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Effect of the carbon-nitrogen ratio on the co-production of polyhydroxyalkanoates and exopolysaccharides by *Enterobacter soli*

Efecto de la relación carbono-nitrógeno en la coproducción de polihidroxialcanoatos y exopolisacáridos por *Enterobacter soli*

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Abstract

The pollution generated by the indiscriminate use of conventional plastics has caused severe damage to the environment, so there is a need for alternatives such as the production of bioplastics from renewable sources. In the present work, the effect of different carbon to nitrogen (C/N) ratio (3, 7 and 11) and three carbon souces (sucrose, glucose and fructose) on co-production of polyhydroxyalkanoates and exopolysaccharides by *Enterobacter soli* in submerged culture was evaluated. The results showed that nitrogen limitation promoted the accumulation of polyhydroxyalkanoates, since with a C/N ratio of 11 the highest concentration was obtained (33 mg L^{-1}). On the other hand, high concentrations of nitrogen result in increased exopolysaccharides production (reported as precipitate g L^{-1}) with a C/N ratio of 3 (1.09 g·L^{-1}). Considering the results obtained, the production of biopolymers and consumption of sucrose were evaluated by means of a growth kinetics adjusting the C/N ratio to 11. The consumption of sucrose, glucose, and fructose substrate is consistent with the production of biomass, PHAs, and exopolysaccharides. The characterization of the biopolymers showed that *E. soli* is capable of co-producing polyhydroxybutyrate and inulin (recovered precipitate), such biopolymers were characterized by FTIR and mass spectrometry, respectively.

Keywords: Biopolymer, bioplastic, co-production, submerged cultivation.

Resumen

La contaminación generada por el uso indiscriminado de plásticos convencional ha generado severos daños al medio ambiente por lo que existe la necesidad de alternativas como la producción de bioplásticos a partir de fuentes renovables. En el presente trabajo se evalúo el efecto de diferentes relaciones C/N (3, 7 y 11) y tres fuentes de carbono (sacarosa, glucosa y fructosa) sobre la coproducción de polihidroxialcanoatos y exopolisacáridos por *Enterobacter soli* en cultivo sumergido. Los resultados mostraron que la limitación de nitrógeno promovío la acumulación de polihidroxialcanoatos, ya que con una relación C/N de 11 se obtuvo la mayor concentración de estos (33 mg·L^{-1}). Por otra parte, las altas concentraciones de nitrógeno permitieron la mayor producción de exopolisacáridos (reportados como precipitado g·L^{-1}) con una relación C/N de 3 (1.09 g·L^{-1}). Considerando los resultados obtenidos, se evaluó la producción de biopolimeros y consumo de sacarosa mediante una cinética de crecimiento ajustando la relación C/N a 11. El consumo de sustrato sacarosa, glucosa y fructosa concuerdan con la producción de biomasa, PHAs y exopolisacáridos. La caracterización de los biopolímeros demostró que *E. soli* es capaz de coproducir polihidroxibutirato e inulina (precipitado recuperado), dichos biopolímeros se caracterizaron por FTIR y espectrometría de masas, respectivamente.

Palabras clave: Biopolímero, bioplástico, coproducción, cultivo sumergido.

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1 Introduction

Conventional plastics have become an essential part of daily human life due to their physical, mechanical, and chemical properties that allow them to be a used in a wide range of sectors (Kumar *et al*., 2020). However, due to their uncontrolled use and fossil origin and inadequate management, plastics have had a negative impact on the environment (Amaro *et al*., 2019; Koller & Obruča, 2022). Indeed, the destination of plastics is often aquifers and terrestrial environments. Moreover, plastics take a long time to degrade (years) and they only disintegrate into smaller particles (micro- and nanoplastics) that negatively affect the environment (Saratale *et al*., 2021). However, although there are reports of the ability to degrade conventional plastics (Narciso-Ortiz *et al*., 2020, Narciso-Ortiz *et al*., 2023), it depends on environmental conditions, whether terrestrial or soil, where water availability and temperature can be more variable than in marine environments (Beltrán-Sanahuja *et al*., 2021). Their decomposition from plastic waste can take up to a thousand years when they are dumped into the environment or landfills. Due to this alarming problem, in recent years there has been an advocate of replacing synthetic plastics with biodegradable or bioplastics, which are biodegradable or compostable (Goel *et al*., 2021).

The environmental pollution generated by conventional plastics has increased interest in the production of green materials that can replace synthetic plastics. Bioplastics have emerged as a possible biotechnological alternative and solution to the negative environmental impacts of plastics (Naser *et al*., 2021) due to, there are reports that indicate that the degradation time of PHAs is shorter (1.5 to 3 years) compared to that of conventional plastics (Dilkes-Hoffman *et al*., 2019; Folino *et al*., 2020). Bioplastics can be synthesized from natural sources and have properties similar to synthetic plastics. Among bioplastics, polyhydroxyalkanoates (PHAs) (Koller & Obruča, 2022) are microbial polymers synthesized intracellularly under adverse growth conditions (Shahid *et al*., 2020). These biopolymers are recognized for their high biodegradability, biocompatibility, and, above all, their physicochemical and mechanical properties that are comparable to that of thermoset synthetic plastics (Khatami *et al*., 2021; Tripathi *et al*., 2021) e. g. insolubility in water, hydrophobicity, melting point, glass transition temperature, and degree of crystallinity (Samrot *et al*., 2021).

Even though PHAs represent a green alternative to replace synthetic plastics, high production costs prevent the establishment of an economically competitive microbial bioplastics industry relative to the already established conventional plastics industry (Khatami *et al*., 2021; Kumar *et al*., 2020). However, there are currently low-cost PHAs production alternatives that include strategies such as the use of industrial waste as a substrate, new methods of extraction and purification of biopolymers, and most recently, the co-production of metabolites (Kumar & Kim, 2018; Yadav *et al*., 2021). Co-production has emerged as an alternative that promotes the integral use of substrates in order to obtain multiple products. This approach is intended to generate economic benefits by using biomolecules that are produced in smaller quantities (Yadav *et al*., 2021) to co-synthesize molecules with high added value, including exopolysaccharides (EPS), biosurfactants, carotenoids, proteins, and amino acids, among others (Kumar and Kim, 2018). The co-product that can be generated depends on the physiology of the microorganism and the source of carbon present in the environment (Vega-Vidaurri *et al* 2022).

The genus *Enterobacter*, in addition to being reported as a producer of PHAs (Giraldo-Montoya *et al*., 2020), has also been recognized as one of the main producers of EPS. These biopolymers have been derived from *Enterobacter* clocacae (Shyam *et al*., 2021), *Enterobacter* ACD2 (Almutairi & Helar, 2020), *Enterobacter* luidwigii (Paikra *et al*., 2022), and *Enterobacter* sp. (Sampaio *et al*., 2021). EPS are synthesized by microbial cells as a protective mechanism during different stress conditions as a osmotic pressure, heating, low temperatures, oxidative pressure, UV radiation, heavy metals, H2O2, ethanol, nutrient balance among others (Obruca *et al* 2018;Jayakrishnan*et al* 2020; Obrucar *et al*., 2021). They are currently considered valuable biopolymers because they possess physiological and functional properties that have industrial applications in food, cosmetics, and medicine (Zhao *et al*., 2021). EPS can provide added value to the production of PHAs; moreover, the synthesis of both biopolymers occurs under stress conditions, with the difference being that EPS are extracellular metabolites (Kopperi *et al*., 2021; Kumar *et al*., 2020). Yadav *et al*. (2021) indicated that there is a better economic balance of co-production when intracellular biomolecules are co-produced with extracellular products. Considering these bioproducts compete for the carbon source during their formation, it is very important to consider the carbon- nitrogen (C/N) ratio because this factor directs the flow of carbon between both biopolymers (Cui *et al*., 2017). It has been reported that high C/N ratios promote EPS synthesis (Hernández-Rosas *et al*., 2021), while nitrogen or carbon limitation (according of the carbon necesario for the optimal cell growth that is depend of the microorganism used) for example in the medium induces PHAs accumulation. The aim of this study was to determine the effect of the C/N ratio

on the synthesis of PHAs and EPS by *Enterobacter soli* in submerged culture.

2 Materials and methods

2.1 Identification of the isolated bacterial strain

The bacterial strain used in the present work was isolated from the local sugar agroindustry and it is part of the Microbial Biotechnology Laboratory of the College of Postgraduates Campus Córdoba. The strain was characterized morphologically (macro- and microscopically) and was molecularly identified by amplifying the 16S ribosomal RNA (rRNA) gene with the oligonucleotides 27F and 1492R by using the conditions described by Lane (1991).

2.2 Inoculum preparation and strain conservation

The strain was reactivated in inclined tubes with nutrient agar (23 g L^{-1}) supplemented with glucose (10 g L^{-1}) and was maintained at 28 °C for 24 h. The reactivated strain was inoculated in 250-mL Erlenmeyer flasks containing 100 mL of medium composed of nutrient broth (8 g L^{-1}) and glucose (20 g L^{-1}). The flasks were incubated at 32 °C for 24 h at 150 rpm; the biomass produced was considered the inoculum (Vega-Vidaurri *et al*., 2022).

For conservation, the strain was inoculated in inclined tubes of nutrient agar (23 g L^{-1}) supplemented with glucose (10 g L^{-1}) and incubated at 32 °C for 24 h. The biomass was harvested with 5 mL of sterile Tween 80 at 0.01% and the suspension was inoculated (to increase biomass) in Erlenmeyer flasks containing nutrient broth. The culture was incubated at 32 °C for 24 h at 150 rpm. Then, the biomass was recovered by centrifugation at 10,000 g for 15 min. The pellet was washed twice with water and resuspended in 1 mL of water. Finally, the suspension was placed in cryovials containing 50% glycerol and sterile glass spheres and kept at -20 °C until use (Castilla-Marroquín *et al*., 2020). The conserved strain was tested for viability at least every 3 months and was conserved periodically.

2.3 Co-production of polyhydroxyalkanoates and exopolysaccharides by submerged culture

The effect of three C/N ratios 3, 7, and 11 on the co-production of PHAs and EPS was evaluated, according of the literature C/N rates reported for biomass and EPS production and considering as reference of the general composition of E. coli (49.67% C, 6.65% H, 24.77% 0, 15.22% N, 0.79% S, 2.90% P) (Duboc *et al*., 1995; Lizardi-Jimenez *et al*., 2012; Hernández-Rosas *et al*., 2021). The co-production of both biopolymers was carried out in submerged culture using 125-mL flasks containing 75 mL of culture medium composed of nutrient broth (8 g L^{-1}), commercial sucrose (20 g L^{-1}), KH2PO4 (1.5 g L^{-1}), and $(NH_4)_2SO_4$ as a source of nitrogen, with the amount adjusted according to the C/N ratio to be evaluated. Each flask was inoculated with 2×10^5 colony-forming units (CFU) mL^{-1} and incubated at 32 °C and 150 rpm.

2.4 Polyhydroxyalkanoate extraction

PHAs were extracted by digestion as described by Meneses *et al*. (2022) with some modifications. The biomass produced in the system was separated from the culture medium by centrifugation at 10,000 g for 15 min, washed twice with distilled water, and freeze-dried. Digestion was performed by resuspending the biomass in 3 mL of 5% sodium hypochlorite solution (v/v). The mixture was incubated at room temperature for 3 h and then centrifuged. The recovered pellet was washed with distilled water. Finally, PHAs were purified by washing with cold isopropanol and dried by freeze-drying for analysis.

2.5 Exopolysaccharide recovery

EPS were recovered by precipitation from the supernatant of the previous stage with cold absolute ethanol, adding 2.5 volumes of ethanol for each volume of supernatant; the mixture was incubated at 4 ºC for 24 h (Anguluri *et al*., 2022). Precipitated EPS were recovered by centrifugation at 10,000 x g, washed twice with 80% ethanol (v/v), and freeze-dried for analysis (Aramsangtienchai *et al*., 2020).

2.6 Growth kinetics and co-production of polyhydroxyalkanoates and exopolysaccharides

The C/N ratio was adjusted according to the biopolymer co-production analyses, and the growth kinetics was evaluated over 48 h using the culture medium for the co-production of biopolymers and 2×10^6 CFU mL^{-1} of the bacteria. The system was incubated at 32 °C and 150 rpm and sampled at 7, 21, 24. 42, 45 and 48 h.

2.7 Sugar quantification of by high-performance liquid chromatography

A Thermo Scientific Finnigan Surveyor high-performance liquid chromatograph was used to

quantify sugars. The high-performance liquid chromatography (HPLC) system consisted of a Surveyor LC Plus pump, an autosampler, and an RI Surveyor Plus detector. Separation was carried out using a 300 × 7.8 mm Phenomenex Rezex RNM-carbohydrate Na^{+2} column, with Milli-Q double-distilled water as the mobile phase. The temperature of the column was maintained at 80 °C, the detector temperature was 37 °C, and the flow rate was 0.4 mL min^{-1}. Samples were diluted and filtered prior to injection using PHENEX PTFE acrodisc filters (25 mm, 0.20 μm pore). The sugar concentrations in the samples were determined from calibration curves prepared with a mixture of analytical grade sucrose, glucose, and fructose standards (Sigma-Aldrich) from 200 to 1000 ppm. The results are reported in g L^{-1}.

2.8 *Characterization of polyhydroxyalkanoates and exopolysaccharides by Fourier-transform infrared spectroscopy*

PHAs and EPS produced by *E. soli* were characterized using Fourier-transform infrared spectroscopy. The method involved attenuated total reflection (ATR) with zinc selenide crystal. The measurement region of the purified samples was 4000 to 650 cm^{-1}, with 150 scans and a resolution of 4 cm^{-1}. The Origin 8 program was used to analyze the spectra. The commercial standards were poly[(R)-3-hydroxybutyric acid] (PHB, SIGMA®) for PHAs and Dahlia tubers inulin (SIGMA®) for EPS.

2.9 *Inulin determination by mass spectrometry*

For mass spectrometry analysis, a Waters Xevo TQ-S micro instrument coupled to an ASAP (Atmospheric Solids Analysis Probe) was used. The analysis was performed at the Corona voltage (kV): 12.30; Cone voltage: 10V; Fountain temperature: 150; desolvation temperature: 650 °C; a gradient of 100 - 450°C was applied to the probe temperature over a period of 5 min. Mass spectra were recorded in full scan mode over a range of 50 m/z to 2048 m/z. A capillary tube sealed at both ends was directly immersed in an aliquot of the liquid sample and the capillary was then loaded into the ASAP probe. MassLynx V4.1 software (Waters, Milford, MA, USA) was used for data acquisition and processing. Tentative identification was assigned according to that reported in published literature and public databases (Pacheco *et al*., 2023.

2.10 *Statistical analysis*

A one-way analysis of variance was performed to detect significant differences between the three evaluated C/N ratios evaluated. Tukey's test was for *post hoc* pairwise comparisons (α = 0.05). To perform the analysis of variance, the results were previously evaluated to corroborate that they complied with the assumptions of normality, homogeneity of variance and independence of the samples. The Shapiro-Wilk and Battlet tests were performed to verify the assumptions of normality and homoelasticity of variance, respectively. Both tests were performed with a 95% confidence interval, while the independence assumption was verified by randomizing the application of the treatments to the experimental units.

3 Results and discussion

3.1 *Characterization of the isolate*

Macroscopic analysis of the isolate revealed that the colonies that grew on nutrient agar showed a circular shape with convex elevation, white coloration, and a smooth surface. Microscopic analysis revealed that the bacterium is a gram-negative coccobacillus. The observed characteristics are like those reported for *Enterobacter* species that produce PHAs (Giraldo-Montoya *et al*., 2021; Rakkan *et al*., 2023). Phylogenetic analysis based on the 16S rRNA gene showed that the strain had maximum homology with *E. soli*, with 99% identity (Figure 1).

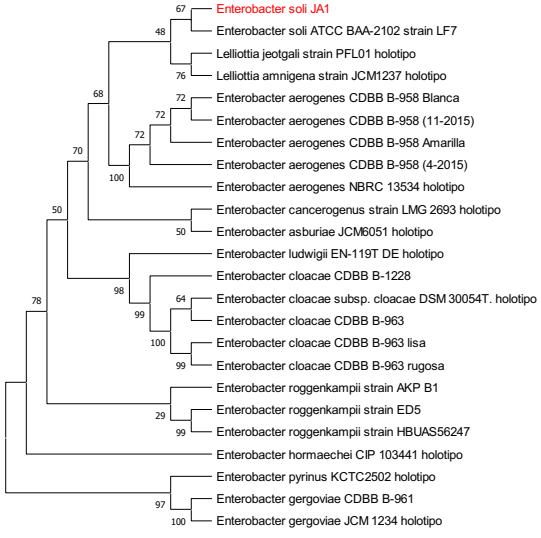

Figure 1. Phylogenetic similarity tree of the 16S ribosomal consensus sequence of isolated strain.

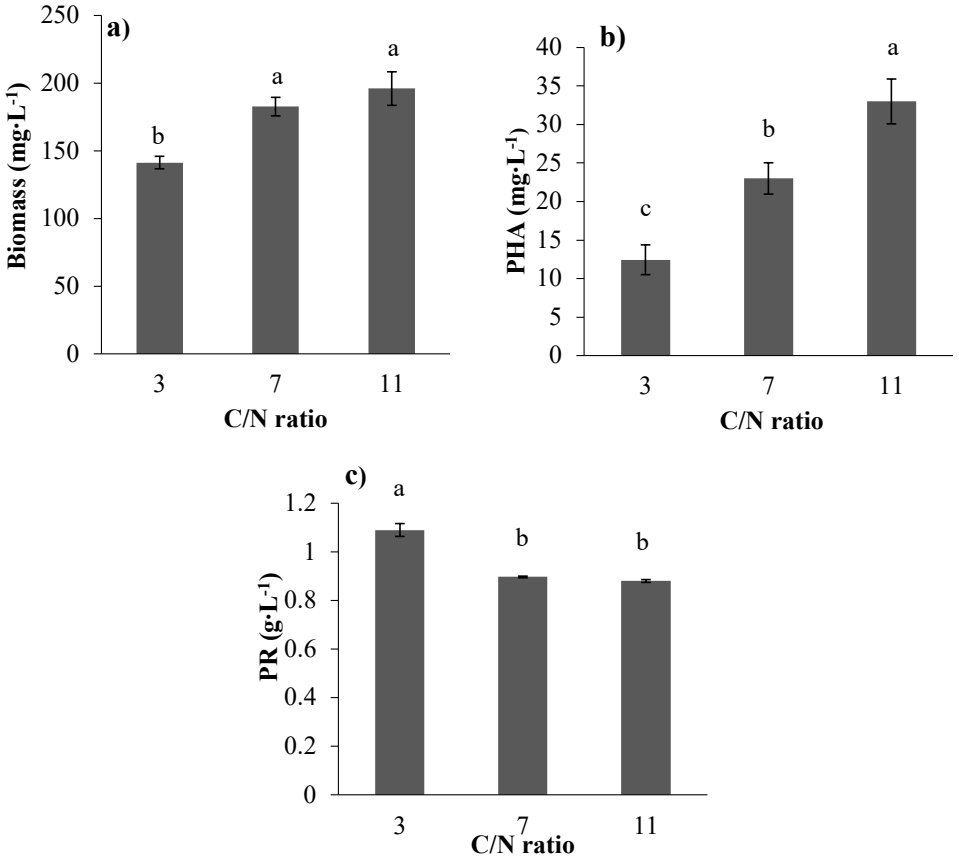

Figure 2. Effect of the C/N ratio on (a) microbial growth and co-production of (b) PHAs and (c) EPS (reported as the RP) ($p \geq 0.05$).

3.2 Effect of the carbon-nitrogen ratio on the co-production of polyhydroxyalkanoates and exopolysaccharides

The effect of the C/N ratio on the co-production of PHAs and EPS as well as biomass production was determined. Figure 2 shows that there is a directly proportional relationship between the C/N ratio with biomass and the synthesis and accumulation of PHAs: As the C/N ratio increases, the PHAs concentration also increases. The highest biomass and PHAs concentrations were obtained with a C/N ratio of 11 (182.23 and 33 mg L^{-1}, respectively), this result is consistent with the results reported by Lizardi-Jimenez *et al*., in 2012 who indicated that the C/N for biomass production (anabolism) was 5 and an imbalance of this either below or above favored catabolism, being the case of this work since PHAs are considered energy reserve.

The results are consistent with what has been reported in the literature: Researchers have determined that increasing the C/N ratio (20 for by *Pseudomonas putida* KT2440 and 40 for microbial consortium, respectively) promotes the accumulation of PHAs (Xu *et al*., 2019; Zhou *et al*., 2022). This is probably since cell growth stops when nitrogen is limited, because the concentration of acetyl-CoA increases as the energy demand is reduced, causing activation of the enzyme 3-ketothiolase and, consequently, accelerated synthesis of PHAs (Schmid *et al*., 2021).

In the present research EPS production was reported as recovered precipitate (RP) and the results was expresed in g L^{-1} because it is likely that there are other molecules with EPS in the precipitate (Figure 2c). In contrast to PHAs production, the RP yield increases as the N concentration increases. Thus, the C/N ratio of 3 produced the highest RP concentration (1.09 g L^{-1}). Cui *et al*. (2017) reported similar findings: An excess of N (C/N ratio of 5) produced the highest EPS concentration (733.58 mg L^{-1}) . Bathia *et al*. (2022) studied the impact of the C/N ratio on the co-production of PHB and EPS in *Sphingobium yanoikuyae* BBL01. They obtained the highest EPS concentration of 3.24 g L^{-1} at C/N ratio of 5, while the maximum accumulation of PHB occurred with the C/N ratio of 25 (47% w/w). Soto *et al*. (2021) suggested that a low C/N ratio promotes protein synthesis, so the production of EPS may be favored because more of the enzyme responsible for the polymerization of monosaccharides present in the environment is produced.

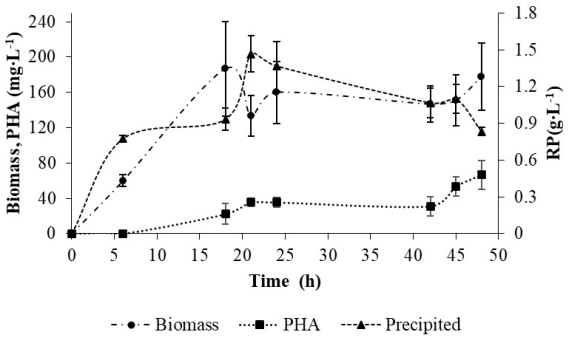

Figure 3. Kinetics of PHAs and EPS co-production by *E. soli* in submerged culture under a C/N ratio of 11.

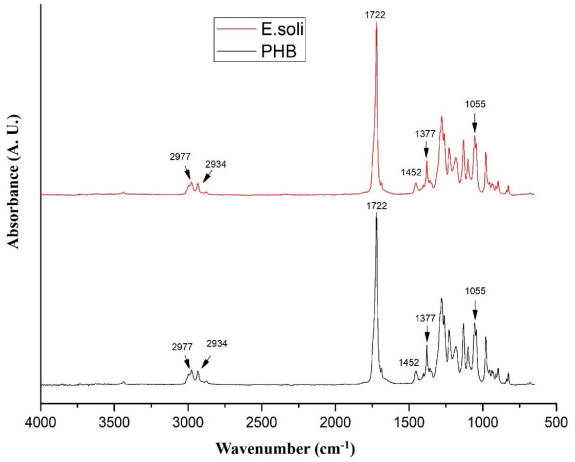

Figure 4. Fourier transform infrared spectra of the polyhydroxyalknoates produced by *E. soli* and poly [(R)-3-hidroxybutiric acid] as commercial reference.

3.3 Growth kinetics and co-production of polyhydroxyalkanoates and exopolysaccharides in submerged culture

Some authors mention that an adequate increase in the C/N ratio should be sought to improve PHAs accumulation; therefore, the C/N ratio that is established should not inhibit microbial growth (Zhao *et al*., 2021). In the present work, growth kinetics were evaluated with the C/N ratio of 11 for three main reasons: (i) the microbial growth of the microorganism was not affected, (ii) the yield of the RP was within the range reported in other studies, and (iii) it yielded the highest PHAs concentration. Figure 3 shows that maximum biomass production occurred after 18 h (186 mg L^{-1}), and microbial growth remained constant until 42 h. The PHAs production results showed that the accumulation of this biopolymer began at 7 h and reached its maximum after 48 h (66.6 mg L^{-1} and 37.5% w/w with respect to biomass). The time for obtaining the maximum concentration of PHAs in the present work was less than those reported by Muneer *et al*. (2021), who observed that with sucrose as a carbon source, *Pseudomonas* sp. AK-3 produced the maximum PHAs concentration at 72 h (1.08 g L^{-1}). Similarly, Choi *et al*. (2021) reported the highest accumulation of PHAs (5% w/w concerning the dry cell mass) by *Pseudomonas* sp. B19-6 after 120 h (2 g L^{-1}). This accumulation of PHAs over time may be because these molecules are synthesized under the limitation of essential nutrients such as nitrogen, phosphorus, and oxygen, among others, so they are usually considered as a secondary energy reserve (Huang *et al*., 2018; Vicente *et al*., 2023).

The EPS production results contrast the behavior of PHAs production by *E. soli*: EPS (reported as the RP in g L^{-1}) were produced faster than PHAs. The maximum concentration occurred at 9 h (1.46 g L^{-1}), after which time the RP concentration decreased until 48 h (0.83 g L^{-1}). The decrease in RP coincides with the slight increase in cell synthesis during the time from 42 to 48 h, suggesting that the EPS contained in the RP are consumed as a carbon source. Indeed, according to Zhao *et al*. (2021), EPS are considered a primary energy reserve, so it is synthesized and consumed earlier than PHAs.

3.4 Quantification of sugars by high-performance liquid chromatography

HPLC was used to quantify sugars. At the beginning of the culture (0 h), the concentration of total sugars (glucose + fructose + sucrose) was 22.4 g L^{-1}: 7.0 g L^{-1} for sucrose, 9.8 g L^{-1} for glucose, and 5.7 g L^{-1} for fructose. The results of the monitoring indicated that when the maximum biomass production was reached (18 h), glucose was most consumed at 1 g L^{-1} (8.8 g L^{-1} quantified), followed by fructose at 0.7 g L^{-1} (5.0 g L^{-1} quantified) and finally sucrose at 0.3 g L^{-1} (6.7 g L^{-1} quantified). From 6 to 9 h (maximum EPS production), sucrose was no longer observed and 0.3 and 0.4 g L^{-1} of glucose and fructose were consumed, respectively. Finally, after 48 h of submerged culture, a total of 0.3 g L^{-1} of sucrose, 1.1 g L^{-1}, and 1.5 g L^{-1} of glucose and fructose, respectively, were quantified. The results of the quantification of sugars may indicate that during the first 18 h the sugar that is consumed in the highest proportion by *E. soli* is glucose, which translates into a rapid production of biomass, because this sugar is easily assimilated and requires relatively little energy consumption to be incorporated into the metabolism (Aguilar, 1998; Nair & Salma, 2021). Likewise, Brückner and Titgemeyer (2002) indicated that when more than one carbon source is present in a culture medium, bacteria generally use one carbon source at a time and leave the other carbon sources for later use, which is likely to be the phenomenon presented in the paper. However, quantification of sucrose indicated that there has been minimal consumption, perhaps

due to the presence of the enzyme inulosucrase that can be induced by the hydrolysis of sucrose and its subsequent conversion to EPS. These data indicate that *E. soli* tends to metabolize monosaccharides over sucrose first. On the other hand, the low yields obtained for the production of biomass, PHAs, and the RP can be associated with low substrate consumption, probably due to the transport mechanisms of sugars (Rawoof *et al*., 2021) that, when facilitated, do not activate metabolic pathways to store energy as PHAs.

3.5 Polyhydroxyalkanoate and exopolysaccharide characterization by FTIR

The results of FTIR spectra for the PHAs produced in the present work for *E. soli* are shown in the Figure 4. The characteristic bands of the spectrum show the functional groups -CH at 2934 cm^{-1}, -C=O at 1722 cm^{-1}, -CH_2 and -CH_3 at 1456 cm^{-1}, -CH_3 at 1377 cm^{-1}, and -C-O cm^{-1} 1055 cm^{-1}, all of which are also present in the standard. These results are similar to those reported in other studies for the PHB (Etxabide *et al*., 2022; Nygaard *et al*., 2021; Vega-Vidaurri *et al*., 2022). Therefore, it is concluded that the FTIR spectrum of the PHAs produced by *E. soli* corresponds to the spectrum of commercial PHB.

Regarding the characterization of RP, in a previous study, the EPS produced by *E. soli* was characterized as inulin, so this fructooligosaccharide was used as a commercial reference. The RP showed some spectral bands similar to those reported for inulin (Figure 5a), including 3268 cm^{-1} assigned to O-H stretching vibrations and 2931 and 1428 cm^{-1} characteristic of C-O-H (Arruda *et al*., 2020). However, other characteristic bands of inulin were not observed in the sample spectrum, including 1039 cm^{-1} corresponding to stretching vibrations of -C-O and -C-O-C of the furanose ring and 931 cm^{-1}, which is attributed to the glycosidic (2 → 1) bond (El-Kholy *et al*., 2020). Instead of these bands, there was a predominant signal at 1117 cm^{-1} that may be masking the fingerprint of fructooligosaccharide. According to Redondo-Cuenca *et al*. (2021), such a band may indicate C-O and C-C stretching vibrations in the pyranose ring.

Based on the above-mentioned issues, it was not possible to identify inulin in the RP by means of FTIR spectroscopy, so the sample was analyzed by mass spectrometry (Figure 5b). The chromatogram shows four samples: the RP at 0 h, the RP at 48 h, the inulin standard, and the solvent in which the samples were dissolved, in this case water. The molecular mass of the samples was determined in the negative ion mode. The chromatograms of the inulin standard and the RP at 48 h had a peak with an m/z of 322.28, which corresponds to the

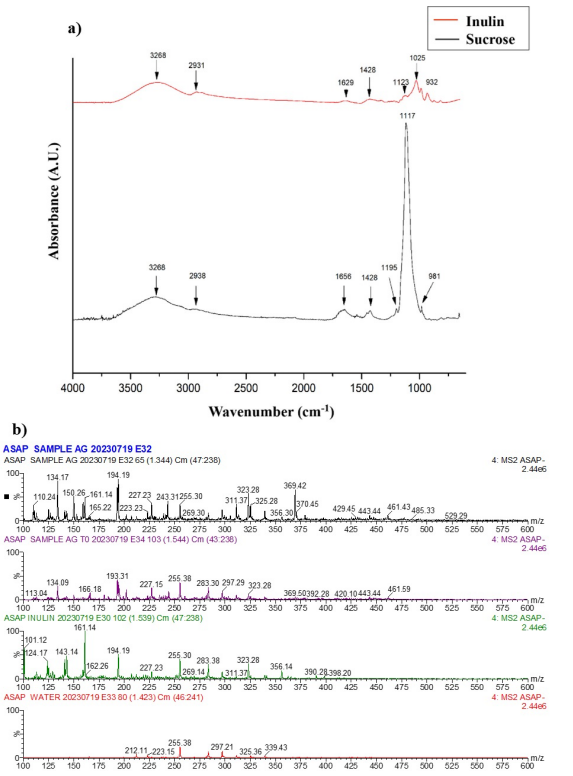

Figure 5. Characterization of the EPS present in the RP. a) Fourier transform infrared spectra of the RP after fermentation by *E. soli* in submerged culture and inulin as commercial reference. b) Representative mass spectra of the RP at 0 h, the RP at 48 and, inulin and water using the negative ion mode.

weight of two fructose molecules (Li *et al*., 2014). These data corroborate that the EPS found in the PR corresponds to the fructooligosaccharide inulin.

Conclusions

E. soli co-produce PHB and inulin, a commercially important fructooligosaccharide, under nitrogen-limiting conditions. High C/N ratios promoted PHAs accumulation, while low C/N ratios directed metabolic carbon flux toward EPS production. In this study, the C/N ratio that promoted the highest PHAs accumulation was 11, where the concentration of EPS produced (reported as recovered precipitate) remained within the range of what has been reported in the literature.

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