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Broth rheology and morphological analysis of *Solanum chrysotrichum* cultivated in a stirred tank

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Abstract

Solanum chrysotrichum cell cultures were grown in a stirred tank bioreactor and their rheological and morphological behaviour were evaluated. The culture broths exhibited non-Newtonian and shear-thinning characteristics. Pseudoplasticity of the broths was governed by their biomass concentration. The roundness of aggregates measured as the elliptical form factor (EFF) had important changes. At the beginning of the culture the aggregates with an EFF lower than 2 represented 52% of the population, but in stationary phase the proportion increased to 77%. Whereas the size of aggregates did not change 80% of the population had an area lower than 0.1 mm^2. Overall, these results indicate that the shape of the aggregate therefore needs to be considered when studying plant broth rheology.

Introduction

Cell morphology in the fermentation process is important because it determines the flow behaviour of broths (Atkinson & Mavituna 1983). Generally, plant cell broths exhibit non-Newtonian shear-thinning characteristics (Rodríguez-Monroy & Galindo 1999). The biomass concentration has been considered as the principal responsible of this pseudoplasticity of the broths. However, plant cell cultures are composed of a mixture of single cells and aggregates of different shape and sizes (Kieran *et al.* 1997). Usually, individual cells and aggregates are counted or measured manually. Reports indicate that in a sample of the same culture it is possible to find cells oscillating between 10 to 120 μm diameter, whereas the individual aggregates may measure several millimeters. The relation of plant cell morphology and broth rheology may be more complex than that studied in microbial systems (Atkinson & Mavituna 1983). Image analysis may be considered as a powerful tool to measure the microscopic characteristics of plant cells (McDonald *et al.* 2001). Recently, this technique was used to obtain data of morphology of suspension cultures of *Oryza sativa, Nicotiana benthamiana* and *Trichosanthes kirilowii* (McDonald *et al.* 2001) and to evaluate the pigment production in cell cultures of *Fragaria ananassa* (Miyanaga *et al.* 2000).

Cell suspension cultures of *Solanum chrysotrichum* may be considered as a potential alternative to produce the antifungal saponin SC-1. Cell suspension cultures for biomass growth and SC-1 production from this species have been reported (Villarreal *et al.* 1997). The objective of this work was to study the broth rheology of *S. chrysotrichum* grown in a stirred tank and to characterize the size and shape of the aggregates using image analysis.

Material and methods

Cell cultures

Cell suspension cultures of *Solanum chrysotrichum* were obtained according to the methodology reported by Villarreal *et al*. (1998). Cells were grown on Murashige & Skoog (1962) medium, supplemented with sucrose (30 g l^{-1}), 2,4 dichlorophenoxyacetic acid (2 mg l^{-1}) and kinetin (2 mg l^{-1}). The pH of the medium was adjusted to 5.7 prior to sterilization.

Bioreactor cultures

A 2 l fermenter (Applikon) with a jacketed glass vessel and a multiport, stainless steel head plate was used. Configuration of the fermentor and the operation conditions were the same as reported by Rodríguez-Monroy & Galindo (1999). The fermentation vessel (containing 1.5 l) was inoculated with 150 ml of 7 days-old suspension culture grown in a 500 ml shake flask. Every three days, a sample of 30 ml was removed from the vessel for its analysis.

Analytical methods

Biomass

Dry weight was determined gravimetrically by filtration of 3 ml samples through a paper filter. Biomass concentrations reported were average of two independent runs.

Protein

Determined by the Bradford assay.

Viscosity measurement

The viscosity of the whole broth and the filtrate was measured using a Haake viscometer (Rotovisco RV20) and the methodology reported by Rodríguez-Monroy & Galindo (1999).

Image analysis

Images of cells were obtained using a microscope (Nikon, Alphaphot-2 YS2) with a charge-coupled device camera (Nikon, Coolpix 900). Magnification of the image was 4×. The image analysis software was Meta Imaging series for Microsoft Windows (version 4.0, Universal Imaging Corporation, USA). In this program, basic image processing tools were used to count aggregates and to measure their areas (mm^2), length (mm), breadth (mm) and the elliptical form factor (EFF), which was calculated as the ratio of length/breadth, where an EFF of 1 represented a round aggregate, whereas an EFF higher than 1 indicated an elongated aggregate. For each sample, the morphology of 200 aggregates was measured. Standard errors of image analysis were less than 0.5%.

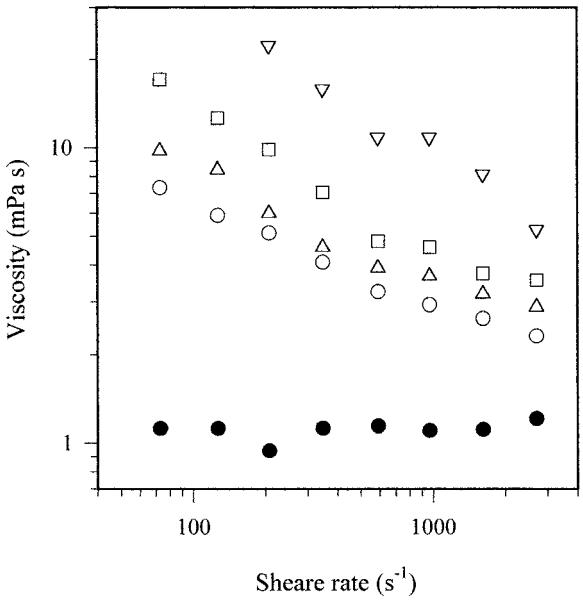

Fig. 1. Rheograms of *S. chrysotrichum* broths with different biomass concentrations (2 g l^{-1}, ○; 4 g l^{-1}, △; 8 g l^{-1}, □; and 10 g l^{-1}, ▽) and filtered medium in the stationary phase (●).

Results and discussion

Figure 1 shows that broth of *Solanum chrysotrichum* presents a pseudoplastic flow behaviour, which increased proportionally to its biomass concentration. The flow behaviour of the filtrated medium obtained from the cultures in the stationary phase, exhibited Newtonian characteristics with a viscosity similar to that of water (≈ 1 cP). This result indicated that the biomass concentration was responsible for the pseudoplastic characteristics of the broth. These results agree with those obtained with other plant broths: *Vinca rosae* (Tanaka 1992), *Catharanthus roseus* (Jolicoeur *et al.* 1992), *Daucus carota* (Curtis & Emery 1993), *Nicotiana tabacum* (Curtis & Emery 1993), and *Beta vulgaris* (Rodríguez-Monroy & Galindo 1999). On the other hand, flow rheology of filtered medium of *N. tabacum* (Kato *et al.* 1987) and *B. vulgaris* (Rodríguez-Monroy & Galindo 1999) grown in stirred tank fermentor also showed pseudoplastic character-

Table 1. Aggregates area (mm^2) and elliptical form factor (EFF) of *Solanum chrysotrichum* aggregates for different biomass concentrations in cultures grown in a stirred tank.

Biomass (g dry wt l^{-1})	Aggregates (% for the population)							
	Aggregates area (mm^2)			EFF				
	<0.1	>0.1<0.2	>0.2	>1<2	>2<3	>3<4	>4<5	>5
2	97	3	0	52	31	11	4	2
4	79	14	7	67	24	5	2	2
8	90	8	2	75	20	3	2	0
10	89	9	2	77	18	4	0	1

istics. These reports indicated that the accumulation of extracellular proteins secreted by the cells were responsible for the shear-thinning behaviour. The analysis of extracellular proteins in filtered medium of *S. chrysotrichum* showed that no protein was secreted by the cells. The accumulation of extracellular proteins reported previously for other species growing in stirred tanks may be therefore a function of the plant species.

In order to analyse the influence of aggregates morphology of *Solanum chrysotrichum* on the flow behaviour, the size and the shape of aggregates corresponding to different biomass concentrations were evaluated. Results given in Table 1 showed that the sizes of the aggregates of *S. chrysotrichum* were constant during development of the culture on the stirred tank. Aggregates of *S. chrysotrichum* lower than 0.10 mm^2 were the predominant population for all the samples with values of 79 to 97% of the whole population. Aggregates between 0.1 to 0.2 mm^2 represented 3 to 14% of the whole population and aggregates bigger than 0.2 mm^2 were between 2 to 7% of the whole population. In contrast, the shape of *S. chrysotrichum* aggregates evaluated by the elliptical form factor (EFF) value had an important change as a function of the biomass concentration (Table 1). At the beginning of the culture (2 g dry wt l^{-1}), groups of aggregates with EFF values between 1 and 2 represented 52% of the population, but in the stationary phase (10 g dry wt l^{-1}) the proportion increased to 77%. Simultaneously, aggregates with an EFF higher than 2 tended to decrease. For example, aggregates with an EFF between 2 and 3 decreased from 31% to 18% of the population as the biomass increased from 2 to 10 g dry wt l^{-1}. These results showed a clear tendency of the cell aggregates of *S. chrysotrichum* to change during culture in a stirred tank, from an elongated to a round form. Figure 2 shows the contrast of

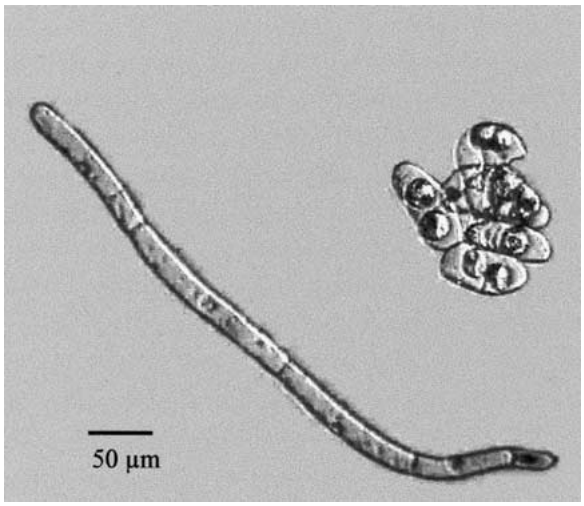

Fig. 2. Microphotographs of cell aggregates of *S. chrysotrichum* (4×) cultivated in a stirred tank bioreactor. Aggregates have the same area but different elliptical form factor (EFF) values. Cells on the left have an EFF value of 5.5 while the cells on the right have an EFF value of 1.2.

aggregates with the same area but with extreme values of EFF.

The results found in this work, suggest that in addition to biomass concentration and the size of the aggregates, the shape of plant cell aggregates could be affecting the flow behaviour of plant cell broths. These results are in agreement with those reported by Curtis & Emery (1993), who found that the morphology of the plant cells was important in flow behaviour of the broth, since round cells generated Newtonian broths, while elongated cells produced non-Newtonian fluids with shear-thinning behaviour. These phenomena could be similar to that observed with fungi cultures, in which the rheology of broths is strongly affected by the mycelia morphology; elongated free dispersed mycelia can generate more viscous broths than that

obtained with pelleted mycelia (Thomas & Paul 1996). Aggregates of *S. chrysotrichum* with high EFF may be equivalent to elongated mycelia, whereas round cell aggregates could represent the pelleted mycelia (Figure 2). Further experiments are necessary in order to find the exact relationship of different shapes of aggregates with the pseudoplasticity of plant cell broths.

Conclusion

Solanum chrysotrichum broths obtained in a stirred tank bioreactor exhibited non-Newtonian and shear-thinning characteristics. Pseudoplasticity of the broth was governed by the biomass concentration. The aggregates shape changed from elongated to round aggregates as a function of the biomass concentration, while the aggregates area did not show changes. Therefore, the role of aggregates morphology should be considered to understand the rheological characteristics of plant cell broths.

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