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SHORT COMMUNICATION

Spirulina platensis growth estimation by pH determination at different cultivations conditions

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Spirulina platensis **is a cyanobacterium that has a high protein content and therefore, a high nutritional value. It can be cultivated either in a liquid or in a solid culture. When cultivated in aqueous culture the cell growth can be determined by following the optical density. On the other hand, when produced by solid cultivation the growth can be determined only indirectly, such as, through determination of the protein content of the fermenting solids. In this work the possibility of estimating cell growth by pH determination was verified. From the results it was concluded that pH and protein production (solid or surface culture) or cell content (liquid culture) correlate well, therefore pH determination seems to be a good method to determine cell growth.**

Spirulina platensis is a filamentous cyanobacterium that is biotechnologically important due its high nutritional value. The nutritional value derives from its high protein content (about 70%) and its type of lipids (γ-linolenic acid) (Ciferri and Tiboni, 1985; Henrikson, 1989). This microorganism also finds application in environmental technology (Pulz and Scheibenbogen, 1998).

Generally it is produced in open ponds in liquid culture (Henrikson, 1989; Pulz and Scheibenbogen, 1998), but recently, its production in solid-state cultivation systems has been studied (Senecal et al. 1992; Cozza et al. 1999; Pelizer et al. 1999; Pelizer et al. 2000; Pelizer et al. 2002).

During production in liquid cultivation systems cell growth is followed by measuring the optical density of the culture medium. In solid cultivation estimation of cell growth is made difficult by the problems of separating cells from the cultivated medium. As a result of these difficulties, biomass

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levels in solid-state fermentation systems are typically determined indirectly through the measurements of cell constituents (Hesseltine, 1972; Huang et al. 1978; Abdullahet al. 1985; Gutiérrez-Rojas et al. 1995).

Desgranges et al. 1991a and Desgranges et al. 1991b, compared four methods for biomass estimation during growth of *Beauveria bassiana* in solid-state fermentation systems: they determined glucosamine, ergosterol, total sugar levels and determined the CO_2 evolution rate. There was a good correlation between the cell growth and the indirect methods for biomass determination.

The aim of this work was to verify the possibility of using pH determination to obtain rapid estimates of cell growth.

Materials and Methods

Microorganism and inoculum

The microorganism used was *Spirulina platensis*. The inoculum was obtained by liquid cultivation using a mineral medium (Paoletti et al. 1975) in 500 mL Erlenmeyer flasks for seven days. The cultivation conditions were: agitation, 160 rpm, temperature, 30ºC and luminosity (luminance), 6.0 Klux. The inoculum concentration was determined by spectrofotometry using a standard curve.

Culture conditions

Three types of experiments were done.

Solid culture was done in with 200 mL Erlenmeyer flasks containing 50 g of a solid medium containing sugar cane bagasse, mineral solution (Paoletti et al. 1975) and 8 g L^{-1} nutrient agar. Table 1 shows how the luminosity (provided with fluorescent lamps), the inoculum concentration and the moisture content were varied in these experiments.

Tray fermentations were also done with 500 g of this culture medium. Table 2 shows how the luminosity and inoculum concentration were varied. The moisture content for all experiments was 95.8%.

Surface culture was done in 200 mL Erlenmeyer flasks with 50 g of a medium consisting of mineral solution with 8 gL^{-1} nutrient agar. Table 3 shows how the luminosity and inoculum concentration were varied.

Liquid culture was done using mineral solution in 5 L tanks. In this case the only variable was inoculum concentration, as shown in Table 4.

Analytical procedures

pH was determined directly in solid and liquid media by a pH meter (SENTRON 1001).

In solid media cell growth was determined by optical density. For surface and solid cultivation growth it was followed indirectly by determination of the protein content of the cultivated material by the Kjeldahl method (Aoac,1984).

Results and Discussion

The protein produced during the various cultivation processes was correlated with the evolution of the pH and the equations for each experiment are shown in Table 1, Table 2, Table 3 and Table 4. For each type of culture medium one general equation was done plotting the results of all experiments together (Figure 1, Figure 2, Figure 3 and Figure 4). Reasonably high correlation coefficients were obtained for all experiments.

For experiments done in solid or surface culture the equations obtained are similar and, except for experiments 06 and 07, they can be grouped by experiments done using the same luminance (luminosity). For experiments using sugar cane bagasse that had a moisture content of 95.8% the equations were similar but in this case experiments using other luminosities were not done.

Experiments done with liquid medium presented almost the same equations and they were also carried out using only one luminosity.

These results show that pH determination can be used as an indicator of microbial growth. If an standard experiment is done to have a calibration curve for each process condition, especially luminosity (luminance), the cell growth can be rapidly estimated by culture pH.

For liquid culture growth estimation can also be done very rapidly by spectrophotometer. In the case of solid cultivation it is very difficult to separate the cells from the cultivation medium, meaning that the optical density method cannot be used. Cell growth can be determined indirectly by the determination of cells constituents such as protein content as done in the present work. However these methods are mostly time-consuming and do not allow on-line monitoring the process.

Concluding Remarks

These results show that pH determination can be used to predict *Spirulina platensis* growth in the types of processes studied in the present work.

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APPENDIX

Tables

Table 1. Results of experiments carried out in Erlenmeyer flasks using sugar cane bagasse and mineral media with nutrient agar as the culture medium.

Experiments	Luminosity Klux	Inoculum g cells. Kg wet cult. media $^{-1}$	Moisture % wet basis	*Equation*	Correlation coefficient
1	3.8	0.05	94.8	y = 1.6x - 15.6	0.979
2	3.8	0.25	94.8	y = 1.7x - 16.1	0.9607
3	6.0	0.05	94.8	y = 1.1x - 10.2	0.8518
4	6.0	0.15	94.8	y = 1.2x -11.1	0.99
5	6.0	0.25	94.8	y = 1.0x - 8.6	0.9608
6	8.2	0.05	94.8	y = 1.0x -9.5	0.99
7	8.2	0.25	94.8	y = 1.7x - 15.8	0.9907
8	6.0	0.05	95.8	y = 1.5x - 14.5	0.9788
9	6.0	0.15	95.8	y = 1.5x - 14.4	0.9632
10	6.0	0.25	95.8	y = 1.3x - 12.2	0.8618
General				y = 1.3x - 12.4	0.8309

Table 2. Results of experiments carried out in tray type bioreactor using sugar cane bagasse and mineral media with nutrient agar as culture medium.

Experiments	Luminosity Klux	Inoculum g cells. Kg wet culture media $^{-1}$	Equation	Correlation coefficient
11	6.0	0.05	y = 5.6x - 53.9	0.8383
12	6.0	0.15	y = 4.1x - 39.2	0.9898
13	6.0	0.25	y = 4.9x - 46.4	0.9543
14	8.2	0.05	y = 2.4x - 22.0	0.7375
15	8.2	0.15	y = 2.8x - 26.7	0.9611
16	8.2	0.25	y = 2.8x - 26.0	0.9117
General			y = 3.0x - 28.4	0.7263

Table 3. Results of surface culture experiments carried out in Erlenmeyer flasks using mineral media with agar nutrient as the culture medium.

Experiments	luminosity Klux	Inoculum g cells. Kg wet cell culture $^{-1}$	Equation	Correlation coefficient
17	**3.8**	**0.05**	**y = 1.6x -14.0**	**0.6599**
18	**3.8**	**0.25**	**y = 1.9x - 16.2**	**0.6454**
19	**6.0**	**0.01**	**y = 1.0x - 8.5**	**0.6181**
20	**6.0**	**0.15**	**y = 1.4x - 11.9**	**0.8299**
21	**6.0**	**0.29**	**y = 1.3x - 11.6**	**0.7789**
22	**8.2**	**0.05**	**y = 2.8x - 26.6**	**0.9466**
23	**8.2**	**0.25**	**y = 2.4x - 22.4**	**0.9286**
General			**y = 1.5x - 12.7**	**0.6024**

Table 4. Results of experiments carried out in mini-tanks using mineral solution as the culture medium.

Experiments	Inoculum g cells. Kg wet cell culture $^{-1}$	Equation	Correlation coefficient
24	0.05	y´ = 0.8x - 7.2	0.8868
25	0,10	y´ = 0.9x - 8.2	0.9241
26	0.15	y´ = 0.8x - 7.6	0.8995
27	0,20	y´ = 0.8x - 7.3	0.8526
General		y´ = 0.8x - 6.9	0.7624

Figures

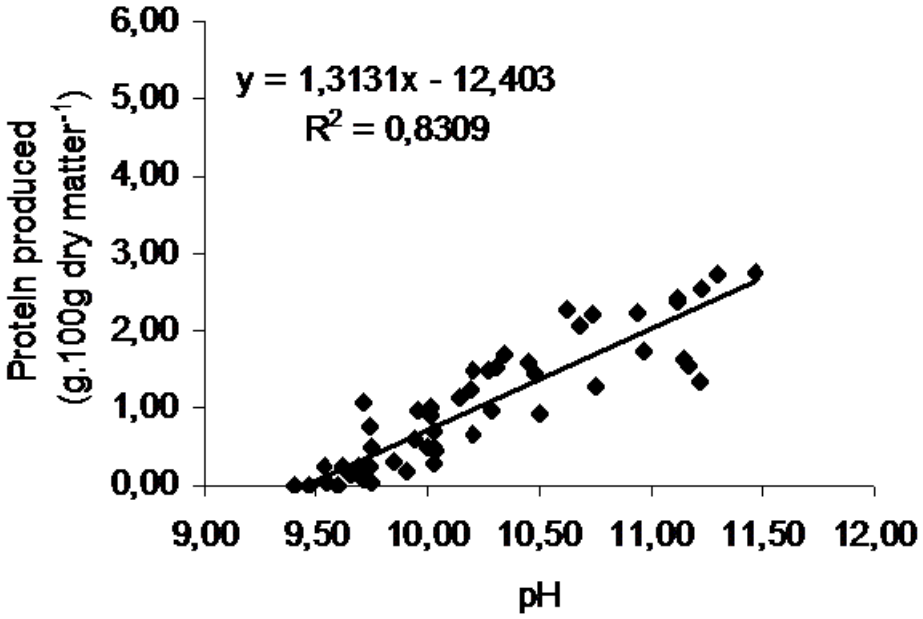
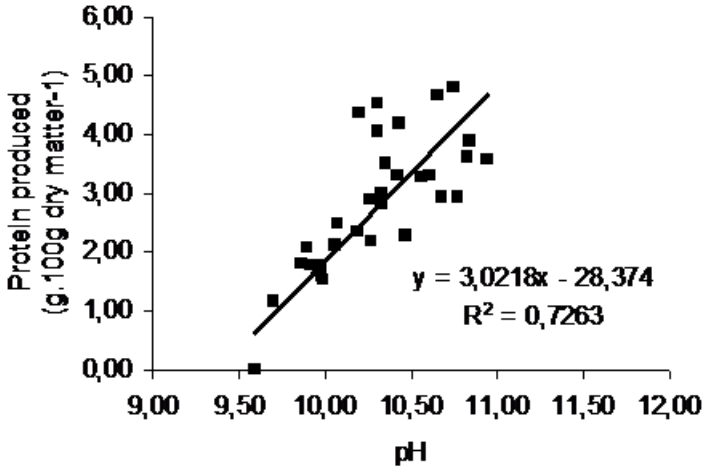
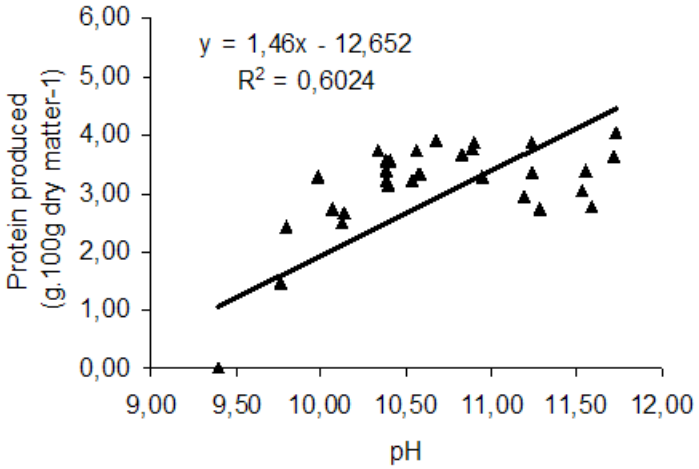


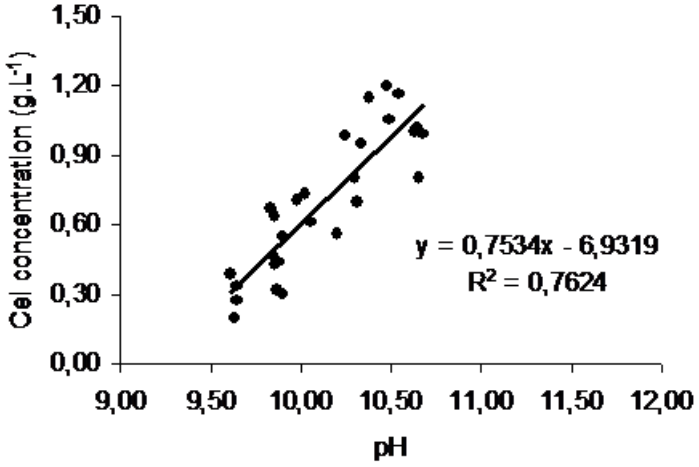
Figure 1. General curve for experiments carried out in Erlenmeyer flasks using sugar cane bagasse and mineral media with nutrient agar as the culture medium.



Figue 2. General curve for experiments carried out in tray type bioreactor using sugar cane bagasse and mineral media with nutrient agar as the culture medium.



Figue 3. General curve for surface culture experiments carried out in Erlenmeyer flasks using mineral media with agar nutrient as the culture medium.



Figue 4. General curve for experiments carried out in mini-tanks using mineral solution as the culture medium.