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ORIGINAL ARTICLE

New technologies from the bioworld: selection of biopolymer-producing microalgae

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Abstract

Microalgae are studied because of their biotechnological potential. The growth of microalgae aims at obtaining natural compounds. Due to the large amount of accumulated polymer waste, one of the solutions is the use of biodegradable polymers. The objective of this work was to select biopolymer-producing microalgae and to study the cell growth phase in which maximum production occurs. Microalgae *Cyanobium* sp., *Nostoc ellipsosporum*, *Spirulina* sp. LEB 18 and *Synechococcus nidulans* were studied. The growth was carried out in closed 2 L photobioreactors kept in a chamber thermostated at 30 °C with an illuminance of 41.6 $\mu mol_{photons}.m^{-2}.s^{-1}$ and a 12 h light/dark photoperiod. The biopolymers were extracted at times of 5, 10, 15, 20 and 25 d. The microalgae that had the highest yields were *Nostoc ellipsosporum* and *Spirulina* sp. LEB 18 with crude biopolymer efficiency of 19.27 and 20.62% in 10 and 15 d, respectively, at the maximum cell growth phase.

Keywords: *cyanobacteria, biopolymer, polyhydroxyalkanoate, productivity.*

1. Introduction

Cyanobacteria were the first phototrophic organisms capable of producing oxygen. They are responsible for the conversion of Earth's atmosphere from anoxic to oxic[1]. For the production of biomass with specific characteristics, manipulation of the culture conditions is a key factor[2].

Cyanobacteria are used for various purposes, e.g., for food supplements for humans[3] and animals[4]. Some cultures are used in wastewater treatment[5], in fixing carbon dioxide and in biocompound synthesis[6,7]. The biomass of *Spirulina* has been investigated for its hypocholesterolemic potential[8], as a source of biofuels[9] and for biopolymer production[10-12]. Several genera and species of cyanobacteria, such as *Dunaliella tertiolecta*[11], *Aulosira fertilissima*[12], *Nostoc muscorum*[13], *Spirulina subsalsa*[14], *Synechocystis* sp.[15], *Spirulina platensis*[16] and *Synechococcus* sp.[17], are used for the production of biopolymers.

Bacteria and cyanobacteria have the capacity to produce polyhydroxyalkanoates (PHAs)[13,18], which are biodegradable polyesters with potential use as polymeric materials[19]. Biodegradable polymers are alternative replacements for petrochemical polymers[20].

Reducing the consumption of plastic materials is difficult because of their versatile properties. However, it is possible to replace the petrochemical polymers with alternative materials that have similar polymer properties but show rapid degradation after disposal[20].

PHAs may positively change the scenario of global climate impact by reducing the amount of non-biodegradable polymers used[20]. Mixed cyanobacterial and bacterial cultures to produce PHAs are emerging due to the potential residuary use for growth and low installation cost towards a profitable production of polyhydroxyalkanoates. The growth of microalgae does not require large amounts of land and can occupy areas unsuitable for agriculture, thus not competing with food production, due to the possibility of using photobioreactors that maximize biomass production[21,22].

The objective of this work was to select biopolymer-producing microalgae and to study the phase of cell growth in which maximum production occurs.

2. Materials and Methods

2.1 Microorganisms and culture medium

The microalgae used were *Cyanobium* sp., *Nostoc ellipsosporum*, *Spirulina* sp. LEB 18 and *Synechococcus nidulans*. The microalgal strain *Nostoc ellipsosporum* (B1453-79) was provided by the University of Göttingen (Germany). The cyanobacteria *Cyanobium* sp.[23], *Spirulina* sp. LEB 18[24] and *Synechococcus nidulans*[7] belong to the Collection of Strains of the Laboratory of Biochemical Engineering of the Federal University of Rio Grande (FURG). *Spirulina* sp. LEB 18 was isolated from Mangueira Lagoon (33°30'12" S, 53°08'58" W) located in Santa Vitoria do Palmar/RS (Brazil). The cyanobacterium *Synechococcus nidulans* was isolated from a stabilization pond of the President Medici Thermoelectric Power Plant, located in Candiota/RS (Brazil) (24º36'13"S, 52º32'43"W). Inocula of *Cyanobium* sp. and *Nostoc ellipsosporum* microalgae were maintained in BG-11 culture medium[25], and *Spirulina* sp. LEB 18 and *Synechococcus nidulans*

microalgae were maintained in Zarrouk culture medium[26]. All inoculations were adapted to their respective culture media for 30 d before the start of the experiments.

2.2 Culture conditions

The cultivations were performed in closed 2 L photobioreactors with a working volume of 1.5 L and continuous agitation by the injection of sterile air to avoid the precipitation of the biomass. For *Nostoc ellipsosporum*, *Spirulina* sp. LEB 18 and *Synechococcus nidulans,* the initial concentration was 0.15 $g.L^{-1}$, but for *Cyanobium* sp., the initial concentration was 0.2 $g.L^{-1}$. The triplicate cultures were kept in a thermostated chamber at 30 °C for 5, 10, 15, 20 and 25 d, for a total of 15 experiments for each microalgae. The illuminance used was 41.6 $\mu mol_{photons}.m^{-2}.s^{-1}$ with a 12 h light/dark photoperiod maintained by 40 W fluorescent lamps.

2.3 Analytical determinations

Daily samples were collected aseptically for the monitoring of the cell concentration and pH. Cell concentration was determined by optical density at 670 nm in a spectrophotometer (Quimis Q798DRM, Brazil) with a calibration curve relating the optical density to the dry weight of the microalgal biomass[27]. The pH determination was performed in digital pH meter (Quimis Q400H, Brazil) following AOAC methodology[28].

2.4 Determination of the crude biopolymer yield

The crude biopolymer yield (Y_{CB}) was calculated according to Equation 1, where C_{cbt} is the concentration of crude biopolymers ($g.L^{-1}$) at time t (d), C_{cb5} is the concentration of crude biopolymers ($g.L^{-1}$) at time 5 d, t is the time (d), and t_5 is the time at 5 d.

$$Y_{CB} = (C_{cbt} - C_{cb5}) / (t - t_5) \quad (1)$$

2.5 Extraction of crude biopolymers

After 5, 10, 15, 20 and 25 d of experiment, the cultures were centrifuged at 7500 rpm for 20 min at room temperature (Hitachi, Japan) to separate the wet biomass from the biopolymer of the culture medium. Later, for every 1 g of dry biomass, 100 mL of distilled water and 25 mL of sodium hypochlorite (10-12% active chlorine (w/v)) were added to the wet biomass, and the solution was kept under stirring for 10 min. The resulting suspension was centrifuged (7500 rpm for 20 min at room temperature). Then, the supernatant was discarded, and the precipitate was washed with 100 mL of distilled water. The sample was centrifuged again, and the supernatant was discarded. This process was repeated adding 50 mL of acetone. The final precipitate (crude biopolymers) was dried at 35 °C for 48 h. The efficiency (η) of crude biopolymers in relation to microalgal biomass (%) was calculated using Equation 2, where m_{cb} is the final mass of crude biopolymer obtained from the microalgal biomass (g), and m_{ma} is microalgal biomass (g).

$$\eta = (m_{cb} * 100) / m_{ma} \quad (2)$$

2.6 Statistical analysis

The results were processed by analysis of variance (ANOVA) and Tukey's test to compare the means of the parameters analyzed with a 95% confidence level.

3. Results and Discussions

The growth curves of cyanobacteria *Cyanobium* sp., *Nostoc ellipsosporum*, *Spirulina* sp. LEB 18 and *Synechococcus nidulans* (Figure 1) showed different behaviors in spite of each species having its own specific growth characteristics and different culture media. In preliminary tests, it was observed that when the microalga *Cyanobium* sp. was grown at low biomass concentrations (0.15 $g.L^{-1}$), it showed photoinhibition in its growth; therefore, the assays were carried out with an initial biomass concentration of 0.2 $g.L^{-1}$, thereby preventing cell death and providing the lag phase of growth.

Spirulina sp. LEB 18 (Figure 1c) showed early stationary growth phase after 20 d of culture. For *Cyanobium* sp., *Nostoc ellipsosporum* and *Synechococcus nidulans,* the stationary phase of growth was not observed by the end of the 25 d of culture. To verify the growth phases of the microalgae *Cyanobium* sp., *N. ellipsosporum* and *S. nidulans*, it would be necessary to grow the cultures for a longer period. For large-scale production, such a long culture period is impractical for the production of biopolymers. Sharma and Mallick[29] cultivated *Nostoc muscorum* microalgae in BG-11 medium with a phosphorus deficiency and addition of exogenous carbon sources and found an increase in the production of PHB. Yields of up to 8.6% (PHB) were found when the extraction of the polymer was performed in the early stationary phase of growth of the microalgae (21 d of culture), whereas in log phase, the yield was 6.1%. Samantaray and Mallick[12] cultivated the microalga *Aulosira fertilissima* during 14 d and observed an accumulation of 6.4% of PHB at the end of logarithmic growth phase.

The microalga *Nostoc ellipsosporum* presented a different behavior in its cell growth compared to the other microalgae under study. During the first 8 d of culture, it showed cell growth, then ceased and remained constant until the 17th d, after which it presented new cell growth. This growth pattern may have occurred because when the microalgae are under a particular nutrient limitation, they use a substrate from its own cell as a nutrient, enabling continued growth. If there is a lack of carbon, the microorganism can consume the biopolymer itself. In this case, it is believed that the biopolymer may have been consumed, because after the 10th d of cultivation, the yield of biopolymers was reduced (Table 1). Another nutrient that may have had an influence was nitrogen, whose release in the culture medium from amino acids of phycobiliproteins and chlorophyll can possibly allow cell maintenance to occur[30,31].

The cyanobacterium *Nostoc ellipsosporum* presented a cell concentration less than the others but had higher efficiency (Table 1) and crude biopolymer yield (Table 2).

Among the microalgae under study, *Nostoc ellipsosporum* and *Spirulina* sp. LEB 18 stood out. These microalgae showed the higher efficiency of crude biopolymers (PHB) and did not differ significantly ($p<0.05$) each other from 15 d. However, *Nostoc ellipsosporum* reached a crude biopolymer

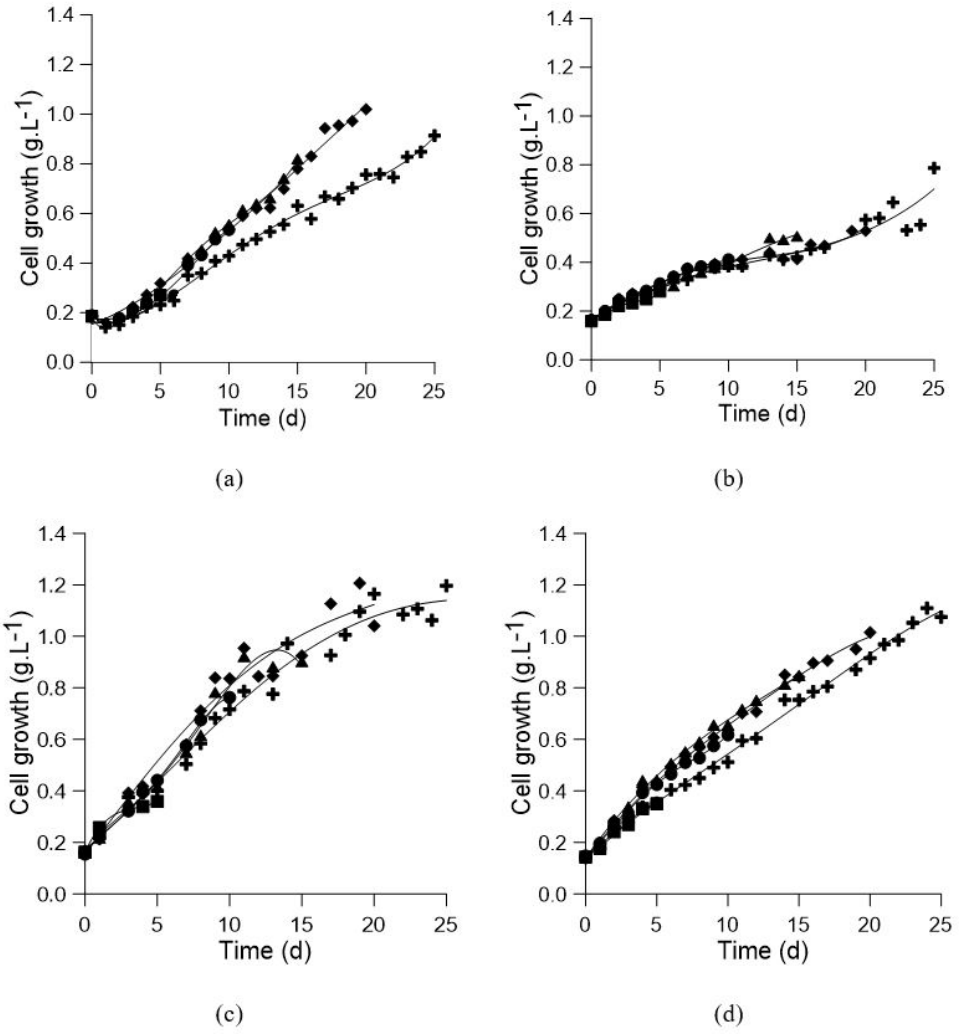

Figure 1. Growth curves of microalgae *Cyanobium* sp. (a) *Nostoc ellipsosporum* (b), *Spirulina* sp. LEB 18 (c) and *Synechococcus nidulans* (d) with 5 (■), 10 (●), 15 (▲), 20 (♦) and 25 (+) d of culture.

Table 1. Crude biopolymer efficiency (%, w/w*) for microalgae at different culture times.

Time (d)	Microalgae			
	***Cyanobium* sp.**	***N. ellipsosporum***	***Spirulina* sp. LEB 18**	***S. nidulans***
5	3.68 ± 0.23^{aAB}	9.04 ± 3.24^{aC}	5.82 ± 2.02^{aB}	1.18 ± 0.23^{aA}
10	3.17 ± 0.26^{abA}	19.27 ± 1.18^{bB}	10.23 ± 0.93^{aC}	8.83 ± 0.06^{bC}
15	2.75 ± 0.40^{bA}	17.79 ± 1.32^{bB}	20.62 ± 3.17^{bB}	1.00 ± 0.33^{aA}
20	2.91 ± 0.15^{abA}	13.41 ± 3.80^{abB}	11.83 ± 1.67^{aB}	10.21 ± 1.95^{bcB}
25	3.12 ± 0.30^{abA}	10.69 ± 2.84^{aB}	11.86 ± 2.43^{aB}	11.01 ± 1.49^{cB}

For the same letters, the averages do not differ significantly ($p<0.05$) by Tukey test. Lowercase letters compare the results in columns. Uppercase letters compare the results in the rows. *Values correspond to averages of results obtained in triplicate with their respective standard deviations.

Table 2. Crude biopolymer yield (Y_{cb}, $g_{cb}.L^{-1}.d^{-1}$) for microalgae at different culture times.

Time (d)	Microalgae			
	***Cyanobium* sp.**	***Nostoc ellipsosporum***	***Spirulina* sp. LEB 18**	***Synechococcus nidulans***
5	-	-	-	-
10	<0.01	2.05	0.88	1.53
15	<0.01	0.87	1.48	<0.01
20	<0.01	0.29	0.40	0.60
25	<0.01	0.08	0.30	0.49

efficiency of 19.27% in 10 d and *Spirulina* sp. LEB 18 reached 20.62% in 15 d of culture. The crude biopolymer efficiency of *Nostoc ellipsosporum* was 2.05 $g.L^{-1}.d^{-1}$ at 10 d, where as that of *Spirulina* sp. LEB 18 was 1.48 $g.L^{-1}.d^{-1}$ at 15 d (Table 2). Panda et al.[15] found that the cyanobacterium *Synechocystis* sp. PCC 6803 accumulated biopolymer PHB in its cells. It has been found that when cultured in BG-11 medium under phosphorus and/or nitrogen deficiency with the addition of exogenous carbon sources, this microalgae showed a higher yield (4.5%) of PHB in the early stationary growth phase (at 21 d cultivation), while in the logarithmic phase, the yield was 2.9%.

The microalga *Cyanobium* sp. did not achieve significant results ($p>0.05$) for the production of crude biopolymers. The cyanobacterium *Synechococcus nidulans* showed the highest PHB efficiency (11.01±1.49%) at a greater time of growth (25 d) in relation to the microalgae *Spirulina*sp. LEB 18 and *Nostoc ellipsosporum*. Therefore, its use is less interesting compared to *Nostoc ellipsosporum* and *Spirulina* sp. LEB 18. Lower yields (3%) of PHB were found by Sankhla et al.[32] in the stationary phase of growth when studying the production of PHB by *Brevibacillus invocatus* MTCC 9039.

The lowest yields obtained in culture times greater than 10 d (*Nostoc ellipsosporum*) and 15 d (*Spirulina* sp. LEB 18) may be due to the depletion of nutrients from the medium, especially carbon, which leads to consumption of the biopolymers for cell growth and maintenance. The results showed the effect of culture time on the production of biopolymers. This difference in yield is associated with the fact that the production of the polymer depends on the availability of the source of carbon and energy, which vary as a function of the culture time. Bhati and Mallick[13] studied the microalga *Nostoc muscorum* for the production of PHB-HV with yields of 16.6% in 10 d of incubation. For the same microalga, yields of different biopolymers were observed at different times using different carbon sources. When BG-11 medium was used with the addition of propionate, the highest yield was 12.6% in 21 d and 16.6% in 10 d with the addition of valerate. The highest yields were in the late exponential phase of growth. Mallick[33] studied the production of PHB-HV in *Nostoc muscorum* using BG-11 medium with the addition of propionate yielding 28.2% of biopolymer in 14 d of culture (late exponential growth phase).

Several microalgae, especially cyanobacteria, are able to accumulate intracellular biopolymers, especially poly-3-hydroxybutyrate and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) belonging to the group of polyhydroxyalkanoates. By modifying the culture conditions, particularly the nutrients, one can divert the metabolic pathways, causing the microorganism to synthesize larger amounts of biopolymers.

Studies are being carried out with photosynthetic mixtures of bacteria and algae that accumulate PHA in conditions with different concentrations of nutrients, and these studies have achieved PHB yields of 20%. The use of mixed photosynthetic culture (bacteria and microalgae) has emerged as an alternative system for the production of PHA, potentially minimizing feed costs through the use of solar energy[34].

The defatted biomass of microalgae *Dunaliella tertiolecta* was used for the production of biopolymers in different salt concentrations, obtaining a yield of 82%[11]. High yields of biopolymers can be achieved using microalgae. It is possible to conclude that many microalgae are able to intracellularly accumulate PHB granules. However, different behaviors are observed due to the use of different microalgal sources and concentrations of nutrients and growth conditions.

4. Conclusions

This study showed that in order to produce biopolymers from microalgal cultures, the microalgae *Spirulina* sp. LEB 18 and *Nostoc ellipsosporum* would be the best candidates. Both microalgae had higher concentrations of biopolymers at short growth times (*Spirulina* sp. LEB 18, 20.62% in 15 d; *Nostoc ellipsosporum*, 19.27% in 10 d). Combining the growth of microalgae and biopolymer production is a strategy with the potential to significantly reduce environmental pollution problems, through both the use of industrial waste as a source of nutrients for the culture medium and the replacement of petrochemical origin polymers by biopolymers degradable and compostable when disposed of in the environment.

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6. References

1. Madigan, M. T., Martinko, J. M., Dunlap, P. V., & Clark, D. P. (2010). *Microbiologia de Brock*. Porto Alegre: Artmed.
2. Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: a review. *Renewable & Sustainable Energy Reviews*, *14*(1), 217-232. http://dx.doi.org/10.1016/j.rser.2009.07.020.
3. Morais, M. G., Miranda, M. Z., & Costa, J. A. V. (2006). Biscoitos de chocolate enriquecido com *Spirulina platensis*: características físico-química, sensorial e digestibilidade. *Alimentos e Nutrição*, *17*(3), 333-340.
4. Silva, M. J., Figueiredo, B. R. S., Ramos, R. T. C., & Medeiros, E. S. F. (2010). Food resources used by three species of fish in the semi-arid region of Brazil. *Neotropical Ichthyology*, *8*(4), 825-833. http://dx.doi.org/10.1590/S1679-62252010005000010.
5. Córdoba, L. T., Bocanegra, A. R. D., Llorente, B. R., Hernández, E. S., Echegoyen, F. B., Borja, R., Bejines, F. R., & Morcillo, M. F. C. (2008). Batch culture growth of *Chlorella zofingiensis* on effluent derived from two-stage anaerobic digestion of two-phase olive mill solid waste. *Journal of Biotechnology*, *11*(2), 1-8. http://dx.doi.org/10.2225/vol11-issue2-fulltext-1.
6. Morais, M. G., & Costa, J. A. V. (2008). Bioprocessos para remoção de dióxido de carbono e óxido de nitrogênio por microalgas visando a utilização de gases gerados durante a combustão do carvão. *Química Nova*, *31*(5), 1038-1042. http://dx.doi.org/10.1590/S0100-40422008000500017.
7. Radmann, E. M., Camerini, F. V., Santos, T. D., & Costa, J. A. V. (2011). Isolation and application of SO_x and NO_x resistant

microalgae in biofixation of CO_2 from thermoelectricity plants. *Energy Conversion and Management*, *52*(10), 3132-3136. http://dx.doi.org/10.1016/j.enconman.2011.04.021.

8. Colla, L. M., Muccillo-Baisch, A. L., & Costa, J. A. V. (2008). *Spirulina platensis* effects on the levels of total cholesterol, HDL and triacylglycerol in rabbits fed with a hypercholesterolemic diet. *Brazilian Archives of Biology and Technology*, *51*(2), 405-411. http://dx.doi.org/10.1590/S1516-89132008000200022.
9. Oltra, C. (2011). Stakeholder perceptions of biofuels from microalgae. *Energy Policy*, *39*(3), 1774-1781. http://dx.doi.org/10.1016/j.enpol.2011.01.009.
10. Martins, R. G., Gonçalves, I. S., Morais, M. G., & Costa, J. A. V. (2014). Bioprocess engineering process aspects of biopolymer production by the cyanobacterium *Spirulina* strain LEB 18. *International Journal of Polymer Science*, *2014*, 1-6. http://dx.doi.org/10.1155/2014/895237.
11. Goo, B. G., Baek, G., Choi, D. J., Park, Y. I., Synytsya, A., Bleha, R., Seong, D. H., Lee, C. G., & Park, J. K. (2013). Characterization of a renewable extracellular polysaccharide from defatted microalgae *Dunaliella tertolecta. Bioresource Technology*, *129*, 343-350. PMid:23262010. http://dx.doi.org/10.1016/j.biortech.2012.11.077.
12. Samantaray, S., & Mallick, N. (2012). Production and characterization of poly-β-hidroxybutyrate (PHB) polymer from *Aulosira fertilissima. Journal of Applied Phycology*, *24*(4), 803-814. http://dx.doi.org/10.1007/s10811-011-9699-7.
13. Bhati, R., & Mallick, N. (2012). Production and characterization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) co-polymer by a N_2-fixing cyanobacterium, *Nostoc muscorum* Agardh. *Journal of Chemical Technology and Biotechnology*, *87*(4), 505-512. http://dx.doi.org/10.1002/jctb.2737.
14. Shrivastav, A., Mishra, S. K., & Mishra, S. (2010). Polyhydroxyalkanoates (PHA) synthesis by *Spirulina subsalsa* from Gujarat coast of India. *International Journal of Biological Macromolecules*, *46*(2), 255-260. PMid:20060853. http://dx.doi.org/10.1016/j.ijbiomac.2010.01.001.
15. Panda, B., Jain, P., Sharma, L., & Mallick, N. (2006). Optimization of cultural and nutritional conditions for accumulation of poly-β-hydroxybutyrate in *Synechocystis* sp. PCC 6803. *Bioresource Technology*, *97*(11), 1296-1301. PMid:16046119. http://dx.doi.org/10.1016/j.biortech.2005.05.013.
16. Jau, M.-H., Yew, S.-P., Toh, P. S. Y., Chong, A. S. C., Chu, W.-L., Phang, S.-M., Najimudin, N., & Sudesh, K. (2005). Biosynthesis and mobilization of poly(3-hydroxybutyrate) [P(3HB)] by *Spirulina platensis. International Journal of Biological Macromolecules*, *36*(3), 144-151. PMid:16005060. http://dx.doi.org/10.1016/j.ijbiomac.2005.05.002.
17. Nishioka, M., Nakai, K., Miyake, M., Asada, Y., & Taya, M. (2001). Production of poly-β-hydroxybutyrate by thermophilic cyanobacterium, *Synechococcus* sp. MA19, under phosphate-limited conditions. *Biotechnology Letters*, *23*(14), 1095-1099. http://dx.doi.org/10.1023/A:1010551614648.
18. Mohammadi, M., Hassan, M. A., Phang, L.-Y., Shirai, Y., Man, H. C., & Ariffin, H. (2012). Intracellular polyhydroxyalkanoates recovery by cleaner halogen-free methods towards zero emission in the palm oil mill. *Journal of Cleaner Production*, *37*, 353-360. http://dx.doi.org/10.1016/j.jclepro.2012.07.038.
19. Laycock, B., Halley, P., Pratt, S., Werker, A., & Lant, P. (2014). The chemomechanical properties of microbial polyhydroxyalkanoates. *Progress in Polymer Science*, *39*(3-4), 397-442. http://dx.doi.org/10.1016/j.progpolymsci.2013.06.008.
20. Chanprateep, S. (2010). Current trends in biodegradable polyhydroxyalkanoates. *Journal of Bioscience and Bioengineering*, *110*(6), 621-632. PMid:20719562. http://dx.doi.org/10.1016/j.jbiosc.2010.07.014.
21. Satyanarayana, A. B., Mariano, A. B., & Vargas, J. V. C. (2011). A review on microalgae, a versatile source for sustainable energy and materials. *International Journal of Energy Research*, *35*(4), 291-311. http://dx.doi.org/10.1002/er.1695.
22. Nonhebel, S. (2005). Renewable energy and food supply: will there be enough land? *Renewable & Sustainable Energy Reviews*, *9*(2), 191-201. http://dx.doi.org/10.1016/j.rser.2004.02.003.
23. Henrard, A. A., Morais, M. G., & Costa, J. A. V. (2011). Vertical tubular photobioreactor for semicontinuous culture of *Cyanobium* sp. *Bioresource Technology*, *102*(7), 4897-4900. PMid:21295968. http://dx.doi.org/10.1016/j.biortech.2010.12.011.
24. Morais, M. G., Reichert, C. C., Dalcanton, F., Durante, A. J., Marins, L. F., & Costa, J. A. V. (2008). Isolation and characterization of a new *Arthrospira* strain. *Zeitschrift für Naturforschung*, *63*(1-2), 144-150. PMid:18386504.
25. Rippka, R., Deruelles, J., Waterburry, J. B., Herdman, M., & Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology*, *111*, 1-61. http://dx.doi.org/10.1099/00221287-111-1-1.
26. Zarrouk, C. (1966). *Contribution à l'étude d'une cyanophycée: influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de Spirulina maxima Geitler* (Ph.D. Thesis). University of Paris, France.
27. Costa, J. A. V., de Morais, M. G., Dalcanton, F., Reichert, C. C., & Durante, A. J. (2006). Simultaneous cultivation of *Spirulina platensis* and the toxigenic, cyanobacteria *Microcystis aeruginosa. Zeitschrift für Naturforschung*, *61*(1-2), 105-110. PMid:16610226.
28. Association of Official Analytical Chemists – AOAC. (2000). *Official methods of analysis of the Association of Official Analytical Chemists*. 17th ed. In W. Horwitz (Ed.), Maryland: Association of Official Analytical Chemists.
29. Sharma, L., & Mallick, N. (2005). Accumulation of poly-β-hydroxybutyrate in *Nostoc muscorum*: regulation pH, light-dark cycles, N and P status abd carbon sources. *Bioresource Technology*, *96*(11), 1304-1310. PMid:15734319. http://dx.doi.org/10.1016/j.biortech.2004.10.009.
30. Jiang, L., Luo, S., Fan, X., Yang, Z., & Guo, R. (2011). Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO_2. *Applied Energy*, *88*(10), 3336-3341. http://dx.doi.org/10.1016/j.apenergy.2011.03.043.
31. Wu, G. F., Wu, Q. Y., & Shen, Z. Y. (2001). Accumulation of poly-β-hydroxybutyrate in cyanobacterium *Synechocystis* sp. PCC6803. *Bioresource Technology*, *76*(2), 85-90. PMid:11131804. http://dx.doi.org/10.1016/S0960-8524(00)00099-7.
32. Sankhla, I. S., Bhati, R., Singh, A. K., & Mallick, N. (2010). Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) co-polymer production from a local isolate, *Brevibacillus invocatus* MTCC 9039. *Bioresource Technology*, *101*(6), 1947-1953. PMid:19900805. http://dx.doi.org/10.1016/j.biortech.2009.10.006.
33. Mallick, N., Gupta, S., Panda, B., & Sen, R. (2007). Process optimization for poly(3-hydroxybutyrate-co-3-hydroxyvalerate) co-polymer production by *Nostoc muscorum. Biochemical Engineering Journal*, *37*(2), 125-130. http://dx.doi.org/10.1016/j.bej.2007.04.002.
34. Fradinho, J. C., Domingos, J. M. B., Carvalho, G., Oehmen, A., & Reis, M. A. M. (2013). Polyhydroxyalkanoates production by a mixed photosynthetic consortium of bacteria and algae. *Bioresource Technology*, *132*, 146-153. PMid:23399498. http://dx.doi.org/10.1016/j.biortech.2013.01.050.

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