged with 200 ml of the enzyme extract prepared *supra*, and allowed to stand at ambient temperature for 144 hours. The resulting glucan solids were collected on a Buchner funnel using a 325 mesh screen over 40 micron filter paper. The filter cake was re-suspended in deionized water and filtered twice more as above to remove sucrose, fructose and other low molecular weight, soluble by-products. Finally two additional washes with methanol were carried out, the filter cake was pressed out thoroughly on the funnel and dried in vacuum at room temperature.

The yield was 403 grams of white flaky solids. The polymer so prepared is herein designated P1.

Number and weight average molecular weights were found to be 64,863 and 168,120 Daltons respectively.

25-30 mg of the polymer were dissolved in 1mL of deuterated DMSO. The ^{13}C NMR spectrum (Bruker Avance 500 MHz NMR spectrometer equipped with a CPDul cryoprobe) showed the presence of resonance peaks at 98.15, 73.57, 71.63, 70.17, 65.79 and 60.56, ppm due to incorporation of dextran primer and resonances consistant with the six expected discrete carbon atoms for poly ($\alpha(1\rightarrow3)$ glucan) at 99.46, 81.66, 72.13, 71.09, 69.66, and 60.30 ppm . These resonances were consistent with the presence of poly($\alpha(1\rightarrow3)$ glucan) containing about 5% dextran.

Preparation of poly(α(1→3) glucan) Spinning Solution

In a drybox, a 100 mL wide mouth glass bottle was charged with 8 g of Polymer P1, and 46 g of anhydrous N-methylmorpholine N oxide (NMMO). To the mixture so-formed were added 21 g of deionized water containing 0.344g of gallic acid propyl ester and 0.086 g of hydroxylamine sulfate. The container was fitted with a cap through which a polypropylene stirring rod had been fitted through a septum. The contents were then heated to 110 °C with intermittent manual mixing performed for about 5 minutes every hour over a period of 6 hours. After 1 hour, vacuum was applied to remove water while the contents continued to be mixed. After 6 hours, 0.6 g of water had been removed resulting in a fiber-forming light amber solution of 10.75 % poly(α(1→3) glucan) solids that could be extruded into fiber under the conditions shown below.

Poly(α(1→3) glucan) Fiber Spinning

The apparatus depicted in Figure 1, as described *supra*, was modified by removal of the driven roll, **10**, from the filament pathway. Spin stretch was attained by running the windup faster than the jet velocity. The spinning solution thus prepared was fed at a rate of 0.30 ml/min through a spin pack having a filter

assembly consisting of 100 and 325 mesh screens to a one hole spinneret with a diameter of 0.003 in.. The extruded filament was passed through an air gap of 1.75 in. (Examples 1 and 2) or 0.75 in. (Example 3), before being immersed in and traversing a 2.5 ft. long coagulation bath containing glacial acetic acid at the temperature indicated in Table 1. Upon removal from the coagulation bath the thus coagulated filament was directed to a tension-controlled wind-up with a traverse rod, at a wind-up speed shown in Table 1.

Physical properties such as tenacity, elongation and initial modulus were measured using methods and instruments conforming to ASTM Standard D 2101-82, except that the test specimen length was one inch.

Table 1 shows the properties of the thus prepared filaments. These include the denier of the fiber produced, and the physical properties such as tenacity (T) in grams per denier (gpd), elongation to break (E, %), and initial modulus (M) in gpd were measured using methods and instruments conforming to ASTM Standard D 2101-82, except that the test specimen length was one inch. Results shown in Table 1 are averages for 3 to 5 individual filament tests.

Comparative Examples A – D

Preparation of cellulose spinning solution

In a drybox, a 100 ml wide mouth glass bottle was charged with 5g of cellulose derived from shredded Whatman #1 filter paper and 54 g of anhydrous NMMO. To the mixture so formed were added 7.6 g of deionized water containing 0.13g of gallic acid propyl ester and 0.033 g of hydroxylamine sulfate. The container was fitted with a cap through which a polypropylene stirring rod had been fitted through a septum. The contents were then heated to 115 °C with occasional (5-10 minutes/hour) manual mixing over a period of 4 hours. At that time dissolution was complete yielding a fiber-forming light amber solution at 7.5 % cellulose solids that could be extruded into fiber under the conditions shown below.

Cellulose Fiber Spinning

Cellulose filaments were prepared using the apparatus and procedures employed in Examples 1 - 3, as described *supra*, except that the feed rate of the spinning solution to the spinneret was 0.2 ml/min, and the air gap was 1.25 in. (Comparative Examples A - C) or 1.75 in. (Comparative Example D). The coagulation bath was 4.8 ft. in length, and contained water only. The coagulated cellulose fiber was wrapped around driven roll, **10**, depicted in Figure 1. The remaining conditions are shown in Table 1.

Physical properties were determined as in Examples 1 – 3. Results are shown in Table 1.

TABLE 1

Examples	BATH TEMP (C)	Jet Velocity (fpm)	Roll Speed (m/min)	Wind-up Speed (fpm)	T (gpd)	E (%)	M (gpd)	dpf
1	23	50	na	70	0.8	15.4	41.3	17.3
2	24	50	na	90	0.8	11.8	13.5	13.5
3	25	50	na	70	0.8	16.2	16.2	16.2
Comp.Ex. A	10	30	22	30	1.5	4.2	97	23.1
Comp.Ex. B	10	30	35	44	1.7	6.4	105	17.4
Comp.Ex. C	11	30	49	50	1.4	8.8	84	15.5
Comp.Ex. D	11	30	49	56	1.5	2.3	128	12.3

EXAMPLES 4 - 17 AND COMPARATIVE EXAMPLES E - M

PREPARATION OF SPINNING SOLUTIONS

Solubiliity Determination

Solubility was determined by visual inspection of the solution in the vial after the dissolution process, described in the examples, *infra*, was complete. If by visual inspection no particles or haziness was observed, the poly(α(1→3)

glucan) was said to completely dissolved. Detection of any particles or haziness was considered to be an indication of incomplete solubility.

From the standpoint of preparing solutions suitable for fiber spinning, the homogeneity imparted by complete solubility is very highly preferred.

In the data tables, *infra*, solubility is indicated by "S," meaning completely dissolved, or "N," meaning not completely dissolved.

Polymer Synthesis

Polymer P2

Three liters of an aqueous solution containing 15% sucrose, 9g of Dextran T-10, 300 ml of undenatured ethanol, and 50 ml of 1 molar KH_2PO_4 pH 6.8 – 7.0, were combined in a vessel. The pH was adjusted with 10 % KOH, and the volume brought up to 3 liters with de-ionized water. The solution was then charged with 20.1 ml (.67 volume per cent) enzyme prepared supra and allowed to stand at ambient temperature for 144 hours. The resulting glucan solids were collected on a Buchner funnel using a 325 mesh screen over 40 micrometer filter paper. The filter cake was suspended in deionized water and filtered twice more as above to remove sucrose, fructose and other low molecular weight, soluble by products. Finally two additional washes with methanol were carried out, the filter cake was pressed out on the funnel and dried in vacuum at room temperature. Yield was 25.5 grams of white flaky solids. The polymer so prepared is herein designated P2.

P3

Three liters of an aqueous solution containing 15% sucrose, were combined in a vessel with 9g of Dextran T-10, 300 ml of undenatured ethanol, and 150 ml of potassium phosphate buffer adjusted to pH 6.8 – 7.0 using 10 %KOH. The volume was brought up to 3 liters with deionized water. The solution was then charged with 30 ml (1 vol%) enzyme prepared supra and allowed to stand at ambient temperature for 72 hours. The resulting glucan solids were collected on a Buchner funnel using a 325 mesh screen over 40

micron filter paper. The filter cake was suspended in deionized water and filtered twice more as above to remove sucrose, fructose and other low molecular weight, soluble by products. Finally two additional washes with methanol were carried out, the filter cake was pressed out on the funnel and dried in vacuum at room temperature. Yield was 55.4 grams of white flaky solids. The polymer so prepared is herein designated P3.

P4

Glucan Primer

25 grams of ground polymer P3 was suspended in 500 ml of 37% HCl (EMD HX0603-4) with a magnetic stir bar in a 500 ml Erlenmeyer flask and allowed to hydrolyze for 2 hours. The acid was neutralized slowly using NaOH solids with 50 ml of water added to keep the hydrolyzed glucan in solution while being cooled in an ice bath. The solution was then dialyzed using 500 MW cut off membrane (Specta/Por Biotech Cellulose Ester (CE) MWCO 500-1,000D) with tap water flowing at a low level overnight to remove salts. The solution was then placed in a rotovap, and the material was dried under vacuum at room temperature. The material so prepared is herein designated P3-H

The materials and procedures employed for preparing polymer P1 were repeated except that 4.6 g of P3-H was employed, and the Dextran was omitted. The polymer so prepared is herein designated P4. Yield was 309 grams of white flaky solids.

P5

In a 150 gallon glass lined reactor with stirring and temperature control approximately 394kg of an aqueous solution was prepared by combining in a vessel 75 kg of sucrose, 500 g of Dextran T-10, 3.4 kg of potassium phosphate buffer adjusted to pH 7.0 using 10 % KOH, and 50 liters of undenatured ethanol. The solution was then charged with 32 units/liter of enzyme prepared supra followed by an additional 1 liter of de-ionized water. The resulting solution was mixed mildly at 25 °C for 72 hours. The resulting glucan solids was transferred to

a Zwag filter with the mother liquor removed. The cake was washed via displacement with water 3 times with approximately 150 kg of water. Finally two additional displacement washes with 100 liters of methanol were carried out. The material was dried under vacuum with a 60 °C jacket. Yield: 6.6 kg white flaky solids. The polymer thus prepared is herein designated P5

P6

The materials and procedures for preparing polymer P3 were replicated except that 2.0 g of P3-H were employed and the Dextran was omitted. Yield was 68 grams of white flaky solids. The polymer so prepared is herein designated P6.

Example 4

0.5 g of Polymer P2 was added to a mixture formed by combining of 8g of a 50/50 by weight mixture of anhydrous NMMO and water with 0.15 ml of an aqueous solution of propyl gallate (0.08M) and hydroxylamine sulfate (0.026 M). The thus combined ingredients were charged to a 40 ml glass vial. After charging, the vial was capped with a silicone septum and the vial was weighed. The septum was then fitted with a stirring rod. The vial was placed into a heating block preheated to 110 °C and kept there for 30 minutes with occasional manual stirring. After 30 minutes, vacuum was applied while continuing to heat at 110 °C to remove water to the level shown in Table 2. Final water content was determined by weighing the amount that was distilled off. Distillation of NMMO was negligible. The polymer was fully dissolved and was light amber in color. Final solids content was 8.9%.

Example 5

1.0 g of polymer P4 was suspended in 8.5 g of a 50/50 by weight mixture of anhydrous NMMO and water, to which was added 0.15 ml of an aqueous solution of propyl gallate (0.016M) and hydroxylamine sulfate (0.005 M). The ingredients were charged into a 40 ml glass vial fitted with a silicone septum.

After charging the vial, its contents were weighed. A stirring rod was then inserted through the septum. The vial was then placed into a heating block preheated to 110 °C and held there for 60 minutes with occasional manual stirring. After 60 minutes, vacuum was applied while heating at 110 °C was continued, to remove water to the level shown Table 2. The polymer was fully dissolved and was light amber in color. Final solids content was 8.1 wt-%.

Example 6

8.0 g of polymer P1 was suspended in a mixture containing 46 g anhydrous NMMO, and 21 ml of an aqueous solution of propyl gallate (0.08M) and hydroxylamine sulfate (0.026 M) . The ingredients were charged into a 100 ml wide mouthed glass vial. After charging, the vial was capped with a septum/stirrer and the assembly was weighed. The mixture was then heated at 110 °C for 30 minutes with occasional manual mixing. After 30 minutes vacuum was applied while continuing to heat at 110 °C to remove water to the level shown in Table 2. The polymer was fully dissolved and light amber in color. Final solids content was 10.9%.

Comparative Example E

The materials and procedures of Example 6 except that 10.0 g of polymer P1 was suspended in the NMMO/aqueous solution mixture. The polymer was not fully dissolved. Final solids content was 13.7%.

Comparative Example F

The materials and procedures of Example 6 were reproduced except that the NMMO/H_2O ratio was adjusted to a different value as shown in Table 2. The resulting solutionwas light amber in color. The presence of some particulate indicated that the polymer was not fully dissolved. Final solids content was 11.0%.

Comparative Example G

The materials and procedures of Example 6 were reproduced except that the NMMO/H_2O ratio was adjusted to a different value as shown in Table 2. The resulting solutionwas light amber in color. The presence of some particulate indicated that the polymer was not fully dissolved. Final solids content was 10.8%.

Comparative Example H

The materials and procedures of Example 6 were reproduced except that the NMMO/H_2O ratio was adjusted to a different value as shown in Table 2. In addition, following the vacuum distillation of water, the vacuum was turned off, the mixture was blanketed with nitrogen, and allowed to continue heating at 110 °C for an additional 60 minutes with occasional mixing. The resulting solution was light amber in color. The presence of some particulate indicated that the polymer was not fully dissolved. Final solids content was 10.8%.

Example 7

0.5g of polymer P3 was suspended in a mixture containing 6g of NMMO and 6 ml of an aqueous solution of propyl gallate (0.08M) and hydroxylamine sulfate (0.026 M) . The ingredients were charged into a 40 ml glass vial fitted with a silicone septum and stirring rod. After charging the vial and its contents were capped and weighed. The mixture was then heated at 110 °C for 30 minutes with occasional manual mixing. After 30 minutes vacuum was applied while heating at 110 °C to remove water to the level shown in the table. following the vacuum extraction of water, the vacuum was turned off, the mixture was blanketed with nitrogen, and allowed to continue heating at 110 °C for an additional 3 hours with occasional mixing. The resulting solution was completely clear and was light amber in color. Final solids content was 5.6%

Example 8

0.5g of polymer P3 was suspended in a mixture containing 5g NMMO and

5 ml of an aqueous solution of propyl gallate (0.08M) and hydroxylamine sulfate (0.026 M) . The equipment and procedures of Example 7 were repeated. The resulting solution was completely clear and was light amber in color. Final solids content was 6.3%

Example 9

0.5 g of polymer P3 was suspended in a mixture 4g NMMO and 4 ml of an aqueous solution of propyl gallate (0.08M) and hydroxylamine sulfate (0.026 M) . The equipment and procedures of Example 7 were repeated. The resulting solution was completely clear and was light amber in color. Final solids content was 8.7%

Comparative Example I

0.5 g of polymer P3 was suspended in a mixture containing 3g NMMO and 3 ml of an aqueous solution of propyl gallate (0.08M) and hydroxylamine sulfate (0.026 M) . The equipment and procedures of Example 7 were repeated. After 3 hours the glucan polymer was gel like with some particulate and was light amber in color. Final solids content was 10.1%

Example 10

3.17 g of 97 % NMMO was transferred to a tared 20 x 125 mm tissue culture tube. 1.63 g (excess) de-ionized water was added to the tube. The tube was capped with a septum, and a plastic stirring rod was inserted through a pre-bored Teflon®-coated silicone septum. The mixture so formed was stirred for approximately 1 minute. After stirring, 0.12 ml of a stabilized aqueous solution containing 0.4 wt % hydroxylamine sulfate and 1.7 wt % propyl gallate was added to the tube and further mixing was conducted for 2 to 5 minutes. 0.25 g of Polymer P5 was added to the tube and the resulting mixture was mixed at room temperature for an additional 2 to 5 minutes, forming a slurry.

Behind a glass shield, the tube was placed in a Pierce Reacti-therm heating module (Pierce Biotechnology, Rockford,IL) at 50 °C. The contents of

the tube were blanketed with nitrogen admitted through a needle inserted through the septum. The tube was thus heated in the block at 50 °C for 30 to 45 minutes, stirring intermittently by hand every 5 to 10 minutes. The polymer solids were observed to have been thoroughly wetted. The temperature was then raised to 100 °C over a period of 15 minutes and then held at 100 °C for 30 to 60 minutes to begin dissolution while mixing intermittently. Maintaining stirring, the temperature was then increased to 115 °C and excess water was removed under vacuum, stirring intermittently, to the concentration shown in Table 2, and to complete formation of the solution. The final composition was as shown in Table 2. The polymer was completely dissolved. Solids content of 6.84wt% was verified by weight loss of water and confirmation by GC-MS that the distillate contained a negligible amount of NMMO.

Examples 11 – 17 and Comparative Examples J - P

The materials and procedures employed in Example 10 were repeated with the changes indicated in Table 2. Results are shown in Table 2.

Table 2

Example	Polymer		Results					
	Designation	Amount (g)	DP	NMMO Content (final, g)	H_2O Content (final,g)	NMMO/ water (wt/wt)	Solids (%)	Solution Forming?
Ex. 4	P2	0.5	870	4	1.13	3.54	8.88	yes
Ex. 5	P4	1	255	8.5	2.84	2.99	8.1	yes
Ex. 6	P1	8	403	46	19.3	2.38	10.91	yes
Comp. Ex.E	P1	10	403	46	16.9	2.72	13.72	no
Comp. Ex.F	P1	8	403	46	18.6	2.47	11.02	no
Comp. Ex.G	P1	8	403	46	20.25	2.27	10.77	no
Comp. Ex.H	P1	8	403	46	20.4	2.25	10.75	no
Ex. 7	P4	0.5	255	6	2.48	2.42	5.57	yes
Ex. 8	P4	0.5	255	5	2.48	2.02	6.27	yes
Ex. 9	P4	0.5	255	4	1.28	3.13	8.65	yes
Comp. Ex. I	P4	0.5	255	3	1.47	2.04	10.06	no
Ex. 10	P5	0.25	372	3.17	0.38	8.34	6.84	yes
Ex. 11	P5	0.3	372	3.18	0.37	8.59	8.06	yes
Ex. 12	P5	0.54	372	3.18	0.35	9.09	14.82	yes
Ex. 13	P5	0.3	372	4.52	1.26	3.59	5.15	yes
Ex. 14	P5	0.36	372	3.04	0.29	10.48	10.15	yes
Ex. 15	P5	0.42	372	3.06	0.37	8.27	11.92	yes
Ex. 16	P5	0.35	372	3.05	0.26	11.73	9.89	yes
Ex. 17	P5	0.43	372	3.1	0.35	8.86	12.07	yes
Comp. Ex. J	P6	0.68	110	3.4	1.7	2	17.21	no
Comp. Ex. K	P5	0.69	372	3.09	0.55	5.62	19.37	no
Comp. Ex. L	P5	0.88	372	3.02	0.48	6.29	25.26	no
Comp. Ex. M	P5	0.23	372	2.26	2.26	1	4.91	no
Comp. Ex. N	P5	0.47	372	2.27	2.27	1	10.08	no
Comp. Ex. O	P5	0.7	372	2.27	2.27	1	15.18	no
Comp. Ex. P	P5	0.93	372	2.28	2.28	1	19.92	no

Claims

What is claimed is:

1. A solution comprising N-methylmorpholine-N-oxide (NMMO), water, and poly(α(1→3) glucan) wherein the concentration of poly(α(1→3) glucan) is in the range of 5 – 20 % by weight with respect to the total weight of the solution, wherein the poly(alpha(1→3) glucan) is characterized by a number average molecular weight (M_n) of at least 10,000 Da; and, wherein the weight ratio of NMMO to water is in the range of 12 to 1.6.

2. The solution of Claim 1 in the form of an isotropic solution.

3. The solution of Claim 1 wherein, in the poly(α(1→3) glucan), at least 90 mol-% of the repeat units in the polymer are glucose repeat units and at least 50% of the linkages between glucose repeat units are α(1→3) glycoside linkages.

4. The solution of Claim 3 wherein, in the poly(α(1→3) glucan) 100 mol-% of the repeat units in the polymer are glucose repeat units and at least 100 % of the linkages between glucose repeat units are α(1→3) glycoside linkages.

5. The solution of Claim 1 wherein the concentration of poly(α(1→3) glucan) is in the range of 10 to 15 % by weight.

6. The solution of Claim 1 wherein the number average molecular weight of the poly(alpha(1→3) glucan) is in the range of 50,000 to 70,000 Daltons.

7. A process for preparing a poly(alpha(1→3) glucan) fiber, comprising the steps of: dissolving in a mixture of N-methylmorpholine-N-oxide (NMMO) and water, 5 to 20 % by weight of the total weight of the resulting solution of poly(alpha(1→3) glucan) wherein the poly(alpha(1→3) glucan) is characterized by a number average molecular weight (M_n) of at least 10,000 Da, to form a solution, wherein the weight ratio of NMMO to water in said solution is in the range of 12 to 1.6; causing said solution to flow through a spinneret, forming a fiber thereby, using a liquid coagulant to extract the NMMO from the thus formed fiber.

8. The process of Claim 7 wherein the solution is in the form of an isotropic solution.

9. The process of Claim 7 wherein at least 90 mol-% of the repeat units in the poly(alpha(1→3) glucan) are glucose repeat units, and at least 50% of the linkages between glucose repeat units are α(1→3) glycoside linkages.

10. The process of Claim 9 wherein 100 mol-% of the repeat units , in the poly(α(1→3) glucan) are glucose repeat units and at least 100 % of the linkages between glucose repeat units are α(1→3) glycoside linkages.

11. The process of Claim 7 wherein the concentration of poly(α(1→3) glucan) in the solution is in the range of 10 to 15 % by weight.

12. The process of Claim 7 wherein the number averager molecular weight of the poly(alpha(1→3) glucan) in the solution is in the range of 50,000 to 70,000 Daltons.

13. The process of Claim 7 wherein the liquid coagulant is glacial acetic acid.

14. The process of Claim 7 wherein the liquid coagulant is a mixture of N-methylmorpholine N oxide and water having a water concentration of at least 75 % by weight.

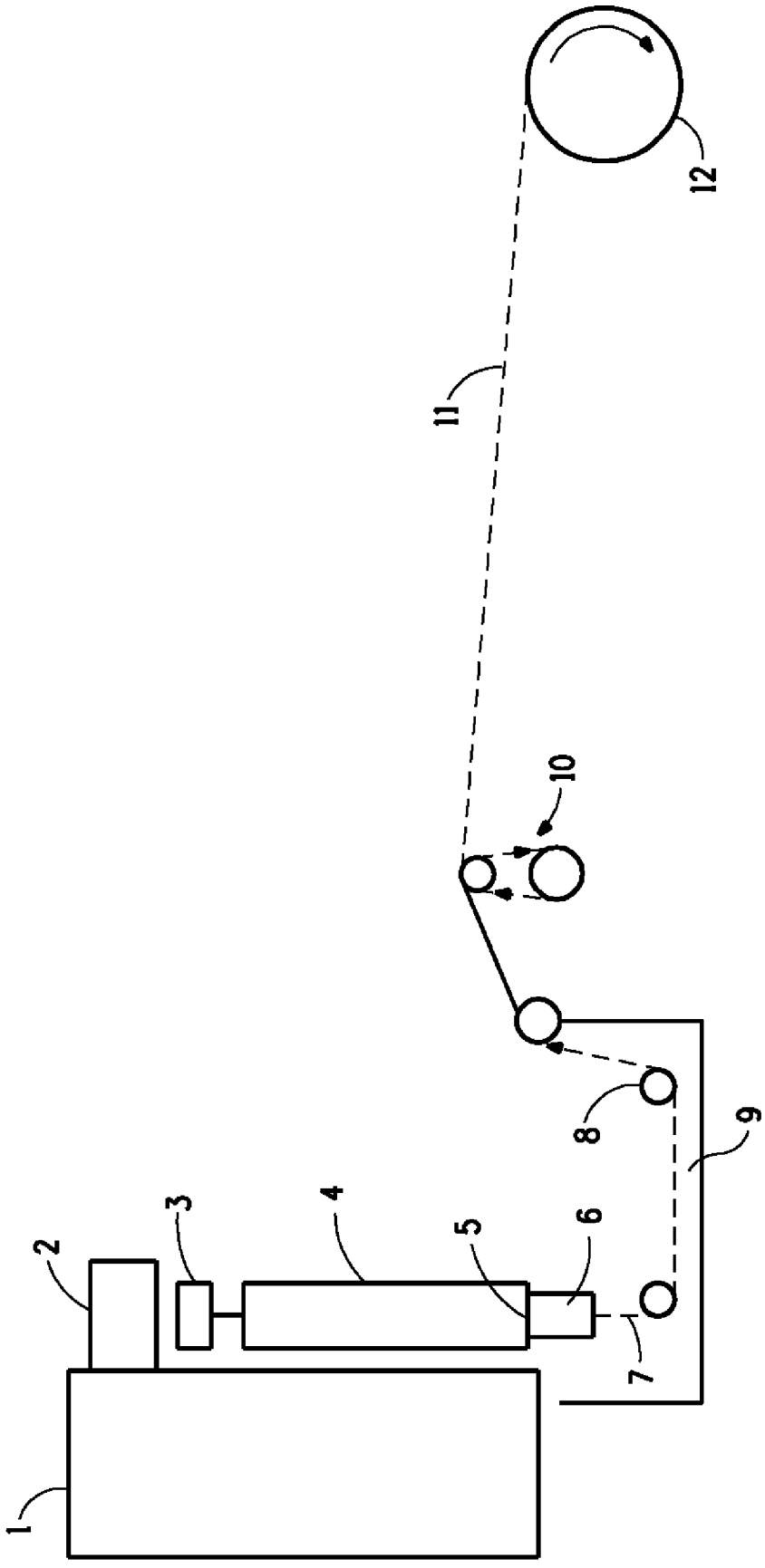


FIG. 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/058850

A. CLASSIFICATION OF SUBJECT MATTER
INV. D01F9/00 C08L5/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
D01F C08L C08B D06M A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZHANG P ET AL: "Effects of urea and sodium hydroxide on the molecular weight and conformation of alpha-(1->3)-d-glucan from Lentinus edodes in aqueous solution", CARBOHYDRATE RESEARCH, PERGAMON, GB, vol. 327, no. 4, 7 August 2000 (2000-08-07), pages 431-438, XP004213354, ISSN: 0008-6215, DOI: 10.1016/S0008-6215(00)00077-X table 1 paragraph [002.] page 431, column 2 page 435, column 1 ----- -/--	1-6

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 January 2013	19/02/2013

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/058850

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/058850

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