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Pentoses Used in Cultures of *Synechococcus nidulans* and *Spirulina paracas*: Evaluation of Effects in Growth and in Content of Proteins and Carbohydrates

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HIGHLIGHTS

- Pentose promotes *Synechococcus* and *Spirulina* growth.
- For the first time pentoses were related with protein accumulation in cyanobacteria.
- Xylose and arabinose can be used as carbon sources for *Synechococcus* and *Spirulina*.

Abstract: The biological assimilation of the sugars present in lignocellulosic residues has gained prominence since these residues are the most abundant and economic residues in nature. Thus, the objective of this work was to determine whether the use of D-xylose and L-arabinose as sources of carbon in *Synechococcus nidulans* and *Spirulina paracas* cultures affects the growth and production of proteins and carbohydrates. Kinetic growth parameters, pentose consumption, protein content and carbohydrates were evaluated.

Synechococcus nidulans and *Spirulina paracas* consumed all concentrations of pentose used. The highest cellular concentration (1.37 g.L^{-1}) and the highest protein productivity ($54 \text{ mg.L}^{-1}.\text{d}^{-1}$) were obtained for *Spirulina paracas*, which was submitted to the addition of 38.33 mg.L^{-1} D-xylose and 1.79 mg.L^{-1} L-arabinose. The use of pentose promoted the accumulation of proteins for the studied microalgae. This is one of the first works to report protein bioaccumulation as a result of pentose addition.

Keywords: arabinose; proteins; *Spirulina*; *Synechococcus*; xylose.

INTRODUCTION

New strategies to reformulate specific culture growth conditions are of great interest to raise the productivity of different species of microalgae. In the search for alternative carbon sources for microalgae culture, pentoses are interesting [1] since the most abundant global renewal biomass source is lignocellulosic material, which contains significant amounts of pentose [2].

With a higher interest in the conversion of vegetal biomass to bioproducts, studies on pentose catabolism, which includes the catabolism of L-arabinose and D-xylose, have drawn attention, and only for the last few years, wild species that are capable of using pentose have been isolated and studied [3]. The utilization of pentose by microalgae is an in-development technology due to scarce studies related to the metabolic routes involved in the process of assimilation. Zheng et al [4] showed that the assimilation of xylose increases gradually with the activation of intermembrane transporters.

Recently associated with the use of pentose, studies with modification of the cultures' physical conditions, such as light intensity, have been developed [5], as well as the use of a combination with other carbon sources, such as carbon dioxide (CO_2) in *Chlorella minutissima* [6]. For microalgae of the *Chlorella* genus, studies involving modifications in the protein profile resulting from the addition of pentose have also gained visibility [5, 6]. The use of pentose has also been reported as responsible for lipid bioaccumulation by microalgae [7, 8].

Therefore, the use of pentose, which is considered a low-value sub product, for the production of compounds with high added value in microalgae collaborates the development of sustainable production systems. The number of studies about the use of pentose in microalgae is still low, and they have been focused on species from the Chlorophyta division (especially microalgae from the *Chlorella* genus)[5, 6, 8, 9].

Thereby, it is necessary to assess the pentose usage capacity of other species with different cellular structures. Thus, the objective of this work was to assess whether the use of D-Xylose or L-Arabinose as a carbon source in *Synechococcus nidulans* and *Spirulina paracas* cultures affects the growth and production of proteins and carbohydrates.

MATERIAL AND METHODS

Microalgae and culture conditions

To perform the assays, *Synechococcus nidulans* and *Spirulina paracas* from the Collection of the Laboratory of Biochemical Engineering of the Federal University of Rio Grande (FURG), Rio Grande do Sul, Brazil, were used.

Zarrouk medium [10] with no carbon source (NaHCO_3), $\text{pH}=9,5$ (correction with solutions of NaOH) and reduced nitrogen concentration ($0.125 \text{ g.L}^{-1} \text{ NaNO}_3$) was used for the cultures with pentose additions. For the control culture, Zarrouk medium [10] without alterations was used.

To evaluate the microalgae growth, cultivations were conducted in Erlenmeyer-type photobioreactors with a total volume of 2 L and a working volume of 1.8 L and performed in duplicate, with a triplicate of the analysis. The temperature was 30° C , the photoperiod was

12 h light/12 h dark, and an illuminance of 33.75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was provided by fluorescent lamps (40 W). The cultures were maintained until the stationary phase of growth.

The addition of D-xylose and L-arabinose (Vetec Quimica, Sigma, Aldrich Corporation) was performed using a synthetic broth, which had concentrations of pentoses that were equivalent to their concentrations in the hydrolyzate broth of pretreated sugarcane bagasse (Table 1), as previously proposal by Freitas *et al* [9].

Table 1. Concentrations of D-xylose and L-arabinose in Zarrouk medium.

Synthetic broth	D-xylose (mg.L ⁻¹)	L-arabinose(mg.L ⁻¹)
1%	3.833	0.179
5%	19.164	0.897
10%	38.328	1.793
20%	76.656	3.586
30%	114.984	5.379

Growth parameters

Cell concentration was determined by spectrophotometry using a previously established standard curve for *Synechococcus nidulans* and *Spirulina paracas*. These curves were obtained at 670 nm using a spectrophotometer (QUIMIS Q798DRM, Diadema, SP, Brazil) and correlated the relative optical density and dry biomass weight, as previously proposed by Costa *et al* [11].

The biomass productivity (P_{max} , $\text{g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) was obtained from the equation $P_{\text{max}} = (X_t - X_0)/(t - t_0)$, where X_t is the biomass concentration ($\text{g}\cdot\text{L}^{-1}$) at time t (d), and X_0 is the biomass concentration ($\text{g}\cdot\text{L}^{-1}$) at time t_0 (d). The maximum specific growth rate (μ_{max} , d^{-1}) was determined using an exponential regression applied to the logarithmic growth phase.

Determination the consumption of pentoses

Pentose consumption was determined using the methodology proposed by Somogyi [12] and was applied to the supernatant obtained by centrifugation of the cultures at 27,000 \times g for 10 min.

Harvesting biomass

The biomass was separated from the culture medium and the washing water by centrifugation at 15,000 \times g and 15 °C for 20 min. Then, the precipitate was dried for 24 h at 50 °C.

Carbohydrate and protein content

The total carbohydrate concentration in biomass ($\%w\cdot w^{-1}$) was determined using the method 3.5 -DNS [13].

The proteins were quantified by the micro-Kjeldahl method according to the methodology described by AOAC [14].

The carbohydrate (P_{CHO} , $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) and protein (P_{PROT} , $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) productivities were calculated based on the dried biomass, as described in Equations 1 and 2, respectively, where X_f is the final biomass concentration of a culture ($\text{g}\cdot\text{L}^{-1}$), CHO is the carbohydrate content (%), PROT is the protein content (%), and Δt is the cultivation time (d).

$$P_{\text{CHO}} (\text{g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}) = \frac{\text{CHO}\cdot X_f}{100\cdot\Delta t} \quad (1)$$

$$P_{\text{PROT}} (\text{g.L}^{-1} \cdot \text{d}^{-1}) = \frac{\text{PROT} \cdot X_f}{100 \cdot \Delta t} \quad (2)$$

The results of the characterization of the biomass obtained from cultures with pentoses (CC5) relative to that obtained from control cultures (CC) were compared according to the relationship $R = ((\text{CC5} - \text{CC}) / \text{CC}) \cdot 100$, where R (R_C or R_P) corresponds to the percentage difference in the results obtained with pentoses relative to the results obtained in the control cultures.

Statistical analyses

An analysis of variance and Tukey's test were performed, with a confidence level of 95% ($p > 0.05$), to determine the differences between the means in each assay.

RESULTS AND DISCUSSION

Higher concentrations of D-xylose and L-arabinose provided to the cultures of *Synechococcus nidulans* as a carbon source delayed a possible stationary phase due to the use of the traditional carbon source in Zarrouk medium (NaHCO_3) (Figure 1a), while for *Spirulina paracas*, lower concentrations of pentose (1, 5 and 10%) were capable of promoting growth for longer periods of time (Figure 1b). This behavior can be related to the maintenance of the enzymes that either delay or modify the arrival into the stationary growth phase, which occur with the use of different carbon sources at adequate conditions and concentrations [15].

For the assimilation of pentose, specific metabolic routes that aid in the pentose intermembrane transport, such as D-xylose [4], have been reported. Other forms of pentose absorption by microalgae can also happen through the activation of sugar transporters [16]. For *S. paracas* and *S. nidulans*, at every pentose concentration, a quick consumption of those sugars was verified. The total consumption of the lowest concentrations (1 and 5% C5) occurred at the first day of culture. At the end of the fourth day, the highest concentrations had already been consumed by the strains of cyanobacteria. The fast consumption of these nutrient sources is related to the low pentose concentrations used in replacement of sodium bicarbonate from the Zarrouk medium. Leite et al [7] also report a quick adaptation and pentose consumption by microalgae from the *Scenedesmaceae* and *Chlorophyceae* families. Freitas et al [5, 6] report the consumption of 20 mg.L⁻¹ pentose for a maximum of 3 days for *Chlorella minutissima*.

S. paracas showed a higher cellular concentration (1.37 g.L⁻¹) for the assays with the addition of 20% of pentose and reduction of 50% in the nitrogenous component, even in the face of controlling conditions. That higher biomass production is a result of the pentose positive contribution in the C/N relation, which was already demonstrated by Freitas et al [1] and Freitas et al [5] when the nitrogen source is reduced.

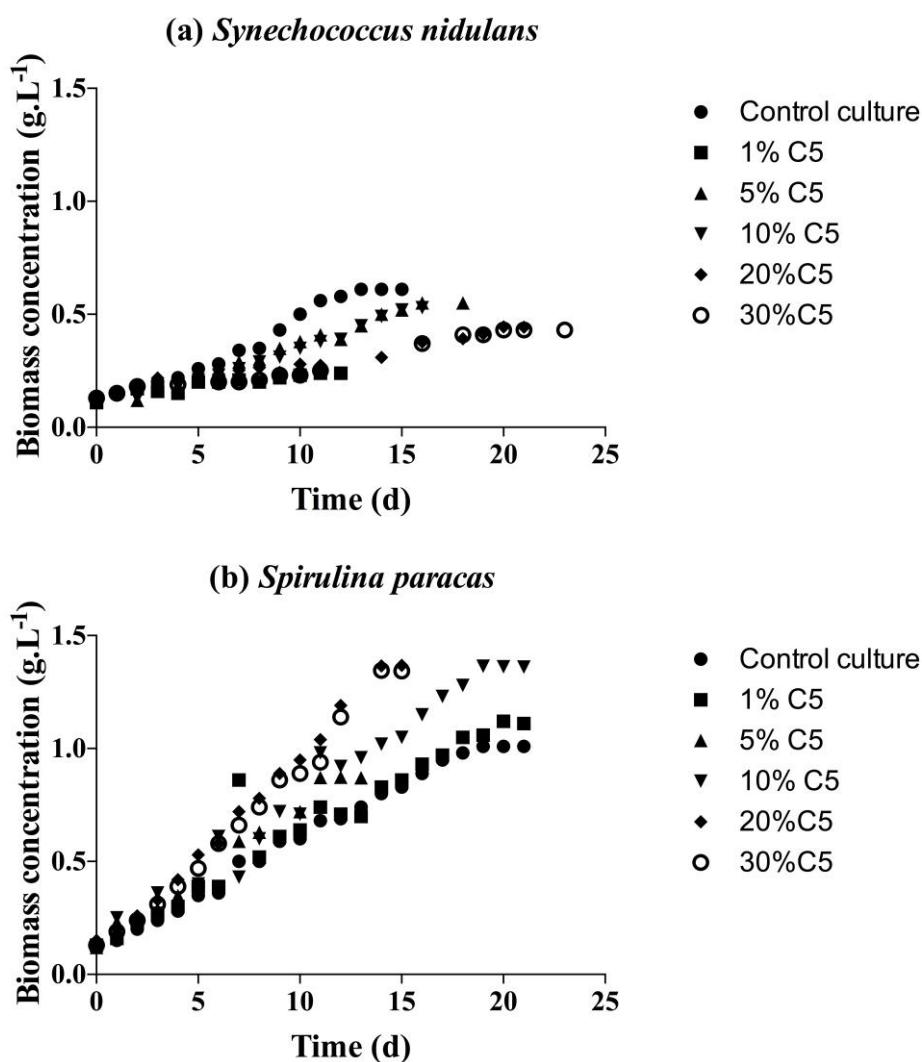


Figure 1. Growth of *Synechococcus nidulans* and *Spirulina paracas* under different culture conditions.

The highest productivities and specific growth rates for *S. paracas* were $0.13 \text{ g.L}^{-1}.\text{d}^{-1}$ and 0.24 d^{-1} , respectively, and both in cultures with 10% C5 (Table 2). For *S. nidulans*, control cultures resulted in better productivity responses and growth rates (Table 2).

The addition of 30% C5 resulted in a larger decline in biomass productivity for *S. nidulans* (Table 2). Many studies showed that a difference in the productivity between similar species occurs when cultures are started at different concentrations [17, 18]. Since all experiments started with a cellular concentration of 0.2 g.L^{-1} in this study, the addition of pentose can be considered the limiting factor for the difference between the obtained results for the studied strains.

The addition of pentose created a proper environment for the synthesis of proteins, which is observed with the positive (Rp) effect from the use of pentose for *S. nidulans* and *S. paracas* shown in Table 2. The highest content of determined protein was 62.9% for *S. nidulans* that was grown with the addition of 10% C5, and this protein content was the result of a raise of 46.3% (RP) in comparison to the control assay (Table 2). The use of 20% pentose induced the accumulation of 52.9% of proteins by *S. paracas* (Table 2). Since macromolecule productivity is related to the biomass production, the higher protein productivity determined for *S. paracas* ($54 \text{ mg.L}^{-1}.\text{d}^{-1}$) followed the higher cellular growth (1.37 g.L^{-1}) found for 20% C5 (Table 2).

The effects of xylose in the growth of three wild strains of *Chlorella vulgaris* were reported by Leite et al [7]. For these authors [7], the addition of xylose presented similar results for those three *Chlorella* strains, inducing a rapid accumulation of lipids under mixotrophic conditions, suggesting that relatively low quantities of xylose can act as an intensifier of lipid production. The accumulation of carbohydrates and the concentration reduction of proteins in *Chlorella minutissima* cells caused by the addition of pentose were reported by Freitas et al [5]. In this study [5], the use of 20 mg.L⁻¹ arabinose raised the carbohydrate content to 53%, whereas the use of xylose and arabinose combined resulted in a loss of 38% in the protein content of *C. minutissima*.

Unlike what has been reported for *Chlorella* cells by Leite et al [7] and Freitas et al [5], for *S. nidulans* and *S. paracas*, the pentoses acted as inducers in the biomass conversion for protein production. This biomolecule production difference can be related to cellular organization and the differences in the complexity of molecular mechanisms utilized by different groups since cyanobacteria (*Synechococcus* and *Spirulina*) are prokaryotes, whereas microalgae from the *Chlorella* genus are eukaryotes. Since differences in photosynthetic metabolism can directly affect nutrient assimilation and biomolecule production, one of the factors that can explain the difference in the response to the pentose addition is that cyanobacteria contain two photosynthetic systems that operate in sequence and are able to perform oxygenic photosynthesis. Other groups contain only one type of reaction center, I or II, depending on the taxon [19, 20].

Table 2. Results for the maximum cell concentration (X_{max} , g.L⁻¹), maximum productivity (P_{max} , g.L⁻¹.d⁻¹), maximum specific growth rate (μ_{max} , d⁻¹), carbohydrate (%w.w⁻¹) and protein (%w.w⁻¹) contents, productivity of protein (P_{PROT}) and carbohydrates (P_{CHO}) (mg.L⁻¹.d⁻¹) (mean±standard deviation) and difference in carbohydrate (R_C -%) and protein (R_P -%) contents generated by the addition of pentoses under different *Synechococcus nidulans* and *Spirulina paracas* culture conditions compared to the control assays.

Assays	X_{max} (g.L ⁻¹)	P_{max} (g.L ⁻¹ .d ⁻¹)	μ_{max} (d ⁻¹)	Proteins (%w.w ⁻¹)	R_P (%)	P_{PROT} (mg.L ⁻¹ .d ⁻¹)	Carbohydrate s (%w.w ⁻¹)	R_C (%)	P_{CHO} (mg.L ⁻¹ .d ⁻¹)
<i>Synechococcus nidulans</i>									
Control	0.62±0.02 ^b	0.06±0.01 ^a	0.19±0.01 ^a	43.0±0.80 ^d	**	17.63±0.43 ^{a,b}	8.9±0.35 ^c	**	3.67±0.09 ^b
1% C5	0.24±<0.01 ^a	0.03±0.02 ^a	0.15±0.01 ^a	49.3±0.99 ^a	(+)14.7	9.97±0.10 ^a	4.3±0.14 ^a	(-)51.8	0.87<0.01 ^a
5% C5	0.55±0.14 ^{a,b}	0.03±0.03 ^a	0.18±0.04 ^a	56.7±0.98 ^c	(+)31.6	17.38±3.07 ^{a,b}	4.1±0.14 ^a	(-)53.8	1.26±0.29 ^a
10% C5	0.53±0.05 ^{a,b}	0.03±<0.01 ^a	0.14±0.02 ^a	62.9±0.07 ^e	(+)46.3	20.84±1.78 ^b	4.9±0.06 ^{a,b}	(-)44.6	1.64±0.14 ^a
20% C5	0.45±0.04 ^{a,b}	0.03±0.01 ^a	0.19±0.02 ^a	55.2±0.07 ^{b,c}	(+)28.3	9.90±1.07 ^a	5.9±0.08 ^b	(-)34.4	1.24±0.09 ^a
30% C5	0.44±0.04 ^{a,b}	0.02±0.02 ^a	0.13±0.01 ^a	52.1±0.78 ^{a,b}	(+)21.2	9.93±0.94 ^a	6.1±0.37 ^b	(-)32.0	1.15±0.12 ^a
<i>Spirulina paracas</i>									
Control	1.01±0.02 ^a	0.11±0.02 ^a	0.21±0.02 ^a	41.2±0.70 ^a	**	19.81±0.39 ^a	11.9±0.45 ^b	**	5.70±0.33 ^c
1% C5	1.12±0.03 ^a	0.11±0.02 ^a	0.22±0.03 ^a	37.7±0.59 ^b	(-)8.5	19.91±0.24 ^a	5.8±0.44 ^a	(-)105.2	3.05±0.04 ^a
5% C5	0.87±0.03 ^a	0.11±0.02 ^a	0.21±0.11 ^a	41.2±0.32 ^a	(+)0.2	30.02±1.04 ^b	5.1±0.74 ^a	(-)132.9	3.70±0.13 ^{a,b}
10% C5	1.36±0.06 ^a	0.13±0.01 ^a	0.24±0.06 ^a	38.9±0.92 ^{a,b}	(-)5.4	25.19±2.29 ^{a,b}	6.1±0.92 ^a	(-)93.5	3.95±0.36 ^{a,b}
20% C5	1.37±0.01 ^a	0.09±0.01 ^a	0.18±0.02 ^a	59.2±0.36 ^d	(+)43.7	54.00±0.35 ^d	5.5±0.77 ^a	(-)117.6	4.97±0.03 ^{b,c}
30% C5	1.35±0.01 ^a	0.09±0.01 ^a	0.16±0.03 ^a	48.2±0.01 ^c	(+)16.9	43.12±3.39 ^c	6.7±0.32 ^a	(-)77.6	5.97±0.48 ^c

*For each strain with different pentose concentrations, the presence of the same superscript letter in the same column indicates that the means are not significantly different at a 95% confidence level ($p>0.05$).

**Assays without addition of pentoses. (+)Increase in the response relative to that of the control culture; (-) Decrease in the response relative to that in the control culture

The carbohydrate content of *S. nidulans* and *S. paracas* was reduced at all of the studied pentose concentrations. This effect is suggested through the negative values obtained for the effect of the pentose (R_C - Table 2). Another factor that demonstrates that pentose has a negative effect on carbohydrate production in cyanobacteria is that the highest carbohydrate productivities were obtained for the control culture conditions (without substituting NaHCO_3 for pentose) for both microalgae (Table 2). The most evident reduction in the content of carbohydrates (-132,9%) was verified for *S. paracas* under the addition of 5% pentose (Table 2).

Both cyanobacteria strains studied showed a capacity for modifying the cells' biochemical composition and increasing their growth rates through the variation in culture conditions resulting from the use of D-xylose and L-arabinose. Those results indicate that the addition of these sugars can positively collaborate with the photosynthetic rates, converging on protein accumulation alongside the growth of the studied strains.

CONCLUSION

The cellular growth and the protein productivity of *Spirulina paracas* were positively influenced by the addition of 20% pentose, reaching 1.37 g.L^{-1} biomass and $54 \text{ mg.L}^{-1}.\text{d}^{-1}$ proteins. For *Synechococcus nidulans*, the highest stimulus in biomolecule production resulted from the addition of 10% pentose, which led to a protein content of 62.9%. The usage of pentose in place of traditionally employed carbon sources in cyanobacteria cultures can be considered a viable strategy for the growth and the production of bioproducts as proteins for *Synechococcus nidulans* and *Spirulina paracas*.

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