XANTHAN PRODUCTION ON CRUDE GLYCEROL-BASED MEDIUM: OPTIMIZATION OF INOCULUM PREPARATION

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Introduction

Xanthan is a non-toxic, biocompatible and biodegradable polysaccharide of microbiological origin. This biopolymer possesses exceptional rheological characteristics that make it widely used in the food, pharmaceutical, petrochemical, chemical and textile industry. Xanthan is produced industrially by submerged aerobic cultivation of reference strain *Xanthomonas campestris* ATCC 13951 on the medium of appropriate composition and under optimal conditions. Although glucose and sucrose are the predominantly used carbon sources in cultivation media for xanthan production, the rise in prices and the increasing demands for these sugars indicate the need for exploration of alternative substrates with lower market value. Crude glycerol proved to be one of the most promising alternative substrate for the production of various high-value products including xanthan. The development of biotechnological process for the production of xanthan on crude glycerol-based media is still in initial stages due to variation in the tolerance of different *Xanthomonas* strains on the impurities present in this effluent. In order to eliminate the mentioned difficulties, it is necessary to optimize all phases of the xanthan production process for each selected *Xanthomonas* strain. The first step towards that goal involves optimization of the inoculum preparation procedure, which includes definition of the time for producing strain incubation.

Objective

The objective of this work was to optimize inoculum preparation for xanthan production on crude glycerol based-medium in terms of incubation time of applied producing microorganism.

Methods

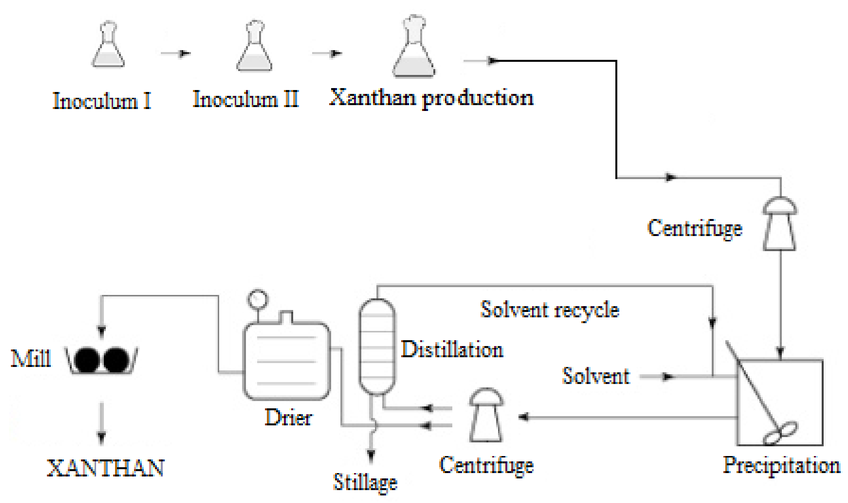

Figure 1. Schematic representation of xanthan production in applied experimental conditions

Inoculum preparation was divided into two stages: inoculum I and inoculum II preparation. Experiments were performed according to a 3-level factorial design to evaluate the effects of two independent variables, i.e. incubation time of inoculum I and incubation time of inoculum II on xanthan quantity in crude glycerol-based production medium at the end of bioprocess. The commercial medium (YMB®) was used for inoculum I preparation and crude glycerol-based growth medium was used for inoculum II preparation. Both inoculums were prepared in aerobic conditions at temperature of 25°C and agitation rate of 150 rpm. For optimization of incubation time, the inoculated flasks of growth media were incubated at different times (24 h, 36 h and 48 h). The xanthan production was performed by cultivation of *Xanthomonas* PL4 strain, isolated from pepper leaves, in 300 mL Erlenmeyer flasks with 100 mL of the crude glycerol-based medium. The biosynthesis was performed under aerobic conditions at temperature of 30°C and agitation rate of 150 rpm for 168 h. Bioprocess efficacy was estimated based on the xanthan concentration in medium at the end of biosynthesis.

Results

The illustrated results (Figure 2) show the predicted effect of incubation times of the inoculum I and inoculum II on the xanthan concentration in crude glycerol-based production medium at the end of bioprocess. According to the presented response surface plot, the applied *Xanthomonas* strain is the most productive if the incubation time of inoculum I is between 32 h and 40 h, and the incubation time of inoculum II in the range from 40 h to 48 h.

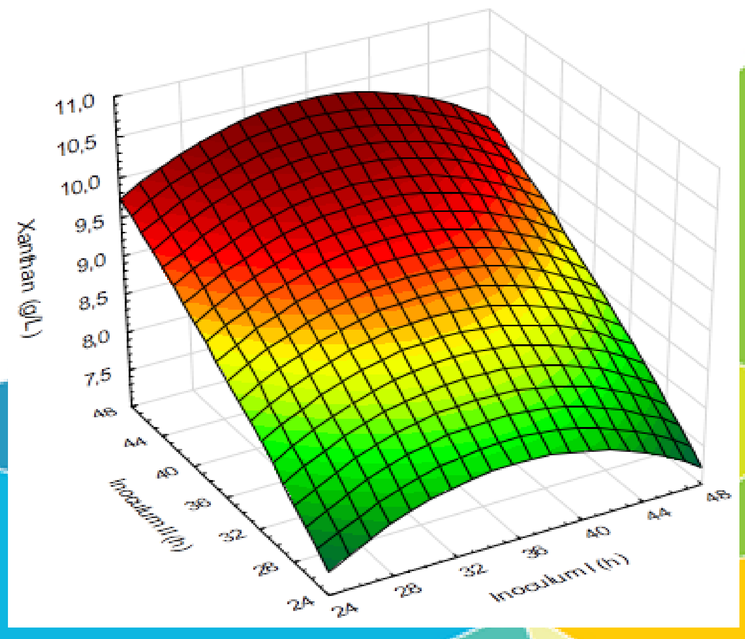

Figure 2. The effect of incubation time of inoculum I and inoculum II on xanthan content

Conclusions

The change in the duration of the second phase of inoculum preparation significantly affects the xanthan production on crude glycerol-based medium in applied experimental conditions. The developed model predicts that the maximum xanthan concentration of about 10.5 g /L can be achieved if the incubation times of inoculum I and inoculum II are 36 h and 48 h, respectively. Results obtained in this research may be a suitable background for future investigations and optimization of the economically justified production of xanthan.

ACKNOWLEDGEMENTS

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