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Oilfield Carbonated Produced Water Recycling Coupled to Exopolysaccharide Transformation by *Lelliottia amnigena*

Igor Carvalho Fontes Sampaio[1,4] · Joalene de Azevedo Santos Ferreira[1] · Pedro Jorge Louro Crugeira[3] · Ian Mascena da Silva Oliveira[1] · Jacson Nunes dos Santos[1] · Josilene Borges Torres Lima Matos[1] · Antonio Luiz Barbosa Pinheiro[2] · Paulo Fernando de Almeida[1]

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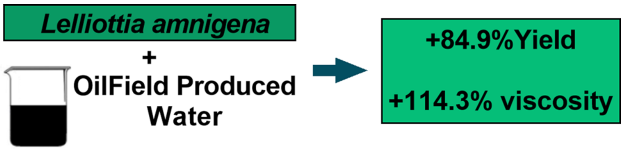
Abstract

It was tested the production of a viscous exopolysaccharide (EPS) by *Lelliottia amnigena* using different concentrations (10, 15, 25, 50 and 100%) of produced water (PW) and Dialyzed PW (DPW) at 100% concentration, with a nutrient medium based on glycerin and sucrose as carbon sources in 44 h batches. There was an increase in EPS recovery when used PW up to a concentration of 25%. The viscosity of the EPS solutions was maintained with DPW and up to 15% of PW, such a condition resulted in 9.34 gL^{-1} of EPS, representing an increase of 84.9% compared to the control ($P < 0.0001$). The PW 15% increased the viscosity per produced liter by 114.3% ($P < 0.0001$). Scanning electron microscopy and Energy-dispersive X-ray spectroscopy analysis showed that EPS adsorbed mineral constituents of the substrate and its characterization by Fourier Transform Infrared Spectroscopy was similar to an external control of a commercial EPS produced by an *Enterobacter A47* strain. PW at 15% becomes an alternative to increase the EPS production process and can contribute to the mitigation of environmental damage through sustainable PW management in the oilfield chain.

Graphical Abstract



Keywords *Lelliottia amnigena* · Produced water · Exopolysaccharide · Recycling · FT-IR

Statement of Novelty

The oil extractive chain will continue to generate huge volumes of produced water (PW) waste, with costs for its treatment and environmental implications. Additionally, the demand for biopolymers (BP) for oil extraction will also increase, given that fossil fuels will continue to be the main energy source for the next years.

This research aimed to use PW as a substrate for the production of a BP with properties that make it a strong candidate for application in the petroleum extractive chain. Thus, the biotechnological recycling of PW and its valuation in the form of a biopolymer of bacterial origin was achieved.

✉ Igor Carvalho Fontes Sampaio
igorsampaio@outlook.com

[1] Laboratory of Biotechnology and Ecology of Micro-organisms, Institute of Health Sciences, Federal University of Bahia, Reitor Miguel Calmon Ave, S/N, Salvador, BA 40110-060, Brazil

[2] Center of Biophotonics, Federal University of Bahia, 62, Araujo Pinho Ave, Canela, Salvador, BA 40110-150, Brazil

[3] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança 5300-253, Portugal

[4] Biotransformation and Organic Biocatalysis Research Group, Department of Exact Sciences, Santa Cruz State University, Ilhéus 45654-370, Brazil

The production with PW showed higher yield, confirming the biotechnological potential and applications arising from this work.

Introduction

Produced water (PW) is an effluent generated during oil extraction. Its composition depends mainly on the geological characteristics of the reservoir and can be composed of solid elements, and dissolved organic and inorganic constituents [1]. The destiny given to the PW involves its disposal, injection into oil wells and reuse. Worldwide, 281.7 million barrels of PW are produced daily [2] and in Brazil, 8.6 million barrels are produced [3]. The global PW treatment market is expected to reach close to $10 billion by 2024 given strict regulations regarding the disposal limits for this by-product [4]. Although biological treatment systems of PW have already been described in the literature [5, 6] few studies aimed to use it as substrate for generating valuable products [7–13].

Among PW organic constituents, some can serve as substrate to microbial activity, including hydrocarbons, a possible C source [14, 15], sulfate and phosphorus that act as macronutrients [16] and metals such as Mg, Fe and Ca, known as enzymatic cofactors [17–20]. On the other hand, in some cases, PW may present high salinity [21], a deleterious characteristic to essentially non-halophilic organisms.

The oil industry faces a high waste production problem as it's expected the water-to-oil ratios will reach 10 in the next 5 to 10 years [22, 23]. Despite it's possible to reuse the PW there is a reduction in the water injectivity. Therefore, there is a need for new approaches to PW utilization, especially regarding your use as substrate to production of substances applied in the oil extraction chain.

The production of microbial exopolysaccharides (EPSs) has drawn industry attention, given its properties of water retention, emulsifier, thickener, gelling and pseudoplastic behavior of its solutions, with applications in the food, pharmaceutical and cosmetics industries [24, 25]. In recent years some EPSs, such as Xanthan gum (XG), have been used in the oil industry, as an agent of microbial enhanced oil recovery (MEOR) and in drilling fluids [26, 27]. The global market for soluble polymers was valued at about $35 billion in 2023 and is believed to continue to grow through 2030 [28, 29], given the growing applications in drilling, enhanced oil recovery [30], mining, wastewater treatment [31], agriculture [32, 33], medical [30, 34] and food activities [35]. About 9.4% of this market is represented by the oil and gas industry and 31.3% by wastewater treatment [28].

EPSs are made up of repeated units of monosaccharides, and may or not be bound to glycoproteins, glycolipids, acids or even extracellular DNA [36] and other constituents of the extracellular polymeric substance matrix, such as inorganic ions [37]. *Enterobacter* strains (such as the reclassified *Lelliottia amnigena*) can use crude glycerin as a carbon source for heteropolymer production composed of glucose monomers, galactose, fucose, mannose, glucuronic acid and pyruvyl. *Enterobacter* strains are found in water, soil, sewage and in some cases, they may cause nosocomial infections. The presence of metabolically active strains in petroleum-contaminated environments was previously reported [38].

Furthermore, the metabolic pathways for obtaining energy and generating bioproducts are equally diverse, being reported EPS production in conditions of low or high oxygen saturation in a varied yield of productions [39, 40] and generation of other products in anaerobiosis [41]. Considerable effort is made in developing strategies to increase the production and quality in the chemical profile of these molecules, either by the use of new strains [24], nutritional strategies and operational parameters [40, 42].

Given the high cost of substrates commonly used in microbial productions (e.g. glucose, sucrose), low-cost carbon sources such as industrial waste or by-products have been sought [25]. Among these sources, crude glycerin from the biodiesel industry has been shown to be a possible alternative [43].

It is hypothesized that by-products from the oil (PW) and biodiesel (crude glycerin) industry can be used efficiently as substrates in the production of EPS in order to reduce production costs and consequently provide the generation of valuable products. Given the hydrocolloid characteristics of EPS, it is also suggested that they can be used for improved oil recovery and as drilling lubricants in place of conventionally used XG. The present work aimed to use effluent from the oil industry (PW) in the production of EPS by *Lelliottia amnigena* in a medium containing sucrose and crude glycerin produced by the biodiesel industry as carbon sources.

The EPS prroduced under different PW ratios was analyzed for its viscosity. Fourier transform infrared spectroscopy (FTIR) [24] was used to identify the functional groups, while energy-dispersive X-ray spectroscopy (EDS) was employed to determine the elements present in the EPS [36]. Furthermore, microscopy techniques, such as SEM, provided information on the surface of polymers [36]. Therefore, it was possible to verify whether there have been any changes in the EPS produced under different operating conditions by using these techniques.

Materials and Methods

Produced Water and Crude Glycerin

The PW was collected from a Brazilian carbonate oil field in the primary recovery stage. The PW was kept in a sterile glass container and kept refrigerated at 4 °C. The physicochemical characteristics of the PW were determined (pH, oxidation/reduction potential—ORP, salinity, chlorides, total dissolved solids, total alkalinity—$CaCO_3$, total petroleum hydrocarbon TPH, sulfate, total phosphorus and metals by their respective standard methods (SM-4500 H^+ B; SM-2580 B; SM-2520 B; SM-4500-Cl^- B; SM- 2540 C; USEPA 310,1 / SM-4500 CO_2 D; USEPA 8015D; SM-4500 SO_4 E; SM-4500 P C; EPA-6020 A) [44, 45].

PW dialysis was carried out in pure regenerated natural cellulose membranes with 1000 to 50,000 molecular weight cut-off points (Spectra/Por™ 3 Dialysis Tubing 3500 Dalton MWCO, Fisher Scientific, England) against distilled water, which water was changed at every 8 h, until a conductivity of less than 300 µS/cm was obtained (FiveEasy Benchtop FP30, conductivity Meter, Powai Mumbai, India) [46]. Crude glycerin (CG) was obtained from a Biodiesel production plant (SENAI/CIMATEC, Salvador, BA, Brazil) and was produced from the transesterification of soybean oil and animal fat (8:2), using sodium methoxide (0.6%) and methanol (17% in excess).

EPS Production and Extraction

The *Lelliottia amnigena* strain was obtained from the Culture Collection of the Laboratory of Biotechnology and Ecology of Microorganisms (LABEM) from the Institute of Health Sciences of the Federal University of Bahia, Salvador, Brazil. The preparation of the microbial inoculum was carried out by inoculating a cryotube of cryoconserved strain in 20 mL of Trypticase Soy Broth medium (Becton Dickinson GmbH, Heidelberg, Heidelberg, Germany) contained in a 125 mL Erlenmeyer vial and incubated for 24 h at 30 °C under orbital agitation of 150 rpm (New Brunswick Scientific Co I26 Incubator Shaker Series, San Diego-CA, USA). After 24 h, 10% peak (v/v) was performed and 16 h of incubation at 30 °C and 150 rpm was made for production.

EPS production by *Lelliottia amnigena* was carried out in triplicate using five concentrations of PW (10, 15, 25, 50 and 100%), Dialyzed PW (DPW) and distilled water (dW) acted as control. Production was started by inoculating 5 mL of the culture in 45 mL of modified mineral salts medium (MSM) [47] in the concentrations of (w/v): Na_2HPO_4 (2.77 g/L); KH_2PO_4 (1.0 g/L), $Ca(NO_3)$ $2.4H_2O$ (0.0713 g/L), $(NH_4)_2SO_4$ (1.0 g/L), $MgSO_4.7H_2O$ (0.2 g/L), yeast extract (0.5 g/L), Sucrose (25 g/L), and crude glycerin (20 g/L). Depending on the study group, the MSM medium was prepared using dW, dialyzed PW or diluted PW. The pH was adjusted to seven with a NaOH 5 M solution and pasteurized at 65 °C/30 min.

EPS productions were performed in 250 mL Erlenmeyer flasks containing 45 mL of modified MSM medium, under orbital agitation of 250 rpm, temperature of 30 ± 0.25 °C in a rotational incubator (I26 Incubator Shaker Series, New Brunswick Scientific, Edison, New Jersey, US) for 44 h and the inoculum volume was 5 mL (10% of final volume). After the production, the contents of each Erlenmeyer were centrifuged at 10.000×*g* for 30 min at 4 °C (Eppendorf 5804 R, Westbury-NY, USA). Subsequently, 150 mL of 99.5% ethyl alcohol at 4 °C was added to the supernatant and packed at 4 °C for 16 h for EPS insolubilization [48]. The unsolved material was then recovered by centrifugation at 10.000×*g* for 30 min at 4 °C. The EPS was then dried in an oven at 30 °C until it reached constant weight, measured with an ATY224 analytical scale (Shimadzu, Kyoto, Japan).

Gravimetric Polysaccharide and Viscosity Analyses

The samples were dried in an oven (Biopar, Porto Alegre-RS, Brazil) until they reached constant weight, crushed with the aid of an adjustable grinder (model 80393BZ, Hamilton Beach, Glen Allen, USA), quantified by gravimetry (Shimadzu, Kyoto, Japan) and packed for further physicochemical analyses (EDS, MEV, FT-IR). Viscosity was analyzed in a Brookfield MVD-8 digital rotational viscometer (Marte Científica, Santa Rita do Sapucaí, Minas Gerais, Brazil) in triplicate solutions of 1.0% polymer at 25 °C under 60 rpm as described by Sampaio et al. [47].

Statistical Analysis

The results were analyzed using the Student's t-test and ANOVA with Tukey's multiple comparison post-test.

Scanning Electron Microscopy and Energy Dispersive X-ray Analysis

Scanning electron microscopy (SEM) was performed to investigate the details of the EPS surface [24, 49] obtained with PW. The samples were metallized in gold (10 min, 2 mbar) in the Desk V preparation system (Denton Vacuum, NJ, US) and observed by SEM in the JSM-6610LV equipment (JEOL Brazil Instrumentos Científicos, Mirandópolis, São Paulo) at 10 kV and magnifications of 100 and 2000×. The elemental analysis [50] of the samples was performed by energy dispersion X-ray spectroscopy (EDS) in a 20 mm^2 X-Max detector (Oxford Instruments, Oxfordshire, England).

Analysis by FT-IR

A Fourier transform infrared spectrometer was used (VERTEX 70 v, Bruker Corporation, Billerica, Massachusetts) to

Table 1 Physical-chemical characteristics and metallic determinations of the produced water

Variables	Results
pH (25 °C)	6.3
Oxidation-reduction potential-ORP Eh (mV)	60.3
Salinity (g/L)	41.2
Total dissolved solids (g/L)	41.4
Total alkalinity—CaCO3 (g/L)	0.115
Total petroleum hydrocarbon—TPH (g/L)	0.8
Sulfate (g/L)	0.230
Total phosphorus (g/L)	0.109
Unresolved complex mixture—UCM (mg/L)	0.6
Manganese (mg/L)	2.1
Iron (mg/L)	8.1

obtain the characterization of the EPS [24, 49]. Dried samples were arranged in an ATR diamond (A225/Q Platinum ATR unit, Bruker Corporation, Billerica, Massachusetts) and the spectra were obtained in a spectral range of 400 to 4000 cm^{-1} with acquisition of spectra through 32 scans with a resolution of 4 cm^{-1}, at temperature of 22 ± 2 °C and controlled humidity. Fucose containing exopolysaccharide produced by *Enterobacter* A47 (Department of Chemistry, Universidade Nova de Lisboa) was used as external control.

Results and Discussion

Physicochemical Analysis of Produced Water

The physicochemical determination of the PW (Table 1) showed the presence of Fe, Ca and Mn in concentrations of 8.1, 115 and 2.1 mg/L respectively. The detected salinity was 41.2 g/L and the total petroleum hydrocarbon (TPH) content was 0.8 mg/L.

The metals iron, calcium and manganese found in produced water (Table 1) play roles in their free forms or as enzymatic cofactors. Fe acts in fundamental functions such as respiration, central metabolism and DNA repair [17]. Also, many enzymes that interconvert phosphoglycerate, pyruvate and oxaloacetate intermediates are strictly dependent or highly stimulated by Mn, suggesting as well as Fe, a key role in carbon metabolism [18].

Moreover, Ca is involved in the maintenance of cell structure, motility, transport, differentiation processes such as the formation of spores, and may also act in signal transduction [19, 20].These elements were present in an EPS production medium [42], strengthening the hypothesis that such PW constituents may be beneficial in production by strains of the *Lelliottia amnigena*.

Sodium molybdate is a non-toxic and environmentally acceptable corrosion inhibitor used by the petroleum industry [51–53], however the produced water analysis showed that Molybdenum was below quantification levels or in an undetectable form.

Furthermore, studies have identified hydrocarbon degrading *Enterobacter* strains (former classification of *Lelliottia amnigena*), such as various alkanes, cyclohexane and naphthalene derivatives [14], hexadecanes, heptadecanes, tridecanes and docosanes[15] as well as crude oil (total petroleum hydrocarbons—TPH) [36, 51]. These data suggest the use of microorganisms of this genus to carry

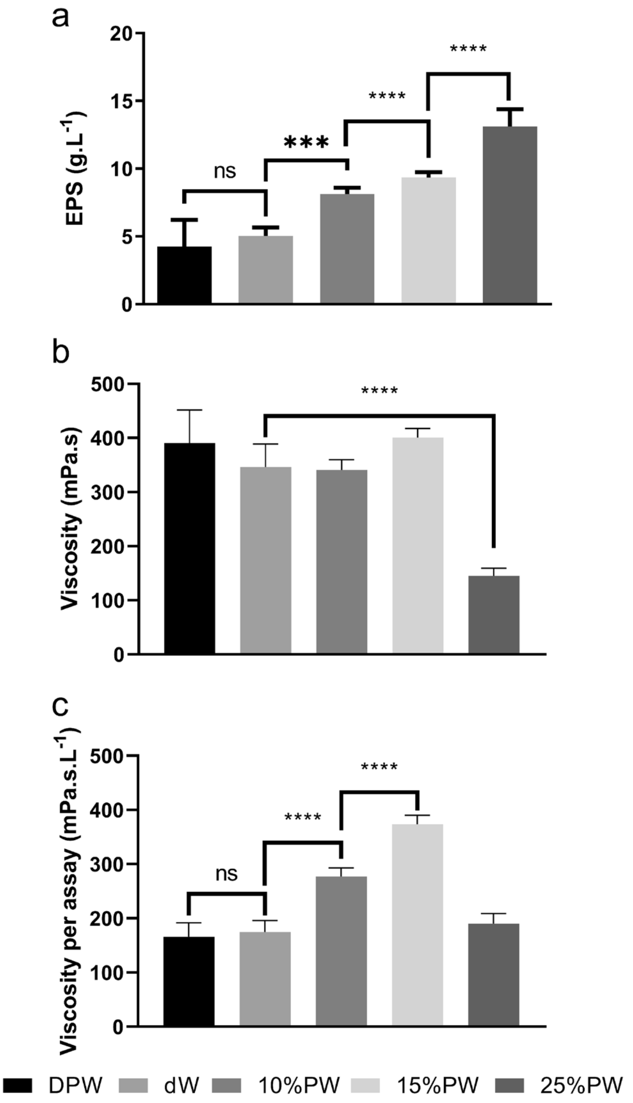


Fig. 1 Production of exopolysaccharide by *Lelliottia amnigena* in dialyzed produced water (DPW), distilled water (dW) and different concentrations of produced water (PW). **a** Gravimetry of productions. **b** Viscosity. **c** Viscosity per L of production. ***$p < 0.001$, ****$p < 0.0001$

out the biological treatment of wastes containing hydrocarbons, such as the PW used in this study.

EPS Production Essay

The gravimetric analysis (Fig. 1a) demonstrated increased recovery of the EPS with the use of no-dialyzed PW and up to the concentration of 25%. Productions with 10 and 15% PW did not differ statistically from each other, 8.14 and 9.34 g/L, respectively. The production with 25% PW resulted in a recovery of 13.1 g/L and presented the highest production, an increase of 159.4% in relation to control with dW ($p < 0.0001$). The use of DPW did not differ statistically in relation to the control (dW), 4.25 and 5.05 g/L, respectively ($p > 0.05$). Since the DPW medium had the salts of produced water removed and have the TPH and UCM, it's suggested that these TPH and UCM are not the major stimulation compounds for the production of EPS by *L. amnigena*. Furthermore, the culture medium used in this work (see "EPS production and extraction" section. EPS production and extraction) contains 45 g/L of carbon source (25 g/L of sucrose and 20 g/L of crude glycerin). This concentration is more than 50-fold higher than that of the TPH (0.8 g/L) and unresolved complex mixture—UCM (0.6 mg/L) present in PW (Table 1). Therefore, it is more plausible that the enzymatic machinery and metabolism of the *L. amnigena* were driven towards these more available substances. In this case, it is suggested that the PW salts (up to PW25%), may have acted as a stress factors to enhance the EPS productivity.

In this regard, the salt present in PW can induce osmotic stress in the bacterial environment. To counteract this, *L. amnigena* responds by synthesizing and releasing EPS, which helps maintain cellular osmotic balance. EPS molecules have a strong binding capability to cations [31], acting as osmoprotectants [54]. They decrease the availability of cations [55] and aid in biofilm formation, restricting the entry of excess salt into bacterial cells [56, 57].

Furthermore, salt stress can also trigger the upregulation of genes involved in polysacharide and exopolysaccharide production [58, 59] and excretion through transcriptional regulators such as RcsA and RcsB [60, 61]. Additionally, as we already discussed in the previous section, Fe and Mn metals found in the produced water can act as enzymatic cofactors to enhance the production of EPS.

There was no recovery of EPS when using either 50 or 100% PW in the culture medium, which could be attributed to the high salinity of PW, which is harmful to the unfitted *Lelliottia amnigena* (Table 1). Microbial quantification assays through MPN [47] confirmed the absence of microbial growth in the production assays with PW50% and PW100% (data not shown). However, cultures with PW up to 25% exhibited a slight lag phase of 4 h. Following this initial lag phase, the samples rapidly grew from an initial microbial concentration of 10 Log_{10} MPN to 11 Log_{10} MPN, reaching the same microbial count as the control (dW). This observation of a lag phase solely in PW samples reinforces the theory of saline stress promoted by the produced water.

The literature reports productions of 13.3 g/L after 168 h in a fed-batch system and using crude glycerin as a C source [24] and 7.6 g/L after 72 h [43]. Freitas et al. [62] using the *Enterobacter* A47 strain and 10 to 20 g/L of glucose in a fed batch, reported a production of 13.4 g/L after 96 h. Using xylose at a concentration of 40 to 70 g/L as a source of C, the same authors reach a production of 5.39 g/L after 96 h. Additionally, Alves et al. [63] using a *Enterobacter sp.* with 200 g/L of glycerol as C source in fed batch, reported a production of 13.28 g/L after 96 h of culture and Prasertsan et al. [40] using *Enterobacter cloacae* WD7 and 3% of sucrose as C source, obtained about 5.0 g/L after 72 h. Interestingly, Scheepe-Leberk et al. [64] using *Enterobacter sakazakii* and just 14 g/L glycerol as carbon source reach a yield of 8 g/L after 24 h of culture. Also, it was reported the use of oil field carbonated produced water to produce 11.6 g/L of xanthan gum by *Xanthomonas campestris* in 120 h (10% PW, crude glycerin and sucrose as carbon sources) [47] and 10.8 g/L of Levan by *Zymomonas mobilis* in 48 h (50%DPW, sucrose and as carbon sources) [65]. Therefore, the *Lelliottia amnigena* used in the present work yielded a relevant exopolysaccharide production in 44-h of batching and using produced water as a constituent of the production medium.

Furthermore, the use of PW can reduce the consumption of freshwater in the production of EPS for non-food grade and cosmetics applications. The higher yield of exopolysaccharides, combined with reduced freshwater usage, has the potential to significantly impact industrial costs and enhance competitiveness in this field. Additionally, the reuse of oil industry effluents represents a great achievement in environmental conservation and effluent management, adding a green label to the portfolio of technologies employed by oil industries.

When analyzing the viscosity of the production (Fig. 1b) it was found that the viscosity of the solutions with DPW and 10% PW did not differ statisticaly significantly from the control (dW, 346.5 mPa s). Although the viscosity of PW15% was higher than that of dW, the difference was not statistically significant.

On the other hand, when using 25% PW the viscosity was significantly reduced to 145.3 mPa s ($p < 0.0001$) in comparison to the control. Differently, the total viscosity (per liter) provided by each production (Fig. 1c), considering the viscosity measured in 1.0% solutions and the total production in grams, resulted values of 175, 166, 277.5, 375 and 190.3 mPa s/L for productions with dW, DPW, PW10%, PW15% and PW25%, respectively.

The viscosities of the produced EPS solutions can be easily controlled through dilution or concentration to achieve ideal drilling fluid applications. Drilling fluids for oil recovery applications typically have viscosities ranging from 5 to 25 mPa s and deep drilling wells often require more viscous fluids. Adding exopolysaccharides is a well-explored approach to enhance the viscosity in such cases [66].

In solutions of low ionic strength, polyelectrolyte molecules (such as the EPS produced by *Lelliottia amnigena*) have high conformational expansion, which collapses to a compact form when increasing salt concentration. This change in the hydrodynamic dimension of polyelectrolyte molecules is directly dependent on the attractive/repulsive electrostatic forces and promotes a reduction in the viscosity of their solutions [67–69]. Accordingly to the viscosity results (Fig. 1), it is proposed that the EPS molecule produced by *L. amnigena* in PW maintains its hydrodynamic volume and solution viscosity until the saline concentration present in about 15% PW, reducing its hydrodynamic volume when PW25% was used.

The slight enhancement of EPS viscosity in a 15% concentration of produced water (PW 15%) can be attributed to several mechanisms. Firstly, the presence of salts in this specific concentration of PW 15% may shield the charges or specifically screens the repulsive electrostatic interactions on the EPS molecules [70], reducing repulsion between them and promoting more extensive polymer chain associations, resulting in increased viscosity [71, 72].

Additionally, the formation of complexes between EPS and metal ions can also influence the EPS's rheological behavior [73]. For instance, metals like iron promote the formation of monocomplexes and point-like cross-links, leading to the production of dimers [70]. These bonds between different EPS chains form a more intricate three-dimensional network, consequently making the EPS matrix more interconnected and leading to increased viscosity. All these aspects shed light on how the produced water not only affects EPS production but also influences its properties.

It was demonstrated that using 15% of PW is possible to produce 9.34 g/L of EPS with a viscosity comparable to that obtained with dW. However, producing 13.1 g/L of EPS using PW at a 25% concentration results in a polymer with lower viscosity. This may require a dialysis or purification pretreatment of the EPS to enhance its viscosity. Alternatively, using the EPS at a higher concentration can be considered to achieve a viscosity similar to the control. The analysis of total viscosity per assay provided insights into the choice of PW concentration for real-world applications. Without a dialysis pretreatment, PW15% produced a higher amount of EPS and increased the total viscosity by 114.3% compared to the control ($P < 0.0001$), as shown in Fig. 1.

Scanning Electron Microscopy

EPS presented a three-dimensional surface with irregularities that were accentuated with the increase of the concentration of PW (Fig. 2). Crystalline geometric structures with different shapes and sizes were visualized in all productions. In the productions with DPW (Fig. 2a) and dW (Fig. 2b) the crystalline geometric structures presented a smaller amount and size of about 1 µm. Crystals around 5 µm were observed when 10% PW (Fig. 2c) was used. The amount of these crystals increased when using 15 (Fig. 2d) and 25% PW (Fig. 2e). It was also possible to observe that the EPS produced with DPW (Fig. 2a) presented a "rougher" aspect than the produced with dW (Fig. 2b).

Exopolysaccharides analyzed by scanning electron microscopy showed varied results, ranging from smooth to rough surfaces, as well visual indication that part of the substrate from the production medium remained in the polymer after the extraction process [74] and the presence of pores on its surface [75, 76]. The samples analyzed in this work also showed this heterogeneous aspect. The distinct morphologies and topography of polysaccharides may be due to the method of production, extraction and also the physicochemical properties of polysaccharides [77]. The presence of crystalline structures, better visualized with the samples produced with PW diluted to 10, 15, 25% (Fig. 2c–e) clearly indicates the constituents of the production medium, especially salts arising from the PW and incorporated by the EPS.

It has been suggested [78] that the presence of bulky pores at the expense of a continuous surface may indicate lower attractive forces in exopolysaccharides. Thus, a lower attractive force can result in a greater hydrodynamic volume of the exopolysaccharide molecules in solution, and consequently, higher viscosity.

Energy Dispersive X-ray Analysis

EDS analysis is a useful tool for qualitative and semiquantitative determinations of elements [79]. Furthermore, EDS has been used for the elemental analysis of different polymeric compounds [76, 80, 81]. The elemental composition (Fig. 3) presented C, O, Na, P and Mo in all samples. The absence of Mo in the PW (Table 1) suggests that this element was acquired through the culture medium. The production with DPW (Fig. 3a), PW10 and PW15% (Fig. 3c–e) presented Cl, but it did not appear in the control with dW (Fig. 3b). The productions with 10, 15 and 25% PW presented additionally Ca. The element K appeared in the productions DPW (Fig. 3a) and dW (Fig. 3b), but in negligible levels in the samples produced with diluted PW (lower than 0.9%). Also, the element Mg appeared just in the samples

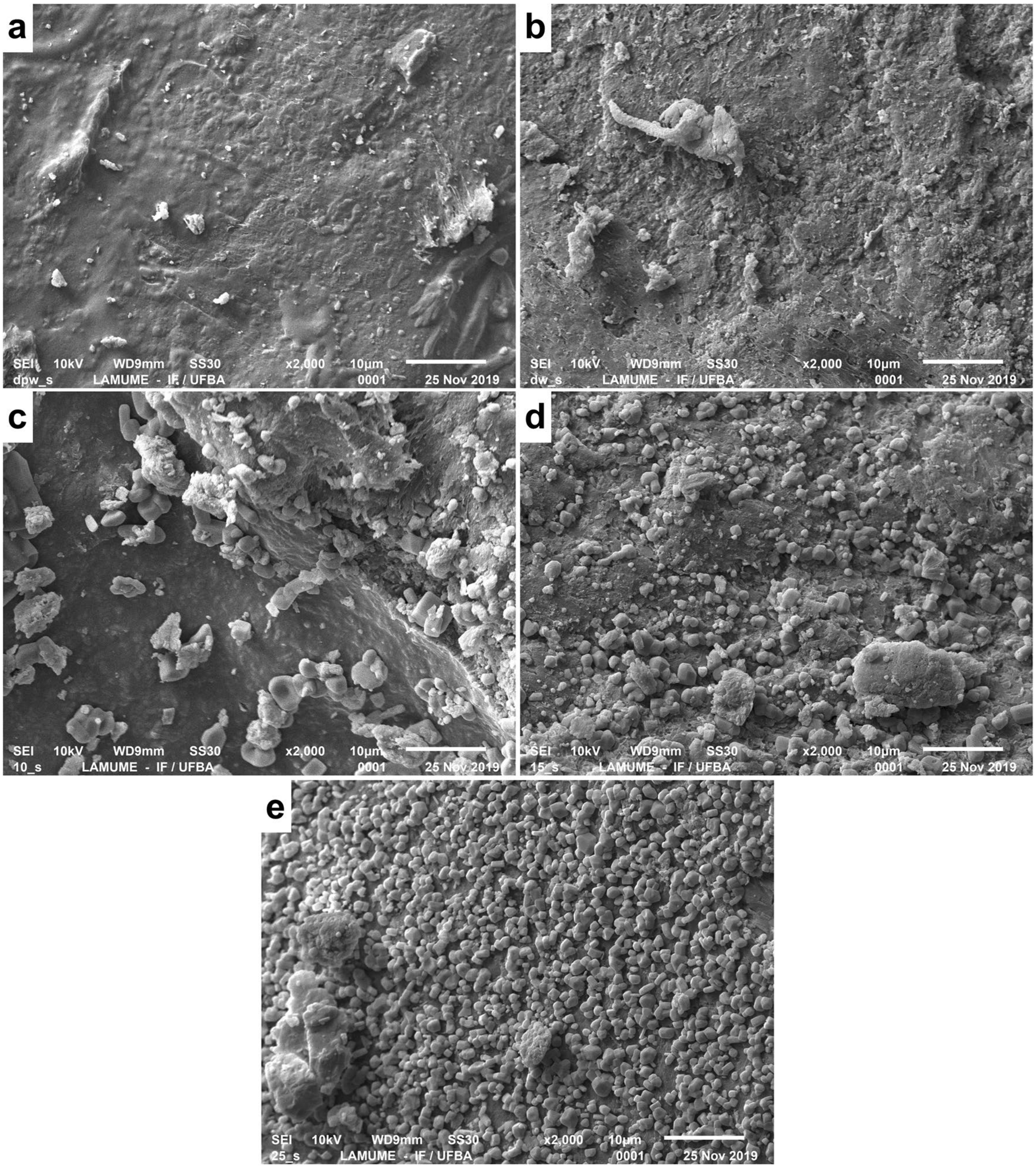


Fig. 2 Scanning electron microscopy of the EPSs produced using: **a** dialyzed produced water (DPW), **b** distilled water (dW), **c** Produced water at 10% (PW10%), **d** Produced water at 15% (PW15%) and **e** Produced water at 25% (PW25%)

PW10% and PW15% in levels lower than 0.5% (Fig. 3c and d).

The elemental composition determined by EDS (Fig. 3) provided information regarding the content (wt%) of C and O (exopolysaccharide indicators), and salts and metals (potential viscosity modifiers of EPS solutions), showing a difference in the elemental composition of the EPS ($p < 0.0001$) between the control (dW) and tests with DPW and with the different concentrations of PW (Fig. 4).

For the control (dW), 79.5% of C and O and 20.5% for salts and metals were found. In the productions with DPW, and 10% and 15% PW, values between 51.2 and 58.7% of C and O and 48.7 and 41.3% of salts and metals were obtained. In PW25%, there were verified values of 44.4% for C and O and 55.6% for salts and metals.

The production PW25% showed the smallest amount of C, O and the higher amount of salts and metals. Also, it was the only production that showed a reduction in viscosity. These results suggest the existence of a limiting ratio

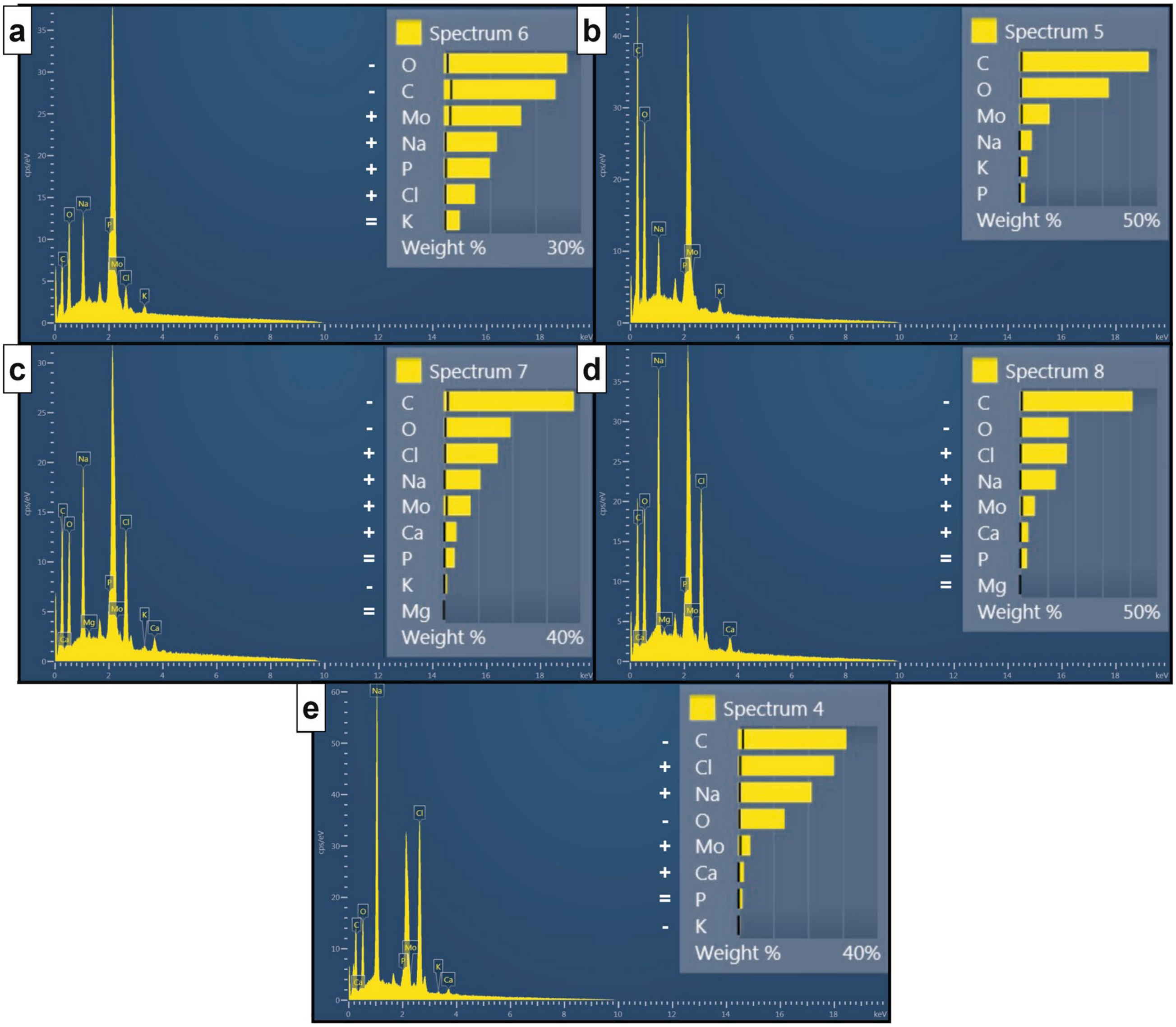


Fig. 3 EDS of the EPSs produced using: **a** dialyzed produced water (DPW), **b** distilled water (dW), **c** Produced water at 10% (PW10%), **d** Produced water at 15% (PW15%) and **e** Produced water at 25% (PW25%). + and − signals mean a significant increase and reduction, respectively, related to dW signal means no significant difference related to dW

between the EPS (C and O) and salts and metals, which, after being exceeded, has a negative effect on viscosity.

The presence of salts is not a limiting characteristic, but it is essential for the optimal functionality of exopolysaccharides used in the oil extraction chain, such as xanthan gum [82]. Additionally, good coordination and tolerance to salts are beneficial for drilling applications [83]. It has been reported that the creation of thermoviscosifying xanthan is useful for the oil industry after the addition of metals from produced water [73]. For instance, a patent report on exopolysaccharides for drilling applications discusses the composition of drilling fluids, which consists of 11% of a combination of inorganic salts (KCl, NaCl, $MgCl_2$, and $CaCl_2$) [84].

Freitas et al. [24] reported a reduction of about 40% in the gravimetric values of the produced EPS after performing purification by dialysis, which corroborates the data obtained by EDS (Fig. 3) for a high mineral content in the EPS produced by *Lelliottia amnigena*. The results of this research show the possibility of using the produced EPS without purification, as shown in the experiment with PW15% (Fig. 1, see "EPS production essay" section. EPS production essay), which resulted in EPS with a suitable viscosity that did not differ from the control in dW.

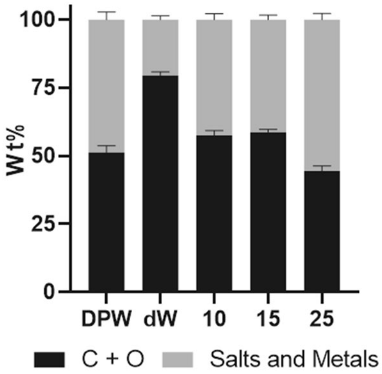


Fig. 4 Individual values of EDS for carbon and oxygen (C + O, representing the carbohydrates of the EPS) and salts and metals (possible viscosity modifiers of the EPS viscosity) of the produced samples

FTIR

To verify the chemical profile of the EPS produced by *Lelliottia amnigena*, a FTIR was performed. As the internal control was considered the EPS produced with dW and as external control was used an EPS produced by *Enterobacter* A47 [24].

The spectra obtained from exopolysaccharides by FT-IR (Fig. 5) corresponding to the external control group—a EPS produced by *Enterobacter* A47 (Fig. 5a), and productions with PW25%, PW10%, DPW, dW and PW15% (Fig. 5b–e) showed a wide peak of absorption at ~ 3300 cm^{-1}, which is corresponding to the elongation of the vibration of the hydroxyl group [24, 85]. Furthermore, the peak ~ 2930 cm^{-1} is attributed to anti-symmetric regions and stretches of C–H of the symmetrical methylene [86] which gives a good allusion to the sugar content of the samples [87] and a small displacement was observed in the samples produced in this work (~ 2925 cm^{-1}). FT-IR spectra also demonstrated peaks in the region of ~ 1720 cm^{-1}, representative of acetyl carbonyl elongation (C=O) [88, 89] or carboxyl groups [87].

The peak of ~ 1607 cm^{-1} of external control (Fig. 5a) was shifted to ~ 1630 cm^{-1} in PW productions (Fig. 5b–e) and it is indicative of asymmetric elongation of carboxylates [90]. The last peak (~ 1630 cm^{-1}) is also a possible binding site for bivalent cations [91]. Additionally, the peak in the region ~ 1550 cm^{-1} (Fig. 5a) is due to the antisymmetric elongation of succinate vibrations [24, 92] showing a displacement to the region of ~ 1537 cm^{-1} in samples of the produced EPS. Furthermore, the peak of ~ 1405 cm^{-1} (Fig. 5a) is an indicative of symmetrical elongation of carboxylate [90] and along with the peak ~ 1607 cm^{-1} are indicative of EPS containing fucose. In the samples produced in the present experiment, a disturbance was observed near the ~ 1405 cm^{-1}.

The band corresponding to ~ 1250 cm^{-1} indicates vibration C–O–C of acyls [24, 87], being very sharp in external control (Fig. 5a) and with a lower intensity in the productions of this experiment (Fig. 5b– e). Also, the peak ~ 1220 cm^{-1}, which may correspond to the vibration of the C–O–C elongations [87] or phosphodiester functional groups of the structures of the DNA/RNA framework showed lower intensity in the external control (Fig. 5a), being well accentuated in the samples of the produced EPS (Fig. 5a–e).

Finally, the region corresponding to ~ 1200 and ~ 900 cm^{-1} is considered the "fingerprint" of the sample and represents the C–O skeleton and also C–C vibration bands of glycosidic bonds and pyrenoid ring [87], more specifically in the region between ~ 1050 and ~ 1150 cm^{-1} there is an axial deformation of C–O, typical of ethers, alcohols, phenols, fatty acids, esters and anhydride [93].

The FT-IR spectra (Fig. 5) demonstrated similarity between the absorbance bands between the spectrum produced by the external exopolysaccharide control and the productions of this work [24, 91, 94]. In order to reduce production costs for application in the oil industry, no purification of the extracted EPS was carried out.

Although the FT-IR spectra are noticeably similar, differences are mainly observed regarding the intensity of EPS external standard (Fig. 5) peaks and the presence of noise in the samples produced, with or without produced water. Other works also reported spectral differences (Raman spectroscopy) in exopolysaccharides produced with PW [95]. This can be explained by the greater amount of impurities in relation to the external standard, since no additional steps were performed to purify the EPS produced by *Lelliottia amnigena* as was showed a significant amount of salts and metals in the composition of the EPS through EDS evaluation (Fig. 3, "Energy Dispersive X-ray Analysis" section). Furthermore, the peak observed in ~ 1250 cm^{-1} that indicates the vibration of C–O–C in acyls [24] appears greatly reduced in the other spectra. In the same way that the peak at ~ 1220 cm^{-1} also only observed in the other samples and it's believed to represent phosphodiester functional groups of DNA/RNA polysaccharide backbone structures or phosphorous-containing carbohydrates only present in the EPS produced in this work [78, 79].

PW10% (Fig. 5b) showed the closer spectral similarity with the external standard sample, because it presents greater similarity at the peak at ~ 1250 cm^{-1} (fingerprint), followed by sample PW25% (a), dW (d), DPW (c) and PW15% (e). The latter one showed small signal amplitudes compared to the other samples, but in similar regions and deformations.

Despite the differences (signal amplitude, noise, distinct regions), there is significant conformity between the spectra produced by the Fucose containing EPS pattern (*Enterobacter* A47 strain) and the samples produced in this work by

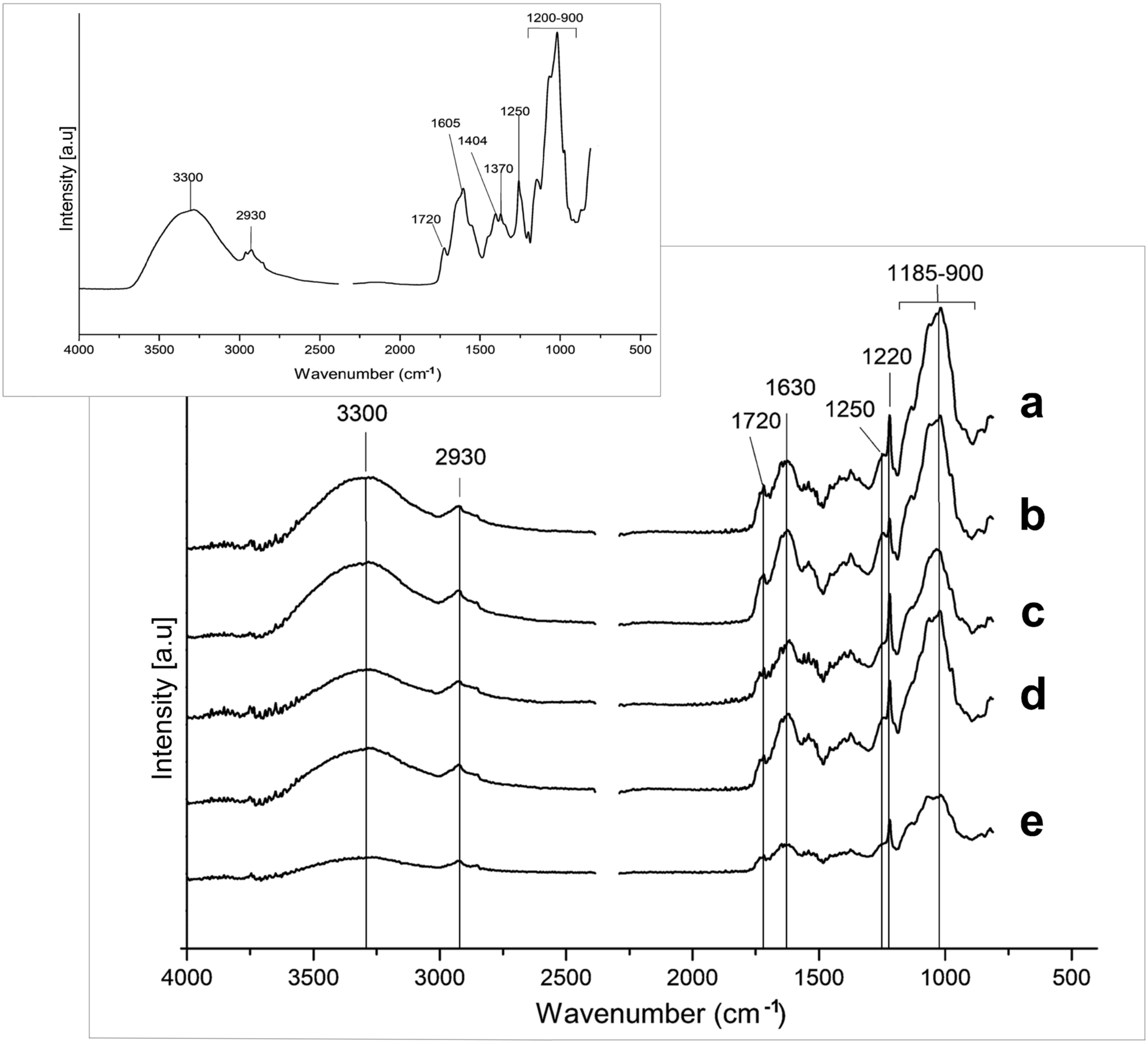


Fig. 5 FT-IR spectroscopy of **a** commercial control of an EPS produced by *Enterobacter* A47 and EPS samples produced in this work by *Lelliottia amnigena*. **b** Produced water at 25% (PW25%), **c** Produced water at 10% (PW10%), **d** dialyzed produced water (DPW), **e** distilled water (dW) and **f** Produced water at 15% (PW15%)

Lelliottia amnigena, as well as with IR spectra presented in the literature [63, 91, 94].

Conclusions

This research showed the production of EPS using industry by-products (PW and CG) as a nutritional supplement in the culture medium by *Lelliottia amnigena*. At the concentration of 15% of PW, a greater amount of EPS was recovered, approximately 84.9% ($P < 0.0001$) which maintained the productive quality. Under these conditions, the total viscosity provided by a liter of production was 114.3% higher ($P < 0.0001$) than the control produced with dW. FT-IR spectra indicate similarity of the EPS to an external exopolysaccharide control produced by *Enterobacter* A47. The simultaneous reuse of PW and CG proved to be advantageous, with economic and environmental implications in the scope of waste management in the oil industry, in the reduction of potable water to be used in EPS production and higher yields.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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