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Production of Gellan Gum by *Sphingomonas paucimobilis* on Crude Sweet Whey Using Different Bioreactor Feeding Strategies

Rawia, F. Gamal[1], Hemmat, M. Abdel-hady[1], Shimaa, A. Amin[1*] and Shimaa, K. Ali[1]

[1]Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors.

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Original Research Article

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ABSTRACT

Aims: Production of gellan gum by *Sphingomoas paucimobilis* from whey was optimized by different fermentation techniques.
Study Design: Study the growth behavior of *Sphingomoas paucimobilis* was cultivated on 40% sweet whey medium in the bioreactor as a batch, fed batch and continuous culture and effect of aeration and agitation speed on gellan production.
Place and Duration of Study: Microbiology Dept., Fac. of Agriculture, Ain Shams Univ., Cairo, Egypt, 2016/ 2017.
Methodology: Using *Sphingomoas paucimobilis* on sweet whey in the bioreactor as a batch, fed batch (pulsed & continuous) and continuous culture. Among the four levels of air saturation and four levels of agitation speeds.
Results: Using the continuous feeding of sugar sweet whey at 1.53 $gl^{-1}h^{-1}$during 12 h was favorable than pulsed feeding for gellan production in fed-batch culture. In continuous culture addition of 40% SW at 0.055 h^{-1} dilution rate (110 ml h^{-1}), the values of gellan parameters recorded by

**Corresponding author: E-mail: dshimaaamin@gmail.com;*

Sphingomoas paucimobilis were 24.34%, 26.54% & 0.337 $gl^{-1}h^{-1}$ for gellan yield, conversion coefficient and gellan productivity during 24 h. At 28ºC. This technique increments the gellan production ($gl^{-1}h^{-1}$) by 3.3 & 2.2-2.5 and 1.5- 1.6 fold as compared to that produced by using batch & fed- batch pulsed and fed-batch continuous techniques respectively. The emulsifying capability of the partially purified gellan was 100% whereas it was 95% for xanthan gum, as well as its high flocculating activity than xanthan. A tough worm-like gel or firm gel were formed when 10% calcium chloride solution or 0.5 g sodium chloride were added to gellan solution.

Conclusion: The maximum gellan yield and lower fermentation period were obtained with air saturation of 60% at 750 rpm agitation speed. The continuous feeding at 1.53 $gl^{-1}h^{-1}$ was favorable than pulsed feeding for gellan production in fed-batch culture, while the maximum gellan productivity was obtained by using a continuous culture technique at 0.055 h^{-1} dilution rate.

Keywords: Gellan gum; Sphingomoas paucimobilis; batch culture; fed-batch; pulsed feeding; continuous feeding; continuous culture.

1. INTRODUCTION

In more recent years, microbial fermentation has played an important role in the production of the polysaccharide. The quality and supply of traditionally used plant and seaweed-derived gums are affected by environmental factors such as seasonal variation and eutrophication [1,2]. Microorganisms offer a more attractive alternative source of gums as they can be grown under controlled condition and they greatly extend. Among these exopolysaccharides (EPSs), gellan gum has been commercially produced in high yield by the non-pathogenic strain *S. paucimobilis.*

[3] reported that gellan production yield compositions structures and properties are genetically determined; it is possible to influence these factors by modifying culture conditions (such as temperature & dissolved oxygen tension) and growth medium composition (i.e., carbon or nitrogen source used and concentration of cations).

Comparison of gellan biosynthesis by *S. paucimobilis* ATCC31461 was carried out by [4] in media containing glucose, lactose (5to30 gl^{-1}) and sweet whey. They found that altering the growth medium can markedly affect the polysaccharide yield, acyl substitution level, polymer rheological properties and susceptibility to degradation. Depression of gellan production from lactose compared with gellan production from glucose (approximately 30%) did not appear to occur at the level of synthesis of sugar nucleotides, which are the donors of monomers used for biosynthesis of the repetitive tetrasaccharide unit of gellan [5]. Gellan production has been the subject of investigations by using batch culture [6,7] and fed-batch culture [8,2]. [2] reported that intermediate feeding of glucose overcame the catabolic repression and supported the higher gellan production. Gellan has unique characteristics and has many applications, particularly in the food, pharmaceutical and biomedical fields. Moreover, it has been widely employed as gelling agent in plant biotechnology, in place of agar in bacterial culture media and in making electrophoresis gels [9]. However, very commonly there is no market niche, because production costs may be very high, product quality may be difficult to maintain and to guarantee or the product may not achieve regulatory acceptability [10].

In Egypt, gellan is not commercially produced and only a few researchers have been devoted in this respect, so, the current work was designed to evaluate the potential of using local bacterial isolate *S. paucimobilis* for gellan production in a bioreactor using different feeding strategies.

2. MATERIALS AND METHODS

2.1 Bioreactor Experiments

In the present studie, 3L dished bottom bioreactor Z610/code (Cole Parmer Instrument) was used, which consists of a 3-liter vessel equipped with lip seal stirrer assembly, automatic pH controller, automatic dissolved oxygen, CO2 controller, automatic temperature controller, foam controller and multi-channel peristaltic pump for feeding. The gellan producing bacteria *S. paucimobilis* was grown in the bioreactor contains 40% sweet whey (1.84% sugar) under different culture techniques.

2.2 As a Batch Culture

1800 ml of sterile sweet whey (40%) was inoculated with 200 ml of standard inoculum ($9x10^8$/ml) to get 2L working volume incubated at 28ºC and 250 rpm. During fermentation (72 h), pH was automatically recorded and samples were withdrawn from the fermentation vessel. The samples were centrifuged at 8000 xg for 30 min at 4ºC. The biomass was washed twice with distilled water and then dried at 70ºC to constant weight. Gellan gum was precipitated with acetone and determined an as dry weight according to the method employed by [11]. The residual sugar and nitrogen were determined in the supernatant according to [12,13], respectively.

2.3 As Fed- batch Culture

The initial volume was 1000 ml of sweet whey and inoculated by 200 ml of standard inoculum. Initial pH was adjusted to 7.0 without controlled during the fermentation period. The speed of agitation and percentage of air saturation were adjusted at the selected optimal degree. In this experiment, two types of feeding (pulsed & continuous) were conducted. In pulsed feeding 800 ml of 40% SW were added by four incremented additions at 4 h intervals during the first 12 h or seven intervals during first 24 of incubation. In Continuous feeding sugar, the nutrients were fed continuously for 72 h at two rates (1.53 and 0.77 $gl^{-1}h^{-1}$ sugar) the final working volume was 2L at the end of the feeding period. Samples were taken periodically under the septic condition to determine growth and gellan parameters.

2.4 As Continuous Culture

The culture in the vessel was allowed to grow up as a batch culture for 48 h. After this period the fresh medium was pumped to the fermentation medium at different flow rates being 70, 90, 110 and 130 ml/h to give dilution rate 0.035, 0.045, 0.055 and 0.065 h^{-1}respectively. Cultivation of each dilution rate was kept for at least 24 intervals. Samples were taken aseptically at each steady state to determine gellan and growth parameters.

2.5 Gellan Determination

It was estimated gravimetrically according to [11]. The culture broth was diluted 30 times and centrifuged at 8000 xg for 30 min to sediment the cells. The polymer was precipitated from the supernatant with 3 volumes of acetone then dried at 50ºC.

2.6 Properties of Gellan Gum

Emulsifying effect and flocculating test of gellan were carried out according to the method recommended by [14], whereas, solubility test was performed according to [15].

2.7 Statistical Analysis

The correlation coefficient (r) was carried out according to the method described by [16].

3. RESULTS AND DISCUSSION

3.1 The Biological Activity of *S. paucimobilis* in the Bioreactor as a Batch Culture

S. paucimobilis (obtained from the department of agricultural microbiology, Faculty of Agriculture, Ain Shams University) was cultivated on 40 % sweet whey medium in the bioreactor as a batch culture to study the growth behavior, effect of aeration and agitation speed on gellan production. Results in Fig. 1 showed that increasing the fermentation period led to a gradual increase in cell dry weight of *Z. paucimobilis* and decreased the residual nitrogen concentration till depletion after 54 h to recording the maximum biomass production being 2.91 gl^{-1}. During propagation slight decrease in pH value from 7.0 to 6.3 was observed. On the other hand, the amount of consumed sugar was increased during the fermentation period to record the highest figure (14.99 gl^{-1}) after 72 h resulting the highest figures of gellan concentration and gellan yield being 2.53 gl^{-1} and 13.90%, respectively. With respect to gellan parameters, the highest values of conversion coefficient 16.88% (amount of gellan per consumed sugar) was attained after 72 h of fermentation, but gellan productivity (amount of gellan per time) was attained after 30 h of fermentation being 0.043$gl^{-1}h^{-1}$.

3.2 Effect of Aeration

The influence of aeration on cell growth and gellan production was studied in the range of air saturation between 20-80%, the illustrated data in Fig. 2 and Table 1 show that *S. paucimobilis* dry weight was increased exponentially during the

first 48 hours of incubation at different air saturation levels and recorded the highest specific growth rate being 0.057 h^{-1} at 60% air saturation. The highest figures of gellan concentration and yield were recorded after 54 h at 60% air saturation being 3.24 gl^{-1} and 27.8%, respectively. It could be noticed that increasing the air saturation from 20 to 60% led to increasing the gellan productivity after 24 h about 1.16 fold. These results were in line with those obtained by [17] they suggested that higher oxygen transfer condition favored gellan production, whereas [6] reported that, moderate aeration level gives highest gellan concentration.

3.3 Effect of Agitation Speed

Data revealed in Figs. (3&4) that gellan concentration (gl^{-1}) increased during fermentation period to give the highest value being 7.36 gl^{-1} at the end of fermentation period at 750 rpm and highest gellan productivity (0.213 gl^{-1} h^{-1}) after 30 h, whereas the doubling time of *S. paucimobilis* increased to reach 15.4 h under the same level of agitation. The low and high agitation speed (250 and 1000) recorded approximately the same value of gellan dry weight, gellan yield and productivity after 48 hours were 3.65 & 3.64 gl^{-1} h^{-1}, 20.05 & 20.00 and 0.08 & 0.08 gl^{-1} h^{-1},

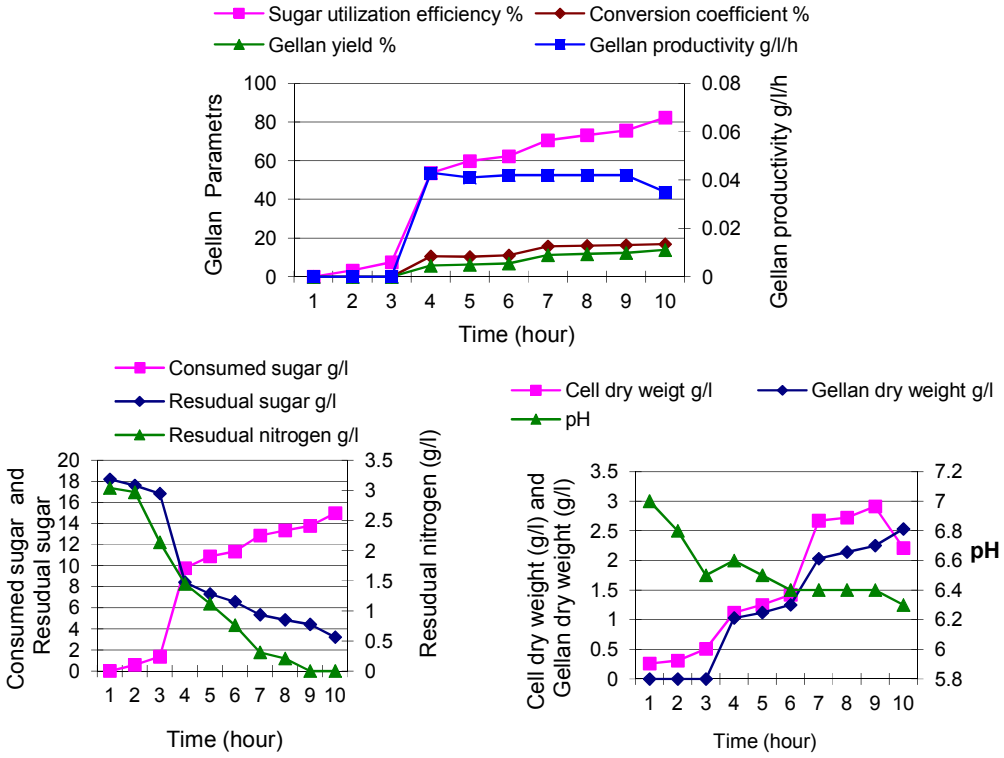

Fig. 1. Biological activity of *S. paucimobilis* and gellan production on 40% crude sweet whey during 72h incubation at 28°C using bioreactor as a batch culture

Table 1. Growth parameters of *S. paucimobilis* grown in 40%crude sweet whey at 28°C during 72h at different air saturation using bioreactor as a batch culture

Air saturation (%)	Growth parameters		
	Doubling time (t_d) (h)	Number of generation (N)	Specific growth rate (μ) (h^{-1})
20	0.051	3.53	13.59
40	0.056	3.88	12.38
60	0.057	3.95	12.16
80	0.053	3.67	13.08

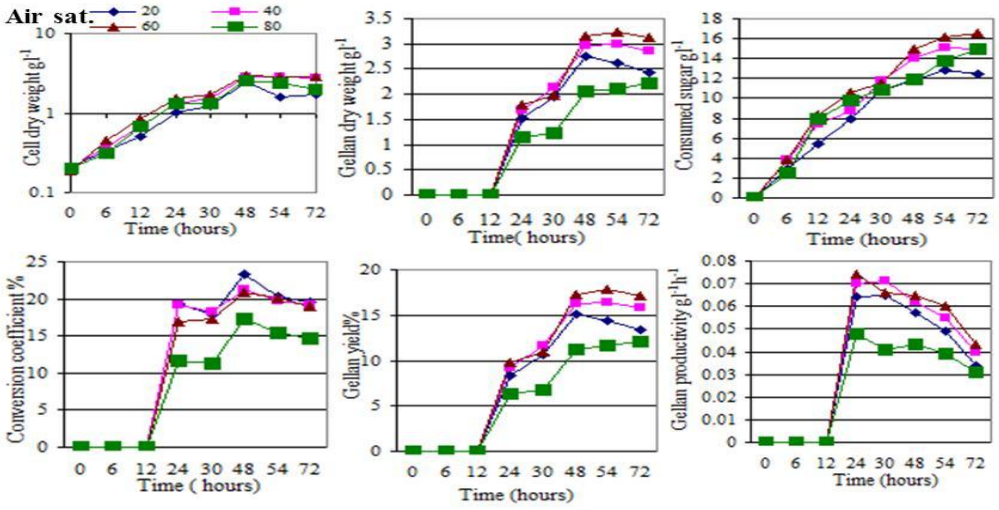

Fig. 2. Growth curves and gellan production by *S. paucimobilis* on 40% crude sweet whey as influenced by different levels of air saturation (%) during 72h at 28ºC using bioreactor as a batch culture. *Sat.= saturation*

respectively. While the efficiency of S. paucimobilis to use the sugar and converted to gellan (conversion coefficient) at 250 rpm was higher than at 1000 rpm agitation speed. From the above mentioned results, it could be concluded that using bioreactor technique as a batch culture with initial air saturation 60 % at 750 rpm agitation speed reduced the fermentation period from 72 to 48 h and enhanced the gellan yield and productivity by *S. paucimobilis* to 3.09 and 4.63 fold, respectively as compared with that obtained in shake flasks technique in our experimental studies. [6] reported that the gellan concentration was the highest at an agitation rate of 500 rpm, regardless of the aeration level (at either 1 or 2 vvm). At 1 and 2 vvm aeration rates, gellan reached 12.3 and 12.4 gl^{-1}, respectively. At 1000 rpm – 1 vvm, gellan concentration was less than 9.0 gl^{-1}, despite the improved cell growth, while at 250 rpm and 1 vvm aeration rate, the maximum gellan concentration dropped to 4.1 gl^{-1}. Under low agitation condition (250 rpm) an increase in aeration enhanced gellan formation and biomass.

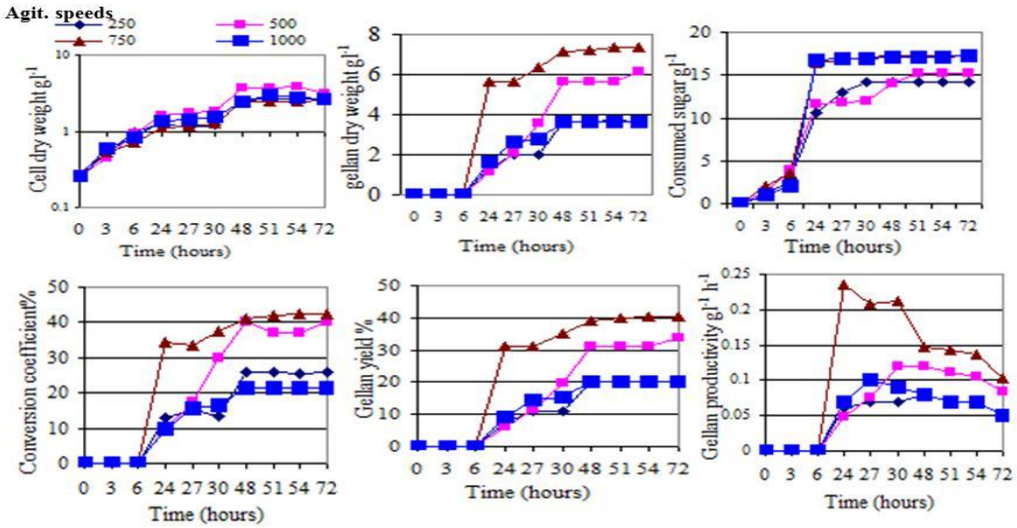

Fig. 3. Growth curves and gellan production by *S. paucimobilis* on 40% crude sweet whey as influenced by different levels of agitation speeds (rpm) during 72 h at 28°C using bioreactor as a batch culture. *Agit.= Agitation*

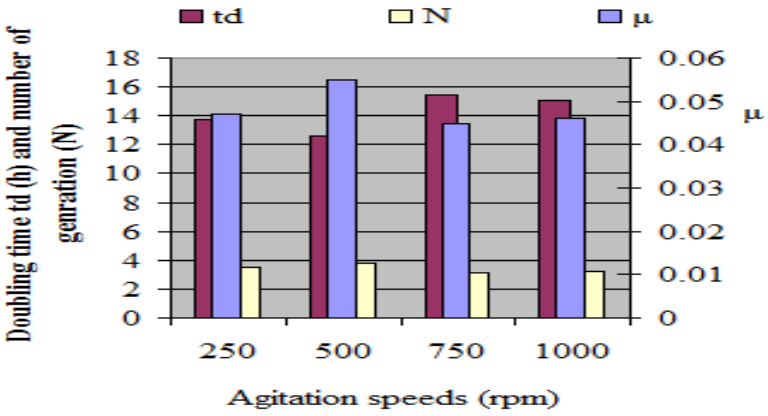

Fig. 4. Growth parameters of *S. paucimobilis* strain grown in 40% crude sweet whey at 28°C at different agitation speed using bioreactor as a batch culture

3.4 Fed-batch Culture

In fermentation processes where cell growth and/or product formation is inhibited by high substrate concentration or by the accumulation of a byproduct, the substrate is intermittently fed to the culture system in order to maintain the substrate concentration below a certain level for enhancement of biological and metabolic activity. Empirical feeding policies have been developed to achieve high cell density culture. [7,18] stated that physic-chemical properties, as well as production efficiency of microbial polysaccharide, are related to the fermentation process. So, *S. paucimobilis* was grown in the bioreactor as a fed- batch culture using two feeding strategies including pulsed and continuous feeding of sugar to determine the best technique for high gellan production by these selected bacteria.

3.5 Pulsed Sugar Feeding

The illustrated data in Fig. 5 show the effect of pulsed sugar addition (crude sweet whey) during 12 h or 24 h on the growth and gellan production by *S. paucimobilis*. The highest figures of gellan concentration (gl^{-1}) and cell dry weight (gl^{-1}) by using both pulsed additions of whey were recorded after 54 h of incubation. The corresponding figures were 8.15 & 2.92 gl^{-1} and 7.42 & 3.02 gl^{-1} with pulsed addition during 12 & 24 hours, respectively. At the same period the values of sugar utilization (%), conversion coefficient (%), gellan yield (%) and productivity ($gl^{-1}h^{-1}$) were 86.85% , 51.10%, 44.29% & 0.151 $gl^{-1}h^{-1}$ and 88.53%, 45.58%, 40.34% & 0.137 $gl^{-1}h^{-1}$, respectively. Also, it could be calculated that the highest figures of specific sugar consumption rate (μs) were recorded at 4 – 8 h of fermentation period being 0.30 h^{-1} and 0.38 h^{-1} with pulsed addition during 12 & 24 hours, respectively. The corresponding figures of specific production rate (μ_p) being 0.14 and 0.14 was obtained during period12 – 24 h and 12 – 16 h fermentation period for 12 and 24 h pulsed addition, respectively. Concerning, gellan gum productivity values throughout 12 h & 24 h pulsed feeding values were 0.321 and 0.189 $gl^{-1}h^{-1}$ after 24 h and 30 h, respectively.

3.6 Continuous Sugar Feeding

Data illustrated in Fig. (6) show the biological activity of *S. paucimobilis* grown on 40% crude sweet whey as fed-batch culture using continuous feeding. In this technique, whey was fed continuously at a rate of 1.53 and 0.77 $gl^{-1}h^{-1}$ (sugar/ liter/hour) during the first 12 and 24 h of fermentation period, respectively. Data clearly show that cell dry weight and consumed sugar increased gradually during the fermentation period at both rates of feeding. The maximum gellan concentration was obtained after 48 h of incubation, at both the rate of feeding being 10.87 gl^{-1} and 9.76 gl^{-1} at rates of 1.53 and 0.77 gl^{-1} h^{-1}, respectively. The corresponding figures of conversion coefficient, gellan yield and productivity were 62.26%, 59.14% & 0.226 gl^{-1} h^{-1}at rate of 1.53gh^{-1} and 54.43%, 52.93% & 0.203 $gl^{-1}h^{-1}$ at rate of 0.77 $gl^{-1}h^{-1}$, respectively. Moreover, the highest specific production rate (μ_p) of gellan being 0.2 & 0.27 were recorded during the first 12-24 h at rates of 1.53 & 0.77 $gl^{-1}h^{-1}$ respectively, whereas the highest specific consumption rate of sugar (μ_s) being 0.35 & 0.42 h^{-1} were recorded during 3 - 6 of fermentation

period at rates of 1.53 & 0.77 gl^{-1} h^{-1}, respectively.

From the previous data, it could be noticed that the continuous feeding at 1.53 $gl^{-1}h^{-1}$ was favorable than pulsed feeding for gellan production in fed–batch culture, as it increased the gellan productivity by *S. paucimobilis* 1.35 fold than pulsed feeding and about 1.53 fold than that produced in batch bioreactor technique at 60% air saturation and 750 rpm agitation speeds, after 48 h fermentation period.

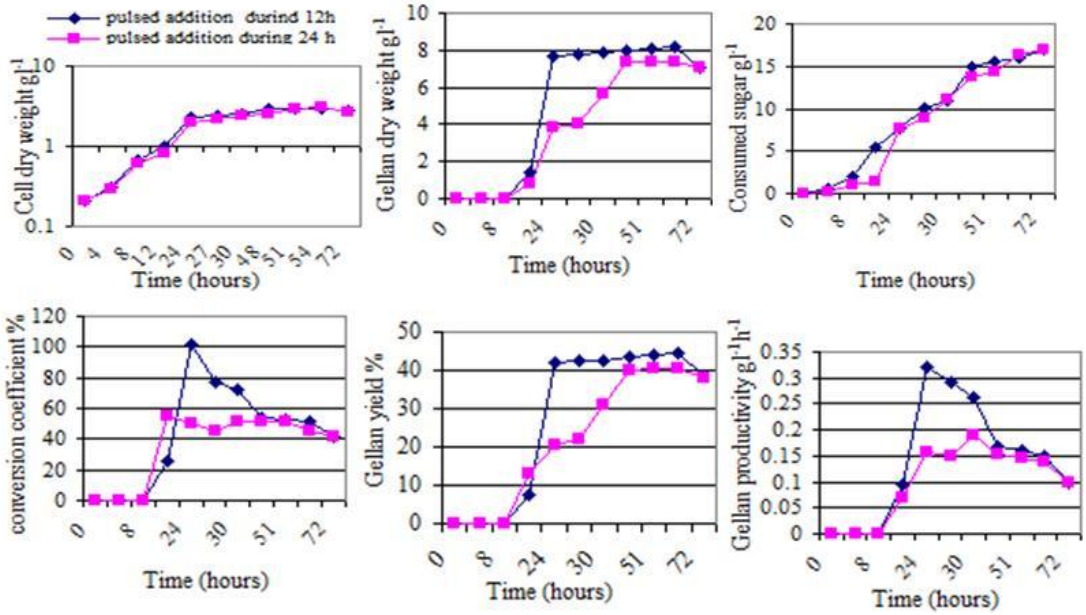

Fig. 5. Gellan production by *S. paucimobilis* on 40% crude SW using bioreactor as a fed–batch culture with the pulsed addition of whey during 12 or 24 h

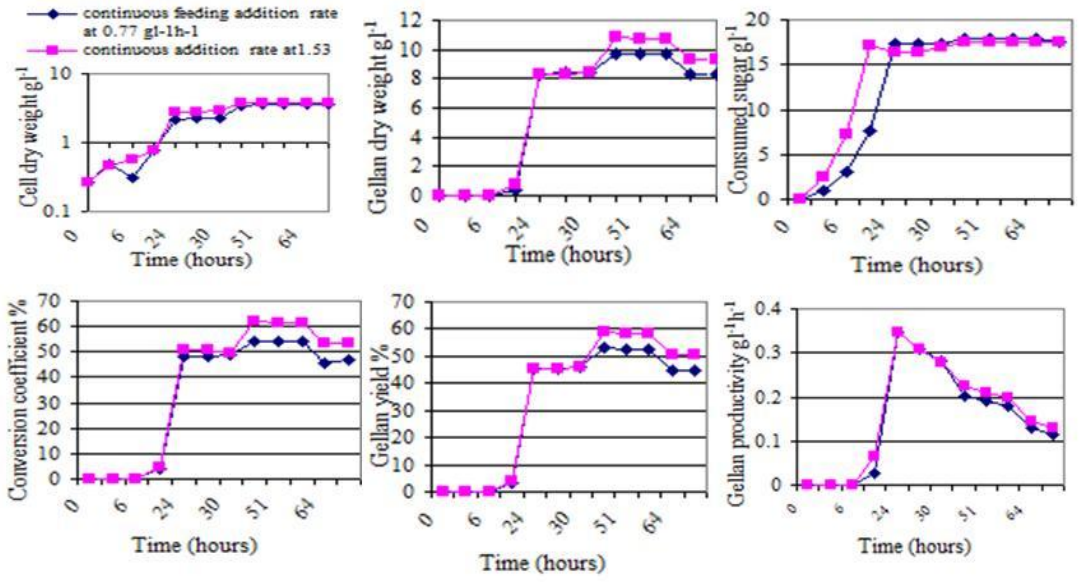

Fig. 6. Gellan production by *S. paucimobilis* on 40% crude sweet whey using bioreactor as a fed–batch culture with the continuous addition of whey at rates of (16.67 and 33.3 ml/l) 0.767 & 1.53 $gl^{-1}h^{-1}$

Table 2. Cultivation of *S. paucimobilis* on productive medium using bioreactor as a continuous culture at 0.035 h^{-1} dilution rate (70* ml medium / h flow rate / 2000 ml culture)

Time (h)	Suger in let (gh^{-1})	Suger out let (gh^{-1})	Consumed sugar (gh^{-1})	Cell dry Weight outlet (gh^{-1})	Cell dry weight out let (gl^{-1})	Gellan dry Weight out let (gh^{-1})	Gellan dry Weight out let (gl^{-1})	Gellan Yield (%)	Conversion Coefficient (%)	Gellan Productivity (gl^{-1} h^{-1})
**0	1.29	0.08	1.21	0.097	1.93	0.33	6.67	25.58	27.27	0.278
3	1.29	0.02	1.27	0.082	1.64	0.29	5.78	22.48	22.83	0.241
6	1.29	0.03	1.26	0.080	1.59	0.29	5.89	22.48	23.01	0.245
9	1.29	0.03	1.26	0.078	1.35	0.30	5.97	23.25	23.80	0.249
12	1.29	0.02	1.27	0.077	1.54	0.29	5.87	22.48	22.83	0.245
24	1.29	0.03	1.26	0.078	1.56	0.29	5.87	22.48	23.01	0.245
Means	**1.29**	**0.04**	**1.26**	**0.082**	**1.55**	**0.29**	**6.00**	**23.13**	**23.79**	**0.251**
r			**0.44**		**-0.64**		**-0.48**			

** D = μ = 0.035, D = F / V, 0.035 = F /2000 ml, F = 70 ml.*

***Data recorded at zero time were obtained after batch cultivation for 48 h using bioreactor.*

r correlation coefficient between time and each of consumed sugar, cell dry weight and gellan dry weight

Table 3. Cultivation of *S. paucimobilis* on the productive medium using bioreactor as a continuous culture at 0.045 h^{-1} dilution rate (90* ml medium / h flow rate / 2000 ml culture)

Time (h)	Suger in let (gh^{-1})	Suger out let (gh^{-1})	Consumed sugar (gh^{-1})	Cell dry Weight out let (gh^{-1})	Cell dry weight out let (gl^{-1})	Gellan dry Weight out let (gh^{-1})	Gellan dry Weight out let (gl^{-1})	Gellan Yield (%)	Conversion coefficient (%)	Gellan Productivity (gl^{-1} h^{-1})
**0	1.66	0.13	1.53	0.11	2.13	0.34	6.81	20.48	22.22	0.284
3	1.66	0.15	1.51	0.12	2.34	0.37	7.45	22.28	24.50	0.310
6	1.66	0.14	1.52	0.11	2.25	0.38	7.55	22.89	25.00	0.315
9	1.66	0.11	1.55	0.12	2.31	0.38	7.61	22.89	24.51	0.317
12	1.66	0.14	1.52	0.12	2.32	0.38	7.62	22.89	25.00	0.318
24	1.66	0.15	1.51	0.12	2.38	0.38	7.64	22.89	25.16	0.318
Means	**1.66**	**0.13**	**1.52**	**0.11**	**2.28**	**0.37**	**7.44**	**22.39**	**24.40**	**0.310**
r			**- 0.28**		**0.55**		**0.65**			

** D = μ = 0.045, D = F / V, 0.045 = F /2000 ml, F = 90 ml.*

***Data recorded at zero time were obtained after batch cultivation for 48 h using bioreactor.*

r correlation coefficient between time and each of consumed sugar, cell dry weight and gellan dry weight

Table 4. Cultivation of *S. paucimobilis* on productive medium using bioreactor as a continuous culture at 0.055 h^{-1}dilutionrate (110* ml medium / h flow rate / 2000 ml culture)

Time(h)	Suger in let (gh^{-1})	Suger out let (gh^{-1})	Consumed sugar (gh^{-1})	Cell dry Weight out let (gh^{-1})	Cell dry weight out let (gl^{-1})	Gellan dry Weight out let(gh^{-1})	Gellan dry Weight out let (gl^{-1})	Gellan Yield (%)	Conversion coefficient (%)	Gellan Productivity (gl^{-1} h^{-1})
**0	2.02	0.14	1.88	0.10	1.99	0.34	6.71	16.83	18.09	0.280
3	2.02	0.15	1.87	0.12	2.45	0.50	7.83	24.75	26.74	0.326
6	2.02	0.14	1.88	0.14	2.78	0.52	8.34	25.74	27.66	0.348
9	2.02	0.15	1.87	0.14	2.81	0.53	8.51	26.24	29.78	0.355
12	2.02	0.14	1.88	0.14	2.84	0.53	8.52	26.24	28.78	0.355
24	2.02	0.13	1.89	0.15	2.91	0.53	8.54	26.24	28.19	0.356
Means	**2.02**	**0.14**	**1.87**	**0.13**	**2.63**	**0.49**	**8.08**	**24.34**	**26.64**	**0.337**
r			**0.66**		**0.75**		**0.70**			

** D = μ = 0.055, D = F / V, 0.055 = F /2000 ml, F = 110 ml.*

***Data recorded at zero time were obtained after batch cultivation for 48 h using bioreactor.*

r correlation coefficient between time and each of consumed sugar, cell dry weight and gellan dry weight

Table 5. Cultivation of *S. paucimobilis* on productive medium using bioreactor as a continuous culture at 0.065 h^{-1} dilution rate (130* ml medium / h flow rate / 2000 ml culture)

Time(h)	Suger in let(gh^{-1})	Suger out let (gh^{-1})	Consumed sugar (gh^{-1})	Cell dry Weight out let (gh^{-1})	Cell dry weight out let(gl^{-1})	Gellan dry Weight out let(gh^{-1})	Gellan dry Weight out let(gl^{-1})	Gellan Yield (%)	Conversion coefficient (%)	Gellan Productivity (gl^{-1} h^{-1})
**0	2.39	0.56	1.83	0.09	1.89	0.34	6.81	14.22	18.57	0.283
3	2.39	0.79	1.60	0.09	1.75	0.30	6.01	12.55	18.75	0.250
6	2.39	0.98	1.41	0.07	1.42	0.27	5.31	11.29	19.14	0.221
9	2.39	1.43	0.96	0.06	1.12	0.21	4.21	8.78	21.87	0.175
12	2.39	1.78	0.61	0.05	1.02	0.20	4.01	8.36	32.78	0.167
24	2.39	1.89	0.50	0.04	0.88	0.11	2.14	4.60	22.00	0.089
Means	**2.39**	**1.23**	**1.15**	**0.06**	**1.34**	**0.23**	**4.74**	**9.97**	**22.19**	**0.198**
r			**- 0.91**		**- 0.89**		**- 0.98**			

** D = μ = 0.065, D = F / V, 0.065 = F /2000 ml, F = 130 ml.*

***Data recorded at zero time were obtained after batch cultivation for 48 h using bioreactor.*

r correlation coefficient between time and each of consumed sugar, cell dry weight and gellan dry weight

Table 6. Comparative studies between different fermentation methods for gellan production

Fermentation methods	Batch culture	Fed batch culture				Continuous culture			
	Time (hours)								
	72	54	54	48	48	24	24	24	24
		Pulsed feeding		Continuous feeding		At different dilution rates			
		** 24 h	** 12h	1.53* gh^{-1}	0.77* gh^{-1}	0.035 h^{-1}	0.045 h^{-1}	0.055 h^{-1}	0.065 h^{-1}
Gellan concentration (gl^{-1})	7.36	7.42	8.15	10.87	9.76	6	7.44	8.08	4.74
Gellan yield (%)	40.44	40.43	44.29	59.14	52.93	23.13	22.39	24.34	9.97
Gellan productivity (gl^{-1} h^{-1})	0.102	0.137	0.151	0.226	0.203	0.251	0.31	0.337	0.198

**Addition rate of sugar gh^{-1},*
***Time of added whey feeding during 12 &24 h.*

3.7 Continuous Culture

Four different dilution rates being 0.035, 0.045, 0.055 and 0.065 h^{-1} (70, 90,110 and 130 mlh^{-1} flow rate) were performed during the fermentation of *S. paucimobilis* on 40% crude sweet whey as a continuous culture. Results in Tables (2, 3, 4 & 5) show that varying the dilution rates, resulted in producing changes in the steady state of gellan yield, conversion coefficient and gellan productivity. At steady state levels of 0.035, 0.045 and 0.055 0 h^{-1}gellan production, consumed sugar and cell density remained constant for 24 hours while washing out was observed at 0.065 h^{-1}. It is interesting to record that mean values of the highest amount of gellan concentration either per hour or liter was 0.49 g h^{-1} or 8.08 gl^{-1}were attained at 0.055 h^{-1} dilution rate. At 0.065 h^{-1} dilution rate, where no steady state was observed, gellan outlet and biomass in bioreactor were decreased from 6.81 to 2.14 and from 1.89 to 0.88 gl^{-1} respectively, after 24 h of fermentation period. It is important to point out that the percentage decrement of cell dry weight and gellan dry weight were 53.44 % and 68.58% respectively after 24 hat dilution rate 0.065 h^{-1} (washing out) comparing to the recorded values at zero time of incubation.

Regarding gellan yield, conversion coefficient and gellan productivity, they increased with increasing of dilution rate (sugar input), reaching their maximum at 0.055 h^{-1} being 24.34%, 26.54% & 0.337 $gl^{-1}h^{-1}$, respectively, then decreased at 0.065 h^{-1} dilution rate (where no steady state was observed).

Whereas, the statically analysis for these tables showed that the correlation coefficient between time and consumed sugar was a positive effect ranged 0.44 to 0.66 at 0.035 h^{-1}dilutionrate and 0.065 h^{-1}dilutionrate and was negative effect ranged from – 0.28 to – 0.91 at 45 & 65 h^{-1} dilutionrate, respectively.

While, r between time and each of cell dry weight and gellan dry weight were positive at a dilution rate of 0.045 and 0.055 h^{-1} dilutionrate ranged from 0.55 to 0.75 and 0.65 to 0.70, respectively. and negative effect at dilution rate 0.035 and 0.065 ranged from – 0.64 to – 0.89 and – 0.48 to – 0.98, respectively.

From the above results, it could be concluded that a high correlation coefficient between time and each of consumed sugar, cell dry weight and polymer dry weight were achieved at dilution rates of 0.055 and 0.065 h^{-1} dilutionrate.

Therefore, it could be stated that the maximum dilution rate to be used, is 0.055 h^{-1} for giving maximum gellan production by *S. paucimobilis*. From the previous data which summarized in Table (6), it could be noticed that the highest gellan yield was attained by fed-batch culture with 1.53 $gl^{-1}h^{-1}$ continuous addition rate of whey being 59.14% after 48 h, while the maximum gellan productivity was obtained by using continuous culture technique at 0.055 h^{-1} dilution rate being 0.337 $gl^{-1}h^{-1}$ medium after 24 hours. Therefore, it could be recommended to produce gellan gum by *S. paucimobilis* using continuous culture technique since it was preceded over the other feeding strategies. The percentage of increment was approximately 32.94% comparing to continuous feeding of sweet whey (1.53 $gl^{-1}h^{-1}$) saturated with 60% air in bioreactor incubated at 28ºC and 750 rpm.

3.8 Gellan Gum Properties

The emulsifying capability of gellan produced from S. *paucimobilis* was examined against olive

oil and gave the highest emulsifying capacity than xanthan gum. Moreover, the flocculating activity of gellan against a suspension of activated carbon proved their unique potential as a flocculating agent. The crude gellan gum was soluble in water and forming a viscous solution and insoluble in ethanol and acetone. A tough worm- like gel or firm gel were formed when 10% calcium chloride solution or 0.5 g sodium chloride were added to gellan solution respectively. Generally, it can be recommended to use as gelling or solidification agent in different applications.

4. CONCLUSION

In this current work gellan gum production by *S. paucimobilis* from whey was optimized by different fermentation techniques using bioreactor as batch, fed-batch and continuous as cultures. Gellan production was affected by initial air saturation levels and different agitation speeds in batch culture. The maximum gellan yield and lower fermentation period were obtained with air saturation of 60% at 750 rpm agitation speed. The continuous feeding at 1.53 $gl^{-1}h^{-1}$ was favorable than pulsed feeding for gellan production in fed-batch culture, while the maximum gellan productivity was obtained by using a continuous culture technique at 0.055 h^{-1} dilution rate. It could be recommended to use gellan produce as gelling agent or solidification in the different application according to their emulsifying capability, flocculation activity and firm gel formation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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