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EFFECT OF DETERGENTS ON XANTHAN PRODUCTION DURING BATCH AND CONTINUOUS CULTIVATION OF *XANTHOMONAS CAMPESTRIS* NRRL B-1459

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Abstract. The effect of the addition of detergents Tween 20, Tween 40, Tween 80 and Triton X-100 on xanthan production was investigated during batch and continuous cultivation of *Xanthomonas campestris* NRRL B-1459. Three of the four tested detergents: Tween 20, 40 and Triton-X 100 gave an increased xanthan production in comparison to control cultivation without detergent in the medium. The best results were achieved either with 0.05% or 0.1% concentrations of Triton-X 100. About 1.18 to 1.21 fold higher production of polymer was observed during batch cultivation in the presence of this compound in the medium. The highest xanthan concentrations were observed on day 5 and 4 of continuous cultivation in the presence of 0.05% and 0.1% of Triton X-100 (respectively 1.34 and 1.36 fold higher than control). Toxic effect of 0.1% Triton X-100 on the cells growth of the strain was observed after 5 days of continuous cultivation. In addition many examples of effect of detergents on the production of biotechnological useful compounds by various microorganisms have been presented in the work.

Key words: xanthan production, detergents, *Xanthomonas campestris*, continuous cultivation

INTRODUCTION

Xanthan gum is a microbial exopolysaccharide produced by a phytopathogenic gram-negative bacterium *Xanthomonas campestris* and it is an attractive alternative for the replacement of traditional gums obtained from plants and marinae algae by chemical extraction processes. It is a polymerized pentasaccharide repeating units (linear or unlinear), consisting of cellulosis 1-4-beta-D-glucose backbone, to which trisaccharide side chain that contains two mannose and a glucuronic acid is linked at every second glucose [Udeh et al. 2002]. The molecule of xanthan is partially substituted with acetyl and puryvate radicals at both internal mannose residues at various degrees. Because of its properties xanthan shows a wide range of applications in the oil, pharmaceutical, cosmetic, paper, paint and textile industry. In these applications it is mainly used as gelling and suspending agent, as a flocculant and for viscosity control. Xanthan is also

used in the food industry. It is mainly added as a stabilizing, thickening, gelling and emulsifying agent. Also at the molecular level investigations are still performed in order to improve xanthan production and polymer quality in spite of partial explanation of xanthan biosynthesis. One of the ways is searching for mutants with increased production or gum quality [Rodriquez et al. 1997]. Very important is also optimalization of culture conditions [Roseiro et al. 1992], by using various methods of cultivation like fed-batch [Shamala and Prasad 2001] and continuous culture [Roseiro et al. 1993] and more effective mixing [Herbst et al. 1992]. In this work batch and continuous cultivation were conducted with the addition of various detergents added at different concentrations to the cultivation medium. These types of surfactants are very useful in biotechnology for improving yields and number of enzymes produced by fermentation. Examples of this include ligninases [Asther et al. 1987], cellulases [Stutzenberg 1987], and amylases [Srivastava and Matur 1985]. The mechanism of this phenomenon is not completely clear. In bacteria it is probably connected with changes in membrane fluidity [Umesaki et al. 1977]. In filamentous fungi detergents stimulate formation of intracellular membranous structures [Panda et al. 1987].

The aim of this work was to study the effect of detergents on yield of xanthan produced by *Xanthomonas campestris* NRRL B-1459 during batch and continuous cultivation.

MATERIALS AND METHODS

The strain of *Xanthomonas campestris* NRRL B-1459 used in the studies was from the culture collection of the Department of Food Technology and Storage, University of Agriculture in Lublin, Poland. The strain was maintained on YM/agar medium (in g/dm^3: glucose 20, yeast extract 3, peptone 5, malt extract 3, agar 20, pH = 7.0) at 4°C and monthly transferred on fresh slants. Cultivations of strain were performed in 500 cm^3 Erlenmeyer flasks and on the mineral medium which consisted of (in g/dm^3): glucose 30, $(NH_4)_2SO_4$ 3.4, H_3BO_3 0.0072, $FeCl_3$ x $6H_2O$ 0.0042, KH_2PO_4 7.2, $CaCO_3$ 0.029, $MgSO_4$ x $7H_2O$ 0.24, citric acid 2, $ZnSO_4$ x $7H_2O$ 0.006, yeast extract 0.75, peptone 0.34 at pH = 7.0. The flasks containing 100 cm^3 of medium were incubated in an orbital shaker at 30°C and 250 rpm for 3 days. Batch bioreactor cultivation (pre-cultivation) and continuous cultivation was run in 5 dm^3 capacity bioreactor Bioflo III- New Brunswick (USA) on the same mineral medium. This medium was sterilized (at 0.05 MPa for 30 min), cooled and inoculated with previously prepared 200 cm^3 inoculum. Cultivation was performed at constant pH value of 7.0 adjusted with 5% NH_4OH and 2.5% H_3PO_4. The continuous cultivation started from day 2 of the batch cultivation followed by feeding the culture with the same mineral medium, fortified either with 0.01% or 0.05% or 0.1% Triton X-100. Continuous cultivation was carried out for 6 days at dilution rate 0.07 h^{-1}. Xanthan concentration was estimated in the supernatant after dilution and centrifugation of the culture at 20.000 x g for 40 min, using ethanol precipitation (2:1 v/v) in the presence of 1% KCL and dry-weight determination. Glucose was measured according to the DNS method [Miller 1959]. The biomass was analyzed by drying to a constant weight at 105°C and expressed in g/dm^3.

RESULTS AND DISCUSSION

The results of experiments conducted in shake flasks in order to evaluate the effects of four detergents at three concentrations on the production of xanthan gum by *Xanthomonas campestris* NRRL B-1459 were shown in Tables 1-4. Three of the four tested detergents: Tween 20, 40 and Triton-X 100 increased the xanthan production in comparison to control cultivation without detergent in medium. The best results either with 0.05 or 0.1% concentrations gave Triton-X 100. About 1.18 to 1.21 fold higher production of polymer was observed during cultivation in the presence of this compound in cultivation medium. Tween 20 and Tween 40 showed the mildest effect and improved the xanthan production by 1.11-1.17 fold. The highest biomass concentrations were measured during cultivation of *Xanthomanas campestris* in the presence of Tween 20 (0.1%) and Tween 40 (0.01 and 0.05%).Triton X-100 turned out to have final biomass concentrations 1.84-2.48 g/dm^3 which were lower than that of control experiment with no detergent. Due to its best effect on xanthan production in shake flask experiments Triton X-100 was further investigated and added to the cultivation medium under more controlled conditions in 5-l bioreactor Bioflo III (New Brunswick-USA). Inductive effect of Triton X-100 during continuous bio-reactor fermentation was examined at three levels of concentration of added detergent (Fig. 1-4). The highest xanthan concentration was achieved after on days 5 and 4 of cultivation in the presence of 0.05%

Table 1. Features of xanthan batch cultures with addition of Tween 20 to cultivation medium
Tabela 1. Charakterystyka ksantanowej hodowli okresowej z dodatkiem Tweenu 20 do podłoża hodowlanego

Concentration of detergent Stężenie detergentu g/dm^3	Biomass concentration Zawartość biomasy g/dm^3	Residual glucose Pozostałość glukozy g/dm^3	Concentration of xanthan Stężenie ksantanu g/dm^3
Control* – Kontrola*	2.96	3.7	11.08
0.1	3.34	3.6	11.24
0.5	3.36	3.0	11.7
1.0	5.36	2.8	12.32

*Without detergent.
*Bez detergentu.

Table 2. Features of xanthan batch cultures with addition of Tween 40 to cultivation medium
Tabela 2. Charakterystyka ksantanowej hodowli okresowej z dodatkiem Tweenu 40 do podłoża hodowlanego

Concentration of detergent Stężenie detergentu g/dm^3	Biomass concentration Zawartość biomasy g/dm^3	Residual glucose Pozostałość glukozy g/dm^3	Concentration of xanthan Stężenie ksantanu g/dm^3
Control* – Kontrola*	3.25	3.15	11.1
0.1	4.26	3.10	11.0
0.5	4.0	3.05	11.6
1.0	3.42	3.10	13.0

*Without detergent.
*Bez detergentu.

Table 3. Features of xanthan batch cultures with addition of Tween 80 to cultivation medium
Tabela 3. Charakterystyka ksantanowej hodowli okresowej z dodatkiem Tweenu 80 do podłoża hodowlanego

Concentration of detergent Stężenie detergentu g/dm^3	Biomass concentration Zawartość biomasy g/dm^3	Residual glucose Pozostałość glukozy g/dm^3	Concentration of xanthan Stężenie ksantanu g/dm^3
Control* – Kontrola*	3.25	3.15	11.1
0.1	3.80	3.10	10.65
0.5	3.60	3.05	10.85
1.0	3.56	3.15	10.65

*Without detergent.
*Bez detergentu.

Table 4. Features of xanthan batch cultures with addition of Triton X-100 to cultivation medium
Tabela 4. Charakterystyka ksantanowej hodowli okresowej z dodatkiem Tritonu X-100 do podłoża hodowlanego

Concentration of detergent Stężenie detergentu g/dm^3	Biomass concentration Zawartość biomasy g/dm^3	Residual glucose Pozostałość glukozy g/dm^3	Concentration of xanthan Stężenie ksantanu g/dm^3
Control* – Kontrola*	3.80	2.90	10.60
0.1	2.48	3.20	11.06
0.5	1.84	2.90	12.50
1.0	2.00	2.40	12.80

*Without detergent.
*Bez detergentu.

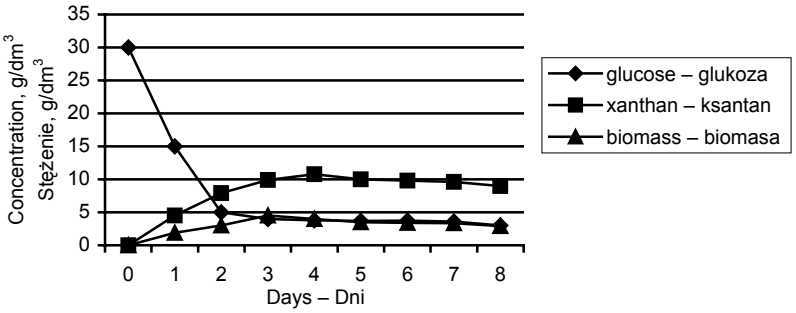

Fig. 1. Continuous cultivation of *Xanthomonas campestris* without addition of detergent to the medium (control)
Rys. 1. Hodowla ciągła *Xanthomonas campestris* bez dodatku detergentu do podłoża hodowlanego

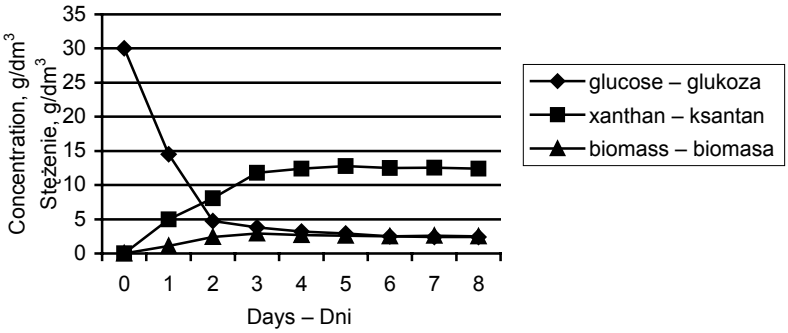

Fig. 2. Continuous cultivation of *Xanthomonas campestris* with 0.1% Triton X-100 addition to the medium
Ryc. 2. Hodowla ciągła *Xanthomonas campestris* z dodatkiem 0,1% Tritonu X-100 do podłoża

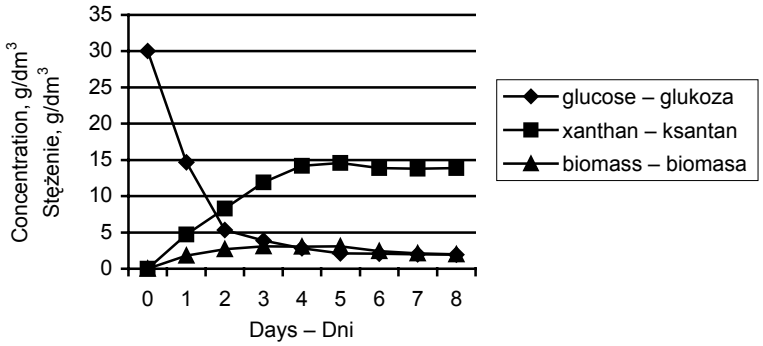

Fig. 3. Continuous cultivation of *Xanthomonas campestris* with 0.5% Triton X-100 addition to the medium
Rys. 3. Hodowla ciągła *Xanthomonas campestris* z dodatkiem 0,5% Tritonu X-100 do podłoża

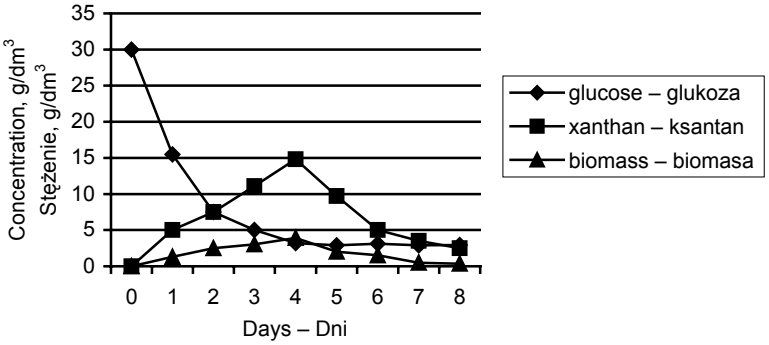

Fig. 4. Continuous cultivation of *Xanthomonas campestris* with 1% Triton X-100 addition to the medium
Rys. 4. Hodowla ciągła *Xanthomonas campestris* z dodatkiem 1% Tritonu X-100 do podłoża

and 0.1% of detergent (respectively 1.34 and 1.36 fold higher than control). Production of exopolysaccharide has rapidly dropped after day 5 of continuous culture with the addition of 0.1% of Triton X-100 to the level of 2.45 g/dm^3 and lysis of the cells of the bacterium was observed under the optical microscope.

Galindo and Salcedo [1996], first used detergents for simultaneous improvement of xanthan production and rheological quality of the polymer. The research tested detergent only during batch cultivation of the strain of *Xanthomonas campestris*. The mechanism of positive effect of detergents on the xanthan production is not clear. Galindo and Salcedo [1996] suggested that detergent could have led to a higher oxygen uptake rate by the cells of microorganisms. According to Ielpi et al. [1993] the action of the detergent could be by interacting with bacterial cell membrane and enhanced the polymerization of the xanthan molecule.

There exist many examples of stimulation of production of biotechnological useful compounds by detergents in various microorganisms. Kruszewska et al. [1990], observed increased secretion of protein by fungus *Trichoderma reesei* QM 9414 in the presence of Tween 80 in the culture medium. Strain QM 9414, grown on media supplemented with Tween 80 exhibited two to threefold higher activity of Dol-P-Man synthase – a key enzyme of O-glycosylation compared to a control lacking this supplement. The effect of Tween 80 on protein secretion by *T. reesei* could be due to stimulation of sythesis and activity of Dol-P-Man synthase, thereby elevating the level of O-glycosylation and protein secretion.

CTAB has been used for permeabilization of baker's yeasts for enhanced activity of *S. cerevisiae* alcohol-dehydrogenase, glucose-6-phosphate-dehydrogenase and hexokinase. CTAB-permeabilized cells could be used for measuring total intracellular enzyme activity and an alternative biocatalyst for analytical and preparative purposes [Gowda et al. 1991].

The same compound was used by Joshi et al. [1987] for increased permeability of the cell membrane of *Kluyveromyces fragilis* for higher secretion of beta-galactosidase. Cellular beta-galactosidase in 0.1% treated cells was 480-fold greater than that of the control cells.

For enhanced penicillin-acylase activity from *E. coli* permeabilization using also CTAB was performed. A maximum activity of enzyme was achieved with 0.2% CTAB treatment for 20 minutes at 5°C [Nagalakshmi and Pai 1994].

Adamczak and Bednarski [1998], obtained the highest activity of endolipase after incubation of biomass of *Rhizomucor miehei* with 0.2% digitonin and of *Yarrowia lipolytica* with 0.2% CTAB. The lipolytic activity of permeabilized cells for both strains was about 2.5 times higher compared with native cells.

Three methods for releasing proteins from the *Pichia pastoris* cells were compared by Naglak and Wang [1990]: chemical permeabilization, mechanical disruption and enzymati lysis. Chemically permeabilized cells using 2M guanidine-HCL and 0.5% Triton X-100 retained their morphology, whereas mechanically disrupted or enzymatically lyzed cells were reduced to a small cell fragment. Only after chemical treatment decrease of cell number was not observed. The same author used 0.4M guanidine plus 0.5% Triton X-100 for permeabilization of *E. coli* cells for rapid protein release under fermentation conditions [Naglak and Wang 1992].

Studies have been carried out to find alternative permeabilizing agents in inducing high level of beta-galactosidase in *Streptococcus thermophilus* ST 128. Triton X-100

displayed 15-times higher levels of beta-galactosidase induction than control cells [Somkuti and Steinberg 1994].

Galabova et al. [1996], permeabilized of *Yarrowia lipolityca* cells by 0.1-0.2% Triton X-100 for the extraction of periplasmic acid phosphatase and alkaline phosphatase. This method was simple, rapid and mild and gave better results than mechanical disruption of the cells. Protein release was maximal during exponential growth and increased slightly in the stationary phase [Christova et al. 1996].

Recovery of periplasmatic proteins (heterologous and homologous) by chemical permeabilization is simpler than osmotic shock and less expensive than using enzymatic digestion. Treatment of *E. coli* (expressing recombinant beta-lactamase of *Bacillus licheniformis* in periplasm) by Triton X-100 with guanidine hydrochloride resulted in the release of cytoplasmic proteins as well as periplasmic proteins and 40-fold purification of the recombinant enzyme. [Naglak and Wang 1990]. A small recombinant protein – staphylokinase has been isolated using permeabilization in Tris/EDTA buffer with addition Triton X-100. This method was advantageous in comparison with the product released by mechanical method because it gave a better the recovery of purer product and shortened the process time [Gehmlich et al. 1997].

Pseudomonas pseudoalcaligenes can only form D-malate from maleate after incubation of the cells with solvents or a detergent for example Triton X-100. The longer the cells were incubated with Triton X-100, the higher was D-malate production activity, until the maximal malease activity was reached. The rate at which the D-malate production activity increased was dependent on the Triton X-100 concentration [Van der Werf et al. 1995].

CONCLUSIONS

Detergents improve xanthan production either in shake flaks (1.18-1.21-fold) or bioreactor (1.34-1.36-fold) cultivation. It is the first report in which detergent was successfully used for improving of xanthan production yield during continuous cultivation. This technique allows lowering the cost of xanthan production and can be adapted to industrial scale. For elucidation of inductive mechanism of detergents on exoplysaccharide biosynthesis further investigations are needed.

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WPŁYW DETERGENTÓW NA PRODUKCJĘ KSANTANU PRZEZ *XANTHOMONAS CAMPESTRIS* NRRL B-1459 PODCZAS HODOWLI OKRESOWYCH I CIĄGŁYCH

Streszczenie. Zbadano wpływ dodatku detergentów: Tweenu 20, Tweenu 40, Tweenu 80 i Tritonu X-100 na produkcję ksantanu przez *Xanthomonas campestris* NRRL B-1459 podczas hodowli okresowych i ciągłych. Trzy spośród czterech testowanych detergentów: Tween 20, 40 i Triton X-100 wpływały na zwiększenie produkcji ksantanu w porównaniu z kontrolną hodowlą okresową bez dodatku detergentu do podłoża. Najlepsze wyniki uzyskano stosując Triton X-100 w stężeniach 0,05% i 0,1%. W obecności tego związku w podłożu hodowlanym zaobserwowano zwiększenie produkcji polimeru podczas hodowli o prawie 1,18 i 1,21 raza. Najwyższe stężenia ksantanu oznaczono w 5 i 4 dniu hodowli ciągłej w obecności 0,05% i 0,1% Tritonu X-100 (odpowiednio 1,34 i 1,36 razy wyższe od kontroli). Stwierdzono toksyczny wpływ 0,1-procentowego Tritonu X-100 na wzrost komórek szczepu w drugiej części (od 5 dnia) hodowli ciągłej. Dodatkowo w pracy przedstawiono wiele przykładów wpływu detergentów na produkcję przez różne mikroorganizmy biotechnologicznie użytecznych substancji.

Słowa kluczowe: produkcja ksantanu, detergenty, *Xanthomonas campestris*, hodowla ciągła

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