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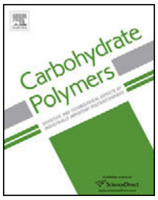

Short communication

New carboxymethyl cellulose tosylate with low biodeterioration

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ABSTRACT

Microbial biodegradation and biodeterioration of cellulose based thickeners is a serious problem in industry. A new tosylic ester of carboxymethyl cellulose (TsCMC) was prepared with anhydride of *p*-toluensulphonic acid. The TsCMC has improved rheological properties, higher viscosity and pseudoplasticity, superior emulsification properties and decreased wettability compared to parental CMC. The biodeterioration of TsCMC was significantly reduced compared to parental CMC or other commercially used modified cellulose thickeners in water based paint industry. Improved rheological properties combined with low biodeterioration make TsCMC a promising new material for industrial applications with a potential to reduce the use of hazardous antimicrobial agents.

1. Introduction

Carboxymethyl cellulose (CMC) is the most important commercial water-soluble cellulose ether derivative. It is inexpensive and widely used in many industrial sectors including adhesives (Hollabaugh, Burt, & Peterson Walsh, 1945), paints (Hollabaugh et al., 1945), leather (Hollabaugh et al., 1945), paper (Tiitu, Laine, Serimaa, & Ikkala, 2006), textiles (Hashem, Refaie, Goli, Smithand, & Hauser, 2009), pharmaceutics (Jones, Woolfson, & Brown, 1997), food (Diftis & Kiosseoglou, 2003), ceramics (Zhu, Jiang, & Tan, 2001), agriculture (Nie, Liu, Zhan, & Guo, 2004), detergents (Black, 1951), oil well drilling (Dolz, Jiménez, Jesús Hernández, Delegido, & Casanovas, 2007), foundry (Ruschev, Stoyanov, & Pinalova, 1988) and mineral processing (Pawlik, Laskowski, & Ansari, 2003). CMC is a non-toxic biodegradable polymer (Orehek, Dogsa, Tomšič, Jamnik, Kočar, & Stopar, 2013). Though from an ecological point of view biodegradability is a desirable property (Sannino, Demitri, & Madaghiele, 2009), it can cause severe biodeterioration problems in a commercial product. For example, in water-based paint industry biodegradation of CMC significantly decreases product viscosity and therefore decreases its commercial value (Tothill, Best, & Seal, 1993; Tothill & Seal, 1993). Due to recent strengthening of the EU legislation where the amount of allowable antimicrobial agents in commercial products was reduced finding CMC replacements with reduced biodegradability becomes an imperative (Biocidal Products Regulation EC 528/2012).

In this work a new tosylic ester of carboxymethyl cellulose (TsCMC) was prepared using CMC and anhydride of *p*-toluensulphonic acid. The new material shows favorable physicochemical properties for applications in water-based paint industry and has significantly reduced biodeterioration compared to parental CMC molecule.

2. Materials and methods

2.1. Synthesis

CMC tosylate (TsCMC) was prepared as an ester of *p*-toluenesulphonic acid (TSA) via free hydroxyl groups on anhydroglucose units of CMC. For derivatization a solution of 16 g NaCMC (90 kDa with DS = 0.7, Aldrich) was dissolved in 350 mL DMSO (Panreac) containing 11 g of TSA (Sigma-Aldrich), 1.0 g of P_4O_{10} (Sigma-Aldrich) and was stirred at 100 °C for 1 h. The water-binding agent P_4O_{10} was used to prevent hydrolysis in the presence of strong acid. An anhydride of TSA was prepared by reacting 10 g of P_4O_{10} with 50 g of TSA in 100 mL of DMSO and stirred at 100 °C for 20 min. In the final step of synthesis the two solutions were mixed together and stirred at 100 °C. When the reaction mixture began to thicken, the reaction was stopped by adding 2000 mL of absolute EtOH (Sigma-Aldrich). The TsCMC was passed through Schleicher & Schuell black ribbon filter paper and washed three times with 1000 mL of absolute EtOH. The neutralization of TsCMC was achieved in 1000 mL of EtOH containing 2 g

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of NaOH pellets by continuous mixing for 30 min. The remaining pellets of NaOH were removed before filtration of neutralized product. After neutralization the TsCMC was washed three times with absolute EtOH and dried at 45 °C to a constant mass.

2.2. Physicochemical characterization of TsCMC

Viscosity was measured on an Anton Paar Physica MCR 301 rotational plate–plate system with a plate diameter of 49.98 mm. The distance between plates was 0.25 mm and the measuring temperature was (25.00 ± 0.01) °C. Approximately 490 μL of sample was applied to fill the gap between the plates. Flow curves in a shear rate ranging from 10 to 1000 s^{-1} were measured in 29 steps with a time delay of 5 s between successive measurements. The results for viscosity measurements are reported at a shear rate of 1000 s^{-1}. The intrinsic viscosities were determined by measuring the viscosity of diluted samples. Samples were diluted in BHM or pure water to a final concentration of 0.05, 0.1, 0.2 and 0.3% (w/v) CMC or TsCMC. The intrinsic viscosity was determined by graphically evaluating the limit of the following equation:

$$[\eta] = \lim_{\gamma \to 0} \frac{\eta - \eta_0}{\eta_0 \gamma} \qquad (1)$$

where $[\eta]$ is the intrinsic viscosity, η viscosity of diluted polymer solution, η_0 viscosity of solvent in the absence of solute, and γ mass concentration of solute in g/cm^3.

IR spectra were recorded on Perkin Elmer Spectrum 100 FTIR spectrophotometer with diffused reflection technique as described previously (Sherman Hsu, 1997).

Elemental analysis of TsCMC was performed according to the following standardized methods: SIST EN 13137 for the determination of total organic carbon (TOC) in waste, sludges and sediments, SIST ISO 609 for hydrogen determination with high temperature combustion method, SIST ISO 351 for determination of total sulfur with high temperature combustion method, and ISO 6878 for determination of phosphorus with ammonium molybdate spectrometric method. The samples for the determination of phosphorous were prepared according to SIST EN 13346—determination of trace elements and phosphorus with aqua regia extraction methods.

For evaluation of emulsifying efficiency solutions of 5 mg CMC or TsCMC in 9 mL of demineralized water were prepared. To these solutions 1 mL of paraffin oil stained with lysochrome Sudan III was added. Emulsions were prepared by hand shaking of closed tubes for 1 min. The evaluations of emulsion stability was performed after 1 h, one day and one week following emulsion preparation (Sroková, Tomanová, Ebringerová, Malovíková, & Heinze, 2004).

The dynamic contact angle measurements were performed on FIBRO DAT 1100 Dynamic Absorption Tester. A testing surface was prepared by soaking filter paper with 1% (w/v) water solution of CMC or TsCMC and dried at 45 °C over two days. 6 μL drops of water solution were placed on the testing surface and contact angle between the basis and water drops was recorded over a period of 50 s.

2.3. Biodegradation of TsCMC

The biodegradation of TsCMC used as a sole carbon source was measured as an increase in reducing sugar concentration during the incubation, by increase in optical density (OD_{650}), and an increase of bacterial colony forming units during the incubation. Reducing sugar concentrations were determined spectrophotometricaly using DNS reagent (Miller, 1959). DNS reagent was prepared as a solution of 10 g 3,5-dinitrosalicylic acid and 300 g of K, Na-tartrate in 1 L of 0.4 M NaOH. Next 1 mL of DNS reagent was added to 1 mL of sample or standard glucose solution. The reaction was carried out in glass tubes incubated at 100 °C for 15 min. After the incubation and colour development, the samples were cooled to room temperature in a water bath. Absorbance at 575 nm was measured and the reducing sugar concentration was determined from a calibration curve, which was freshly prepared from standard glucose solutions (0–5 mM). Biodegradability of TsCMC and other tested cellulose based thickeners was tested on a modified Buschnell Hass growth media (BHM) (Bushnell & Haas, 1941). The modified BHM growth media differs from the original formulation due to the elimination of iron source, which was shown to cause aggregation of CMC. The overnight bacterial culture was washed two times with a sterile physiological solution and 1 mL was inoculated into 100 mL of BHM growth medium.

Table 1
Results of CHSP elemental mass analysis and theoretically calculated elemental composition of CMC and TsCMC.

Element (%)	CMC_t	CMC_d	$TsCMC_t$	$TsCMC_d$
C	40.5	38.8	38.9	38.5
H	4.9	5.0	4.6	5.0
S	0	<0.02	1.3	1.5
P	0	<0.02	1.24	0.8

Legend: CMC_t = theoretical elemental mass composition calculated for CMC with DS value of 0.7; CMC_d = experimentally determined elemental composition; $TsCMC_t$ = theoretically calculated elemental composition for TsCMC with DS of carboxymethylic groups 0.7 and DS of tosylic groups 0.1 in the presence of one molecule of Na_3PO_4 per 10-anhydroglucosic units present as an impurity; $TsCMC_d$ = experimentally determined elemental composition. For theoretical calculations of element compositions it has been assumed that DS of parental CMC (DS = 0.7) did not change during derivatization and that all carboxylic groups are present as Na-salts.

2.4. Biodeterioration of TsCMC

The biodeterioration of TsCMC defined as a process of any undesirable change in the properties of materials caused by organisms was measured as the decrease in viscosity of the growth medium and was tested with cultures of *Bacillus firmus*, *Bacillus mycoides*, *Salmonella typhimurium*, *Azospirillum brasilense* ATCC 29145, *Alcaligenes* sp. NCIB 11015, *Bacillus subtilis* JH 642, *Streptomyces coelicolor* DSM 40233, *B. subtilis* NCIB 3610, *B. subtilis* IS-75, *Chromobacterium violaceum*, *Cellulomonas uda* DSM 20108. All inoculated samples were aerobically incubated at 37 °C in the dark on an orbital shaker (200 rpm) for one week. The biodeterioration of TsCMC or other commercial cellulose based thickeners was estimated as a fraction of residual viscosity remained after the incubation (initial viscosity – final viscosity) × 100.

3. Results and discussion

3.1. Chemical characterization

To the best of our knowledge, the tosylation via ester linkage to CMC has not been performed yet. As given in Fig. 1, the FTIR spectra indicate that global vibrational structures of parental CMC and derived TsCMC are similar. The two spectra, however, differ at several vibrational bands, which were broadened and shifted. Most notably the bands at 816 and 690 cm^{-1} were present only in TsCMC but not in CMC. The two vibrations correspond to out of plane C—H deformation and out of plane ring vibrations of *p*-toluenesulphonic acid, respectively. This suggests that CMC has been tosylated. The element composition of TsCMC is given in Table 1. The TsCMC contained 1.3% sulfur, which allowed calculation of the average degree of substitution of CMC with tosylic groups. On average every tenth anhydroglucose unit of CMC was substituted with tosyl group (DS = 0.1). The results suggest that TsCMC contained few phosphorous impurities, which was likely a result of P_4O_{10} hydrolysis to Na_3PO_4 during the TsCMC synthesis and purification.

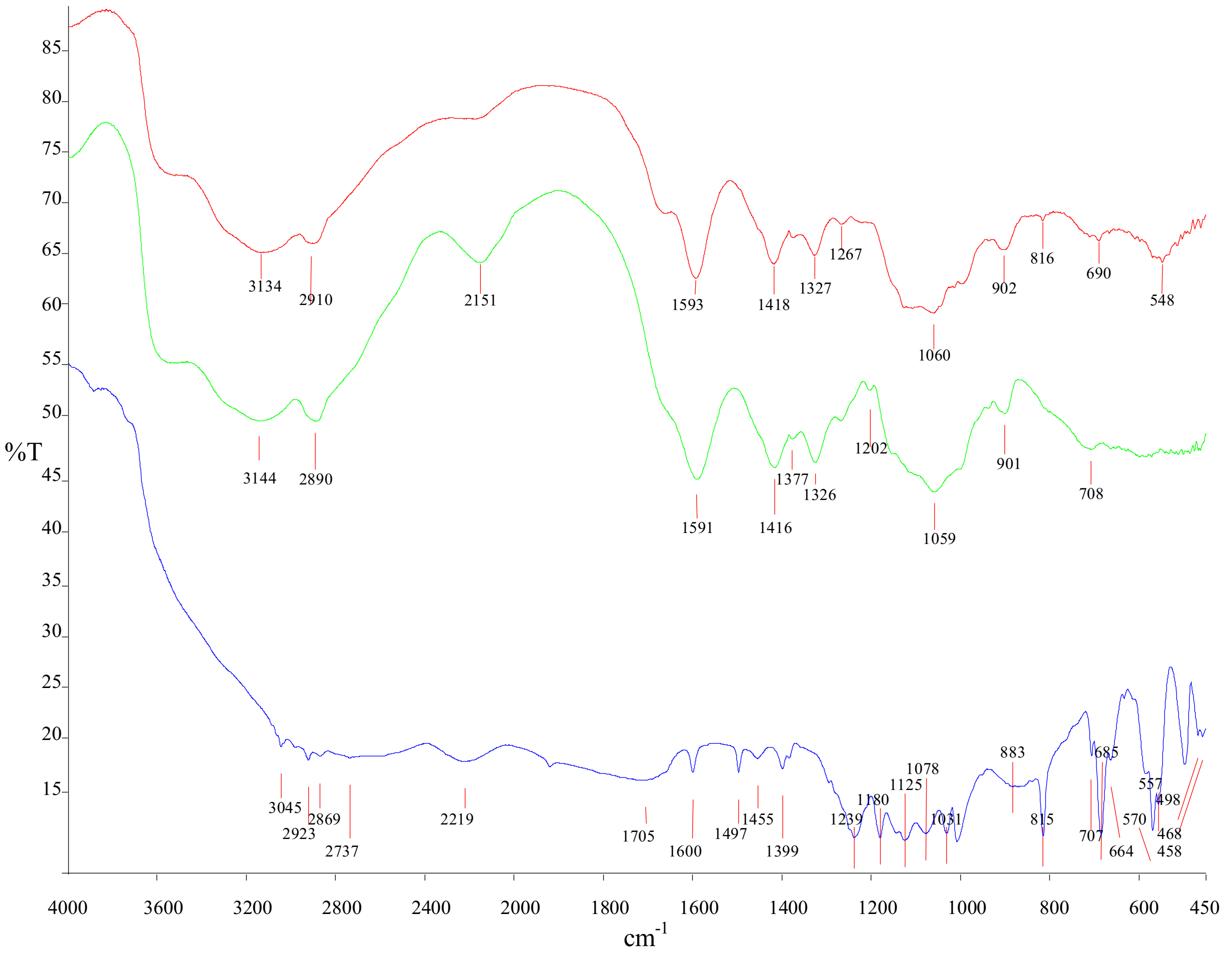

Fig. 1. FTIR spectra of TsCMC (red), CMC (green) and *p*-toluensulphonic acid (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Physical characterization

As given in Fig. 2, the viscosity of TsCMC solution in BHM medium was much higher compared to CMC. For instance, in BHM medium the viscosity of 10 g/L TsCMC solution was 27.2 mPa s compared to 3.5 mPa s in CMC. In addition, tosylation changed rheological behavior of CMC as shown in Fig. 3. Whereas, CMC exhibited approximately Newtonian flow behavior (Fig. 3B), TsCMC displayed pseudoplastic flow behavior (Fig. 3A). The individual polymer chains in TsCMC may interact with neighboring polymer chains via tosylic groups forming inter π–π stacking interactions that result in increased pseudoplasticity (Sinnokrot, Valeev, & Sherrill, 2002; McGaughey, Gagné, & Rappé, 1998). The intrinsic viscosity in BHM growth medium was (350 ± 10) cm^3/g for TsCMC and (330 ± 10) cm^3/g for parental CMC, which suggests that the two polymers have comparable Mw and that no polymerization occurred during the derivatization. The results of the dynamic contact angle measurements for the two materials are given in Fig. 4. The data indicate that TsCMC is less wettable than CMC. The initial contact angle between TsCMC and water was 85° and slowly decreased to 40°. On the other hand in CMC the initial contact angle was 65° that rapidly decreased to 48° and then slowly to 35° to the end of the experiment. This demonstrates that TsCMC is more hydrophobic than CMC, which is consistent with the addition of tosylic groups to the CMC backbone. Emulsion stability of TsCMC was significantly enhanced compared to CMC. After two weeks TsCMC emulsions were still homogenous with only a thin layer of oil fraction on the surface (data not shown). It is likely that tosylic groups promote emulsification by adsorption to oil drops through hydrophobic effect, whereas polar carboxymethyl groups of TsCMC bind water thus preventing the phase separation.

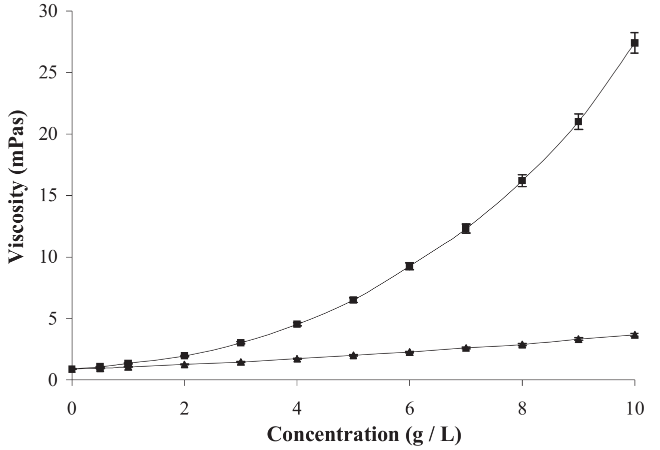

Fig. 2. Viscosity (at shear rate 1000 s^{-1}) of CMC (▲) and TsCMC (■) at different concentrations in BHM growth medium. Mean values and estimated errors are given.

3.3. Biodeterioration

The main focus of this work was to develop CMC derivative with significantly decreased biodegradation and biodeterioration.

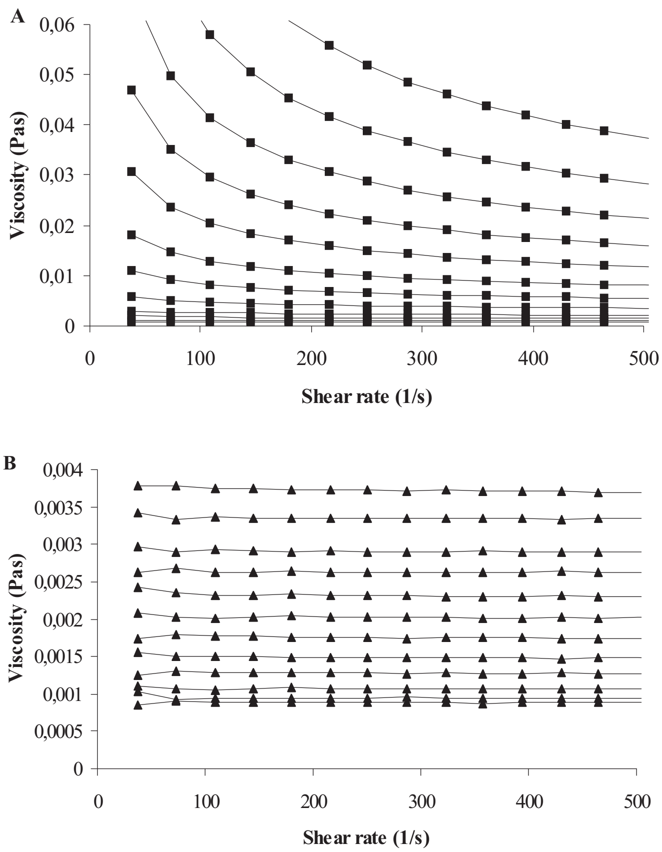

Fig. 3. Flow behavior of TsCMC (■) (panel A) and CMC (▲) (panel B) for 0; 0.05; 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9, and 1% (w/v) polymer concentration in BHM growth medium (from bottom to the top).

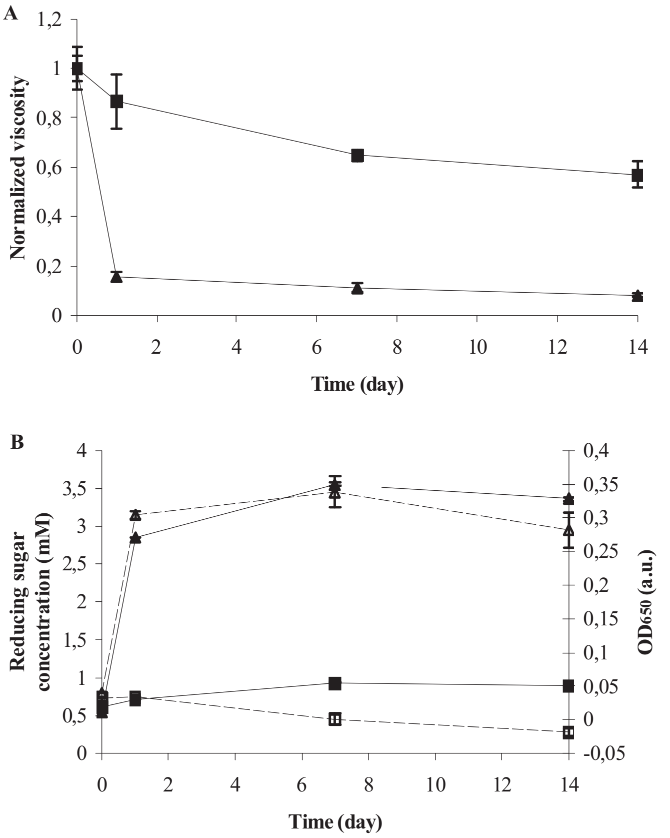

Fig. 5. (A) Normalized viscosity of CMC (▲) and TsCMC (■) during the incubation with *Cellulomonas uda* in BHM growth medium; (B) reducing sugar concentration (filled symbols) and OD_{650} (empty symbols) of CMC and TsCMC during the incubation. Mean values and standard deviations are given ($n = 3$).

The results of incubation experiment with TsCMC or CMC as a sole carbon source are given in Fig. 5. When CMC was used as a sole carbon source, a ten-fold increase in OD_{650} was observed during the first 24 h of incubation, indicating a significant increase in the number of bacteria. Consistently, up to 7-fold increase in the reducing sugars concentration and sharp decrease of viscosity was observed (Fig. 5B). After one day of incubation the viscosity of CMC was comparable to viscosity of BHM solution and degraded CMC could no longer act as a thickener (Fig. 5A). On the other hand, TsCMC used as a sole carbon source did not enable growth of *C. uda*. Bacteria, however, remained viable to the end of experiment and were able to grow when transferred to fresh growth medium or when glucose was added to TsCMC. *C. uda* was able to produce some reducing sugars during the incubation on TsCMC; the increase was fairly small but significant ($p = 0.0096$). An increase in sugar concentration was not coupled to the growth of the bacterium, but correlated with the decrease of viscosity. The initial viscosity of TsCMC decreased for approximately 40% during the incubation. It should be noted, however, that even a single break in the large polymer such as CMC or TsCMC may substantially reduce the initial viscosity. Notwithstanding, the TsCMC at the end of incubation could still be used as a thickening agent (Fig. 5A). No decrease of viscosity of TsCMC was observed in the absence of *C. uda* suggesting that non-biological degradation was negligible under the experimental conditions.

The results of comparison of biodeterioration of cellulose thickeners that are widely used in water-based paint industry and TsCMC are given in Table 2. The selected cellulose derivatives differ in physicochemical properties but have similar thickening properties. The results indicate that all selected cellulose thickeners were liable to *C. uda* biodeterioration. Nevertheless, the residual viscosity after one week of incubation was much higher for TsCMC. This demonstrates that tosylated CMC has significantly improved resistance to biodeterioration compared to commercially available thickeners with comparable rheological properties. The resistance of TsCMC to biodeterioration by different bacteria is given in Fig. 6. The tested bacteria can be grouped into two categories: bacteria that are not able to degrade neither CMC nor TsCMC; and bacteria that degrade CMC and to a much lesser extent TsCMC (i.e. *C. uda*, and *B. subtilis* strains). None of these bacteria, however, were able to completely eliminate TsCMC thickening properties during the incubation. To further test biodeterioration properties of the new TsCMC two products made in water based paint industry were

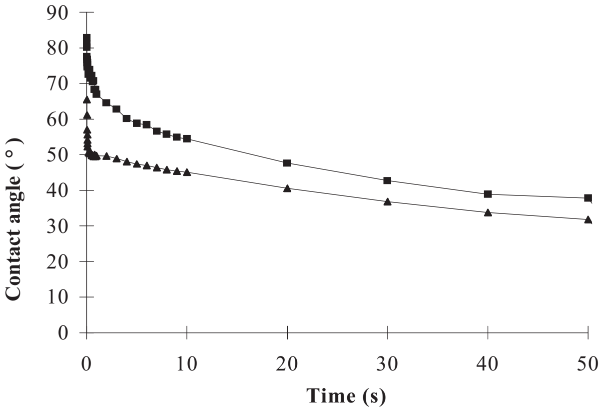

Fig. 4. Dynamic contact angle diagram for CMC (▲) and TsCMC (■). Experiment was independently repeated 2 times.

Table 2
Biodeterioration of various cellulose thickeners by *Cellulomonas uda* grown in BHM growth medium during one week of incubation. Legend: TsCMC = tosylated carboxymethyl cellulose; CMC = carboxymethyl cellulose; HEC = hydroxyethyl cellulose, HMHEC = hydrophobically modified HEC with cetylic side chains; MC = methyl cellulose. The fraction of residual viscosity was calculated as (initial viscosity–final viscosity) × 100. Solvent viscosity was subtracted from all samples.

Cellulose thickener	Initial viscosity at 1000 s^{-1} (mPa s)	Viscosity after one week incubation (mPa s)	% of residual viscosity	Producer
1% TsCMC	26.5	17.2	64.9	Prepared in this work
1% CMC (90 kDa/DS = 0.7)*	2.6	0.22	8.5	Aldrich
1% CMC (250 kDa/DS = 1.2)	18.3	3.11	17.0	Sigma-Aldrich
1% CMC (250 kDa/DS = 0.9)	24.1	0.72	3.0	Aldrich
1% CMC (250 kDa/DS = 0.7)	26.8	0.61	2.3	Aldrich
1% CMC (Blanose CMC 12M31P)	36.5	3.30	9.0	Ashland
0.7% HEC (Natrosol 250 HHR)	53.2	0.23	0.4	Ashland
1% HEC (Natrosol 250 MR)	51.0	0.34	0.7	Ashland
0.7% HMHEC (Natrosol plus 330 PA)	18.8	5.87	31.2	Ashland
1% MC (Culminal 7000 PF)	69.4	0.78	1.1	Ashland

* The parental CMC molecule used for derivatization.

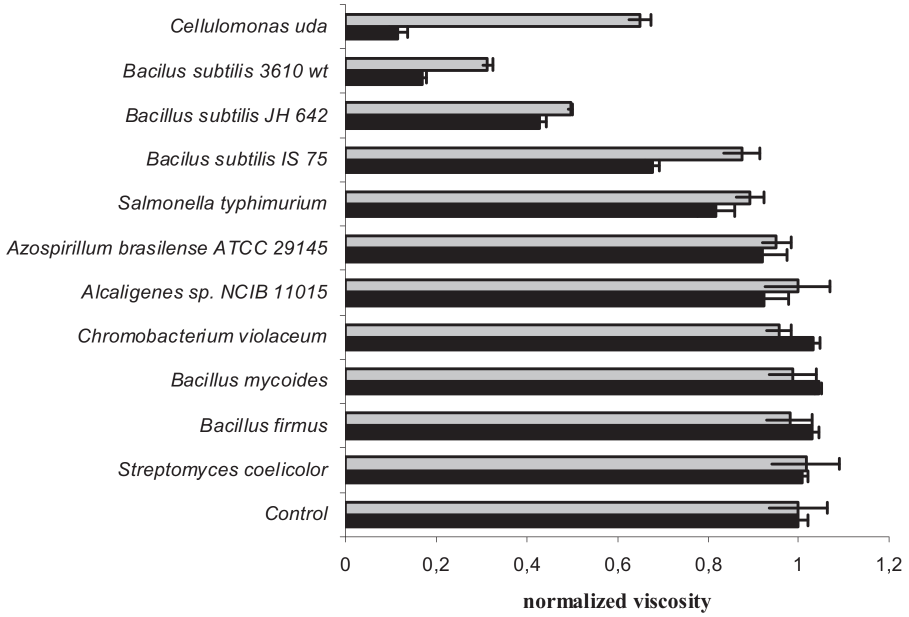

Fig. 6. Biodeterioration of CMC (black bars) and TsCMC (gray bars). Loss of viscosity (normalized relative to control) is given after 7 days of incubation with different bacterial strains. Mean values and standard deviations are given ($n = 3$).

prepared. In these products CMC was replaced with TsCMC, while keeping all the other ingredients unchanged. The results indicate that the same quality of the product was obtained, but with significantly improved shelf life of the TsCMC product (data not shown). Due to superior pseudoplastic behavior reduced concentrations of TsCMC were used. In conclusion, the TsCMC appears to be a promising new polymer for products where bacterial contamination of CMC is an important issue, like for instance in water-based paints.

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