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EXTRACELLULAR POLYMERIC SUBSTANCES PRODUCTION BY RHODOCOCCUS ERYTHROPOLIS ATCC 4277 IN GLUCOSE

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ABSTRACT

Extracellular polymeric substances (EPS) play a crucial role in various biotechnological applications, including metal biosorption in bioremediation processes. This study investigates EPS production by *Rhodococcus erythropolis* ATCC 4277 under different glucose concentrations to optimize conditions for EPS yield per cell. Cultures were grown in a medium containing malt extract, yeast extract, and calcium carbonate supplemented with glucose concentrations ranging from 0 to 100 g/L. Samples were incubated at 24 °C with 180 rpm agitation for 24 and 48 h. Biomass concentration was determined by optical density, and EPS production was quantified using the phenol-sulfuric acid method. Results showed that while higher glucose concentrations generally increased biomass and EPS concentrations, optimal EPS yield per gram of biomass was achieved at intermediate glucose levels (10.0 $g.L^{-1}$ for 24 h and 6.0 $g.L^{-1}$ for 48 h). These findings underscore the importance of balancing substrate availability to maximize EPS production efficiency. The study provides critical insights for future research focused on utilizing EPS from *R. erythropolis* ATCC 4277 as an effective biosorbent for metal ion removal in bioremediation applications.

Keywords: Biosorption. *Rhodococcus erythropolis*. EPS Production. Glucose Concentration. Bioremediation.

1 INTRODUCTION

Extracellular polymeric substances (EPS) are polymers synthesized by microorganisms during metabolic processes and accumulate on the cell surface[1]. They primarily consist of exopolysaccharides but also contain proteins, nucleic acids, and various functional groups such as amines and carboxyls. These components enhance their ability to attract and adsorb metals and organic compounds[2,3,4]. In the natural environment, their primary function is to protect bacterial cells from extreme conditions such as temperature, light intensity, pH variations, and biotic stresses through biofilm formation[5,6].

Several microorganisms have the capability to excrete EPS, including species of the *Rhodococcus* genus[7,8]. *Rhodococcus* is characterized as a gram-positive, immobile, non-sporulating, aerobic species known for its exceptional persistence and stress tolerance, which makes it suitable for various bioprocesses[9,10]. This includes biodegradation of a wide range of pollutants. For example, *R. erythropolis* is notable for its production of biosurfactants and EPS, which play crucial roles in bioremediation processes such as biosorption of metals like copper, aluminum, zinc, and iron[11,12]. Biosorption involves the adherence of molecules, liquids, gases, or dissolved solids to the surface of substances produced by biological entities, including bacterial EPS[13,14]. Additionally, the anionic nature of EPS' outer layer facilitates the capture of minerals and nutrients essential for cell growth, as well as the chelation of metals[15]. Given the involvement of *Rhodococcus* bacteria in bioprocesses producing extracellular polymers, significant efforts have been dedicated to understanding how EPS mediates interactions between the cell surface and various compounds[16].

In previous studies, our group utilized *R. erythropolis* ATCC 4277 in biomining processes of solid metallic waste, and solubilizing metals in a bulk solution. The production of EPS and its adsorption capacity present a promising approach to recovering metals obtained through these methodologies. Given the significant environmental contamination caused by metals and the financial impact of wasting metallic raw materials - estimated at US$57 billion[17] - ecological alternatives for metal recovery such as biomining or biosorption using bacterial EPS are crucial.

This study investigated EPS production by *R. erythropolis* ATCC 4277 under varying glucose concentrations as the main substrate. Glucose was selected based on our prior biomining research, particularly a study by Todescato et al. in 2017. Furthermore, it is barely discussed in the literature how variations in glucose concentration affect EPS production in *R. erythropolis*. Our experiments aimed to optimize the culture medium's composition and process duration to maximize EPS yield. These efforts represent the initial phase of a project focused on utilizing EPS from *R. erythropolis* ATCC 4277 as a biosorbent for metal adsorption from electronic wastes.

2 MATERIAL & METHODS

The experiment aimed to assess EPS production by *R. erythropolis* ATCC 4277 using culture media with varying glucose concentrations. Each culture medium, totaling 300 mL, consisted of 6.1 $g.L^{-1}$ yeast extract, 5.0 $g.L^{-1}$ malt extract, and 1.2 $g.L^{-1}$ calcium carbonate, in addition to different glucose concentrations. Following preparation, the media underwent sterilization in an autoclave (121°C for 20 minutes) before inoculation. Each experimental condition was conducted in triplicate.

The cultures of *R. erythropolis* ATCC 4277 were maintained in an incubator with orbital shaking for 24 h or 48 h, in 25 ºC and 180 rpm. Table 1 outlines the specific glucose concentrations used and the duration of the cultures. After the incubation period, culture samples were withdrawn and centrifuged at 6000 G for 15 minutes.

Table 1 Variations in the culture time and glucose availability in the culture medium.

Culture Time	Glucose Concentration in the Culture Medium ($g.L^{-1}$)													
24 h 48 h	0	0.25	0.50	1.0	2.0	4.0	6.0	8.0	10	20	40	60	80	100

Biomass concentration was determined using optical density measured at a fixed wavelength of 600 nm with UV-Vis spectrophotometry. EPS was extracted by adding 95% ethanol to the samples at a 4:1 ratio. The mixture was then stored at -80 ºC for 30 min, followed by washing with 70% ethanol and resuspension in distilled water. The concentration of exopolysaccharides in the EPS was determined using the phenol-sulfuric acid method, and the samples were measured with a UV-Vis spectrophotometer at a wavelength of 490 nm.

3 RESULTS & DISCUSSION

In the study evaluating EPS production by *R. erythropolis* ATCC 4277 across different glucose concentrations, analysis of variance (ANOVA) was conducted to assess significant differences at a p-value threshold of ≤ 0.05. This analysis aimed to identify conditions yielding the highest EPS production per gram of bacterial biomass. Results indicated significant differences among experimental conditions concerning glucose concentration after 24 and 48 h of cultivation, as shown in Table 2.

Table 2 Summary of ANOVA for 24 h and 48 h assays varying glucose concentration.

Univariate Tests of Significance - Effective hypothesis decomposition in 24h					
Effect	SS	Degree of Freedom	MS	F	p-value
Intercept	3880,727	1	3880,727	1120,008	0,00000
Glucose Concentration	966,845	13	74,373	21,465	0,00001
Error	90,088	26	3,465		
Univariate Tests of Significance - Effective hypothesis decomposition in 48h					
Effect	SS	Degree of Freedom	MS	F	p-value
Intercept	13423,496	1	13423,496	1094,261	0,00000
Glucose Concentration	9298,027	13	715,233	58,304	0,00000
Error	331,214	27	12,267		

Tukey's test was employed to identify specific differences between treatment means. Over a 24-hour cultivation period, the condition yielding the highest EPS productivity was observed with 10 $g.L^{-1}$ of glucose, achieving a ratio of approximately 60:1 grams of EPS per gram of dry biomass of *R. erythropolis* ATCC 4277. Over a 48-hour period, the highest EPS/biomass ratio was observed with 6.0 $g.L^{-1}$ of glucose, resulting in a ratio of 24:1 grams of EPS per gram of dry biomass. Figure 1 illustrates a graph showing the relationship between glucose concentration and EPS/biomass ratio after 24 h and 48 h of culture.

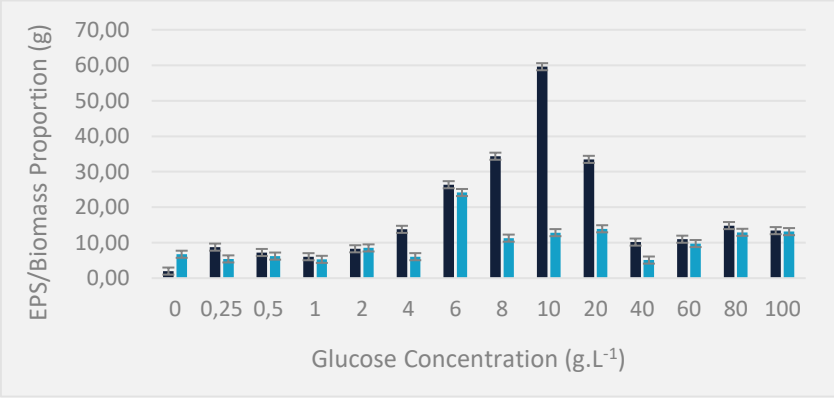

Figure 1. Proportion of EPS produced by *R. erythropolis* ATCC 4277 and its biomass, for the tests carried out for 24 h (■) and 48 (■) h

EPS biosynthesis in bacteria, such as *R. erythropolis* ATCC 4277, commences with the uptake and metabolism of the sugar source, specifically glucose. This process involves several enzymatic steps: hexokinase phosphorylates glucose to form glucose-6-phosphate (G6P), which is subsequently converted to glucose-1-phosphate (G1P) by phosphoglucomutase. UDP-glucose pyrophosphorylase then converts G1P to UDP-glucose, serving as the precursor for exopolysaccharide (EPS) synthesis via an anabolic pathway within the cells[18]. Interestingly, the phenomenon where the highest concentrations of glucose do not necessarily correlate with the highest EPS production can be attributed to allosteric inhibition of hexokinase. This enzyme plays a crucial role in the initial steps of glycolytic pathways in *Rhodococcus*, such as the Embden-Meyerhof-Parnas, Entner-Doudoroff, and pentose-phosphate pathways. Allosteric inhibition occurs when excess G6P slows down the phosphorylation of glucose, thereby influencing EPS production[9,19]

This insight is crucial for optimizing culture conditions to efficiently maximize EPS production and highlights the importance of balancing substrate availability to achieve optimal metabolic conditions for EPS generation by *R. erythropolis*. Understanding these metabolic pathways and their regulatory mechanisms is essential for enhancing bioprocess efficiency and exploring EPS as a valuable biotechnological resource, particularly in applications such as metal adsorption. The relationship between glucose concentration and EPS production in *R. erythropolis* ATCC 4277 remains poorly understood, as mentioned previously. However, by optimizing conditions that maximize EPS production relative to biomass, it is possible to generate more biosorbent material for metal recovery using less biological material, thereby enhancing process efficiency in subsequent stages of this project.

4 CONCLUSION

The strain *R. erythropolis* ATCC 4277 demonstrates the capability to produce EPS within 24 and 48 h of culture, utilizing glucose as a substrate across various concentrations. This aligns with expectations, as this bacterial species is recognized for its proficiency in EPS production using different carbon sources, particularly sugars. Moreover, the study highlighted that optimal EPS yield per biomass was observed at intermediate glucose concentrations tested. Specifically, at 24 h of culture, the highest EPS production per gram of dry biomass (with a ratio of 60:1) occurred with a glucose concentration of 10 $g.L^{-1}$. Similarly, at 48 h of culture, the highest EPS to biomass ratio (24:1) was achieved with a glucose concentration of 6.0 $g.L^{-1}$.

These findings offer valuable insights into the conditions that favor maximum EPS production by *R. erythropolis* ATCC 4277, crucial for future experiments aimed at assessing the adsorption capacity of EPS generated by this bacterium in the presence of metals. Such investigations could further explore the potential of EPS as an efficient biosorbent for metal removal, leveraging the optimized conditions identified in this research...

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