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MICROBIAL SYNTHESIS OF XANTHAN GUM BY REPEATED-BATCH PROCESS

M.A. Qadeer and Shahjahan Baig

PCSIR Laboratories, Lahore-16

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Production of xanthan gum by a locally isolated culture of *Xanthomonas cucurbitae* PCSIR – 52 in sugar cane juice and synthetic sucrose-salt media was studied by submerged fermentation in 1-litre conical flasks and by repeated batch process in a 10 l stirred fermenter. The rate of gum synthesis and the percentage conversion of sugar to biopolymer were greater in the fermenter than in the shake flasks. The efficiency of the culture remained about the same in three cycles of 50 hr. each during repeated batch process. Xanthan gum formation was higher in synthetic sucrose-salt medium (20.0 g/l) than in sugar cane juice (14.0 g/l). The addition of cotton seed meal as "proflo extract" enhanced the rate of gum synthesis.

Key words: Xanthan gum; *Xanthomonas cucurbitae;* Fermentation of sucrose salt.

INTRODUCTION

Xanthan gum, a polyanionic heteropolysaccharide, is commercially produced by fermentation process and finds applications in oil well drilling, ceramic glazing, textile, food and pharmaceutical industries. Silman and Rogovin [1] studied xanthan gum fermentation by *Xanthomonas campestris* NRRL 8-1459 in a single-stage continuous process and showed that production of the biopolymer was a function of dilution rate and pH. However, certain strain variations have been observed in both the continuous and batch-type fermentations [2-4]. Charles and Radjai [5] have studied xanthan gum fermentation using lactose as acid whey substrate with *X. campestris* by repeated batch operations. In this process about 80 % of the fermented broth was replaced by fresh sterilized medium aseptically and three cycles were run successfully.

The present work in continuation of our earlier studies [6] describes the synthesis of xanthan biopolymer by fermentation of sucrose-salt and sugar cane juice media with *Xanthomonas cucurbitae* PCSIR – 52 by repeated batch process.

MATERIALS AND METHODS

Organism. Culture of *Xanthomonas cucurbitae* PCSIR – 52 was maintained on yeast-malt extract agar slants containing (g/l): yeast extract, 3.0; malt extract, 3.0; peptone, 5.0; glucose, 10.0; $CaCO_3$, 1.0 and agar, 20.0. The inoculum was built up according to the procedure described by Burton *et al.* [7] and Haynes *et al.* [8].

Fermentation. Xanthan gum fermentation was carried out in 1 litre conical flasks and a 10-litre glass-stainless steel fermenter containing 100 ml and 6 litre fermentation medium respectively (Table 1). The flasks after inoculation with viable cultures of *X. cucurbitae* PCSIR-52 were rotated at 150 r.p.m. on a rotary shaker, and incubated at 29 ± 1° for 72 hr. The repeated batch experiments were carried out in a 10-litre baffled fermenter, designed and fabricated in the PCSIR Laboratories workshops using QVF pipes with stainless steel plates at both ends and also provided with agitation and aeration systems. The agitation was provided by a, five-bladed open turbine impeller mounted on a centred vertical shaft entering from the top plate. The rates of agitation and aeration were 200 r.p.m. and 1/1/min respectively. The composition of various fermentation media used in the present work is indicated in Table 1. Fresh sugar cane juice was obtained from the market and centrifuged at 3,000 r.p.m. for 15 min. The sugar content in the cane juice was about 11.0 %. All fermentation media were sterilized at 121° for 15 min. $CaCO_3$ suspended in tap water was separately sterilized and aseptically transferred to the basal medium to control the pH near neutral. The initial pH of the fermentation medium was adjusted at 7.0 ± 0.5 with 2.5 N NaOH or H_2SO_4. The culture medium was inoculated at the rate of 5 % v/v by 24 hr old vegetative cells developed in the shake flasks.

Analytical methods. The viscosity of the fermented broth was determined by an Ostwald viscometer at 30° after appropriate dilutions. The residual sugar was esti-

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mated by the method of Lo and Garceau [9]. The dry cell mass and Xanthan gum were determined according to the methods of Jeanes *et al.* [10] and Morain *et al.* [11] respectively.

RESULTS AND DISCUSSION

Selection of fermentation medium. Table 1 shows the composition of different fermentation media (A-F) such as sugar cane juice and synthetic sucrose salt, with or without supplementation of Proflo* extract – a complex organic nitrogenous source obtained from premium quality cotton seed meal. The media were evaluated in shake flasks and categorized on the basis of xanthan formation, prior to utilization in the fermenter for the assessment of the repeated batch process (Fig. 1). It has been shown that the amount of xanthan produced in complex sugar cane juice medium (A) was slightly less at 7.5 g/l than that obtained in synthetic sucrose-salt medium (8.25 g/l). The highest titer of xanthan gum achieved in sucrose-salt medium containing "Proflo extract" (E), is an indication of enhancement in the metabolic efficiency of *X. cucurbitae* PCSIR – 52 because of readily available vitamins, amino-acids and a variety of minerals.

Repeated batch fermentation. The production of xanthan was conducted in a 10 litre stirred fermenter by repeated batch process using three fermentation media (Fig. 2 – 4). These media comprise a complex sugar cane juice and chemically defined sucrose-salt media which were formerly evaluated in Shake flasks (Fig. 1). Comparatively. the bioconversion of sugar to xanthan gum greatly increased in the fermenter than obtained in shake flasks. The reasons for the earlier completion of xanthan fermentation and fast utilization of sugar may be attributed to efficient mass transfer in the stirred fermenter as compared to shake flasks.

In the repeated batch process, the first cycle of xanthan fermentation was run for 50 hr. at the most, and gen-

Table 1. Composition of different production media.

Ingredients (g/l)	Fermentation media					
	A	B	C	D	E	F
Sucrose	–	–	–	–	30.0	30.0
Sugar cane juice (sucrose)	30.0	30.0	30.0	30.0	–	–
$(NH_4)_2HPO4$	–	–	1.5	1.5	1.5	1.5
Na_2HPO_4	–	–	–	–	3.0	3.0
$MgSO_4.7H_2O$	–	0.25	0.25	0.25	0.25	0.25
$CaCO_3$	1.0	1.0	1.00	1.0	1.0	1.0
Proflo* extract (10 % w/v)	–	–	50 ml	–	50 ml	–

*Proflo (cotton seed meal) extract was prepared by refluxing 10 g of 'Proflo" with 100 ml distilled water at 100° for 30 min. and centrifuged at 3000 rpm. The supernatant was used as a nitrogen source.

*Proflo is a product of Traders Protein Division, Texas, USA.

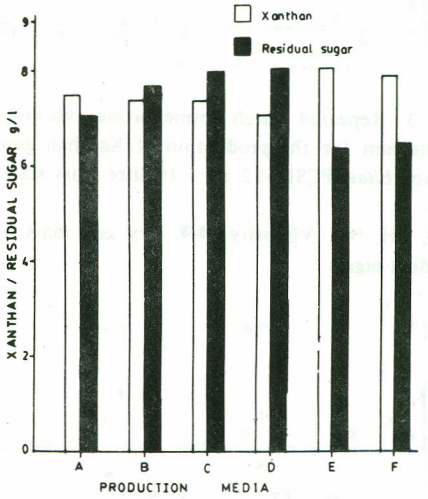

Fig. 1. Effect of production media on the biosynthesis of Xanthan gum by *Xanthomonas cucurbitae* PCSIR-52 in shake flasks. (average of five replicates).

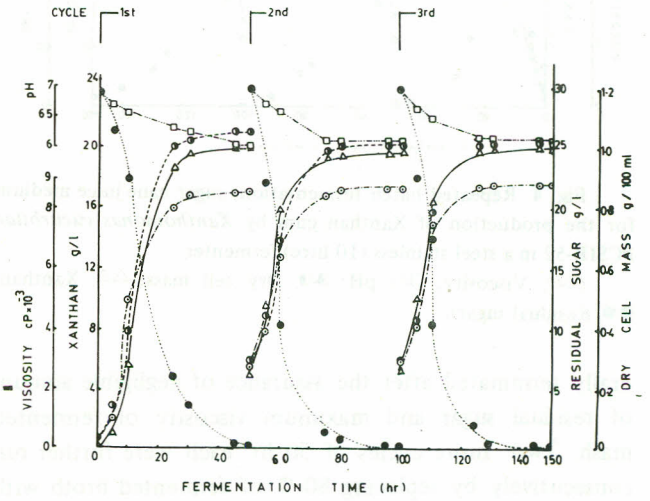

Fig. 2. Repeated batch fermentation; sucrose salt medium for the production of Xanthan gum by *Xanthomonas cucurbitae* PCSIR-52 in a 10 litre glass-stainless steel fermenter.

(□□, pH; ○○, Viscosity; ●●, Dry cell mass; △△, Xanthan; ●●, Residual sugar).

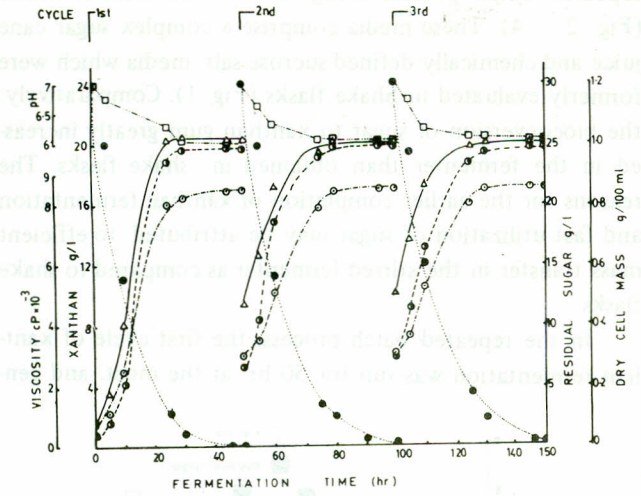

Fig. 3. Repeated batch fermentation; sucrose-salt "Proflo" extract medium for the production of Xanthan gum by *Xanthomonas cucurbitae* PCSIR-52 in a 10 litre glass stainless steel fermenter.

(□-□, pH; ⊙-⊙, Viscosity; ◐-◐, Dry cell mass; △-△, Xanthan; ●-●, Residual sugar).

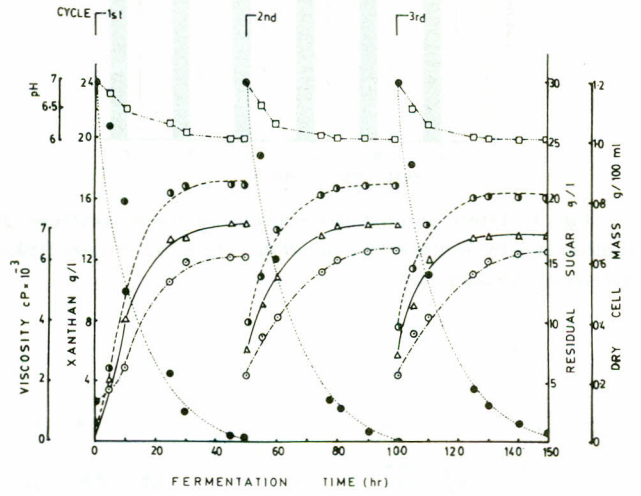

Fig. 4. Repeated batch fermentation; sugar cane juice medium for the production of Xanthan gum by *Xanthomonas cucurbitae* PCSIR-52 in a steel stainless (10 litre) fermenter.

(⊙-⊙, Viscosity; □-□, pH; ◐-◐, Dry cell mass; △-△, Xanthan; ●-●, Residual sugar).

erally terminated after the assurance of negligible amount of residual sugar and maximum viscosity of fermented mash. Two more cycles of 50 hr. each were further run consecutively by replacing 80 % of fermented broth with fresh pre-sterilized fermentation media, and culture was continuously grown for 150 hr.

Fig. 2 shows the pattern of xanthan fermentation in three cycles, using a sucrose-salt medium and indicate the highest titer of product attained (20 g/l) after 40 hr. leaving a negligible quantity of residual sugar. The supplementation of "Proflo extract" with sucrose-salt medium exhibited a pronounced effect on bacterial growth and the rate of xanthan production. Hence, the bacterium efficiently metabolized sugar to polysaccharide and consequently reduced the fermentation period. There is a slight decrease in the dry cell mass in the presence of "Proflo extract" (Fig. 3), than that obtained in the case of sucrose-salt medium (Fig. 2). In the sugarcane juice medium, biopolysaccharide formation of 14 g/l was achieved in 40 hr and remained almost the same in three cycles (Fig. 3). The bacterium did not efficiently utilize sugarcane juice medium as compared to the sucrose-salt or sucrose-salt-"Proflo extract" medium. It follows that sugar cane juice – a rich source of vitamins, organic nitrogenous components and a variety of metallic ions – promoted bacterial growth instead of xanthan synthesis. It is quite interesting to note that the efficiency of *X. cucurbitae* PCSIR-52 was slightly affected in three cycles during repeated batch process without any detectable cultural degeneration. Similar studies of xanthan gum production by repeated batch has also been reported by Charles and Radjai [5] using acid or cheese whey.

CONCLUSION

The important factors for maximum xanthan gum fermentation, by locally isolated culture of *Xanthomonas cucurbitae* PCSIR-52 were, an adequate supply of oxygen (agitation and aeration) and medium composition. The amount of biopolysaccharide produced was better in synthetic sucrose-salt medium than that obtained in cane juice. That is, nutritionally deficient conditions in synthetic sucrose-salt medium, such as minerals, vitamins and growth factors, favour the accumulation of xanthan gum instead of bacterial cell mass.

The bacterial culture did not show any degeneration during the continuous growth by repeated batch process. The period of lag phase or preparation of fresh seed culture for each fermentation run can be eliminated by the repeated batch process.

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