(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 October 2014 (02.10.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/154806 A1

(51) International Patent Classification:
C12N 5/00 (2006.01) *C09K 8/035* (2006.01)

(21) International Application Number:
PCT/EP2014/056163

(22) International Filing Date:
27 March 2014 (27.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
13161745.8 28 March 2013 (28.03.2013) EP
13168889.7 23 May 2013 (23.05.2013) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2014/154806 A1

(54) Title: PROCESS FOR PREPARING PARTIALLY DEGALACTOSYLATED XYLOGLUCAN AND ITS USE FOR OILFIELD APPLICATIONS

(57) Abstract: A method for treating an oil and/or natural gas bearing subterranean formation penetrated by at least one well bore,comprising the steps of (a) providing an aqueous treating formulation which comprises a treating composition,comprising partially degalactosylated xyloglucan having a galactose removal ratio of at least 0.40,based on the total number of β-D-galactopyranosyl residues of xyloglucan;and (b) injecting said aqueous treating formulation into at least one well bore penetrating the oil and/orgas bearing subterranean formation; thereby blocking highly permeable zones in the oil and/or natural gas bearing subterranean formation.

Process for preparing partially degalactosylated xyloglucan and its use for oilfield applications

Description

The present invention relates to a method for treating a subterranean formation penetrated by at least one well bore using partially degalactosylated xyloglucan. The invention also relates to compositions and aqueous formulations comprising said partially degalactosylated xyloglucan as well as their use in oilfield applications. The invention further relates to a process for preparing partially degalactosylated xyloglucan using an enzyme preparation comprising β-galactosidase.

Oil and/or natural gas accumulated within subterranean formations is recovered or produced therefrom through wells, called production wells, penetrating the oil and gas bearing subterranean formation. However, a large amount of the oil and/or natural gas is left in the subterranean formations if produced only by primary depletion, i.e., where only formation energy is used to recover the oil. Where the initial formation energy is inadequate or has become depleted, supplemental operations, often referred to as secondary, tertiary, or enhanced oil recovery, are employed.

In the widely used secondary oil recovery operations, a fluid is injected into the formation by pumping it through one or more injection wells penetrating the subterranean formation. The fluid, generally water or a miscible gas, is primarily employed to maintain the pressure of the reservoir and secondarily to displace additional oil from the reservoir. Thereby, oil is displaced and moved through the subterranean formation, and is produced from one or more production wells penetrating the subterranean formation. In a particular recovery operation of this sort, field water or field brine is employed as the injection fluid and the operation is referred to as water flooding. Although water is the most common fluid, injection fluids can include gaseous or supercritical fluids such as nitrogen, carbon dioxide, and the like.

Conventional water flooding is effective in obtaining additional oil and/or natural gas from oil and/or natural gas-bearing subterranean formations, but the technique does exhibit a number of shortcomings. One shortcoming is the tendency of flooding water to finger through an oil-bearing formation and thus bypass substantial portions thereof. By fingering is meant the development of unstable water fronts which advance toward the production wells more rapidly than the remainder of the flooding water. For example, the injection fluid generally flows along a low resistance route from the injection well to the production well. Accordingly the injection fluid often sweeps through geological

zones of higher permeability and bypasses lower permeability zones of the subterranean formation resulting in a non-uniform displacement of oil. Such higher permeability geological zones of the subterranean formation are commonly called thief zones or high permeability streaks.

To increase the recovery or the production of oil and/or natural gas in secondary and tertiary oil recovery operations, a substantially uniform permeability throughout the whole subterranean formation is desired.

If the formation permeability is heterogeneous, the injection fluids will seek areas of high water permeability, producing channeling and the passage of injection fluid to the producing well. As the more water-permeable zones of the subterranean formation are depleted of oil, the injection fluid has a tendency to follow such channels and increase water production, reflected in a higher water/oil ratio at the producing well. Improved diversion of water through oil bearing rock can be obtained in subterranean formations of non-uniform permeability by permeability corrections of the more water-permeable zones of the subterranean formation.

There are several strategies that can be used to reduce the water permeability of these more permeable zones of the subterranean formation. These involve mechanical blocking devices or chemicals that at least partially plug the more water-permeable zones and achieve reduced water permeability in said zones. One approach of reducing water permeability in said zones and thus increasing the recovery or the production of oil is to use a comparatively low-viscosity formulation whose viscosity rises only under formation conditions, thereby plugging a highly water-permeable zones (regions) of the subterranean formation.

Such formulations are commonly known as “thermogel” or “delayed gelling system”. Said formulation is hereinafter referred to as “delayed gelling formulation”. For plugging highly water-permeable zones of the subterranean formations, the delayed gelling formulation is injected readily into a well bore penetrating the oil and/or natural gas bearing subterranean formation, and its viscosity rises significantly after injection into the subterranean formation.

Two families of delayed gelling formulations are typically used, delayed inorganic gelling formulations and delayed (organic) polymer gelling formulations. Delayed inorganic gelling formulations typically contain a metallic or silicate salt and an activator. The transformation to a gel occurs when the pH of the formulation is modified by reaction of the activator. This process is also triggered by time and temperature and can also be delayed to allow sufficient time for placement into the target zone of the subterranean

formation. Delayed polymer gelling formulations typically contain an acrylamide polymer and a cross linker. The transformation to a gel occurs when the polymer is cross-linked. This process is triggered by time and temperature and can be delayed to allow sufficient time for placement into the target zone of the subterranean formation.

In one approach of reducing water permeability and thus increasing recovery or production of oil, a delayed gelling formulation is injected under pressure into at least one injection well of the oil-bearing subterranean formation. The delayed gelling formulation injected through an injection well tends to sweep through higher permeability zones of the subterranean formation and does not uniformly flow through the lower permeability zones as said formulation naturally follows lower resistance paths to the production well(s). Therefore, the delayed gelling formulation flows preferentially through permeable zones depleted of oil, the so called thief zones.

After the transformation to a gel, the once highly water-permeable zone of the subterranean formation is plugged. As a result, the injection water is forced again to flow through the oil-saturated, low permeability zones of the subterranean formation. Such an approach is known as 'conformance control'. Background information on conformance control can be found in Borling *et al.* "Pushing out the oil with Conformance Control", Oilfield Review 1994, 44.

In another approach, a delayed gelling formulation is injected under pressure into at least one production well penetrating the oil and/or natural gas bearing subterranean formation. As delayed gelling formulations, the same formulations can be used as for the above described injection into the injection well. This approach is also called conformance control or permeability modification. However, it is also frequently called 'water shut off' since its ultimate objective is to shut off the water or at least decrease the water/oil ratio at the production well. General background information on water shut off can be found in Bailey et al. 'Water control', Oilfield Review, 2000, 30.

US 4,844,168 discloses a process for plugging sections of high-temperature mineral oil formations, in which polyacrylamide and a polyvalent metal ion, for example Fe(III), Al(III), Cr(III) or Zr(IV), are forced into a mineral oil formation having a reservoir temperature of at least 60 °C. Under the conditions in the formation, some of the amide groups $-CONH_2$ hydrolyze to -COOH groups, the metal ions crosslinking the -COOH groups formed so that a gel is formed with a certain time lag.

US 2008/0035344 discloses a method for plugging zones in underground formations using delayed gelling formulations, which comprises at least one acid-soluble cross-linkable polymer, for example partly hydrolyzed polyacrylamide; a partially neutralized

aluminum salt, for example an aluminum hydroxychloride; and an activator which can liberate bases under formation conditions, such as, for example, urea, substituted ureas or hexamethylenetetramine. The formulation gels at temperatures above 50 °C within 2 h to 10 d, depending on conditions of use.

RU 2339803 discloses a two-step process for plugging highly permeable zones in subterranean formations. In a first process step, an aqueous formulation of carboxymethylcellulose and chromium acetate as a crosslinking agent is injected. In a second step, an aqueous formulation of polyacrylamide and a crosslinking agent is injected.

L. K. Altunina et al., Oil & Gas Science and Technology-Rev. 2008, 63, 37-48, describe various thermogels and their use for oil production, including thermogels based on cellulose ethers.

Besides delayed gelling formulations it is also possible to use preformed gels like, for example, preformed particle gels, microgels or bright water.

The application of xyloglucan as additive for fluids in oil field applications was already proposed in US 3,480,511. Various other patent documents have disclosed the use of xyloglucan (frequently called tamarind or tamarind gum) as thickening or gelling agent in different oil field applications (see for example US 2009/0149353, US 2009/0093382, WO 2007/031722 and WO 2005/014754).

The use of substituted xyloglucan in oil field applications is mentioned in US 2007/261848 which discloses a loss circulation fluid, comprising an alkali metal formate and a carboxymethyl-tamarind gum as thermally activated self-crosslinkable gel forming material for oil field drilling and completion operations.

US 2006/0142165 discloses a method of treating subterranean formations penetrated by a well bore using treating fluids comprising sulfonated tamarind gum as gelling agent polymer. WO 2007/058814 discloses the use of cationized tamarind gum in well serving fluid compositions. The cationized tamarind gum is thereby prepared by chemical means, e.g. quaternization with various quaternary amine compounds containing reactive chloride or epoxide sites. US 7,271,133 discloses methods of treating subterranean formations using esterified and etherified tamarind gums as gelling agent polymers.

A. K. Andriola et al., Carbohydrate Polymers 2010, 555-562 disclose the production of xyloglucans having different galactose removal ratios by reacting a 2 wt.-% aqueous solution of xylo-glucan with an enzyme preparation comprising β-galactosidase from

Aspergillus oryzae. The enzyme preparation is thereby used as received from supplier. The so observed partially degalactosylated xyloglucans has significantly shorter back bone chain lengths than xyloglucan itself. In general, the higher the galactose removal ratio of the xyloglucan, the shorter becomes the back bone chain length.

However, many compositions or formulations proposed for conformance control are deemed environmentally unacceptable due to their high toxicity, poor biodegradability and enrichment ability. It is also to be expected that public concerns about ground water contamination, mishandling of waste and health effects might become a factor of even greater importance. Thus, it is highly desirable to provide improved non-toxic, biodegradable compositions and formulations that can be used in methods for treating subterranean oil and gas bearing formations penetrated by at least one wellbore.

Therefore, it is an object of the present invention to provide treating compositions and aqueous treating formulations that can be used in methods for treating subterranean oil and/or natural gas bearing formations penetrated by at least one well bore. Another object of the present invention is to provide an improved method for treating a subterranean oil and natural gas bearing formation penetrated by at least one well bore. It is a further object of the present invention to provide a process for preparing said treating compositions and aqueous treating formulations.

The object of the present invention is solved by a treating composition comprising a partially degalactosylated xyloglucan having a galactose removal ratio (GRR) of at least 0.40, based on the total number of β-D-galactopyranosyl residues of xyloglucan.

Surprisingly, it has been found that the use of an aqueous treating formulation which comprises the treating composition has improved thermogelation properties compared to the same formulation comprising xyloglucan instead of the partially degalactosylated xyloglucan. For example, the aqueous treating formulation which comprises the “treating composition” displays sol-gel transition temperatures of from 0 to 100 °C, while the same formulation comprising xyloglucan instead of the partially degalactosylated xyloglucan does not undergo sol-gel transition at all.

Furthermore, the aqueous treating formulation comprising the treating composition also displays higher gel-strenghts and higher viscosities than the same formulation comprising xyloglucan instead of the partially degalactosylated xyloglucan at temperatures of from 0 to 100 °C. At the same time, the partially degalactosylated xyloglucan is equally as good biodegradable and non-toxic as xyloglucan. The thermo-gelation properties allow oil and natural gas-field applications, especially in conformance control.

The treating composition comprises at least the partially degalactosylated xyloglucan and optionally additives like, for example, biocides. The partially degalactosylated xyloglucan comprises a back bone consisting essentially of 1,4-linked β-D-glucopyranose residues like cellulose. Said back bone is hereinafter referred to as xyloglucan back bone. The 1,4-linked β-D-glucopyranose residues of the xyloglucan back bone are partially substituted by 1,6-linked α-D-xylopyranose residues which themselves may be partially substituted by 1,2-linked β-D-galactopyranose residues or, more rarely, α-L-arabinofuranose residues. Furthermore, said β-D-galactopyranose residues can themselves be further substituted by 1,2-linked L-fucopyranose residues.

It is known to the person skilled in the art that the partially degalactosylated xyloglucan may comprise traces of other pyranose residues, furanose residues and the like besides the ones mentioned above. This also applies to the xyloglucans mentioned hereinafter.

The expression 'consists essentially of" means that the xyloglucan back bone consists of more than 90 %, preferably more than 95 %, even more preferred more than 98 %, often more than 99 % by weight of the 1,4-linked β-D-glucopyranose residues.

The partially degalactosylated xyloglucans are generally composed of one out of three different types of repeating units which are all linked through 1,4-glycosidic bonds. However, the number of repeating units may vary and their pyranose or furanose substituents, or the like may differ. One of said three different types of repeating units consists of four consecutive 1,4-linked β-D-glucopyranose residues, wherein two consecutive 1,4-linked β-D-glucopyranose residues are substituted by 1,6-linked α-D-xylopyranose residues which themselves may be further substituted as shown below, and another two consecutive 1,4-linked β-D-glucopyranose residues are unsubstituted.

The second type of repeating unit consists of five consecutive 1,4-linked β-D-glucopyranose residues, wherein two consecutive 1,4-linked β-D-glucopyranose residues are similarily substituted as the ones of type one, and another three consecutive 1,4-linked β-D-glucopyranose residues remain unsubstituted.

The third type of repeating unit consists of four consecutive 1,4-linked β-D-glucopyranose residues, wherein three consecutive 1,4-linked β-D-glucopyranose residues are substituted with 1,6-linked α-D-xylopyranose residues which themselves may be further substituted; and one 1,4-linked β-D-glucopyranose residue remains unsubstituted.

The substitution pattern and the length of the xyloglucan back bone of the partially degalactosylated xyloglucan depends on the plant source of which the xyloglucan has been isolated. The length of the xyloglucan back bone is best expressed by the average number of repeating units (m) and the average molecular weight. The partially degalactosylated xyloglucans have typically a glucopyranose (Glc*p*) : xylopyranose (Xyl*p*) : galactopyranose (Gal*p*) : fucopyranose (Fuc*p*) : arabinofuranose (Ara*f*)-ratio of 4 : 2.1 to 3.3 : 0.0 to 1.7 : 0.0 to 0.6 : 0.0 to 0.6.

The partially degalactosylated xyloglucan according to the present invention has preferably an average molecular weight of from 200 000 to 1 500 000 Da.

Having average molecular weights lower than 200 000 Da, the partially degalactosylated xyloglucan does not have thermogelation properties at all. But with increasing average molecular weight, the viscosity and the gelation strength of the partially degalactosylated xyloglucan increase as well, resulting in a decreased requirement of the partially degalactosylated xyloglucan to achieve the desired viscosity and gelation strength in the aqueous treating formulation. Thus, the partially degalactosylated xyloglucan has more preferred an average molecular weight of from 400 000 to 1 500 000 Da, even more preferred of from 600 000 to 1 500 000 Da, even more preferred of from 800 000 to 1 500 000 Da, most preferred of from 1 00 000 to 1 500 000 Da.

The average molecular weight can be determined by conventional methods, e.g. field flow fractionation (FFF). The average molecular weights given herein have been determined by FFF. Details about FFF can be found, for example, in B. Roda et al. Analytica chimica acta 2009, 635, 132-143, and the literature cited therein.

As contaminants may negatively affect the thermogelation properties of the partially degalactosylated xyloglucan and, thus, the treating composition, the partially degalactosylated xyloglucan has preferably a purity of at least 80 % by weight based on the total weight of the partially degalactosylated xyloglucan.

The partially degalactosylated xyloglucan has more preferred a purity of at least 90 %, even more preferred of at least 95 %, most preferred of at least 98 % based on the total weight of the partially degalactosylated xyloglucan.

The partially degalactosylated xyloglucan has preferably a galactose removal ratio of from 0.40 to 0.90. At higher galactose removal ratios, the partially degalactosylated xyloglucan reveals gelation over a broader temperature range. Moreover, the higher the galactose removal ratio, the higher becomes the viscosity and the gelation strength of the partially degalactosylated xyloglucan. As a result, lower concentrations of partial-

ly degalactosylated xyloglucan are required to achieve the desired viscosity and gelation strength. Thus, the degalactosylated xyloglucan has more preferred a galactose removal ratio of from 0.43 to 0.80, even more preferred of from 0.48 to 0.70, most preferred of from 0.50 to 0.60. The galactose removal ratio (GRR) was determined as:

GRR = (amount of galactose residues in xyloglucan – amount of galactose residues in the partially degalactosylated xyloglucan) / amount of galactose residues in xyloglucan

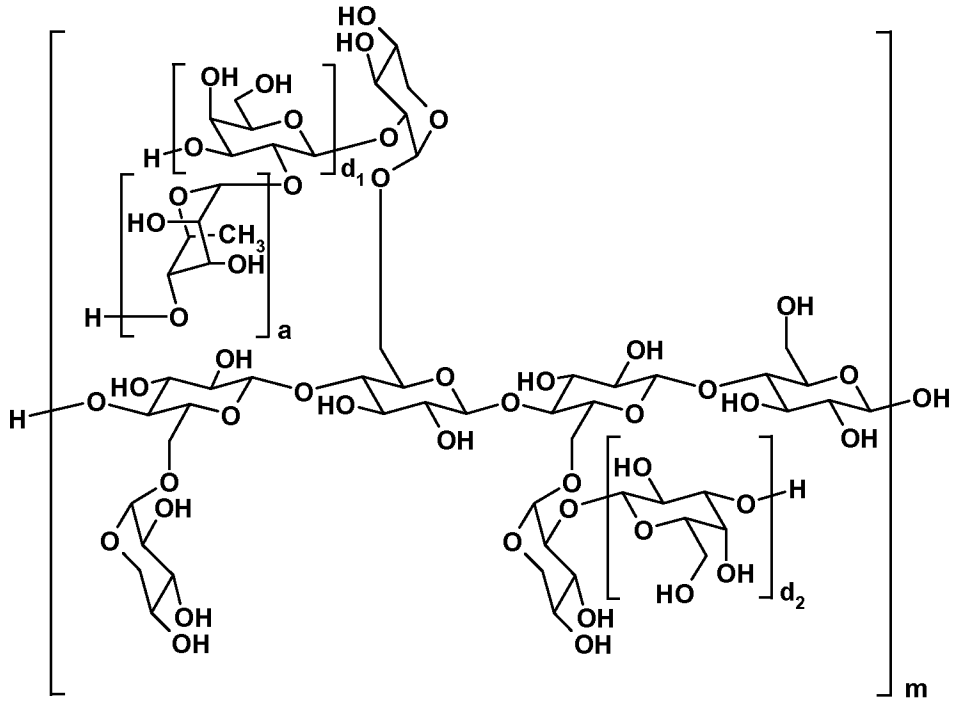
The amount of galactose residues in xyloglucan and the amount of galactose residues in the partially degalactosylated xyloglucan was measured after total hydrolysis by heating the polysaccharides in 2 N sulfuric acid at 100 °C for 3 h according to M. Shirakawa *et al.* (M. Shirakawa et al., Food Hydrocolloids 1998, 12, 25-28).

The thermogelation properties of the aqueous treating formulation comprising the treating composition was evaluated by rheological experiments with Anton Paar MCR Rheometers. Temperature sweep experiments were done in a temperature range between 0°C and 140°C. A sealed geometry (pressure cell) was used with a double gap geometry. Measurements were carried out at a constant shear rate of 10 $s^{-1}$with a heating rate of 0.5 °C/min.

Gel kinetic experiments were done in a concentric cylinder geometry with small amplitude oscillation shear (SAOS) measurements at 1 Hz with a deformation of 5%. The geometry was set to the particular measurement temperature before the sample was filled. The initial sample temperature was about 4°C. Right after the sample was filled into the geometry, the sample was covered with silicone oil. Silicone oil was used to prevent evaporation and salt crust formation at elevated temperatures. In order to have identical conditions, this procedure was kept constant for all kinetic measurements at all temperatures. Due to the overall handling procedure there was a time delay of about 60-90 seconds till the first data point could be collected.

The average number of repeating units (m) of the partially degalactosylated xyloglucan depends on the average molecular mass, the type of repeating unit of the xyloglucan used as well as the galactose removal ratio. The average number of repeating units (m) can easily be calculated from the average molecular mass, the galactose removal ratio and the average mass per repeating unit. The average number of repeating units (m) is from 200 to 1400. Preferably, the average number of repeating units (m) is from 300 to 1400. The average number of repeating units (m) is more preferred from 500 to 1400, even more preferred from 700 to 1400, most preferred from 900 to 1400.

In one particularly preferred embodiment of the present invention, the partially degalactosylated xyloglucan is partially degalactosylated tamarind xyloglucan having general formula (I),



(I)

wherein
the average number of the β-D-galactopyranose residues per repeating unit ($d_1 + d_2$) is from 0.20 to 1.20,
the average number of the α-L-fucopyranose residues per repeating unit (a) is from 0.00 to 0.20, and
the average number of the repeating units (m) is from 200 to 1400.

The treating composition comprises the partially degalactosylated xyloglucan as defined above, and optionally additives like, for example, one or more biocides to avoid degradation, especially degradation of the xyloglucan back bone.

In one embodiment of the present invention, the treating composition comprises the partially degalactosylated xyloglucan as defined above, and one or more biocides.

In another embodiment of the present invention, the treating composition consists of the partially degalactosylated xyloglucan as defined above.

In another embodiment of the present invention, the treating composition is stored and transported in the form of a concentrated aqueous formulation comprising at least 20 % by weight of the treating composition based on the total weight of said concentrated aqueous formulation.

The object of the present invention is further solved by an aqueous treating formulation which comprises the treating composition as defined above.

It has been found that the sol-gel transition temperature as well as the viscosity and the gel-strength of the aqueous treating formulation, comprising the treating composition as defined above, can easily be fine tuned by variation of the amount of salt and the amount of treating composition present in the aqueous treating formulation. This allows the use of the same treating composition for several applications, such as for example, conformance control at different formation temperatures.

The aqueous treating formulation comprises preferably from 0.01 to 20.00 % by weight of the treating composition as defined above, based on the total weight of the aqueous treating formulation. The amount of treating composition present in the aqueous treating formulation affects mainly the viscosity and the gel strength while the sol-gel transition temperature remains almost unaffected. The higher the proportional amount of the treating composition in the aqueous treating formulation, the higher becomes the viscosity and the gel strength.

The aqueous treating formulation comprises more preferred from 0.02 to 10.00 % by weight, even more preferred from 0.05 to 7.00 % by weight, most preferred from 0.1 to 5.00 % by weight of the treating composition as defined above, based on the total weight of the aqueous treating formulation.

The aqueous treating formulation comprises at least the treating composition as defined above and water. The water can be obtained from any acceptable source, and may include brine, fresh water, sea water and the like.

Preferably, the aqueous treating formulation comprises the treating composition and one or more salts. The salt or the salts may already be present in the water and/or may be additionally added into the water. Where used, the aqueous treating formulation comprises of from 0.001 to 40.00 % by weight of one or more salts based on the total weight of the aqueous treating formulation. The aqueous treating formulation comprises more preferred of from 0.02 to 30.00 % by weight, even more preferred of from 0.05 to 20.00 % by weight, most preferred of from 0.5 to 15.00 by weight of one or more salts based on the total weight of the aqueous treating formulation.

The salt content strongly influences the sol-gel transition temperature while the viscosity and the gel strength remain almost unaffected. As a rule, the higher the content of salt, the higher becomes the sol-gel transition temperature. Thus, the lowest sol-gel transition temperature is usually achieved if the water is deionized water. Accordingly, the highest sol-gel transition temperature can usually be achieved at a very high content of salt.

Preferably, the aqueous treating formulation comprises the treating composition and one or more additionally added salts. In general, all kinds of salt may be used as additionally added salt. Preferred additionally added salts are selected from the group consisting of $Al_2(SO_4)_3$, ethylene diamine tetra acetic acid trisodium salt (Na_3EDTA), cinnamic acid sodium salt, $NaBO_2$, $Na_2B_4O_7$, NaCl, $CaCl_2$, $AlCl_3$, $FeSO_4$, $FeCl_3$ and NDIIa.

In a particularly preferred embodiment of the present invention, the one or more additionally added salts are selected from salts having divalent cations. It has been found that the type of salt present in the aqueous treating formulation influences the gel strength and viscosity as well. Accordingly, the higher the ratio of salts having bivalent cations to salts having monovalent cations, the higher becomes the gelling strength and viscosity.

If said salts having monovalent, and divalent and/or trivalent cations are additionally added, their concentration in the aqueous treating formulation is in general of from 0.001 % to 30.0 % by weight based on the total weight of the aqueous treating formulation. Preferably, the additionally added one or more salts are present in concentrations of from 0.05 % to 15.0 % by weight based on the total weight of the aqueous treating formulation.

Preferably, the aqueous treating formulation further comprises one or more acids. More preferred, the aqueous treating formulation further comprises one or more acids selected from the group consisting of kaffeic acid, *trans*-ferulic acid, tannic acid, 2,4-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, boric acid, 2,4,6-trihydroxybenzoic acid, 2,4,6-trihydroxybenzoic acid, cinnamic acid and ricinoleic acid.

Where used, such acids are present in the aqueous treating formulation in concentrations of from 0.001 % to 10.0 % by weight based on the total weight of the aqueous treating formulation. The aqueous treating formulation comprises preferably of from 0.01 to 8.0 % by weight, more preferred of from 0.05 to 5.0 % by weight, most preferred of from 0.05 to 3.0 by weight of such acids based on the total weight of the aqueous treating formulation.

The aqueous treating formulation can further comprise one or more biocides, *inter alia*, to protect both the subterranean formation as well as the aqueous treating formulation from attack by bacteria or fungi. Such attacks may be problematic because they may lower the molecular weight of the partially degalactosified xyloglucan resulting in lower viscosity of the aqueous treating formulation and thus in poorer performance, such as poorer proppant suspension properties, for example. Suitable biocides are known in the art. A person skilled in the art with the benefit of this disclosure will be able to identify suitable biocides for a given application. Suitable biocides include, for example, formaldehyde, sodium hypochlorite, 2,2-dibromo-3-nitrilo-propion-amide, 2-bromo-2-nitro-1,3-propanediol and glutardialdehyde. Preferred biocides are formaldehyde and glutardialdehyde.

Where used, such biocides are present in concentrations sufficiently high to destroy all bacteria and fungi that may be present. Said biocides are usually present in the aqueous treating formulation in concentrations of from 0.001 % to 0.50 % by weight based on the total weight of the aqueous treating formulation. Such biocides are preferably present in concentrations of from 0.001 % to 0.10 % by weight, more preferred of from 0.001 % to 0.01 % by weight based on the total weight of the aqueous treating formulation.

The aqueous treating formulation can further comprise one or more other additives, including those which are common additives in well bore treating. Preferred additives are selected from the group consisting of lignin, chitosan, hydrochinon, hydroxyhydrochinon, sucrose, guar gum, xanthan, schizophyllan, epigalocatechin gallate, pyrogallol, pectin, Bretax C®, Mimosa ME and Tamol® NNOL.

Where used, such additives are present in the aqueous treating formulation in concentrations of from 0.001 % to 15.0 % by weight based on the total weight of the aqueous treating formulation. The aqueous treating formulation comprises preferably of from 0.01 to 10.0 % by weight, more preferred of from 0.05 to 7.0 % by weight, most preferred of from 0.05 to 5.0 by weight of such additives based on the total weight of the aqueous treating formulation.

In a particular embodiment of the present invention, the aqueous treating formulation comprises

(A) 0.5 to 7.0 % by weight of the treating composition as defined above,

(B) 61.0 to 99.249 % by weight of water,

(C) 0.2 to 30.0 % by weight of one or more salts,

(D) 0.05 to 7.0 % by weight of one or more other additives, and

(E) 0.001 to 0.50 % by weight of one or more biocides,

wherein the sum of (A), (B), (C), (D) and (E) adds up to 100 % by weight based on the total weight of the aqueous treating formulation.

The object of the present invention is further solved by a method for treating an oil and natural gas bearing subterranean formation penetrated by at least one well bore comprising the steps of

(a) providing an aqueous treating formulation as defined above, and

(b) injecting said aqueous treating formulation into at least one well bore penetrating the oil and gas bearing subterranean formation.

Regarding the method according to the present invention, the following may be stated specifically:

For carrying out the method according to the present invention at least one well bore had to be sunk in the subterranean formation. This is usually a well bore which had already been used in an earlier stage of oil or natural gas production, for example in the course of water flooding.

According to the invention, the subterranean formation is one which has a minimum temperature of 0 °C, preferably 10 °C, more preferred 20 °C, most preferred 30 °C, and a maximum temperature of 150 °C, preferably of 140 °C, more preferred of 130 °C, most preferred 120 °C.

According to the present invention, the method comprises at least the two steps (a) and (b).

In step (a), the aqueous treating formulation as defined above is provided. The aqueous treating formulation is thereby provided, for example, by mixing water with the treating composition and optionally one or more further components as defined. The treating composition can thereby be mixed in the form of a solid or a concentrated aqueous formulation as defined above. The aqueous treating formulation is typically

transparent in the case of relatively low concentration of the treating composition and becomes opalescent or milky at higher concentrations. In any case, the aqueous treating formulation may be handled as a liquid, which greatly simplifies its preparation and use.

Preferably, one or more biocides are admixed with water before the treating composition as defined above is added.

In step (b), the aqueous treating formulation is injected into at least one well bore penetrating the oil and/or natural gas bearing subterranean formation. The injection of the aqueous treating formulation can be undertaken by means of customary apparatus. Said formulation can be injected into one or more injection wells or into one or more production wells by means of customary pumps.

Said wells are often lined with steel tubes cemented in place in the region of an oil and/or natural gas bearing subterranean formation, and the steel tubes are perforated at the desired point. In a manner known by the one skilled in the art, the pressure applied by means of the pumps fixes the flow rate of the aqueous treating formulation and hence also the shear stress with which the aqueous treating formulation enters the subterranean formation. The shear stress on entry into the subterranean formation can be calculated by the person skilled in the art in a manner known in principle on the basis of the Hagen-Poiseuille law using the flow area on entry into the formation, the mean pore radius and the volume flow rate. The average permeability or porosity of the formation can be determined in a manner known in principle by measurements on drill cores. Of course, the greater the volume flow rate of aqueous formulation injected into the formation, the greater the shear stress.

The rate of injection can be fixed by the person skilled in the art according to the properties and the requirements of the subterranean formation (number of injectors, configuration thereof, etc.).

Preferably, the shear rate on entry of the aqueous treating formulation into the subterranean formation is at least 30 000 s^{-1}, preferably at least 60 000 s^{-1} and more preferred at least 90 000 s^{-1}.

One aspect of the present invention is the use of the treating composition or the aqueous treating formulation as defined above for oil-field applications. Preferably, the oil-field application is conformance control.

Conformance control is a measure in which, for increasing the oil and/or natural gas production, highly permeable zones of the subterranean formation are plugged by injecting, for example, an aqueous gelling formulation which, after being forced into a well bore, forms a highly viscous gel under the influence of the temperature of the subterranean formation. As a result, highly permeable zones having low flow resistance are plugged and the flooding water flow again through the oil and/or gas saturated zones.

The term “gelling” means that the formulation can in principle form gels under certain conditions but that the gel formation does not begin immediately after the mixing of the components of the formulation. Instead, the formation of a gel is delayed and only starts once the gel formation temperature is achieved. It is clear for the person skilled in the art that the speed of gel formation may depend as a rule both on the time and on the temperature. The person skilled in the art can determine the gel formation temperature exactly by measuring the gel formation speed of a certain formulation as a function of the temperature, followed by an extrapolation of the measured curve to a reaction rate at zero. In a pragmatic approach, the person skilled in the art can define the gel formation temperature approximately as the onset of gel formation after a time span relevant in practice. All that is important is that for comparison of the gel formation temperatures of the formulations used in each case, the same method for determining the gel formation temperature is used in each case.

The method according to the present invention can be employed especially in the case of oil and natural gas-bearing subterranean formations with an average permeability of around 100 mD to around 5 D (around 1.0×10^{-13} m² to around 50×10^{-13} m²), preferably around 150 mD to around 2 D (around 1.5×10^{-13} m² to around 20×10^{-13} m²), and more preferably around 200 mD to around 1 D (around 2.0×10^{-13} m² to around 10×10^{-13}).

The permeability of an oil and natural gas-bearing subterranean formation can be determined from the flow rate of a liquid phase in the oil and natural gas-bearing subterranean formation as a function of the pressure differential applied. Details thereof can be found, for example, in K. Weggen, G. Pusch, H. Rischmüller in “Oil and Gas“, pages 37 ff., Ullmann’s Encyclopedia of Industrial Chemistry, Online edition, Wiley-VCH, Weinheim 2010.

Methods for temperature determinations of a subterranean formation are known in principle to those skilled in the art. The temperature is generally undertaken from temperature measurements at particular sites in the formation.

The aqueous treating formulation as defined above may be prepared and provided on the surface and then pumped through tubing into the well bore. While a high viscosity, high gelling strength formulation is highly desirable after the formulation is positioned in the highly permeable zone of the subterranean formation, large amounts of energy are required to pump such formulations through tubing into the formation. Therefore, delayed gelling is desired since it reduces the amount of energy required to pump the aqueous treating formulation through the tubing by permitting pumping of a relatively less viscous formulation having relatively low friction pressures within the well tubing.

Gelling is typically affected when the aqueous treating formulation is placed in the highly permeable zone of the subterranean formation after which the advantageous properties are then available for plugging the permeable zone. Advantageous is a high viscosity and a high gelling strength at the specific temperature of the subterranean formation.

One embodiment of the present invention is therefore a method for treating a subterranean oil and natural gas bearing formation penetrated by at least one well bore comprising the steps of

(a) providing an aqueous treating formulation as defined above,

(b) injecting said aqueous treating formulation into at least one well bore penetrating the oil and natural gas bearing subterranean formation, and

(c) gelling of said aqueous treating formulation under the influence of the temperature of the oil and natural gas bearing subterranean formation.

In a preferred embodiment of the present invention, the aqueous treating formulation is injected in step (b) into at least one well bore, wherein the well bore is an injection well penetrating the oil and natural gas bearing subterranean formation. Such an approach is called conformance control.

In another preferred embodiment of the present invention, the aqueous treating formulation is injected in step (b) into at least one well bore, wherein the well bore is a production well penetrating the oil and gas bearing subterranean formation. Such an approach is also called conformance control or permeability modification. However, it is also frequently called'water shut of'.

The aqueous treating formulation as defined above should be approached on a specific project basis to meet objective in terms of sol-gel transition temperature, gel strength

and viscosity. The sol-gel transition temperature of the aqueous treating formulation is thereby especially dictated by the total amount of salt present in said formulation and can also be affected by the galactose removal ratio of the partially degalactosylated xyloglucan. Typically, the lower the total salt content of the aqueous treating formulation and the higher the galactose removal ratio of the partially degalactosylated xyluglucan, the lower is the sol-gel transition temperature of said formulation. Consequently, the sol-gel transition temperature of the aqueous treating formulation can be very precisely adapted to the formation temperature. Most important, however, is the chain length of the xyloglucan back bone of the partially degalactosylated xyloglucan. The longer the chain length of the xyloglucan back bone of the partially degalactosylated xyloglucan, the higher is the gelling strength and viscosity of the aqueous treating formulation.

In one aspect of the present invention, the at least one well bore penetrating the oil and/or natural gas bearing subterranean formation is, after plugging the permeable zone of the subterranean formation, water flooded to extract or produce oil and natural gas on at least one production well. To execute the method, at least one production well and at least one injection well were sunk into the subterranean formation. In general, an oil and natural gas-bearing formation is provided with several injection wells and several production wells. The term “oil” in this context does not mean only single-phase oil; instead, the term also comprises the customary crude oil-water emulsions. By virtue of the pressure generated by injection of the fluid, the oil and/or natural gas flows in the direction of the production well and is produced via the production well.

In this aspect of the present invention, the method for treating a subterranean oil and natural gas bearing formation penetrated by at least one well bore comprises the steps of

(a) providing an aqueous treating formulation as defined above,

(b) injecting said aqueous treating formulation into at least one injection well penetrating the oil and natural gas bearing subterranean formation,

(c) gelling of said aqueous treating formulation under the influence of the temperature of the subterranean formation, and

(d) water flooding at least one injection well penetrating the oil and natural gas bearing subterranean formation to produce oil and natural gas on at least one production well.

After method steps (a), (b) and (c) the oil and natural gas production is continued through at least one production well. Preferably, the oil and natural gas production can be effected by customary methods by injecting a flooding medium through at least one injection well into the oil and natural gas bearing subterranean formation and producing oil and natural gas through at least one production well. The flooding medium may be in particular water or carbon dioxide. The at least one injection well may be the injection well(s) used for injecting the formulations or suitably arranged other injection wells. However, the oil and natural gas production can of course also be continued by means of other methods known in the art. For example, microorganisms which develop methane or carbon dioxide in the subterranean formation can be used and the pressure can be maintained in this manner. Furthermore, highly viscous formulations of thickening polymers, such as, for example, polyacrylamide or copolymers comprising acrylamide, or certain polysaccharides can be used.

In another aspect of the present invention, the at least one well bore penetrating the oil and natural gas bearing subterranean formation is, after plugging the permeable zone of the subterranean formation, used for the production of oil and natural gas, whereas at least one injection well is water flooded at the same time. To execute the method, at least one production well and at least one injection well were sunk into the subterranean formation. By virtue of the pressure generated by the fluid injection, the oil and natural gas flows in the direction of the production well and is produced via the production well. In this aspect of the present invention, the method for treating a subterranean oil a natural gas bearing formation penetrated by at least one well bore comprises the steps of

(a) providing an aqueous treating formulation as defined above,

(b) injecting said aqueous treating formulation into at least one production well penetrating the oil and natural gas bearing subterranean formation,

(c) gelling of said aqueous treating formulation under the influence of the temperature of the subterranean formation, and

(d) producing oil and natural gas on at least one production well penetrating the oil and natural gas bearing subterranean formation by water flooding at least one injection well.

A particular aspect of the present invention is the use of the treating composition or the aqueous treating formulation as defined above for oil-field applications, wherein the oil-field applications are conformance control measures or water shut off measures.

The object of the present invention is further solved by a process for preparing the partially degalactosylated xyloglucan as defined above which comprises the steps of

(a) providing an aqueous xyloglucan preparation, and

(b) contacting said aqueous xyloglucan preparation with an enzyme preparation comprising β-galactosidase being capable of removing galactose from xyloglucan.

β-galactosidases (E.C. 3.2.1.23) themselves do not degrade the xyloglucan backbone consisting essentially of 1,4-linked β-D-glucopyranose residues. If β-galactosidases are sufficiently pure, they are capable of catalyzing the hydrolysis of β-D-galactopyranosyl-(1→2)-β-D-xylopyranosyl linkages within the xyloglucan without affecting the xyloglucan backbone. As a consequence, partially degalactosylated xyloglucans were formed having high average molecular weights and, thus, improved thermogelation properties.

The term 'without significantly affecting the chain length of the xyloglucan back bone' means that the average number of repeating units of the partially degalactosylated xyloglucan (m) is almost as high or equally high as the average number of repeating units of the xyloglucan (n) used for its preparation.

Regarding the process according to the present invention, the following may be stated specifically:

According to the present invention, the process comprises at least the two steps (a) and (b). In step (a) of the process, an aqueous xyloglucan preparation is provided. Said preparation can comprise xyloglucan isolated from one species; or mixtures of two, three, four or more xyloglucans isolated from different species.

Xyloglucans are widespread in nature. They belong to a group of polysaccharides typically referred to as hemicelluloses and can be found in primary cell walls of different plants, such as for example plants belonging to the class dicotyledons and plants belonging to the sub-class non-graminacious monocotyledons.

A few among these plants (all of which are dicotyledons) use xyloglucan also as a carbohydrate reserve instead of the most common carbohydrate reserve starch. Seeds of these plants have thick cell walls containing vast quantities of xyloglucan.

Examples of said plants are flowering plants of the genus *Nasturtium*, such as *Nasturtium africanum, Nasturtium floridanum, Nasturtium gambelii, Nasturtium microphyllum, Onerow yellowcress* and *Nasturtium officinale*; flowering plants of the genus *Impatiens*, such as *Impatiens balfourii, Impatiens balsamina, Impatiens capensis, Impatiens edgeworthii, Impatiens glandulifera, Impatiens hians, Impatiens marianae, Impatiens niamniamensis, Impatiens noli-tangere, Impatiens parviflora Impatiens platypetala, Impatiens repens*; flowering plants of the genus *Annonas*, such as *Annona amambayensis, Annona acuminata, Annona ambotay, Annona asplundiana, Annona atabapensis, Annona bullata,, Annona biflora, Annona bicolor, Annona brasililensis, Annona cacans, Annona calophylla, Annona campestris, Annona cherimola, Annona chrysophylla, Annona pubescens, Annona tripetala, Annona conica, Annona coriacea, Annona cornifolia, Annona crassiflora, Annona cristalensis, Annona crotonifolia, Annona deceptrix, Annona deminuta, Annona dioica, Annona diversifolia, Annona dolabripetala, Annona dolichophylla, Annona echinata, Annona ecuadorensis, Annona ekmanii, Annona excellens, Annona glabra, Annona palustris, Annona glaucophylla, Annona haematantha, Annona hayesii, Annona hypoglauca, Annona hystricoides, Annona jahnii, Annona jamaicensis, Annona longiflora, Annona lutescens, Annona macrocalyx, Annona malmeana, Annona manabiensis, Annona microcarpa, Annona montana, Annona marcgravii, Annona monticola, Annona muricata, Annona macrocarpa, Annona nitida, Annona nutans, Annona oligocarpa, Annona paludosa, Annona paraguayensis, Annona phaeoclados, Annona praetermissa, Annona purpurea, Annona pygmaea, Annona reticulata, Annona salzmannii, Annona scleroderma, Annona senegalensis, Annona sericea, Annona spinescens, Annona spraguei, Annona squamosa, Annona testudinea, Annona tomentosa, Annona trunciflora* and trees of the genus *Tamarindus* such as *Tamarindus indica.*

Xyloglucan from seeds of one of these plant genuses mentioned is hereinafter referred to as seed xyloglucan.

Xyloglucans comprise a back bone consisting essentially of 1,4-linked β-D-glucopyranose residues like cellulose. Said back bone is hereinafter referred to as xyloglucan back bone. The 1,4-linked β-D-glucopyranose residues of the xyloglucan back bone are either substituted or unsubstituted (subunit **'G'**). The 1,4-linked β-D-glucopyranose residue may be substituted by 1,6-linked α-D-xylopyranose residue (creating subunit **'X'**) which themselves may be further substituted by one or two 1,2-linked β-D-galactopyranose residues (creating subunit **'L'** or **'J'**) or, more rarely, one or two α-L-arabinofuranose residues (creating subunit **'S'** or **'T'**). Furthermore, said 1,2-linked β-D-galactopyranose residue may themself be further substituted by a 1,2-linked L-fucopyranose residue (creating subunit **'F'**).

It is known to the person skilled in the art that xyloglucans may comprise traces of other pyranose residues, furanose residues and/or the like besides the ones mentioned above. Thus, the expression 'consisting essentially of" means that the xyloglucan back bone consists of more than 90 %, preferably more than 95 %, even more preferred more than 98 %, often more than 99 % by weight of the 1,4-linked β-D-glucopyranose residues.

A single letter code is used to simplify representation of the structure of xyloglucan subunits. Said single letter code is shown below:

i. **G** = -4)-β-D-Glc*p*-(1-

ii. **X** = α-D-Xyl*p*-(1-6)-β-D-Glc*p*-(1-
4
↑

iii. **F** = α-L-Fuc*p*-(1-2)-β-D-Gal*p*-(1-2)-α-D-Xyl*p*-(1-6)-β-D-Glc*p*-(1-
4
↑

iv. **L** = β-D-Gal*p*-(1-2)-α-D-Xyl*p*-(1-6)-β-D-Glc*p*-(1-
4
↑

v. **S** = α-L-Ara*f*-(1-2)-α-D-Xyl*p*-(1-6)-β-D-Glc*p*-(1-
4
↑

vi. **T** = α-L-Ara*f*-(1-3)-α-L-Ara*f*-(1-2)-α-D-Xyl*p*-(1-6)-β-D-Glc*p*-(1-
4
↑

vii. **J** = α-D-Gal*p*-(1-2)-β-D-Gal*p*-(1-2)-α-D-Xyl*p*-(1-6)-β-D-Glc*p*-(1-
4
↑

The term 'xyloglucan subunit' used herein relates to one single substituted or unsubstituted β-D-glucopyranose residue of the xyloglucan back bone. Examples of said 'xyloglucan subunits' are **G**, **X**, **F**, **L**, **S**, **T** and **J**, as shown above.

In contrast thereto, the term 'xyloglucan individual unit' relates herein to four or five consecutive 1,4-linked β-D-glucopyranose residues of the xyloglucan back bone, wherein either two or three of said residues are further substituted with a D-xylopyranose residue which themself may be further substituted as shown above. Typical examples of such xyloglucan individual units are XXXG, XXJG, FXXG and LXLG.

Usually, the structure of xyloglucans varies among plant species and also in a tissue specific manner. Furthermore, the structure of seed xyloglucan may also depend on the seeds' maturity. Nevertheless, xyloglucans can be classified in at least three types, namely 'XXXG'-type, 'XXGG'-type and 'XXGGG'-type.

'XXXG'-type xyloglucans have repeating units consisting of three consecutive 1,4-linked β-D-glucopyranose residues, wherein each one of said glucopyranose residues is at least substituted with one 1,6-linked α-D-xylopyranose residue; and a fourth unsubstituted 1,4-linked β-D-glucopyranose residue. 'XXXG'-type xyloglucan consists essentially of individual units connected by β-1,4-glycosidic bonds, wherein the individual units are selected from the group consisting of XXXG, FXXG, LXXG, SXXG, TXXG, JXXG, XFXG, XLXG, XSXG, XTXG, XJXG, XXFG, XXLG, XXSG, XXTG, XXJG, FFXG, FLXG, FSXG, FTXG, FJXG, LFXG, LLXG, LSXG, LTXG, LJXG, SFXG, SLXG, SSXG, STXG, SJXG, TFXG, TLXG, TSXG, TTXG, TJXG, JFXG, JLXG, JSXG, JTXG, JJXG, FXFG, FXLG, FXSG, FXTG, FXJG, LXFG, LXLG, LXSG, LXTG, LXJG, SXFG, SXLG, SXSG, SXTG, SXJG, TXFG, TXLG, TXSG, TXTG, TXJG, JXFG, JXLG, JXSG, JXTG, JXJG, XFFG, XFLG, XFSG, XFTG, XFJG, XLFG, XLLG, XLSG, XLTG, XLJG, XSFG, XSLG, XSSG, XSTG, XSJG, XTFG, XTLG, XTSG, XTTG, XTJG, XJFG, XJLG, XJSG, XJTG, XJJG.

The expression 'consists essentially of' means that more than 90 %, preferably more than 95 %, more preferred more than 98 % by weight of the xyloglucan consists of the individual units selected from the corresponding group mentioned above, based on the total weight of the xyloglucan.

The term 'xyloglucan repeating unit' refers to the smallest recurring unit within the xyloglucan backbone. For example, the repeating unit of the 'XXXG'-type xyloglucans is XXXG. In this case, the backbone consists essentially of: -XXXG-XXXG-XXXG-XXXG-XXXG-XXXG- and so on, wherein the 1,4-linked β-D-glucopyranose residue (**'G'**) is unsubstituted and the 1,6-linked α-D-xylopyranose residue (**'X'**) may be further substituted randomly with the fucose and/or pyranose residues shown above.

The length of the xyloglucan is best expressed by way of the average molecular weight (M_w) and the average number of repeating units (n).

Depending on the xyloglucan source, 'XXXG'-type xyloglucan has typically a glucopyranose (Glc*p*) : xylopyranose (Xyl*p*) : galactopyranose (Gal*p*) : fucopyranose (Fuc*p*) : arabinofuranose (Ara*f*)-ratio of 4 : 2.7 to 3.3 : 1.7 to 2.3 : 0.0 to 0.5 : 0.0 to 0.5 and a weight averaged molar mass (M_w) of from 64 000 to 2 400 000 Da. Moreover, said xyloglucan has typically an average number of repeating units (n) of from 50 to 1500 which corresponds to an average number of subunits of from 200 to 6000.

More details about xyloglucan structures and methods of structure determination can be found in S.F. Fry. J. Expt. Botany 1989, 40, 1-11; A. Mishra et al., J. Mater. Chem. 2009, 19, 8528-8536; W. York et al., Carbohydr. Res. 1990, 200, 9-31; Hoffman et al., Carbohydr. Res. 2005, 340, 1826-1840; W. York et al., Carbohydr. Res. 1996, 285, 98-128; and the literature cited therein.

Similarly, 'XXGG'-type xyloglucans have repeating units consisting of two consecutive 1,4-linked β-D-glucopyranose residues, wherein each one of said glucopyranose residues is substituted with at least a 1,6-linked α-D-xylopyranose residue; and two consecutive unsubstituted 1,4-linked β-D-glucopyranose residues. 'XXGG'-type xyloglucans consist essentially of individual units connected by β-1,4-glycosidic bonds, wherein the individual units are selected from the group consisting of XXGG, FXGG, LXGG, SXGG, TXGG, JXGG, XFGG, XLGG, XSGG, XTGG, XJGG, FFGG; FLGG, FSGG, FTGG, FJGG, LFGG, LLGG, LSGG, LTGG, LJGG, SFGG, SLGG, SSGG, STGG, SJGG, TFGG, TLGG, TSGG, TTGG, TJGG, JFGG, JLGG, JSGG, JTGG, JJGG.

Depending on the xyloglucan source, 'XXGG'-type xyloglucans have typically a glucopyranose (Glc*p*) : xylopyranose (Xyl*p*) : galactopyranose (Gal*p*) : fucopyranose (Fuc*p*) : arabinofuranose (Ara*f*)-ratio of 4 : 1.7 to 2.3 : 1.7 to 2.3 : 0.0 to 0.5 : 0.0 to 0.5, an average molecular weight (M_w) of from 57 000 to 2 300 000 Da, and an average number of repeating units (n) of from 50 to 1500.

'XXGGG'-type xyloglucans have repeating units consisting of two consecutive 1,4-linked β-D-glucopyranose residues, wherein each one of said glucopyranose residue is substituted with at least a 1,6-linked α-D-xylopyranose residue; and three consecutive unsubstituted 1,4-linked β-D-glucopyranose residues. 'XXGGG'-type xyloglucan consists essentially of individual units connected by β-1,4-glycosidic bonds, wherein the individual units are selected from the group consisting of XXGGG, FXGGG, LXGGG, SXGGG, TXGGG, JXGGG, XFGGG, XLGGG, XSGGG, XTGGG, XJGGG, FFGGG; FLGGG, FSGGG, FTGGG, FJGGG, LFGGG, LLGGG, LSGGG, LTGGG, LJGGG, SFGGG, SLGGG, SSGGG, STGGG, SJGGG, TFGGG, TLGGG, TSGGG, TTGGG, TJGGG, JFGGG, JLGGG, JSGGG, JTGGG, JJGGG.

Typically, 'XXGGG'-type xyloglucans have a glucopyranose (Glc*p*) : xylopyranose (Xyl*p*) : galactopyranose (Gal*p*) : fucopyranose (Fuc*p*) : arabinofuranose (Ara*f*)-ratio of 5 : 1.7 to 2.3 : 1.7 to 2.3 : 0.0 to 0.5 : 0.0 to 0.5, an average molecular weight (M_w) of from 65 000 to 1 900 000 Da, and an average number of repeating units (n) of from 50 to 1200.

More details about xyloglucan structures can be found in the above cited literature (S.F. Fry., A. Mishra et al., Hoffman et al, W. York et al., Hoffman et al., W. York et al.) and the literature cited therein.

Various attempts have been made to isolate xyloglucan from plant sources. Most of these attempts include the steps of first crushing or pulverizing the parts of the plant containing xyloglucan, and then treating the crushed or pulverized parts of the plant with air, water, or an organic solvent. Depending on the isolation procedure and the xyloglucan source, the so obtained xyloglucan may still have as main contaminations of from 0 to 40 % by weight of proteins, of from 0 to 20 % by weight of polysaccharides (which are different from xyloglucan), and 0 to 25 % by weight of fats.

Details about the isolation of xyloglucans and their purities can be found, for example, in Y. Kato et al.; Agricultural and Biological Chemistry 1981, 45, 2745-2753; J.-P. Joseleau et al., Plant Physiology, 1984, 74, 694-700; T. Hayashi et al., Plant and Cell Physiology, 1980, 21, 1405-1418; P. S. Rao, H. C. Srivastava, in R L Whistler (ed), Industrial Gums, 2nd ed., Academic Press, New York, 1973, 369-411; G. Sawr et al., J. Biol. Chem. 1947, 172, 501; US 4,895,938 ; and the literature cited therein.

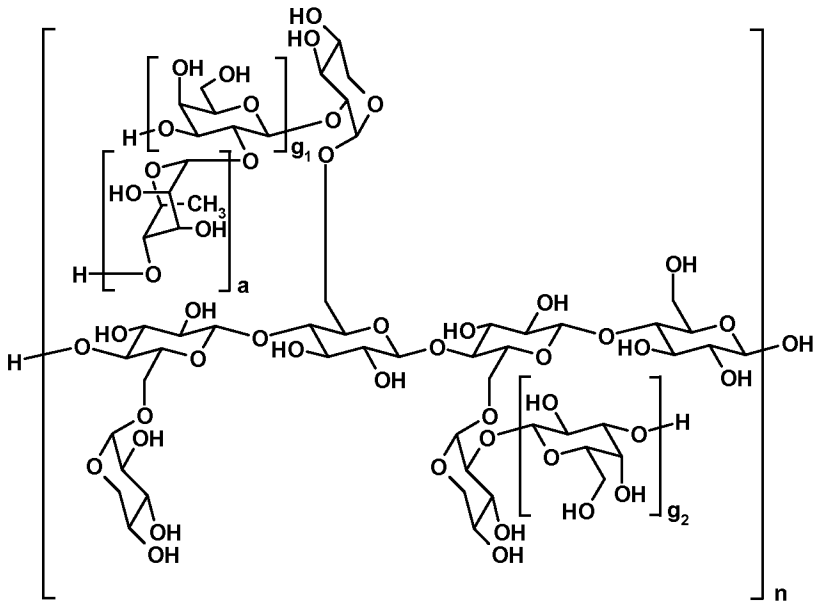
The process according to the present invention can principally be conducted with every xyloglucan type in any purity. However, for preparing the partially degalactosylated xyloglucan as defined above, xyloglucan having a purity of at least 50 % by weight is preferred. Xyloglucans having lower purities may affect, for example, the enzymatic reaction in a negative manner. The xyloglucan has more preferred a purity of at least 80 %, even more preferred a purity of at least 90 %, and most preferred a purity of at least 95 % by weight.

As important as the purity of the xyloglucan is its water solubility. Highly desirable is that xyloglucan and its contaminations dissolve completely thereby forming an aqueous solution. Because a too large proportion of water insoluble particles in the aqueous treating formulation may create problems in most of its possible applications (for example, settling of insoluble particles in containers and/or pipes might damage pumps or result in the blocking of pipes), the amount of insoluble particles is preferably very low.

Therefore, the proportion of water insoluble particles in said aqueous treating formulation is best kept low by using a highly water soluble xyloglucan. Alternatively, the amount of insoluble particles may be reduced, for example, by centrifugation and/or filtration, either before or after the process according to the present invention.

Preferably, the process of the present invention is conducted with an aqueous xyloglucan preparation, wherein at least one xyloglucan is a xyloglucan isolated from seeds of one of the genuses selected from the group consisting of *Nasturtium*, *Impatiens, Annona and Tamarindus.* More preferred, the aqueous xyloglucan preparation comprises one or more xyloglucans isolated from seeds of one of the species selected from the group consisting of *Tamarindus indica, Annona squamosa* and *Annona cherimola.*More preferred, the aqueous xyloglucan preparation comprises xyloglucan isolated from seeds of the species *Tamarindus indica.*

Said Xyloglucan is herein referred to as tamarind xyloglucan. Tamarind xyloglucan belongs to the "XXGG"-type xyloglucan and consists essentially of individual units connected by β-1,4-glycosidic bonds, wherein the individual units are selected from the group consisting of XXXG, XFXG, XLXG, XJXG, XXLG, XXJG, XFLG, XFJG, XLLG, XLJG, XJLG, XJJG. Tamarind xyloglucan can also be illustrated by general formula (II),



(II)

wherein
the average number of the β-D-galactopyranose residues per repeating unit (g_1 + g_2) is from 1.70 to 2.30,

the average number of the α-L-fucopyranose residue per repeating unit (a) is from 0.00 to 0.20, and
the average number of the repeating units (n) is from 200 to 1400.

In a particular embodiment of the present invention, the aqueous xyloglucan preparation comprises tamarind xyloglucan obtained by at least one extraction step and at least one solid-liquid separation step from commercially available tamarind seed flours and/or tamarind seed flakes.

Some suppliers for tamarind flakes and powders are shown in table 1.

Table 1

No.	Xyloglucan suppliers	Country	Xyloglucan form	Purity
1	Vishnu gum and chemicals	India	Deoild flakes & Powder	
2	TCI Germany GmbH	Germany	Pure polysaccharide	95
3	Altrafine Gums	India	Deoild flakes & Powder	
4	Balasanka	India	Deoild flakes & Powder	
5	Ramachandra Pulverisers & Industries	India	Deoild flakes & Powder	
6	The Andhra starch	India	Deoild flakes & Powder	
7	MYSORE	India	Deoild flakes & Powder	
8	Dainippon Sumitomo Pharma	Japan	Pure polysaccharide	98
9	Vishnu Engeneering Works	India	Deoild flakes & Powder	
10	Shree Vinayak Corporation	India	Deoild flakes & Powder	
11	Megazyme	Ireland	Pure polysaccharide	95

Frequently, tamarind flours, flakes and powders comprise around 60 to 80 % by weight of tamarind xyloglucan and 20 to 40 % by weight of fats, proteins, polysaccharides (which are different from xyloglucan) and the like, based on the total weight of said flours, flakes and/or powders. Said flours and flakes typically have wide particle size distributions containing also particles being larger than 50 µm as well as particles being smaller than 1 µm. Especially the particles being larger than 50 µm are frequently poorly water-soluble and therefore dissolve very inadequately resulting in an aqueous suspension. Therefore, said flakes or flours are first extracted, and then, the still remaining insoluble particles are separated off in a solid-liquid separation step.

For the extraction step, an aqueous suspension comprising between 0.5 and 5.0 %, preferably between 1.0 and 4.0 %, more preferred between 1.5 and 3.0 % by weight of

tamarind seed flours and/or tamarind seed flakes based on the total weight of the aqueous suspension is used. The extraction step is preferably carried out at temperatures of from 50 to 100 °C, more preferable of from 80 to 100 °C, most preferable of from 90 to 100 °C at ambient pressure. After an extraction time of around 0.5 to 8 h, most of the suspended particles are dissolved resulting in an aqueous suspension, wherein most of the xyloglucan of said flakes and/or flours is dissolved. In the solid-liquid separation step, the aqueous suspension is separated into a solid fraction and a liquid fraction. Preferably, this step involves centrifugation and/or filtration of said aqueous suspension. After separation, the solid fraction is removed and the liquid fraction is either directly used in the process of the present invention or stored. Said liquid fraction comprises between 0.3 and 4.0 %, preferably between 0.7 and 3.2 %, more preferred between 1.0 and 2.4 % by weight of tamarind xyloglucan based on the total weight of the liquid fraction. Optionally, said liquid fraction is further subjected to evaporation and drying. The drying may involve spray-drying or freeze-drying.

The aqueous xyloglucan preparation comprises at least the xyloglucan as defined above and water.

The aqueous xyloglucan preparation comprises preferably of from 0.1 to 10.0 % by weight, more preferred of from 0.5 to 5.0 % by weight, most preferred of from 1.0 to 3.0 % by weight of xyloglucan as defined above based on the total weight of the aqueous xyloglucan preparation.

Preferably, the aqueous xyloglucan preparation comprises one or more additives. Among these additives are not only buffers, such as for example, sodium citrate, sodium phosphate and/or ammonium sulfate; but also biocides, such as for example, formaldehyde glutardialdehyde and the like.

Where used, such biocides usually have concentrations of from 0.001 % to 0.100 % by weight based on the total weight of the aqueous xyloglucan preparation. Where used, such buffers have typically concentrations of from 0.1 to 20.0 % by weight based on the total weight of the aqueous xyloglucan preparation.

The aqueous xyloglucan preparation may comprise one or more water soluble organic solvents, like for example, alcohols, glycols or polyols. If any soluble organic solvent is present, its concentration is typically between 0.1 and 50 % by weight based on the total weight of the aqueous xyloglucan preparation. Preferably, the aqueous xyloglucan preparation does not comprise water soluble organic solvents.

The aqueous xyloglucan preparation has preferably a pH value of from 3 to 8, more preferred of from 4.5 to 7.5.

The aqueous xyloglucan preparation is provided by mixing water with the xyloglucan as defined above, and optionally one or more further additives and/or one or more water soluble organic solvents, as defined above, in every order. Preferably, the aqueous xyloglucan preparation is provided by mixing water with a biocide and subsequently adding the xyloglucan as defined above. Optionally, the aqueous xyloglucan preparation can be warmed up to between 25 and 75 °C, preferably to between 40 and 60 °C prior to use.

In step (b) of the process according to the present invention, the aqueous xyloglucan preparation is contacted with an enzyme preparation comprising β-galactosidase being capable of removing galactose from xyloglucan.

As regards the selection of suitable β-galactosidases (A2), the main emphasis is on the β-galactosidase activity. Thus, in general, all kinds of β-galactosidases can be used as long as they are capable of removing galactose from xyloglucan. The term 'being capable of removing galactose from xyloglucan' means that the β-galactosidase catalyzes the hydrolysis of β-D-galactopyranosyl-(1→2)-β-D-xylopyranosyl linkages within xyloglucan, therby forming D-galactose (D-galactopyranose) and partially degalactosified xyloglucan. Whether a specific β-galactosidase is capable of removing galactose from xyloglucan can be determined by standard methods. Such methods are, for example, described in X. Zhang, H. Bremer, H., *J. Biol. Chem.* **1995**, *270*, 11181-11189 and the literature cited therein, or in the SIGMA quality control test procedure 'Enzymatic assay of beta-galactosidase' which is available from Sigma-Aldrich.

Suitable β-galactosidases being capable of removing galactose from xyloglucan (A2) are, in general, all kinds of β-galactosidases (β-D-galactoside galactohydrolases, E.C. 3.2.1.23). Such β-galactosidases are, for example, β-galactosidases isolated from fungi like *Trichoderma reesei, Kluyveromyces lactis, Penicillium sp., Aspergillus oryzae, Aspergillus niger, Aspergillus aculeatus, Aspergillus awamori, Aspergillus carbonarius, Aspergillus japonicus, Aspergillus flavus, Kluyveromyces marxianus, Lactobacillus sp., Neurospora crassa, Rhizopus oryzae, Saccharomyces sp., or Saccharomyces sp.*; β-galactosidases isolated from bacteria *like Caulobacter crescentus, Bacillus circulans, Escherichia coli, Bacteroides fragilis, arthrobacter sp., Thermus thermophiles, Alicyclobacillus acidocaldarius, Bifidobacterium sp., Geobacillus stearothermophilus, Pseudomonas sp., Saccharopolyspora rectivirgula, Streptococcus sp., or Thermus sp.*; β-galactosidases isolated from archaea like *Sulfolobus solfataricus*; β-galactosidases isolated from animals like *Mus musculus*; or humans. However, homologues or variants

of the β-galactosidases isolated from the above mentioned source are also within the scope of the present invention.

Preferably, the β-galactosidase being capable of removing galactose from xyloglucan (A2) is selected from the group consisting of β-galactosidases isolated from the fungi *Trichoderma reesei, Kluyveromyces lactis, Penicillium sp., Aspergillus oryzae, Aspergillus niger, Aspergillus aculeatus, Aspergillus awamori, Aspergillus carbonarius, Aspergillus japonicus, Aspergillus flavus, Kluyveromyces marxianus, Lactobacillus sp., Neurospora crassa, Rhizopus oryzae, Saccharomyces sp., or Saccharomyces sp.*; β-galactosidases isolated from the bacteria *Caulobacter crescentus, Bacillus circulans, Escherichia coli, Bacteroides fragilis, arthrobacter sp., Thermus thermophiles, Alicyclobacillus acidocaldarius, Bifidobacterium sp., Geobacillus stearothermophilus, Pseudomonas sp., Saccharopolyspora rectivirgula, Streptococcus sp., or Thermus sp;* and homologues or variants thereof.

More preferred, the β-galactosidase being capable of removing galactose from xyloglucan (A2) is a β-galactosidase isolated from *Aspergillus oryzae, Aspergillus niger, Aspergillus aculeatus, Aspergillus awamori, Aspergillus carbonarius, Aspergillus japonicus, Aspergillus flavus*, or a homologue or variant thereof.

In a particularly preferred embodiment of the present invention, the β-galactosidase being capable of removing galactose from xyloglucan (A2) is the β-galactosidase isolated from *Aspergillus oryzae*, or homologues or variants thereof.

The amount of β-galactosidase used in the process according to the present invention is difficult to determine in absolute terms (e.g. grams), as its purity may vary. Instead, the activitiy is given in terms of the β-D-galactosidase activity. The physical unit of activity is unit (U). One unit (1.0 U) is herein defined to be the amount of β-galactosidase that catalyses the hydrolysis of 1 micromole of *o*-nitrophenyl β-D-galactoside to *o*-nitrophenol and D-galactose per minute at pH 6.0 at 37 °C. The β-D-galactosidase activities mentioned herein have been determined according to SIGMA quality control test procedure ‘Enzymatic assay of beta-galactosidase’.

The quantity of β-galactosidase is preferrably set to an amount of at least 500 U per g Xyloglucan, more preferred set to an amount of at least 750 U per g Xyloglucan, even more preferred set to an amount of at least 1000 U per g Xyloglucan, most preferred set to an amount of at least 1500 U per g Xyloglucan.

In a particularly preferred embodiment of the present invention, the enzyme preparation comprising β-galactosidase is essentially free of contaminants showing cellulase activi-

ty. β-galactosidases themselves do not degrade the xyloglucan back bone. However, β-galactosidases are generally isolated from sources that also contain endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) and/or other enzymes, capable of hydrolyzing cellulose polymers to smaller oligosaccharides, cellobiose and/or glucose. Such enzymes do also catalyze the hyrolysis of β(1→4)-glycoside bonds in the back bone of xyloglucan. As a consequence, partially degalactosylated xyloglucan is being formed having lower average molecular weights and, thus, inferior thermogelation properties. Therefore, it is preferred to use enzyme preparations comprising β-galactosidase being essentially free of contaminants showing cellulase activity.

According to the present invention, the enzyme preparation comprising β-galactosidase is deemed to be essentially free of contaminants showing cellulase activity, if it has a cellulase activity below 2 U/g.

Preferably, the enzyme preparation comprising β-galactosidase has a cellulase activity below 2 U/g. The specific cellulase activity is more preferred below 1 U/g and most preferred below 0.1 U/g.

The term 'cellulase activity' used herein refers to enzyme preparations or solutions containing endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) and/or other enzymes capable of hydrolyzing cellulose polymers to smaller oligosaccharides, cellobiose and/or glucose. A person skilled in the art is familiar with measurement methods of cellulase activity. Measurement methods of cellulase activity have been reviewed several times (see, for example, 'Determination methods of cellulase activity" T. Shuangqi et al., African Journal of Biotechnology 2011, 10, 7122-7125; 'Cellulase activities in biomass conversion: Measurement methods and comparison' M. Dashtban et al, Critical Reviews in Biotechnology 2010, 1-8).

The values of the cellulase activity mentioned herein have been determined according to the azo-xyloglucan assay. One unit (1.0 U) is herein defined to be the amount of enzymes that will catalyse the hydrolysis of 1 micromole of azo xyloglucan to low molecular weight fragments per minute. Said assay is specific for *endo*-1,4-ß-D-glucanase activity present in cellulase preparations. On incubation of azo xyloglucan with cellulase, said azo xyloglucan is depolymerized by an *endo*-mechanism to produce low-molecular weight fragments. After incubation the reaction is stopped by adding methanol. Then, high-molecular weight fragments are removed by centrifugation whereas low-molecular weight fragments remain in the supernatant solution. Said supernatant solution is poured into a spectrophotometer cuvette and the absorbance of blank and low-molecular weight fragment containing supernatant solution is measured at 590 nm.

Endo-1,4-ß-D-glucanase activity is determined by reference to a standard curve to convert absorbance to cellulase activity.

The aqueous xyloglucan preparation as defined above can be contacted with the β-galactosidase as defined above in every suitable way. For example, the β-galactosidase can be added in the form of a solid to the aqueous xyloglucan preparation. Alternatively, the β-galactosidase is first suspended or dissolved in water and subsequently added to the aqueous xyloglucan preparation in the form of an aqueous suspension or aqueous solution comprising the β-galactosidase. Said aqueous suspension or aqueous solution can comprise besides water and β-galactosidase also one or more additives, one or more water soluble organic solvents, and/or the like. Suitable are the same additives and water soluble organic solvents mentioned above with respect to the aqueous xyloglucan preparation. The aqueous suspension or aqueous solution comprising the β-galactosidase has preferably a pH value of from 3 to 8, more preferred of from 4.5 to 7.5. In another alternative, the aqueous xyloglucan preparation as defined above is added to the aqueous suspension or aqueous solution comprising the β-galactosidase. Thereby, the aqueous suspension or aqueous solution comprising the β-galactosidase is preferably preheated to a temperature of between 30 and 70 °C, more preferred to a temperature of between 40 and 60 °C.

Depending on the temperature and the specific activity of the β-galactosidase, the time needed to achieve a galactose removal ratio of at least 0.4 varies between several minutes to several days. For example, the time needed to achieve a galactose removal ratio of 0.4 is between 3 and 6 hours, if the specific activity of the β-galactosidase is set to 2000 U/g and the temperature to 50 °C. It is obvious to the person skilled in the art that differing conditions may result in a galactose removal ratio of at least 0.4 as well. For example, the choice of a lower specific activity and a prolonged reaction time may also result in a galactose removal ratio of at least 0.4.

Subsequently, said aqueous suspension is subjected to filtration, centrifugation and/or the like to remove the supernatant aqueous solution. The gel type precipitate obtained as filter cake, sediment and/or the like can be dried and optionally grinded to receive the partially degalactosylated xyloglucan as defined above.

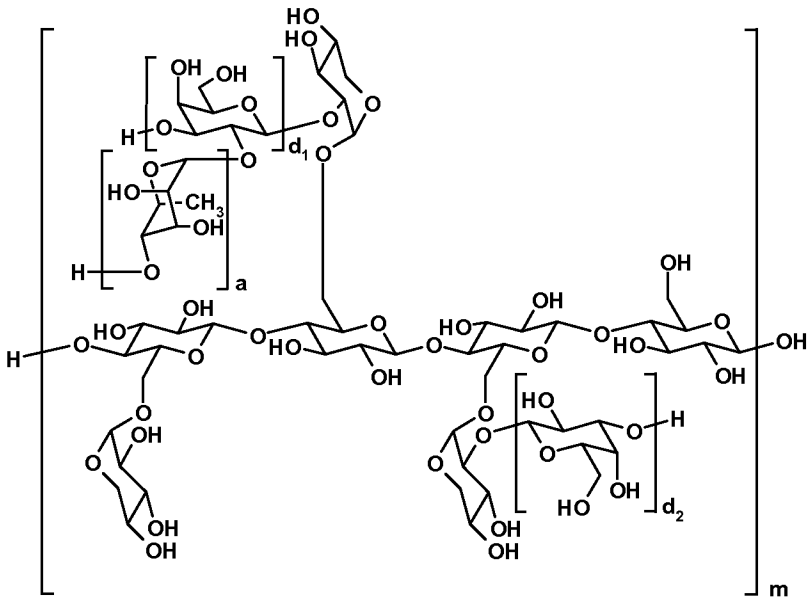
In a preferred embodiment of the present invention, said gel type precipitate is washed at least one times with an organic solvent, such as for example, acetone, ethanol, methanol, isopropanol or n-butanol.

Then, the amorphous precipitate obtained is separated from the organic solvent, dried, and optionally grinded to receive the partially degalactosylated xyloglucan as defined

above having a purity of least 90 % by weight based on the total weight of the partially degalactosylated xyloglucan.

The process of the present invention provides partially degalactosylated xyloglucan, wherein the average number of repeating units of the partially degalactosylated xyloglucan (m) is at least 0.8 times the average number of repeating units of the xyloglucan (n) such that the ratio m/n is from 0.90 to 1 (m/n = 0.90 to 1). The ratio m/n is preferably from 0.95 to 1, more preferred of from 0.98 to 1, most preferred of from 0.99 to 1.

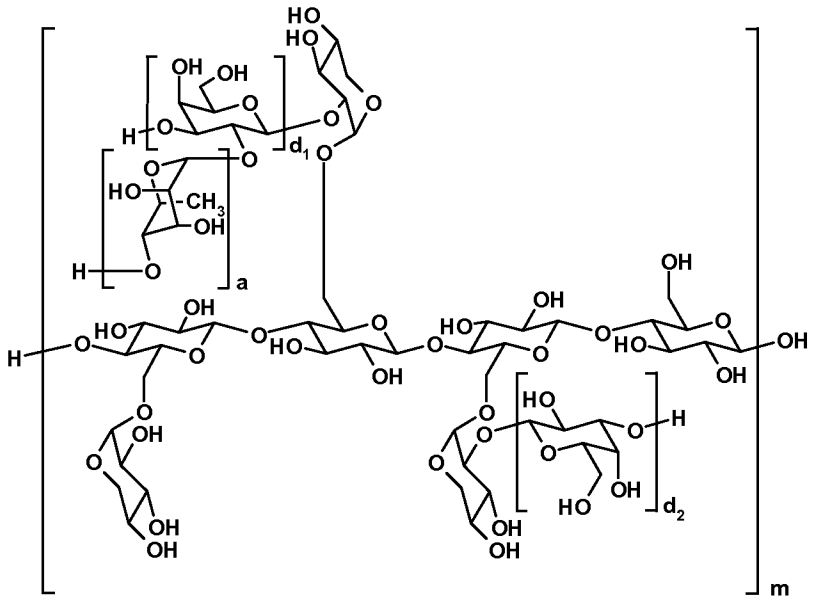
If the xyloglucan of the aqueous xyloglucan preparation is tamarind xylogucan, the process of the present invention provides partially degalactosylated tamarind xyloglucan having general formula (I),



(I)

wherein
the average number of the β-D-galactopyranose residues per repeating unit ($d_1 + d_2$) is from 0.20 to 1.20,
the average number of the α-L-fucopyranose residues per repeating unit (a) is from 0.00 to 0.20, and
the average number of the repeating units (m) is from 200 to 1400.

In a preferred embodiment of the present invention, the partially degalactosylated xyloglucan is partially degalactosylated tamarind xyloglucan having general formula (I),



(I)

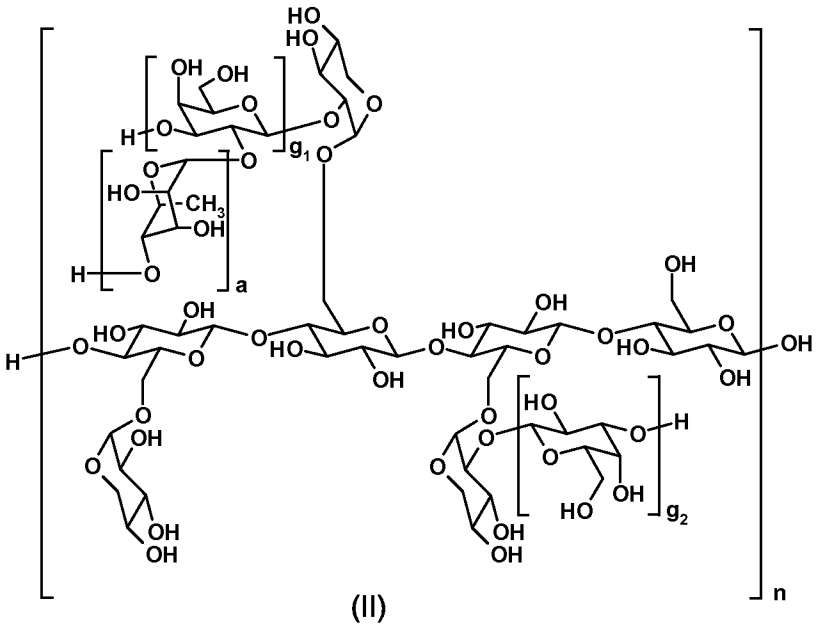
wherein

the average number of the β-D-galactopyranose residues per repeating unit (d_1 + d_2) is from 0.20 to 1.20,

the average number of the α-L-fucopyranose residues per repeating unit (a) is from 0.00 to 0.20, and

the average number of the repeating units (m) is from 200 to 1400,

and the xyloglucan of the aqueous xyloglucan preparation is tamarind xyloglucan having general formula (II),



(II)

wherein
the average number of the β-D-galactopyranose residues per repeating unit (g_1 + g_2) is from 1.70 to 2.30,
the average number of the α-L-fucopyranose rediues per repeating unit (a) is from 0.00 to 0.20, and
the average number of the repeating units (m) is from 200 and 1400.

Another aspect of the present invention is a method for producing enzyme preparations comprising β-galactosidaseas defined above.

This is achieved by a method for producing enzyme preparations comprising β-galactosidase as defined above comprising at least one anion exchange chromatographic step and at least one hydrophobic interaction chromatographic step, wherein said chromatographic steps can be conducted in arbitrary order.

β-galactosidases themselves do not catalyze the hydrolysis of β(1→4)-glycosidic bonds in the back bone of xyloglucan. However, β-galactosidases are generally isolated from sources that also contain endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) and/or other enzymes, capable of hydrolyzing cellulose polymers to smaller oligosaccharides, cellobiose and/or glucose. Such enzymes do also catalyze the hydrolysis of β(1→4)-glycosidic bonds in the back bone of xyloglucan. As a consequence, partially degalactosylated xyloglucan is being formed having lower average molecular weights and, thus, inferior thermogelation properties.

Preferably, the enzyme preparation comprising β-galactosidase has a cellulase activity below 2 U/g. The specific cellulase activity is more preferred below 1 U/g and most preferred below 0.1 U/g.

It has also been found that it is possible to purify industrial enzyme preparations containing of from 0.02 % to 95 % by weight of β-galactosidase, based on the dry weight of said industrial enzyme preparations, by means of an anion exchange chromatography and a hydrophobic interaction chromatography to achieve β-galactosidase as defined above.

The method for producing β-galactosidase as defined above comprises two different chromatographic separation steps, namely the method of anion exchange on the basis of competitive interaction of charged ions and the method of hydrophobic interaction, which is characterized in that the nonpolar surface zones of a protein adsorb to the weakly hydrophobic ligands of a stationary phase at high salt concentrations. To be

distinguished therefrom is the chromatographic separation principle of affinity chromatography which is based on the specific and reversible adsorption of a molecule to an individual matrix-bound bonding partner. The hydroxyapatite chromatography, which is based on the use of inorganic hydroxyapatite crystals, is a further separation method which differs from the anion exchange chromatography and the hydrophobic interaction chromatography.

All these chromatographic principles mentioned can easily be distinguished by the person skilled in the art (see, for example, Bioanalytik, F. Lottspeich, H. Zorbas (ed.), Heidelberg, Berlin, Germany, Spektrum Akad. Verlag 1998).

So called "β-galactosidases" currently available from suppliers are in fact industrial enzyme preparations containing of from 0.02 to 95 % by weight of β-galactosidase, based on the dry weight of said industrial enzyme preparations. Said industrial enzyme preparations containing β-galactosidase are commercially available, for example, Amano (Lactase F), Amano (LOacase 14-DS), Novozymes Lactozym® Pure, andNovozymes Lactozym® Pure 6500L.

For most of the desired applications, the purity of said industrial enzyme preparations containing β-galactosidase is high enough. However, higher purities are required in some particular applications, among them, the method of treating subterranean formations according to the present invention. As discussed above, the commercially available industrial enzyme preparations containing β-galactosidase comprise normally also other enzymes which may cause undesired side reactions, like fission of the β(1→4) glycosidic bond of the xyloglucan back bone. To avoid such side reactions, said industrial enzyme preparations containing β-galactosidase have to be purified. Most important in the context of the present invention is the separation of enzymes showing cellulase activity. The contaminations of the industrial enzyme preparations are mainly cell components, like other enzymes, polysaccharides, DNAs, RNAs etc.

According to the present invention it was found that β-galactosidases can be separated off from the contaminants showing cellulase activity by using at least one exchange chromatographic step and at least one hydrophobic interaction chromatographic step. The method for purification and/or isolation of β-galactosidases allows the separation of β-galactosidase from most of its contaminants present in the industrial enzyme preparations, especially from enzymes showing cellulase activity. As a result, the enzyme preparations comprising β-galactosidase (A2) obtained show specific cellulase activities below 2 U/g.

The term 'contaminants' used herein refers to all kinds of substances in the enzyme preparation being different from β-galactosidases. The contaminants may include substances like, for example, endoglucanases or cellobiohydrolases. The contaminants may also include further substances such as DNAs, RNAs or polysaccharides, etc., and additives which had been used in the purification and isolation from the producing organism.

Anion exchange chromatography primarily retains proteins and other molecules by the interaction of amine groups on the anion exchange matrix resin with aspartic or glutamic acid sidechains, having pKs values of around 4.4. The mobile phase is buffered at pH values greater than 4.4, below which acid sidechains begin to protonate and retention decreases. Above a pH value of 4.4, retention of proteins and other molecules is largely dependent on the number of anionic sidechains present in the proteins and other molecules. Proteins having different numbers of anionic sidechains can often be separated by adjusting the pH value of the mobile phase to between 7 and 10 where histidine is not protonated and lysine begins to deprotonate. A mobile phase having a pH value of greater than 10 is not generally recommended because of possible degradation of the proteins.

Suitable matrices and protocols for conducting the anion exchange chromatography can be taken from the product information of suppliers (for example GE Healthcare: http://www.gelife-science.com, Bio-Rad: http://www.bio-rad.com).

Suitable anion exchange matrices include, for example, DEAE (diethylaminoethyl) sepharose Cl-4B, DEAE Sepharose Fast Flow, Q Sepharose (quaternary ammonium) Fast Flow, Q Sepharose High Performance from GE Healthcare; Preferably, quaternary ammonium matrices are used as matrix for the anion exchange chromatography. More preferred, Q sepharose Fast Flow and Q sepharose High Performance available by GE Healthcare are used. Most preferred Q sepharose Fast Flow is used as matrix for the anion exchange chromatography.

Typically, the chromatography is performed using an aqueous buffer system at pH values of from about 5 to 10 and running a gradient from an aqueous solution containing said buffer system and one or more salts. Suitable buffer systems for the anion exchange chromatography include, for example, N-methyl piperazine/HCl, piperazine/HCl, L-histidine/HCl, Na_2HPO_4/NaH_2PO_4, triethanolamine/HCl, N-methyl-diethanolamine/HCl, diethanolamine/HCl, 1,3-diaminopropane/HCl, ethanolamine/HCl, piperazine/HCl. The preferred buffer systems are L-histidine/HCl, Na_2HPO_4/NaH_2PO_4, triethanolamine/HCl. Most preferred is the buffer system Na_2HPO_4/NaH_2PO_4.

For purifying an industrial enzyme preparation containing β-galactosidase (or prepurified β-galactosidase), the pH value of the buffer system should possibly be between 6.0 and 8.0. Preferably, the pH value is from 6.5 to 7.5. The concentration of the buffer system lies between 5 and 100 mM, preferably between 10 mM and 50 mM.

In the first step of the anion exchange chromatography, an aqueous buffer system having a pH value of between 6.0 and 8.0 is employed for equilibrating and washing the column.

Subsequently to washing, the industrial enzyme preparation containing β-galactosidase (or prepurified β-galactosidase) is injected onto the column under conditions where it will be strongly retained. Afterwards, an aqueous solution containing the buffer system and an increasing amount of one or more salts is applied to elute the industrial enzyme preparation containing β-galactosidase (or prepurified β-galactosidase) from the column. This is effected by means of increasing the ionic strength, which is effected by means of increasing salt concentration in the aqueous solution. Suitable salts are for example NaCl or KCl..

The hydrophobic interaction chromatography can be conducted with conventional resins. Suitable resins are, for example, butyl sepharose, octyl sepharose or phenyl sepharose from GE Healthcare; Macro-Prep® methyl or Macro-Prep® t-butyl from Bio-Rad; Fractogel® EMD Phenyl (S), Fractogel® EMD Propyl (S) from Merck; and TSK-GEL® Ether-5PW (20), TSK-GEL® Phenyl-5PW (20), TSK-GEL® Ether-5PW (30), TSK-GEL® Phenyl-5PW (30) from Tosoh Bioscience LLC. Preferably, the hydrophobic ligands are butyl, phenyl or octyl groups. More preferred are phenyl groups. Most preferred are phenyl sepharose, Fractogel® EMD Phenyl (S), TSK-GEL® Phenyl-5PW (20) and TSK-GEL® Phenyl-5PW (30).

Conventional buffer systems, which are also employed in other types of chromatography, are suitable as buffer systems for the hydrophobic interaction chromatography. A preferred buffer system is Na_2HPO_4/NaH_2PO_4.

In the first step of the hydrophobic interaction chromatography, the column is equilibrated with at least two column volumes of an aqueous solution comprising at least one salt and a buffer system.

Suitable salts are, for example, NH_4SO_4, K_2SO_4, Na_2SO_4, $NH_4OC(O)CH_3$, $KOC(O)CH_3$, $NaOC(O)CH_3$, NH_4Cl, KCl or NaCl. Preferably, the salt is NH_4SO_4, K_2SO_4, Na_2SO_4. More preferred the salt is NH_4SO_4. The salt concentration in said aqueous solution is preferably from 0.5 to 3.0 mol/l, more preferred from 1.0 to 2.5 mol/l. The pH value of

said aqueous solution is usually between 6.0 and 8.0, preferably from 6.5 to 7.5. A preferred aqueous solution for hydrophobic interaction chromatography contains between 0.01 and 0.10 g/ml of a buffer system and 1.5 and 2.0 g/mol of at least one salt.

Subsequently to equilibration, the prepurified β-galactosidase (or the industrial enzyme preparation containing β-galactosidase) is injected onto the column in an aqueous solution having the same composition as the aqueous solution used for equilibration. Then, the prepurified β-galactosidase (or the industrial enzyme preparation containing β-galactosidase) is eluted from the column by reducing the hydrophobic interaction. This can be achieved by reducing the salt concentration in the mobile phase and/or by eluting with a non-polar organic solvent, like for example, ethylene glycol or isopropanol. While the β-galactosidase passes through the column, most of the contaminants of the prepurified β-galactosidase (or the industrial enzyme preparation of β-galactosidase) remain bound to the column or passes through only at decreased salt concentration or high non-polar organic solvent concentration.

More details about hydrophobic interaction chromatography can be taken from the relevant literature, for example from the product information of the suppliers mentioned above (GE Healthcare and Bio-Rad). In general, the person skilled in the art is familiar with the chromatographic principles utilized in the hydrophobic interaction chromatography according to the present invention.

After at least one cation exchange chromatographic step and at least one hydrophobic interaction chromatographic step, an enzyme preparation comprising β-galactosidase may be isolated and dried, wherein said enzyme preparation is obtained as an amorphous solid which shows a specific cellulase activity below 2 U/g.

In one embodiment of the present invention, the method for producing enzyme preparations comprising β-galactosidase as defined above comprises only two chromatographic separation steps, namely one anion exchange chromatographic step and one hydrophobic interaction chromatographic step.

In a preferred embodiment of the present invention, the anion exchange chromatography is conducted as first step, and the hydrophobic interaction chromatography as second step. This preferred method for producing an enzyme preparation comprising β-galactosidase provides an enzyme preparation showing a specific cellulase activity below 2 U/g.

In a preferred embodiment of the invention the enzyme preparation showing a specific celluase activity below 2 U/g comprises at least 98 % by weight of β-galactosidase, preferably at least 98 % by weight.

The present invention is illustrated in more detail by the following examples and patent claims.

Examples

Fig. 1 shows rheological properties of a (partially) degalactosified xyloglucan in water and NDIIa. Gelation of the degalactosified xyloglucan occurs at higher temperature in NDIIa.

Fig. 2 shows rheological differences between a (partially) degalactosified and non-modified xyloglucan. Gelling behaviouor is pronounced solely by (partial) degalactosification of xyloglucan.

Fig. 3 shows a comparison of three different (partially) degalactosified xyloglucans. The higher the galactose removal ratio (GRR), the higher the gel-strength becomes.

Fig. 4 shows a comparison of four different aqueous formulations containing a (partially) degalactosified xyloglucan. Two formulations do contain EGCG (epigallocatechin gallate) as an additive. EGCG shifts the gelling temperature.

Fig. 5 shows viscosity values of two different aqueous (Landau water) well treatment formulations as a function of time (in hours after the formulation's preparation). The formulation, comprising xyloglucan and purified β-galactosidase being essentially free of contaminants showing cellulose activity, does form a stable gel, whereas the formulation comprising non-purified β-galactosidase does not.

Fig. 6 shows a macroscopic picture of a (partially) degalactosified xyloglucan (GRR = 0.53; 1.5 wt% in NDIIa) at different temperatures.

Example 1: Enzyme Purification – Step 1

In the first chromatographic step, anion exchange chromatography is employed. A Q Sepharose Fast Flow column (height (h): 22 cm, diameter (d): 5.0 cm, volume (V): 432 ml) from GE Healthcare is used for chromatographic separation. The chromatography is conducted at pH 7 using the aqueous buffer solutions A and B:

Aqueous buffer solution A: 20 mmol/l Na_2HPO_4/NaH_2PO_4, pH 7.0;
Aqueous buffer solution B: 20 mmol/l Na_2HPO_4/NaH_2PO_4, 0.5 mol/l NaCl, pH 7.0.

55 g Lactase F "Amano" (obtained from Amano) is added to 900 ml water and the pH of the mixture obtained is adjusted to pH 7 by adding an appropriate amount of a 1 M aqueous NaOH solution. The Lactase F "Amano" mixture is loaded onto the Q Sepharose Fast Flow column equilibrated with aqueous buffer solution A. The Lactase F mixture is eluted from the column using a linear gradient to 100 % by volume of aqueous buffer solution B (1200 ml) and afterwards 400 ml aqueous buffer solution B. β-Galactosidase activity of the collected fractions is verified by using p-Nitrophenyl-ß-D-galactopyranosid which is cleaved upon the enzymatic activity whereby the absorption at 405 nm increases (for details see Miller, J.H. 1972. Experiments in Molecular Genetics: Assay of β-Galactosidase. CSH Laboratory Press, Cold Spring Harbor, NY: 352-355.). The β-galactosidase elutes at 60 - 80 % by volume aqueous buffer solution B. The combined β-galactosidase containing fractions (Volume: 300 ml) having a protein content of 7.624 mg/ml (determined by the Bradford assay, for details of said assay see "Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding", M. M. Bradford, Anal. Biochem. 1976, 72, 248–254.).

Determination of cellulase activity

Cellulase activity has been determined using an azo xyloglucan based assay. Therefore, 50 µl of a collected β-galactosidase comprising fraction is mixed with 50 µl of an aqueous azo xyloglucan solution (1.0 % by weight azo xyloglucan, 0.05 M sodium citrate (pH 5.5)). Then, 10 µl of a 1 M aqueous sodium citrate buffer (pH 5.5) is added to keep the pH value constant. After incubation the reaction is stopped by adding 170 µl methanol. The precipitate is removed by centrifugation and the supernatant solution is directly poured into a spectrophotometer cuvette and the absorbance of blank and supernatant solution is measured at 590 nm.

Example 2: Enzyme Purification – Step 2

In the second chromatographic step, hydrophobic interaction chromatography is employed. A TSK-GEL® Phenyl-5PW (20) column (h: 28 cm, d: 5.0 cm, V: 530 ml) from GE Healthcare is used for chromatographic separation. The chromatography is conducted at pH 7 using the aqueous buffer solutions C and D:

Aqueous buffer solution C: 20 mmol/l Na_2HPO_4/NaH_2PO_4, 60 wt.-% $(NH_4)_2SO_4$, pH 7.0;
Aqueous buffer solution D: 20 mmol/l Na_2HPO_4/NaH_2PO_4, pH 7.0.

A β-galactosidase containing solution (V: 619 ml, protein content: 8.562 mg/ml (Bradford), total protein content: 5300 mg) obtained by anion exchange chromatography is supplemented with ammonium sulphate to 60 wt.-% saturation (room temperature) and loaded on the Phenyl-Sepahrose Fast Flow column previously incubated with aqueous buffer solution C.
Whereas the β-galactosidase passes through the column using a linear gradient to 100 % by volume of aqueous buffer solution D (1200 ml) and afterwards 400 ml aqueous buffer solution D, the proteins showing cellulase activity remain, to a large extent, bound to the column. β-Galactosidase activity of the collected fractions is again verified by using p-Nitrophenyl-ß-D-galactopyranosid (for details see Miller, J.H. 1972. Experiments in Molecular Genetics: Assay of β-galactosidase. CSH Laboratory Press, Cold Spring Harbor, NY: 352-355.). The combined β-galactosidase containing fractions (V: 350 ml) having a protein content of 10.363 mg / ml.

Cellulase activity has been determined as described in example 1.

β-galactosidase fractions showing a cellulase activity not exceeding 0.1 U / ml were used for all degalactosification experiments.

Example 3: Preparation of xyloglucan from tamarind kernel flakes (TKF)

Extraction of xyloglucan polysaccharide from tamarind kernel flakes was done in three steps:

1) extraction in stirred tank reactor;
2) centrifugation in Sorvall bucket centrifuge;
3) clear filtration using pressure filter.

Extraction in stirred tank reactor and centrifugation:
In 20 L stirred tank reactor, equipped with propeller stirrer, was filled with tamarind kernel flakes and water (2.0 wt.-% of flakes). Final amount of this suspension was 16.0 L. The suspension was warmed to 95 °C (this took ca. 75 min with mixing at 700 upm) and the resulting temperature was then kept for 35 min. (while stirring). After this time, reaction mixture was divided into 4 equal portions. These were centrifuged in Sorvall bucket centrifuge (Type Sorvall RC-4) as follows: 15 min., C-value 5300. Solid content in a suspension was ca. 0.3 wt.-%.

Clear filtration using pressure filter:
For a clear filtration two pressure filters with a filtration area of 100 cm² were used. These were operated in parallel. As a filter medium a depth filter sheet of type K 300

(5-12 microns) from (PALL) was used. The pressure filters were also heated to 95 °C. The set constant pressure difference was 1 bar.

Xyloglucan concentration in the filtrate was determined to be 1,38 wt.-% and the average molecular weight (M_w) found to be 1300 kDa (recovery rate 85 %).

Average molecular weight determination

Average molecular weights (M_w) for un-modified (Example 3) and partially degalactosified xyloglucans (Examples 4, 5 and Comparison example 1) were determined via field flow fractionation (FFF). Following equipment was used: field flow fractionation apparatus Eclipse 2, light-scattering detector Dawn Eos, concentration detector R.I. Optilab DSP (company Wyatt), spacer: 350 µm, injection pump 0.20 mL/min, RC-membrane 10kDa. For a measurement concentration of 0.05 N $NaNO_3$, pH=6 was used. Polysaccharide samples were prepared as 1.0 g/L solutions and filtrated over 0.45 µm PVDF filter before measurement.

Example 4: Preparation of partially degalactosified xyloglucan

Preparation of partially degalactosified xyloglucan having a galactose removal ratio (GRR) of 0.43 using a solution of β-galactosidase which shows a cellulase activity not exceeding 0.1 U / mL(reaction solution)

A 6.0 L glass reactor is filled with 6000 g of aqueous xyloglucan (XG) preparation, (1.38 wt.-% XG, 83.0 g of XG, purity > 98 %, average molecular weight (M_w) = 1300 KDa stabilized with 500 ppm formaldehyde) followed by 120 mL sodium citrate buffer (1 M, pH = 4.8). Then the temperature is set to 50 °C and 102 mL of purified Amano Lactase F β-galactosidase (102 U / mL(β-galactosidase solution), 1.7 mg / mL(β-galactosidase solution), 2000 U / g(XG) as a solution in 20 mM NaH_2PO_4 / Na_2HPO_4-buffer (pH = 7.0) and 30 - 40 % by weight of $(NH_4)_2SO_4$) is added. The resulting reaction mixture is stirred at a constant temperature of 50 °C for 5.5 h. After this time, the reaction mixture is centrifuged (40 min, 5300 g and 40°C) and the supernatant separated. To a gel type precipitate obtained, 1:1 (v/v) acetone is added and slowly stirred with a mechanical blade agitator. Precipitated material is filtrated using glass filter (porosity No. 2). The filter cake is then suspended in 1.5 L acetone and stirred until a clear-flowing suspension is obtained. The precipitate is filtrated and vacuum dried at 40 °C for ca. 12 h. The partially degalactosified xyloglucan is obtained as an amorphous powder which is grinded and stored in a closed flask at room temperature.

Example 5: Preparation of partially degalactosified xyloglucan having a GRR of 0.50 using a solution of β-galactosidase which shows a cellulase activity not exceeding 0.1 U / mL(reaction solution)

Partially degalactosified xyloglucan having a GRR of 0.50 is prepared according to the procedure described in example 4. Instead of a activity of 2000 U / g(XG), a activity of 1500 U / g(XG) is used. Moreover, the reaction mixture is stirred at 50°C for 24 h.

Comparison example 1: Preparation of partially degalactosified xyloglucan having a GRR of 0.36 using a solution of β-galactosidase which shows a cellulase activity not exceeding 0.1 U / mL(reaction solution)

Partially degalactosified xyloglucan having a GRR of 0.36 is prepared according to the procedure described in example 4. Instead of a activity of 2000 U / g(XG), a activity of 500 U / g(XG) is used. Moreover, the reaction mixture is stirred at 50°C for 20 h.

Average molecular weight found for xyloglucan from TKF after partial degalactosyfication

Sample	Example 4	Example 5	Comparison example 1
GRR	0.43	0.50	0.36
M_w in kDa	1080	1160	1090

The average molecular weight has been determined according to the procedure shown in example 3.

Determination of galactose removal ratio (GRR)

Galactose removal ratio (GRR) was determined according to a slightly modified procedure from Shirakawa, M., Yamatoya, K., & Nishinari, K. (**1998**). Tailoring of xyloglucan properties using an enzyme. *Food Hydrocolloids*, **12**, 25–28: In the first step native xyloglucan was hydrolyzed with 12M H_2SO_4 to obtain the total galactose (GAL) amount available from starting material. Degree of degalactosification was calculated as: **GRR** = GAL amount after enzyme treatment/total GAL amount.

Example 6: Rheology measurements using partially degalactosified xyloglucan from Example 5

Sample preparations

A 100 mL beaker containing 22.92 g synthetic NDIIa water and 31.19 g water was immersed into an ice-bath. The solution was stirred at 1600 rpm using 40 mm dissolver disc and 0.83 g of the partially degalactosified xyloglucan obtained in Example 5 (GRR 0.50) was added. Stirring was continued for 30 min followed by addition of 0.069 mL of formaldehyde (36.5% solution in water). After preparation the samples were kept at +4°C.

Composition of synthetic NDIIa water:	$CaCl_2 \times 2H_2O$:	67.71 g / L
	$MgCl_2 \times 6H_2O$:	26.90 g / L
	NaCl:	158.4 g / L
	Na_2SO_4:	0.32 g / L
	$NaBO_2 \times 4H_2O$	0.46 g / L

Rheological experiments

The rheological experiments were done with Anton Paar MCR Rheometers. Temperature sweep experiments were done in a temperature range between 0°C and 140°C. A sealed geometry (pressure cell) was used with a double gap geometry. Measurements were carried out at a constant shear rate of 10 s^{-1} with a heating rate of 0.5 °C / min.

Gel kinetic experiments were done in concentric cylinder geometry with small amplitude oscillation shear (SAOS) measurements at 1 Hz with a deformation of 5 %. The geometry was set to the particular measurement temperature before the sample was filled. The initial sample temperature was about 4 °C. Right after the sample was filled into the geometry, the sample was covered with silicone oil. Silicone oil was used to prevent evaporation and salt crust formation at elevated temperatures. In order to have identical conditions, this procedure was kept constant for all kinetic measurements at all temperatures. Due to the overall handling procedure there was a time delay of about 60-90 seconds till the first data point could be collected.

Sol-gel transition temperatures from temperature sweep experiments using the partially degalactosified xyloglucan obtained in Example 5 (GRR = 0.50)

Partially deg. xyloglucan (GRR = 0.50) [% by weight]	synthetic NDIIa water / deionized water	EGCG* [ppm]	Sol-gel transition temperature [°C]
1.5	1 : 1	-	39
1.5	100 : 0	-	58
1.5	1 : 1	150	48
1.5	100 : 0	150	58

* EGCG = epigalocatechin gallate

Sol-gel transition temperature were obtained from the minimum in the viscosity-temperature curves (Figures 1 - 4)

Claims

1. A method for treating an oil and/or natural gas bearing subterranean formation penetrated by at least one well bore, comprising the steps of

   (a) providing an aqueous treating formulation which comprises a treating composition, comprising partially degalactosylated xyloglucan having a galactose removal ratio of at least 0.40, based on the total number of β-D-galactopyranosyl residues of xyloglucan, and

   (b) injecting said aqueous treating formulation into at least one well bore penetrating the oil and/or natural gas bearing subterranean formation.

2. The method according to claims 1, wherein the partially degalactosylated xyloglucan has an average molecular weight of from 400 000 to 1 500 000 Da.

3. The method according to claim 1 or 2, wherein the aqueous treating formulation further comprises one or more salts.

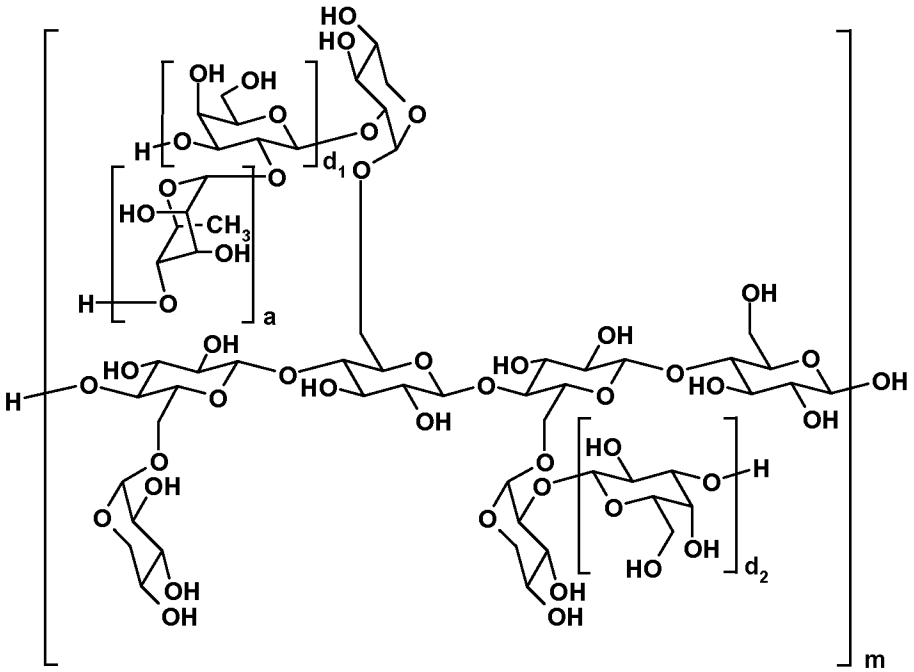
4. The method according to any one of claims 1 to 3, wherein the aqueous treating formulation comprises of from 0.02 to 30.00 % by weight of one or more salts based on the total weight of the aqueous treating formulation.

5. The method according to any one of claims 1 to 4, wherein the aqueous treating formulation further comprises one or more biocides.

6. The method according to any one of claims 1 to 5, further comprising the step of

   (c) gelling of said aqueous treating formulation under the influence of the temperature of the subterranean formation.

7. The method according to any one of claims 1 to 6, wherein the partially degalactosylated xyloglucan is partially degalactosylated tamarind xyloglucan having general formula (I),



(I)

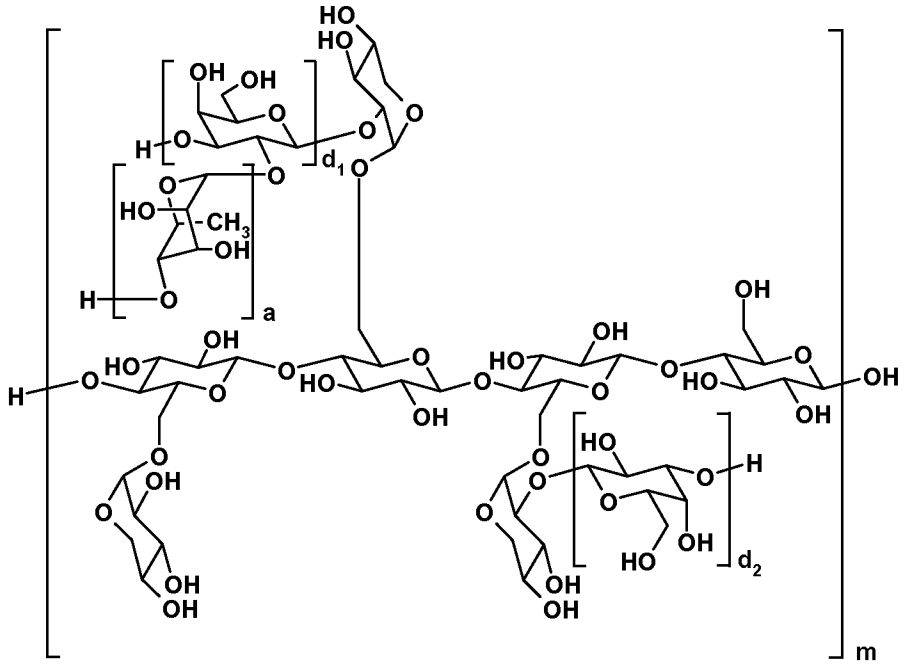
wherein
the average number of the β-D-galactopyranose residues per repeating unit ($d_1 + d_2$) is from 0.20 to 1.20,
the average number of the α-L-fucopyranose residues per repeating unit (a) is from 0.00 to 0.20, and
the average number of the repeating units (m) is from 200 to 1400.

8. The method according to claim 7, wherein the average number of the repeating units (m) is from 400 and 1400.

9. A treating composition comprising partially degalactosylated xyloglucan having a galactose removal ratio of at least 0.40, based on the total number of β-D-galactopyranosyl residues of xyloglucan, and comprising at least one further component.

10. The treating composition according to claim 9, wherein the partially degalactosylated xyloglucan has an average molecular mass of from 800 000 to 1 500 000 Da.

11. The treating composition according to claim 9 or 10, wherein the partially degalactosylated xyloglucan is partially degalactosylated tamarind xyloglucan having general formula (I),



(I)

wherein
the average number of the β-D-galactopyranose residues per repeating unit ($d_1 + d_2$) is from 0.20 to 1.20,
the average number of the α-L-fucopyranose residues per repeating unit (a) is from 0.00 to 0.20, and
the average number of the repeating units (m) is from 700 to 1400.

12. An aqueous treating formulation comprising the treating composition as defined in any one of claim 9 to 11, and water.

13. The aqueous treating formulation according to any one of claims 9 to 12, further comprising one or more salts.

14. The aqueous treating formulation according to any one of claims 9 to 13, further comprising one or more biocides.

15. Use of the treating composition according to any one of claims 9 to 11, or of the aqueous treating formulation according to any one of claims 12 to 14 for oil-field applications.

16. The use according to claim 15, wherein the oil-filed application is a conformance control measure or a water shut-off measure.

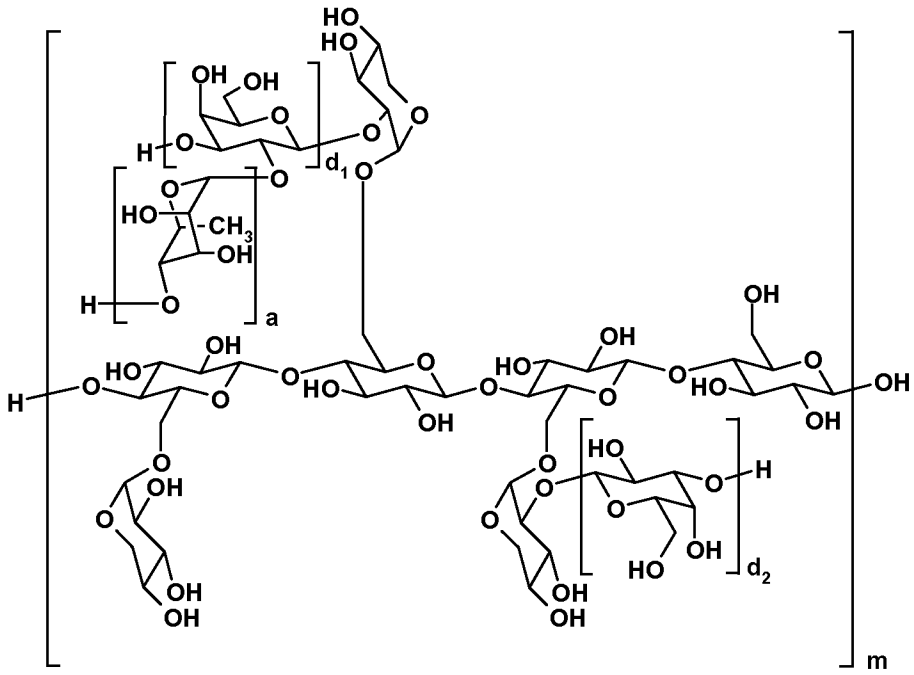
17. A process for preparing the partially degalactosylated xyloglucan as defined in any one of claims 9 to 11 which comprises the steps of

(a) providing an aqueous xyloglucan preparation, and

(b) contacting said aqueous xyloglucan preparation with an enzyme preparation comprising β-galactosidase being capable of removing galactose from xyloglucan.

18. The process according to claim 17, wherein the enzyme preparation has a cellulase activity below 2 U/g.

19. The process according to claim 17 or 18, wherein the partially degalactosylated xyloglucan is partially degalactosylated tamarind xyloglucan having general formula (I),

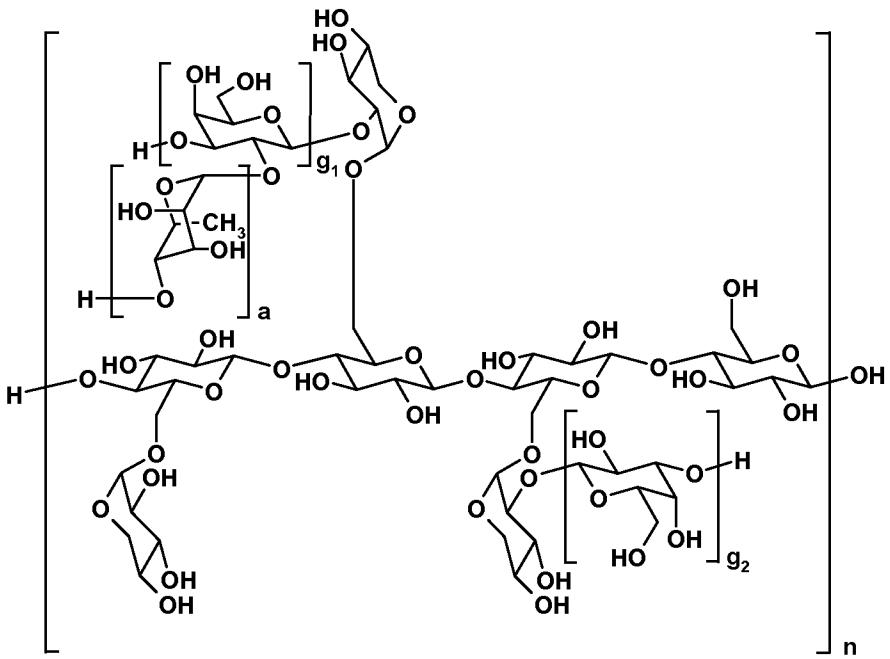


(I)

wherein

the average number of the β-D-galactopyranose residues per repeating unit ($d_1 + d_2$) is from 0.20 to 1.20,
the average number of the α-L-fucopyranose residues per repeating unit (a) is from 0.00 to 0.20, and
the average number of the repeating units (m) is from 200 and 1400;

and wherein the xyloglucan of the aqueous xyloglucan preparation is tamarind xyloglucan having general formula (II),



(II)

wherein
the average number of the β-D-galactopyranose residues per repeating unit ($g_1 + g_2$) is from 1.70 to 2.30,
the average number of the α-L-fucopyranose rediues per repeating unit (a) is from 0.00 to 0.20, and
the average number of the repeating units (n) is from 200 to 1400.

Figure 1

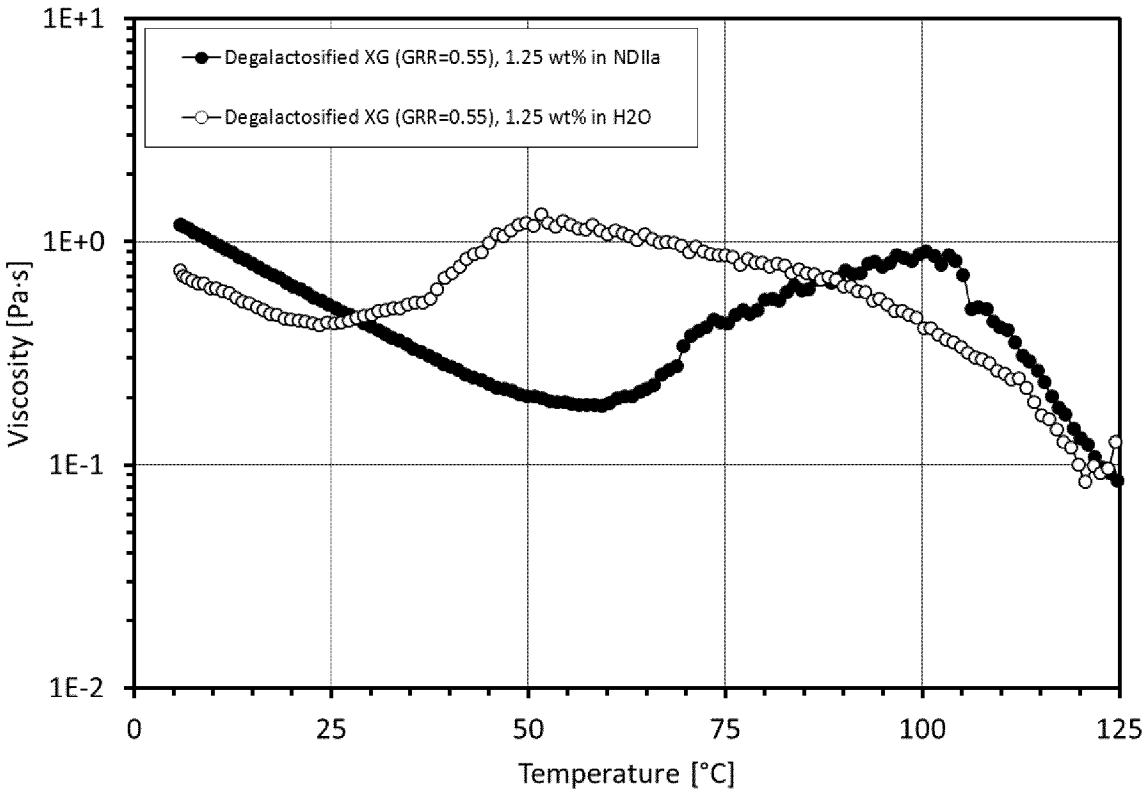

Degalactosified XG (GRR=0.55), 1.25 wt% in NDIIa
Degalactosified XG (GRR=0.55), 1.25 wt% in H2O
Viscosity [Pa·s]
1E+1
1E+0
1E-1
1E-2
0
25
50
75
100
125
Temperature [°C]

Figure 2

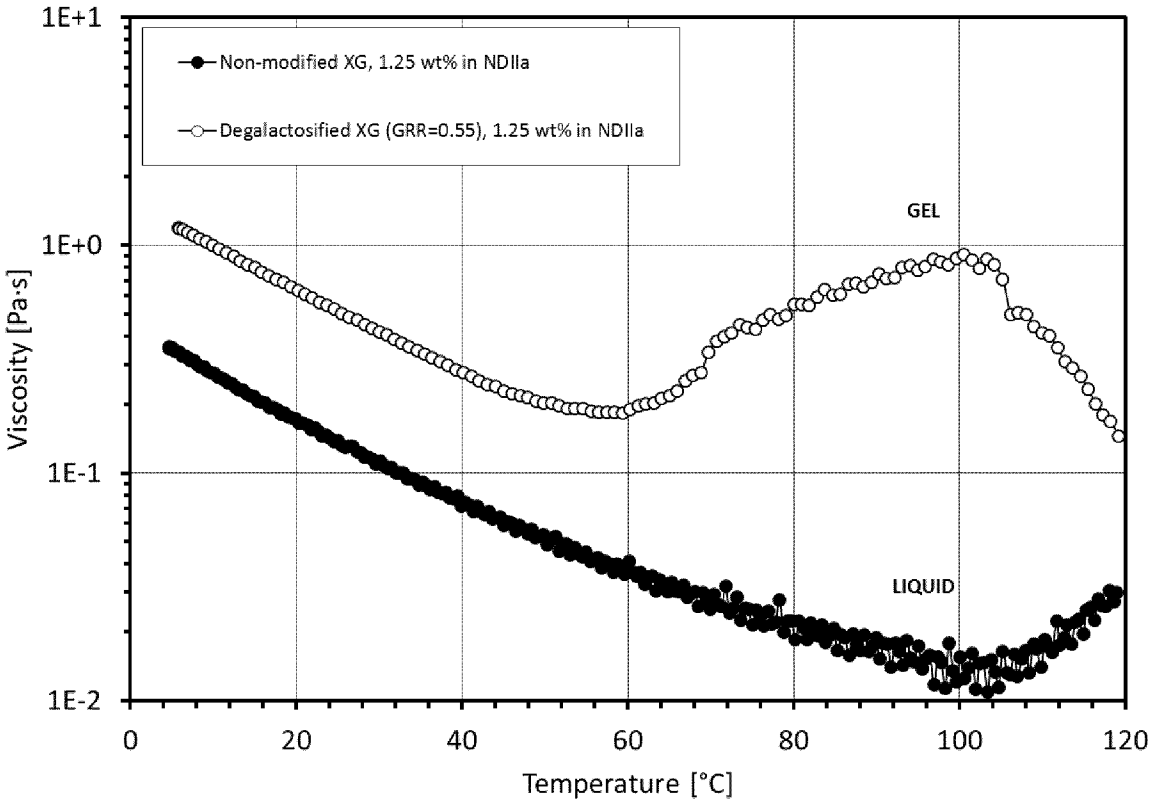

1E+1
1E+0
1E-1
1E-2
Viscosity [Pa·s]
0
20
40
60
80
100
120
Temperature [°C]
Non-modified XG, 1.25 wt% in NDIIa
Degalactosified XG (GRR=0.55), 1.25 wt% in NDIIa
GEL
LIQUID

Figure 3

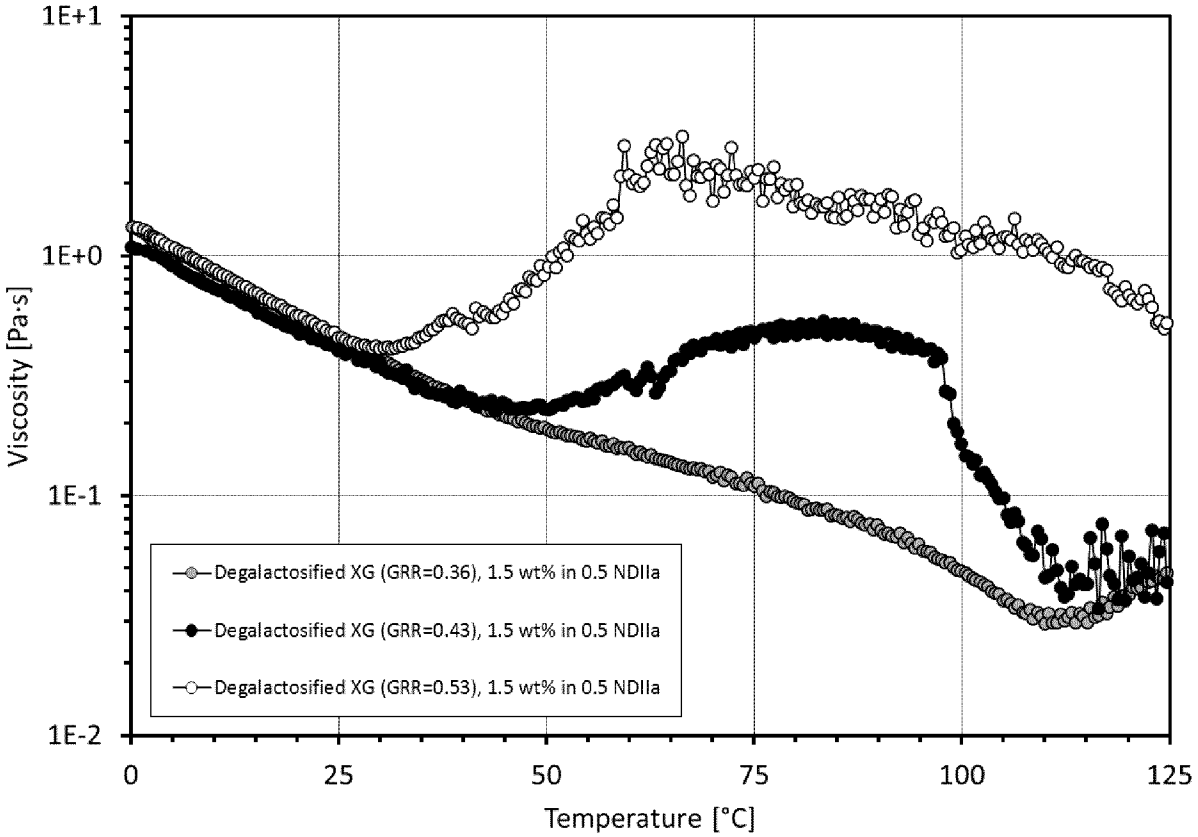

1E+1
1E+0
1E-1
1E-2
Viscosity [Pa·s]
0
25
50
75
100
125
Temperature [°C]
Degalactosified XG (GRR=0.36), 1.5 wt% in 0.5 NDIIa
Degalactosified XG (GRR=0.43), 1.5 wt% in 0.5 NDIIa
Degalactosified XG (GRR=0.53), 1.5 wt% in 0.5 NDIIa

Figure 4

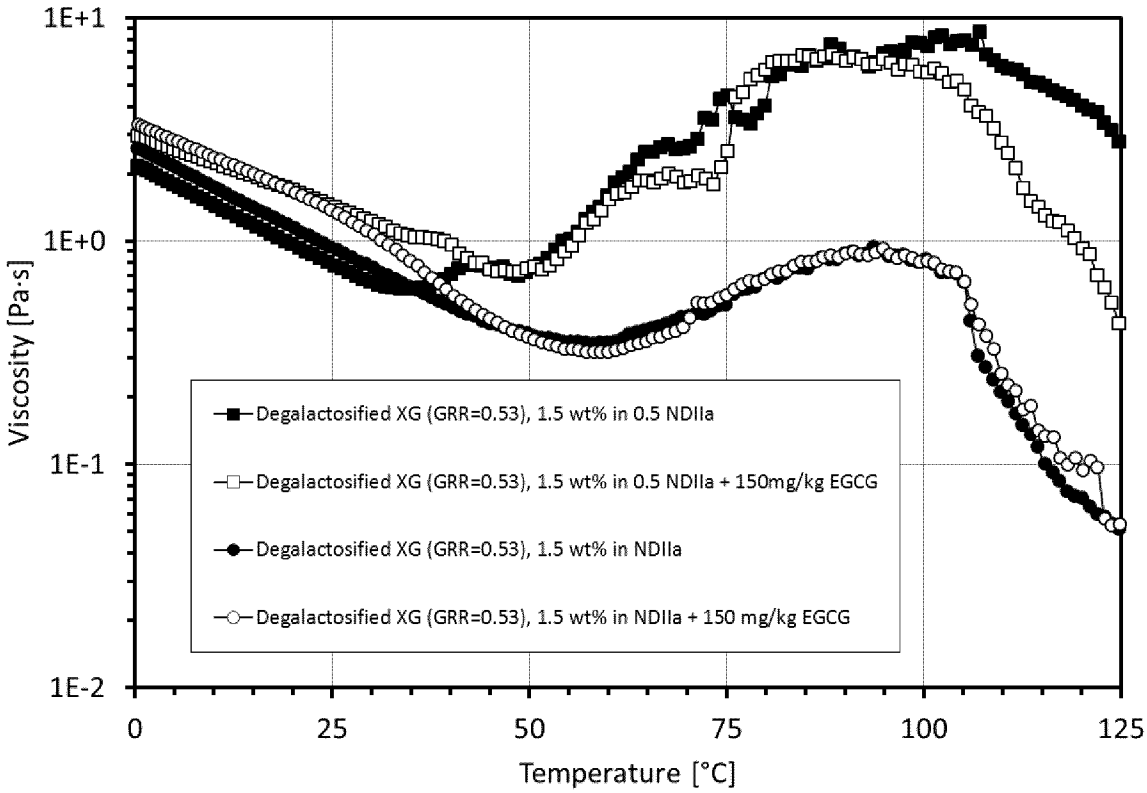

1E+1
1E+0
1E-1
1E-2
Viscosity [Pa·s]
0
25
50
75
100
125
Temperature [°C]
Degalactosified XG (GRR=0.53), 1.5 wt% in 0.5 NDIIa
Degalactosified XG (GRR=0.53), 1.5 wt% in 0.5 NDIIa + 150mg/kg EGCG
Degalactosified XG (GRR=0.53), 1.5 wt% in NDIIa
Degalactosified XG (GRR=0.53), 1.5 wt% in NDIIa + 150 mg/kg EGCG

Figure 5

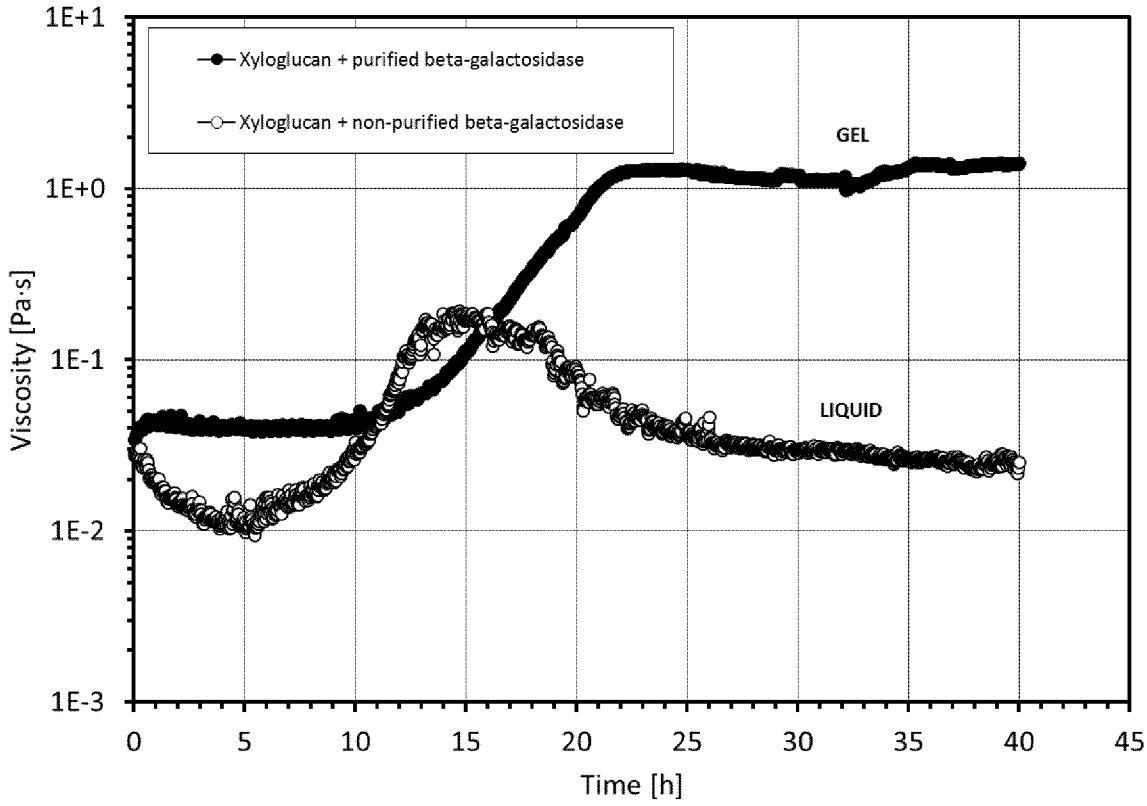
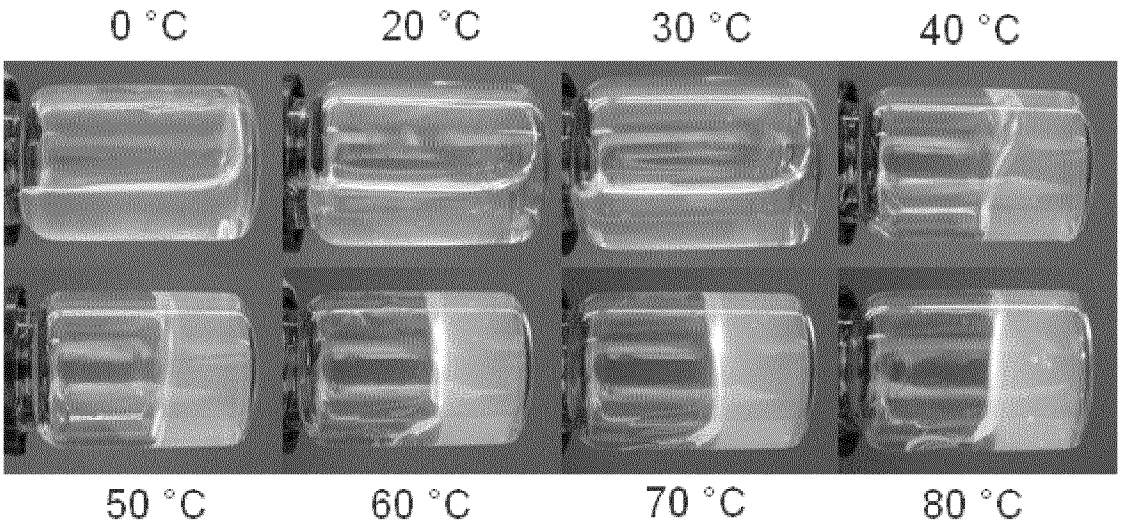

Xyloglucan + purified beta-galactosidase
Xyloglucan + non-purified beta-galactosidase
GEL
LIQUID
Viscosity [Pa·s]
1E+1
1E+0
1E-1
1E-2
1E-3
0
5
10
15
20
25
30
35
40
45
Time [h]

Figure 6



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/056163

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/00 C09K8/035
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)
C12N C09K

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.
X	AMANDA K. ANDRIOLA SILVA BRUN-GRAEPPI ET AL: "Study on the sol-gel transition of xyloglucan hydrogels", CARBOHYDRATE POLYMERS, vol. 80, no. 2, 1 April 2010 (2010-04-01), pages 555-562, XP55121456, ISSN: 0144-8617, DOI: 10.1016/j.carbpol.2009.12.026 3. Results and discussion. up to 85% removal rate. 2.1 Galactosidase reaction 2.2 Thermogelation the whole document ----- -/--	9-14, 17-19

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

[X] See patent family annex.

* Special categories of cited documents :

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"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
5 June 2014	12/06/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Straub, Thomas

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/056163

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MAYUMI SHIRAKAWA ET AL: "Tailoring of xyloglucan properties using an enzyme", FOOD HYDROCOLLOIDS, vol. 12, no. 1, 1 January 1998 (1998-01-01), pages 25-28, XP55121452, ISSN: 0268-005X, DOI: 10.1016/S0268-005X(98)00052-6 p. 27, right column. 58%, 56% removal ratio the whole document	9-14, 17-19
A	WO 2007/031722 A1 (HALLIBURTON ENERGY SERV INC [US]; CURTIS PHILIP ANTHONY [GB]; WALTERS) 22 March 2007 (2007-03-22) claims 6,8,11 the whole document	1-19
A	US 4 466 893 A (DILL WALTER R [US]) 21 August 1984 (1984-08-21) claim 13 the whole document	1-19
A	US 2004/108113 A1 (LUKE KAREN [US] ET AL) 10 June 2004 (2004-06-10) claims 1,3,17 the whole document	1-19
X,P	EP 2 698 429 A1 (CENTRE NAT RECH SCIENT [FR]; GENETHON [FR]) 19 February 2014 (2014-02-19) paragraph [0040] the whole document	9,12,17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/056163

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007031722 A1	22-03-2007	AR 058667 A1	20-02-2008
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EP 2698429 A1	19-02-2014	NONE	