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*International Journal of Current Research*
*Vol. 10, Issue, 03, pp.66882-66885, March, 2018*

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

**ISSN: 0975-833X**

## RESEARCH ARTICLE

# BIODEGRADABILITY OF XANTHAN GUMBY BACTERIA FROMFRACTURING WATERS FROM THE OIL INDUSTRY

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**ARTICLE INFO**

***Article History:***
Received 22nd December, 2017
Received in revised form
29th January, 2018
Accepted 07th February, 2018
Published online 30th March, 2018

***Key words:***

Xanthan gum, Viscosity,
Bacteria, Degradation.

**ABSTRACT**

In oil recovery operations, the application of xanthan biopolymers is considered more environmentally friendly than that of synthetic polymers. However, a decrease in the viscosity of xanthan has sometimes been observed. The objective of the present study was to investigate the decrease in the viscosity of xanthan gum caused by bacterial activity in samples from fracturing waters in the Golfo San Jorge Basin, Argentina. To this end, solutions of xanthan gum dissolved in culture medium were studied by means of viscometry and Fourier Transform infrared spectroscopy. The intrinsic viscosity of xanthan in the original sample decreased in 48-72 h, whereas that in two bacterial communities grown in a previous culture medium was not modified.



**Citation: Baztan Maite and Graciela Pucci, 2018.** "Biodegradability of xanthan gumby bacteria fromfracturing waters from the oil industry", *International Journal of Current Research*, 10, (03), 66882-66885.

## INTRODUCTION

Natural polymers have been utilized for a variety of industrial and environmental applications due to their physicochemical properties and useful functions. These properties include intrinsic biocompatibility, biodegradability, and non-toxicity. In the oil industry inthe San Jorge Basin, Argentina, chemical-enhancedoil-recovery methods have been introduced but have often been considered to be unprofitable because of the low oil prices.One of the most promising chemical-enhancedoil-recovery processes in many reservoirs, due to its lower cost, is the polymer flooding method (Jang et al. 2015, Taylor and Nasr-El-Din 1998). In this method, high-molecular-weight water-soluble polymers can increase the viscosity of the aqueous phase easily, which also results in an increase in the sweep efficiency of the oil recovery. The use of polymers as xanthan is frequent. Xanthan is a biopolymer produced by *Xanthomonascampestrispv. campestris*, widely applied in different industries, including the petroleum industry, due to its viscosity property (García-Ochoa *et al.,* 2000; Katzbauer, 1998; PalanirajandJayaraman, 2011). However, xanthan is a highly stable polysaccharide (McInerney*et al.,* 2005) not easily degraded by most microorganisms (Li *et al.,* 2009).

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Xanthan gum has attracted considerable attention as an agent for chemical-enhancedoil-recovery methods in oil drilling, fracturing, and pipeline cleaning (PalanirajandJayaraman, 2011), due to its strong ability to increase the shear viscosity, coupled with its excellent stability under high salinity(Jang *et al.,* 2015), high temperature and mechanical shear conditions. However, because the viscosity of the displacing fluid in chemical-enhanced oil-recovery methods is vital, it is important to control the viscosity of xanthan gum under given salinity and temperature conditions (Jang *et al.,* 2015). The objective of the present study was to investigate the effect ofbacterial activity on the decrease in xanthan gum viscosity in a sample of fracturing waters from the San Jorge Basin.

## MATERIALS AND METHODS

### Experimental design

The experiment was conducted to verify xanthan biodegradation by culturing in synthetic medium (g $L^{-1}$: NaCl 5, $K_2PO_4$ 1, $KPO_4H_2$ 1, $(NH_4)_2O$ $SO_4$ 1,$MgSO_4$ 0.2, $KNO_3$ 3, yeast extract 0.02 and 1% xanthan gum). The experiment was performed at 30°C in mineral mediumwith xanthan as sole carbon source (1%). The original fracturing waters, a mix of aerobic facultative bacteria from xanthan medium, a mix of aerobic facultative bacteria from CLDE medium (g/L peptone 4, meat extract 3, L-cysteine 0.128, trypteine 4, bromothymolblue 0.02, lactose 10, agar 15), and 16 bacterial

strains were incubated in the medium with xanthan, and followed by optical density, viscosity studies and Fourier transform infrared (FT-IR) spectroscopy at day zero and day 6. Facultative aerobic degraders were aerobically isolated by the plating technique onmineral medium with xanthan and CLDE medium. The culture from the original sample was used as inoculum. Approximately 0.1 ml of culture was transferred to mineral medium with xanthan plates before the volume was spread. The plates were incubated at 28°C. When, growth was detected, single colonies were transferred to new plates by using the streaking technique. The purity and morphology of the culture was controlled by microscopy. When pure colonies were obtained, colonies from the twoplates were transferred to aerobic liquid tube with xanthan and a mixof all pure cultureswas used to control the bacterial communities.

**Viscosity**

The viscosity of the culture fluid was measured in triplicate with a Brookfield DV-E viscometer (Brookfield) at 25 ± 1°C after 1 min of rotation using a spindle #2. The rotation speed was 20 rpm.

speed detector DTGS equal to 10 kHz. Three spectra were sequentially acquired for each sample. These spectra were processed and analyzed with the aid of Resolution Varian software.The region from 700 to 900 waves/cm was used for bacterial fingerprinting.

**Optical density:** The growth of bacterial communities was determined by optical density (OD) at 600 nm.

## RESULTS AND DISCUSSION

The stability of xanthan gum has been discussed in several studies (Li *et al.,* 2008; Qian*et al.,* 2007). These studies have shown that this biopolymer is completely degraded by only a few microorganisms (Cadmus *et al.,* 1982) as *Bacillus* (Nankai*et al.,* 1999, Hashimoto *et al.,* 2003), *Microbacterium*sp.(Yang *et al.,* 2014) and*Paenibacillus* (Ruijssenaars*et al.,* 2000).

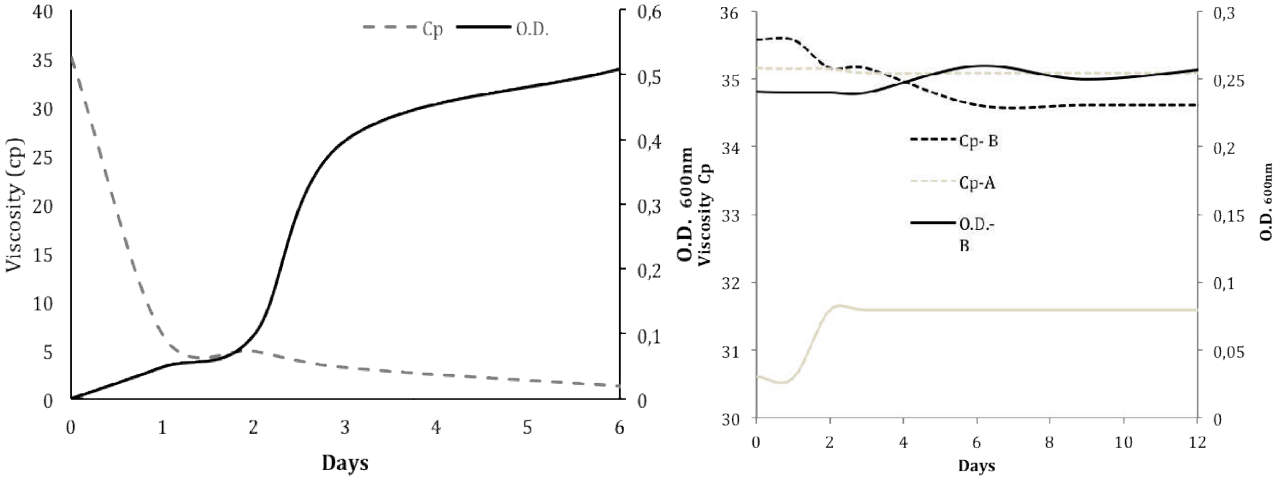


**Fig. 1. Viscosity and optical density (OD) a: medium with xanthan; b:medium for fermenters**

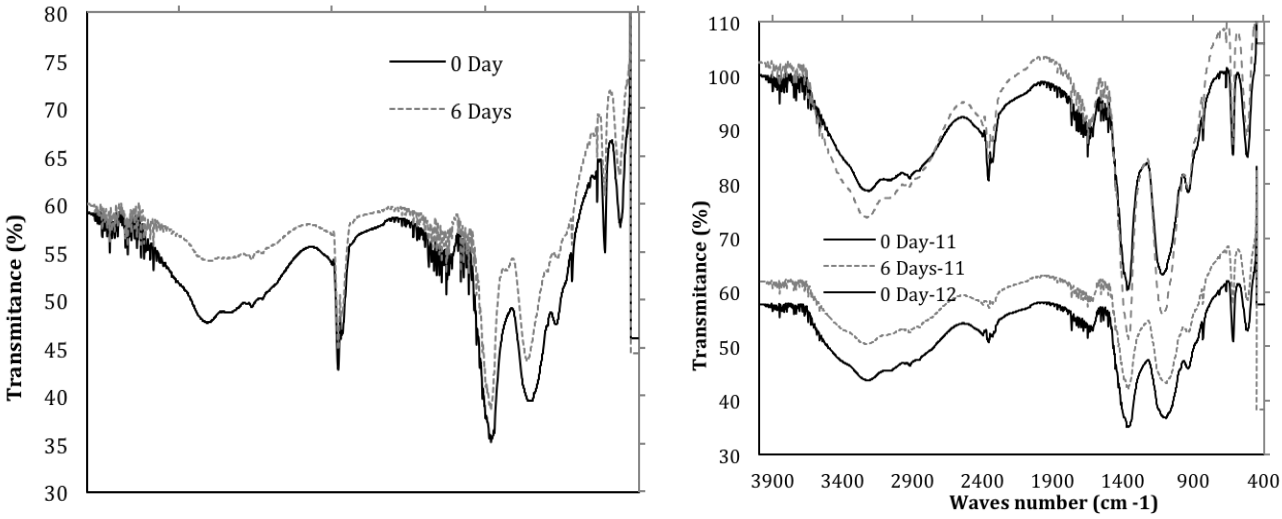


**Fig 2. FT-IR spectrum of xanthan samples**

**FT-IRspectroscopy**

Samples were analyzed on Varian equipment, operated in the spectral window from 400 to 4000 waves/cm with 32scans/sample with a resolution of 4waves/cm and scanning

The truncation of the side chain by lysisleads to a change in the viscosity of xanthan gum (Wang *et al.,* 2013). Theyhave been found in the soil (Garcia-Ochoa *et al.,* 2000) and seawater-injected oil fields (Hovland, 2015). In several studies, xanthan-degrading species were purified from mixed cultures growing

on xanthan. However, these studies did not provide the incubation time spent before xanthan degradation was demonstrated in the initial cultures.In the present study, after isolation of single colonies, 16 bacterial strains were transferred to aerobic mineral media with xanthan and incubated at 28°C. After approximately two weeks, no growth was detected in any of the enrichments. The isolates had a rod-shaped morphologywith a wide variety in lengths, and all were Gram-negative. Results from the viscosity analysis showed no decrease in the viscosity of xanthan in any of the 16 strains. The optical density curves showed no modifications in the values (results not shown).

The growth of the mix of culturable bacteria was also demonstrated in the communities from the mineral medium with xanthan and in those from the CLDE medium, but was less pronounced and not associated with a significant reduction in viscosity compared to the original sample. Growth without xanthan degradation was probably supported by cellular debris and glucose residues from the xanthan production, used as a carbon source by species not able to degrade xanthan. Viscosity measurements (Fig. 1) confirmed that no degradation of xanthan had occurred and prolonged incubation for another 6 days did not change the viscosity.In the original sample, the biodegradation of xanthan was assessed by a decrease in viscosity, an increase in the cell number and shifts in the bacterial communities (Fig. 1b). FT-IR spectroscopy (Fig. 2) is a methodology to detect similarities and differences in the chemical structures of compounds. Samples on days 0 and 6 were analyzed to identify the functional groups present in the structure of xanthan. The region studied included all the spectral bands located in the window between wave numbers 400 and 4000 $cm^{-1}$.Comparison of the FT-IR spectrum at the beginning and at the end of the experiment showed bands around 3400 $cm^{-1}$, 2939 $cm^{-1}$and 990–1200 $cm^{-1}$, common to all polysaccharides, and represent O–H bonds, C–H bonds of the $CH_2$groups and saccharides, respectively. In concordance with the results regarding the viscosity of xanthan, the FT-IR in the original sample loss represents O–H bonds 3400 $cm^{-1}$.

In agreement with previous studies (Hashimoto *et al.,* 1998; Liu *et al.,* 2005; Ruijssenaars *et al.,* 1999), our results showed that xanthan degradation occurred fast, extending for a couple of days. So that, for microorganisms to metabolize biopolymers, the macromolecule is broken down into smaller fragments outside the cell before uptake and further degradation inside the cell (Li *et al.,* 2008, Kreyenschulte *et al.,* 2014). Xanthan is hydrolyzed by only a few enzyme systems, which supports the assumption that xanthan is relatively resistant to biodegradation (Ruijssenaars *et al.,* 1999; Lui*et al.,* 2005).The degradation of the biopolymer was induced when xanthan was the only carbon source in the original communities and not from the culturablecommunities. However, the results showed no changes in the viscosity of the culturable communities probably because the xanthan lyase production was greatly decreased when the xanthan-degrading strains were purified from the mixed cultures they originated (Cadmus *et al.,* 1982, 1989). In fact, when xanthan gum was used in oil recovery in the San Jorge Basin, problems aroused (Bragg *et al.,* 1983; McInerney *et al.,* 2005). Hou *et al.,* (1986) investigated microorganisms that might have been responsible for the loss of viscosity under enhanced oil-recovery operation conditions, whereas Hashimoto *et al.,* (1998) and Ruijssenaars *et al.,* (1999) reported that the cultures growing on xanthan generally produced a mixture of xanthan-degrading enzymes, which was excreted from an aerobe culture that was not inhibited by anoxic conditions or different chemicals and biocides commonly used in enhanced oil-recovery operations.

**Acknowledgements**

We thanks for the financial support of CEIMA and the help of Miriam Robledo and MirtaLeiva for the preparation of the material.

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