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Dairy wastewater treatment employing microencapsulated *Pseudomonas aeruginosa* on low acyl gellan gum

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ABSTRACT

This study assessed the ability of *Pseudomonas aeruginosa*, microencapsulated in gellan gum, to decontaminate dairy wastewater and explored the potential reuse of microcapsules. *P. aeruginosa* was microencapsulated using the internal ionic gelation technique, employing low-acyl gellan gum as the wall material. The free and microencapsulated *P. aeruginosa* were inoculated into 150 mL of sterile wastewater and incubated in a shaking flask (150 rpm) at 30°C. Subsequently, the Baranyi Model was employed to calculate the growth parameters of P. aeruginosa. Concurrently, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were determined. The obtained results indicated that the microencapsulation process reduced the growth rate of the encapsulated microorganism. However, the microencapsulated bacteria achieved COD and BOD reduction percentages of 61.54% and 64.05%, respectively. Similarly, when reusing the microcapsules, removal percentages exceeding 57.00% were achieved. These findings could have significant implications for the industry in terms of reducing effluent contamination caused by substantial amounts of pollutants.

Keywords: dairy wastewater, gellan gum, microencapsulation, *Pseudomonas aeruginosa.*

Tratamento de águas residuais de laticínios utilizando *Pseudomonas aeruginosa* microencapsulada em goma de gelana de baixo acil

RESUMO

O objetivo do presente estudo foi avaliar a capacidade da *Pseudomonas aeruginosa*, microencapsulada em goma de gelana, para descontaminar águas residuais de laticínios e explorar o potencial reuso das microcápsulas. A *P. aeruginosa* foi microencapsulada usando a técnica interna de gelificação iônica, empregando goma de gelana de baixo acil como material de parede. As formas livres e microencapsuladas de P. aeruginosa foram inoculadas em 150 mL de água residual estéril e incubadas em um frasco agitado (150 rpm) a 30°C. Posteriormente, o modelo de Baranyi foi utilizado para calcular os parâmetros de crescimento da *P. aeruginosa*. Ao mesmo tempo, foram determinadas a Demanda Bioquímica de Oxigênio (DBO) e a Demanda Química de Oxigênio (DQO). Os resultados obtidos indicaram que o processo de microencapsulação reduziu a taxa de crescimento do microorganismo encapsulado.

No entanto, as bactérias microencapsuladas alcançaram porcentagens de redução de DQO e DBO de 61,54% e 64,05%, respectivamente. Da mesma forma, ao reutilizar as microcápsulas, foram alcançadas porcentagens de remoção superiores a 57,00%. Essas descobertas podem ter implicações significativas para a indústria em termos de redução da contaminação do efluente causada por quantidades substanciais de poluentes.

Palavras-chave: águas residuais de leite, goma de gelana, microencapsulação, *Pseudomonas aeruginosa.*

1. INTRODUCTION

The rapid expansion of the global economy, coupled with population growth and urbanization, results in a significant increase in waste production worldwide (Awasthi *et al.*, 2022). Every year, the fermentation and food sectors produce a vast quantity of wastewater, amounting to hundreds of millions of tons, which presents considerable environmental hazards (Israni *et al.*, 2020). The dairy industry entails the presence of high levels of organic compounds, such as carbohydrates, proteins, and fats in its wastewater (Wang and Serventi, 2019). For each liter of processed milk, the dairy sector generates 2.5–10 liters of wastewater (Szabo-Corbacho *et al.*, 2021). If this wastewater is disposed of with no prior treatment, it could negatively impact the environment (Silva *et al.*, 2020).

The composition of dairy wastewater is influenced by the specific production processes and the raw dairy materials utilized. For instance, wastewater stemming from milk processing typically exhibits a chemical oxygen demand (COD) of 3,000 mg/L, while that originating from cheese production can reach as high as 50,000 mg/L (Melchiors *et al.*, 2016). Normally, the treatment of dairy wastewater encounters issues associated with high concentrations of proteins and lipids, resulting in pH alterations and elevated levels of COD and BOD (Biochemical Oxygen Demand) (Sarkar *et al.*, 2006). Disposal of this wastewater into the environment causes serious problems due to its high oil content, COD and color. Inadequate management of this effluent from dairy processing poses significant challenges for local municipal sewage treatment systems and can result in severe environmental contamination. Common treatment methods employed for such waste effluents include coagulation, flocculation, and sedimentation as primary treatment (Parihar *et al.*, 2024). Biological treatments for dairy wastewater have been recommended as a cost-effective and environmentally friendly option. Bioremediation is a high-efficient and low-cost technology for wastewater treatment (Deng *et al.*, 2021).

Several microorganisms can help mitigate contaminant compounds in wastewater treatment (Dhouib *et al.*, 2006). *Pseudomonas* strains have been isolated from freshwater environments (Yang *et al.*, 2019; Li *et al.*, 2015) and employed in wastewater treatment (Deng *et al.*, 2021). Sugimori and Utsue (2012) reported that the utilization of *Pseudomonas sp*. exhibits strong promise as an effective strain for the bioremediation of various wastewater types, such as dairy effluents. In this context, dairy wastewater serves as a significant source of protein-rich materials that can be biologically transformed into less biodegradable compounds. The utilization of free bacteria as biocatalysts may have certain drawbacks, including limitations on repeated use, reduced stability in real-world applications, and the unintentional production of byproducts. The microencapsulation of bacteria for the degradation of different compounds in wastewater offers several benefits in the bioremediation process when compared to suspended microbial cells. These advantages include less difficulty in separating biomass, increased efficiency, enhanced resilience to environmental challenges, reduced susceptibility to toxic compounds, and decreased dispersion of microorganisms in the environment. The microencapsulation of cells has been proposed as a strategy to enhance biodegradation efficiency, improve enzyme production, enhance microorganism tolerance to environmental

stressors, and mitigate the inhibitory effects of toxic compounds (Dhanarani *et al.*, 2016). Bernardo *et al.* (2013) microencapsulated *Pseudomonas aeruginosa* for the production of hydroxamic acids. Mohebrad *et al.* (2022) used a polysaccharide for immobilization of *P. aeruginosa* in a microbial electrochemical system for treatment of dairy wastewater. Gellan gum has been widely employed for microencapsulation of bacteria, since it is less toxic than synthetic polymers and easily gelled under mild conditions (Salazar-Montoya *et al.*, 2018). Gellan gum is a bacterial polysaccharide derived from the microorganism *Sphingomonas elodea.* It is characterized as an anionic heteropolysaccharide with a linear structure consisting of a repeating tetra-saccharide unit, which includes glucose, glucuronic acid, glucose, and rhamnose. Additionally, glycerate and acetate side groups are attached to the glucose units (Hu *et al.*, 2023). Native gellan is known as high acyl (HA) gellan because it presents both an acetate group (C6) and a glycerate group (C2) in its glucose residue. When HA gellan is exposed to strong alkali treatment at high temperatures, the acyl groups are hydrolyzed and low acyl (LA) gellan is obtained. The purpose of this paper was to assess the ability of *P. aeruginosa* microencapsulated in gellan gum to decontaminate dairy wastewater and possible reuse of microcapsules.

2. MATERIALS AND METHODS

2.1. wastewater sample

The samples of dairy wastewater were collected from a privately owned dairy industry located near Cartagena City (Colombia). The culture of *Pseudomonas aeruginosa* was obtained from the microbiology laboratory located in the pilot plants of the University of Cartagena. The samples were collected between 6:00 and 7:00 a.m using polyethylene bags. The bags were kept in an icebox and transported to the laboratory. Finally, wastewater was autoclaved at 121°C and 15 psi for 15 min to reduce contamination.

2.2. Culture condition and growth curve

P. aeruginosa was inoculated into a modified culture medium containing 20% dairy wastewater at 120 rpm under aerobic conditions (An air pump, coupled with an aeration diffuser installed in the walls of the bioreactor, was utilized to deliver continuously oxygen to the bioreactor. Typically, a consistent level of 1.8 mg L-1 of dissolved oxygen was maintained within the bioreactor solution) in order to adapt the bacterium to the new environmental conditions. Finally, *P. aeruginosa* was enumerated in Cetrimide medium after incubation at 30°C during 48 h. Results were expressed as the average ± standard deviation (log CFU/g).

2.3. Microencapsulation of *P. aeruginosa*

Low acyl gellan gum dispersions were prepared with deionized water at a 0.4% (w/v). Then calcium was added (30 mM) and dispersed by constant stirring at 90°C for 10 min on a hot plate stirrer. Next, a concentration of *P. aeruginosa* (2.5 Ln CFU/mL) was incorporated to the dispersion, the counting was carried out in culture dishes with agar Cetrimide. The emulsions were prepared via the adding of 0.2% sorbitan monooleate in vegetable oil under constant stirring at 600 rpm on a hot plate stirrer. Then, α-gluconolactone was added until a pH of 4.5 was reached to start the gelation process. Finally, all the oil was removed by adsorption, and the microcapsules contained in the aqueous phase were centrifuged at 5000 rpm for 10 min with saline solution and stored at 4°C until use.

2.4. Growth of *P. aeruginosa* in wastewater

P. aeruginosa, both in free and microencapsulated states, were inoculated into 150 mL of sterile wastewater and incubated in a bioreactor under constant agitation at 150 rpm at 30°C. Growth was monitored by a plating method. Growth curves were constructed by plotting the

logarithm of the number of microorganisms versus time. The Baranyi Model (Baranyi *et al.*, 1996) was used to calculate the growth parameters of *P. aeruginosa* in wastewater (Equation 1).

$$(t) = y_0 + \mu_{max}t + \frac{1}{\mu_{max}} ln\ ln\ (e^{-vt} + e^{-h_0} - e^{-vt-h_0})\ - \frac{1}{m} ln\left[1 + \frac{e^{m\mu_{max}t + \frac{1}{\mu_{max}} lnln\left(e^{-vt}+e^{-h_0}-e^{-vt-h_0}\right)} - 1}{e^{m(y_{max}-y_0)}}\right] (1)$$

Where y(t) is the cellular concentration or colony diameter, y_0 is the initial concentration or diameter, μ_{max} is the maximum specific growth rate (1/h), m is a curvature parameter to characterize the transition of the exponential phase, v is a curvature parameter to characterize the transition the exponential phase, and h_0 is a dimensionless parameter that quantifies the initial physiological state of the cells.

2.5. Physicochemical effluent characterization

The physicochemical analyses were carried out employing the Standard Methods protocols recognized for raw water and wastewater. The fat and oil content was estimated by the Soxhlet method according to the Standard Methods of the APHA *et al.* (2012). Biological oxygen demand (BOD) was determined by preparing the required volume of dilution water with the addition of nutrients and incubating for a period of five days at 20°C, while chemical oxygen demand (COD) was determined according to the dichromate titration (APHA *et al.*, 2012). The COD degradation efficiency was defined as the amount of COD decreased versus the amount of initial COD. The phosphorus content was calculated by acid digestion, using the ascorbic acid method expressed in mg of P/L. Hydrogen potential was determined potentiometrically using a digital potentiometer (Bench pH-Conductivity meter PC 510). All the experiments were performed in triplicate and data presented as means ± SD.

2.6. Statistical analysis

In this study, one-way ANOVA was employed in comparing differences between growth parameters of *P. aeruginosa* and chemical characteristics of dairy wastewater. Statistical analysis was performed using software SPSS 23.0 (SPSS Inc., USA). All tests were considered significant at $\alpha = 0.05$ level.

3. RESULTS AND DISCUSSION.

3.1. Growth of P. aeruginosa

The growth curve of *P. aeruginosa* growing in dairy wastewater shows three phases: (1) lag phase, (2) logarithmic phase and (3) stationary phase (Figure 1). Similar behavior has been observed for *Y. lipolytica* growing in synthetic and dairy wastewater (Tarón-Dunoyer *et al.*, (2021). These findings show the capability of the microorganisms to use some compounds present in the wastewater as a source of carbon, nitrogen and energy. Deng *et al.* (2021) indicated that *Pseudomonas spp* could be used in aquaculture wastewater treatment. The Baranyi Model was chosen to get the growth kinetic parameters. Fitting the Baranyi Model to the growth curves allows determinant growth parameters such as initial count cells (Y_0), maximum growth rate (μ), latency phase (λ) and maximum cell population (Ymax) as can be seen in Table 1.

The initial concentration of *P. aeruginosa* (Y_0) in dairy wastewater was not significantly ($P>0.05$) affected by microencapsulation process, which can be caused by the control on the number of *P. aeruginosa* incorporated at the beginning of the biodegradation process. Y_0 had a value of 2.057 log CFU/g for free microorganisms and 2.043 log CFU/mL for microencapsulated bacteria. Another parameter is Y_{max}, that represents the maximum microbial

concentration achieved at the end of the exponential phase. Y_{max} values are of greater importance when studies on the production of some metabolites are carried out. The highest Y_{max} value (7.254 log CFU/mL) was reached with free bacteria; while, microencapsulated bacteria had 6.724 log CFU/mL. However, it must be noted that microencapsulated bacteria took longer to reach that Y_{max} value. These findings are similar to those reported by Wu *et al.* (2009); although these authors did not calculate Ymax, in the graphs they present, it can be observed that the curves reach different Ymax values at different times than when encapsulating *Y. lipolytica* in sodium alginate.

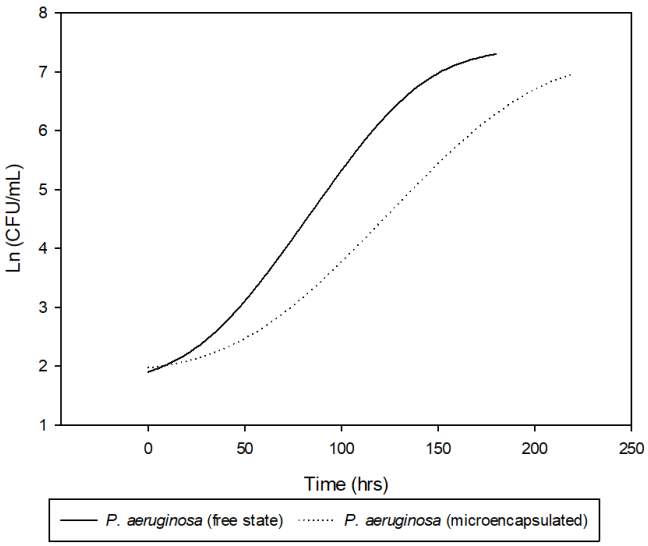

Figure 1. Behavior of *P. aeruginosa* in free and microencapsulated state adjusted to the Baranyi and Roberts Model.

Table 1. Growth parameters of *P. aeruginosa* are calculated from the Baranyi Model.

Growth parameter	Free bacteria	Microencapsulated bacteria	Unit
Y_0	2.057 ± 0.040^a	2.043 ± 0.024^a	(log CFU/g)
Y_{max}	7.254 ± 0.330^a	6.724 ± 0.420^b	(log CFU/g)
μ_{max}	0.052 ± 0.010^a	0.026 ± 0.001^b	(h^{-1})
λ	27.614 ± 0.024^a	37.594 ± 0.020^b	(h)

Rows with no common letter showed a statistically significant difference ($p<0.05$) based on LSD test.

In order to assess the velocity with which a microorganism grows, it is relevant to establish the μ_{max} values. However, it must be considered that μ_{max} values mainly depend on the environmental conditions (Arroyo-López *et al.*, 2012). Free bacteria had μ_{max} values of 0.052 h^{-1} and microencapsulated bacteria had lower values (0.026 h^{-1}). These results indicate a reduction in the growth rate of the microorganism when subjected to microencapsulation. Contrary results were published by Wu *et al.* (2009), who found similar degradation rates of COD when using microorganisms in free form and encapsulated.

The last parameter is λ, which represents the time that microorganisms take to adapt to new environmental conditions (Swinnen *et al.*, 2004). This parameter increased when the bacteria was microencapsulated from 25.614 to 37.594 h. μ_{max} and λ are important parameters to describe the growth behavior of bacteria on different substrates and these findings suggest that *P. aeruginosa* can grow faster in free state than microencapsulated. However, the advantage of using microencapsulated bacteria in biodegradation processes is that they can be reused in

new bioprocesses. In this study, it is significant to note the capability of *P. aeruginosa* immobilization for wastewater treatment.

3.2. Physicochemical effluent characterization

Table 2 presents the physicochemical parameter values of dairy wastewater before and after the fermentation process using *P. aeruginosa* in both free form and microencapsulated with low acyl gellan as a wall material. Additionally, it includes the values obtained through the reuse of the microcapsules in a second biodegradation process.

Table 2. Chemical characteristics of dairy wastewater.

Parameters	Ic	Fc (a):	Fc (b):	Fc(c):	Unit
COD	65248 ±25.43^{a}	23456±18.81^{b}	25093±21.44^{c}	27448±14.9^{d}	mg/L
BOD	22370 ±15.45^{a}	7775 ±10.03^{b}	8042 ± 8.33^{c}	8803±9.01^{d}	mg/L
Fat	6280 ±8.45^{a}	975± 4.93^{b}	1006 ± 3.72^{b}	1073±7.08^{c}	mg/L
pH	8.321 ±0.34^{a}	6.53 ±0.10^{b}	6.88 ± 0.00^{bc}	7.01±0.02^{c}	U of pH
Phosphate	1.4 ±0.01^{a}	1.38 ±0.02^{a}	1.39 ± 0.02^{a}	1.38±0.00^{a}	mg/L

Ic: Initial conditions of the effluent. Fc (a): Final conditions using Free bacteria.
Fc(b): Final condition using Microencapsulated bacteria. Fc(c): Final conditions using reused microcapsules. Rows with no common letter showed a statistically significant difference ($p<0.05$) based on LSD test.

Evaluating COD in decontamination treatments is important as it is a measure used to determine the amount of organic matter and chemical contaminants present in the water that require oxygen to decompose through chemical and biological processes. In this regard, reducing COD is a common goal in wastewater treatment and in the purification of water for human consumption, as high COD levels can indicate the presence of contaminants that may be harmful to the environment or public health. Considering the COD values obtained, it can be stated that dairy residues are highly biodegradable; the highest values were obtained at the beginning of the degradation process (65248 mg/L), while fermentations carried out with the free-state microorganism showed a COD reduction of 64.05% (Figure 2), whereas fermentation with the microencapsulated microorganism resulted in a reduction of 61.54%. These results corroborate those obtained in the growth rate since microencapsulation appears to decrease the bacterial growth rate.

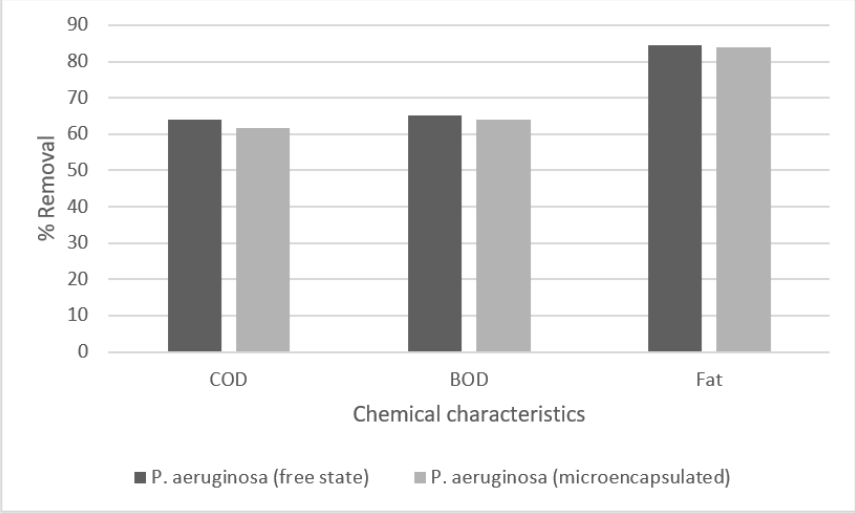

Figure 2. Removal percentages of *Pseudomonas sp.* in free and microencapsulated state.

BOD is another crucial parameter to measure in wastewater treatment as it is a metric used to assess the amount of oxygen required by microorganisms in a wastewater sample to decompose the biodegradable organic matter present over a 5-day period. The highest BOD value was obtained at the beginning of the degradation process, reaching 22370 mg/L. After treatment, reductions of 65.24% and 64.05% (Figure 2) were observed when the free-state microorganism and the microencapsulated microorganism were used, respectively. One-way ANOVA of COD and BOD values revealed significant differences ($p<0.05$) among the initial conditions and the values obtained with free-state and microencapsulated microorganisms. However, it is important to note that when the microcapsules were removed to carry out a second degradation cycle, the microcapsules retained their activity, resulting in reductions of 57.93% and 60.64% for COD and BOD, respectively.

Studies have demonstrated significant decreases in COD and BOD levels in wastewater through the utilization of bacterial isolates, as evidenced by publications from Das and Santra (2010) and Gaikwad *et al.* (2014). Like many other sectors in the agro-industry, the dairy industry generates robust wastewater streams characterized by their high COD and BOD levels, indicative of their substantial organic content. The reduction in COD values could be attributed to the concentration of nutrients, which microbial cultures can utilize for their growth. Our current findings align with the COD reduction reported by Guillen-Jimenez *et al.* (2000), where a maximum COD decrease of approximately 65–70% was observed. A similar reduction in COD, amounting to 99.9%, was documented in dairy wastewater by Cosa and Okoh (2014) through the use of a consortium comprising two marine species. Considering the COD and BOD removal data obtained (>60%), P. aeruginosa in free and microencapsulated states can be used to treat wastewater from the dairy industry, which has high levels of COD and BOD. This is because, according to some standards for industrial wastewater disposal into receiving water bodies, removal percentages for COD and BOD between 60 and 70% are required (CONAMA, 2011; COPAM/CERH-MG, 2008).

With respect to the percentage of fat removal, a greater reduction can be observed when the free-state microorganism was used, with 84.47% reduction, whereas when the microencapsulated microorganism was employed, the removal was 83.98% (Figure 2). The same behavior was observed with the pH level, meaning there is a greater decrease in pH when the free-state microorganism is used due to higher metabolic activity resulting in a greater reduction in COD and BOD values. Conversely, phosphate values did not show any significant variation, remaining between 1.4 and 1.38 mg/L. These studies showed that some *P. aeruginosa* is a good candidate to reduce COD, BOD and fat levels in dairy wastewater.

3.3. COD and BOD degradation by reuse of the microencapsulated cells

The reutilization of microencapsulated cells could offer advantages by potentially reducing cell wastage, saving time, and cutting down cultivation costs. After completing the fermentation process, the microcapsules were separated and washed with sterile distilled water before being reintroduced into 150 mL of fresh dairy wastewater. After several hours of fermentation, COD and BOD were measured (this was considered the second fermentation cycle). In this study, an additional degradation cycle was conducted using the microcapsules employed in the previous fermentation treatment. The second fermentation shows a slight decrease in biodegradation activity. For instance, the percentage of COD reduction was 57.93%, while the reduction of BOD was 60.64%. The percentage of fat removal was 82.91%. These results indicate that *P. aeruginosa* can be microencapsulated in low acyl gellan and can grow in dairy wastewater, using it as a source of carbon. Furthermore, the results suggest that gellan gum might be an ideal carrier for the microencapsulation of *P. aeruginosa*. Wu *et al.* (2009) found that immobilized *Yarrowia lipolytica* cells in alginate can be reused for 12 cycles of 50 hours while maintaining a degradation rate of 77%. The use of immobilized cells has traditionally been employed to reduce the loss or decrease in the viability of bacterial cells, save time, and lower

cultivation costs. Immobilized *Pseudomonas aeruginosa* reduced the dairy wastewater treatment time using the microbial electrochemical system from 12 h to 4 h. (Mohebrad *et al.*, 2022).

4. CONCLUSIONS

Pseudomonas aeruginosa emerged as a viable option for biological treatment of wastewater from the dairy industry, given its ability to achieve reductions exceeding 60% in COD and BOD levels. Additionally, microencapsulation of this bacterium presents itself as a promising avenue for cost reduction in biological wastewater treatment processes. The reusability of microcapsules ensures sustained removal efficiencies comparable to those observed with free-state bacteria, thus enhancing the economic viability of treatment systems.

5. REFERENCES

ARROYO-LÓPEZ, F.; BAUTISTA-GALLEGO, J.; GARRIDO-FERNANDEZ, A. Role of Predictive Microbiology in Food Preservation. In: BHAT, R.; ALIAS, A. K.; PALIYATH, G. **Progress in Food Preservation**. New York: John Wiley & Sons, 2012. p. 389-404.

APHA; AWWA; WEF. Standard Methods for the examination of water and wastewater. 22nd ed. Washington, 2012. 1496 p.**APHA. Standard Methods for the Water and Wastewater. 19th ed. Washington DC. 2012.**

AWASTHI, M. K.; SINDHU, R.; SIROHI, R.; KUMAR, V.; AHLUWALIA, V.; BINOD, P. *et al.* Agricultural waste biorefinery development towards circular bioeconomy. **Renewable and Sustainable Energy Reviews,** v. 158, n. 112122, 2022. https://doi.org/10.1016/j.rser.2022.112122

BARANYI, J.; ROSS, T.; MCMEEKIN, T. A.; ROBERTS, T. A. Effects of parameterization on the performance of empirical models used in "predictive microbiology". **Food Microbiology**, v. 13, n. 1, p. 83–91, 1996. https://doi.org/10.1006/fmic.1996.0011

BERNARDO, M.; PACHECO, R.; SERRALHEIRO, M. L. M.; KARMALI, A. Production of hydroxamic acids by immobilized Pseudomonas aeruginosa cells: Kinetic analysis in reverse micelles. **Journal of Molecular Catalysis B: Enzymatic,** v. 93, p. 28-33, 2013. https://doi.org/10.1016/j.molcatb.2013.03.016

CONAMA (Brasil). Resolução nº 430 de 13 de maio 2011. Dispõe sobre as condições e padrões de lançamento de efluentes, complementa e altera a Resolução nº 357, de 17 de março de 2005, do Conselho Nacional do Meio Ambiente-CONAMA. **Diário Oficial [da] União**: seção 1, Brasília, DF, n. 92, p. 89, 16 maio 2011.

COPAM/CERH-MG. Deliberação Normativa Conjunta n.º 1, de 05 de Maio de 2008. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. **Minas Gerais Diário do Executivo**, Belo Horizonte, 13 maio 2008.

COSA, S.; OKOH, A. Bioflocculant production by a consortium of two bacterial species and its potential application in industrial wastewater and river water treatment. **Polish Journal of Environmental Studies,** v. 23, n. 3, p. 689-696, 2014.

DAS, S.; SANTRA, S. C. Simultaneous biomass production and mixed-origin wastewater treatment by five environmental isolates of Cyanobacteria. **Biologija,** v. 56, n. 1-4, p. 9-13, 2010. https://dx.doi.org/10.2478/v10054-010-0010-7

DENG, M.; ZHAO, X.; SENBATI, Y.; SONG, K.; HE, X. Nitrogen removal by heterotrophic nitrifying and aerobic denitrifying bacterium *Pseudomonas sp.* DM02: Removal performance, mechanism and immobilized application for real aquaculture wastewater treatment. **Bioresource Technology**, v. 322, n. 124555, 2021. https://doi.org/10.1016/j.biortech.2020.124555

DHANARANI, S.; VISWANATHAN, E.; PIRUTHIVIRAJ, P.; ARIVALAGAN, P.; KALIANNAN, T. Comparative study on the biosorption of aluminum by free and immobilized cells of bacillus safensis KTSMBNL 26 isolated from explosive contaminated soil. **Journal of the Taiwan Institute of Chemical Engineers,** v. 69, p. 61–67, 2016. https://doi.org/10.1016/j. jtice.2016.09.032

DHOUIB, A.; ELLOUZ, M.; ALOUI, F.; SAYADI, S. Effect of bioaugmentation of activated sludge with white-rot fungi on olive mill wastewater detoxification. **Letters in Applied Microbiology**. v. 42, n.4, p. 405–411, 2006. https://dx.doi.org/10.1111/j.1472-765X.2006.01858.x

GAIKWAD, G. L.; WATE, S. R.; RAMTEKE, D. S.; ROYCHOUDHURY, K. Development of microbial consortia for the effective treatment of complex wastewater. **Journal of. Bioremediation & Biodegradation,** v. 5, p. 4, 2014. https://dx.doi.org/10.4172/2155-6199.1000227

GUILLEN-JIMENEZ, E.; ALVAREZ-MATEOS, P.; ROMERO-GUZMAN, F.; PEREDA-MARTIN, J. Bio-mineralization of organic matter in dairy wastewater, as Affected by pH. The evolution of ammonium and phosphates. **Water Research,** v. 34, p. 1215-1224, 2000. https://doi.org/10.1016/S0043-1354(99)00242-0

HU, X.; ZHOU, H.; MCCLEMENTS, D. Impact of dispersion conditions and coacervation on fibril formation in gellan gum-potato protein mixtures. **Food Hydrocolloids,** v. 145, n. 109153, 2023. https://doi.org/10.1016/j.foodhyd.2023.109153

ISRANI, N.; VENKATACHALAM, P.; GAJARAJ, B.; VARALAKSHMI, K. N.; SHIVAKUMAR, S. Whey valorization for sustainable polyhydroxyalkanoate production by *Bacillus megaterium:* production, characterization and in vitro biocompatibility evaluation**. Journal Environmental Management,** v. 255, n. 109884, 2020. https://doi.org/10.1016/j.jenvman.2019.109884.

LI, C.; YANG, J.; WANG, X.; WANG, E.; LI, B.; HE, R. *et al.* Removal of nitrogen by heterotrophic nitrification-aerobic denitrification of a phosphate accumulating bacterium *Pseudomonas stutzeri* YG-24. **Bioresource Technology,** v. 182, p. 18–25. 2015. https://doi.org/10.1016/j.biortech.2015.01.100

MELCHIORS, M. S.; PIOVESAN, M.; BECEGATO, V. R.; BECEGATO, V. A.; TAMBOURGI, E. B. T.; PAULINE, A. T. Treatment of wastewater from the dairy industry using electroflocculation and solid whey recovery, **Journal of Environmental Management,** v. 182 p. 574–580, 2016. https://doi.org/10.1016/j.jenvman.2016.08.022

MOHEBRAD, B.; GHODS, G.; REZAEE, A. Dairy wastewater treatment using immobilized bacteria on calcium alginate in a microbial electrochemical system. **Journal of Water Process Engineering,** v. 46, n. 102609, 2022. https://doi.org/10.1016/j.jwpe.2022.102609

PARIHAR, R. K.; BHANDARI, K.; BURNWAL, P.; GHOSH, S.; CHAURASIA, P.; MIDDA, O. Advancing dairy wastewater treatment: Exploring two-stage fluidized bed anaerobic membrane bioreactor for enhanced performance, fouling, and microbial community analysis. **Journal of Water Process Engineering,** v. 58, n. 104917, 2024. https://doi.org/10.1016/j.jwpe.2024.104917

SALAZAR-MONTOYA, J.; GONZÁLEZ-CUELLO, R.; FLORES-GIRÓN, E.; RAMOS-RAMÍREZ, E. Effect of free and microencapsulated *Lactococcus lactis* on composition and rheological properties of Manchego-type cheeses during ripening. **Food Research International,** v. 105, p. 59–64. 2018. https://doi.org/10.1016/j.foodres.2017.10.067

SARKAR, B.; CHAKRABARTI, P.; VIJAYKUMAR, A.; KALE, V. Wastewater treatment in dairy industries – possibility of reuse. **Desalination,** v. 195, p. 141–152, 2006. https://doi.org/10.1016/j.desal.2005.11.015

SILVA, D.V.; QUEIROZ, L. G.; MARASSI, R. J.; ARAÚJO, C. V.; BAZZAN, T.; CARDOSO- SILVA, S. *et al.* Predicting zebrafish spatial avoidance triggered by discharges of dairy wastewater: an experimental approach based on self-purification in a model river, **Environmental Pollution,** v. 66. n. 115325, 2020. https://doi.org/10.1016/j.envpol.2020.115325

SUGIMORI, D.; UTSUE, T. A STUDY of the efficiency of edible oils degraded in alkaline conditions by *Pseudomonas aeruginosa* SS-219 and *Acinetobacter sp.* SS-bacteria isolated from japanese soil. **World Journal Microbiology & Biotechnology,** v. 28, p. 841–848, 2012. https://doi.org/10.4236/aces.2016.61006

SWINNEN, I. A.; BERNAERTS, K.; DENS, E. J.; GEERAERD, A. H.; VAN IMPE, J. F. Predictive modelling of the microbial lag phase: a review. International **Journal of Food Microbiology,** v. 94, n. 2, p. 137-159, 2004. https://doi.org/10.1016/j.ijfoodmicro.2004.01.006

SZABO-CORBACHO, M. A.; PACHECO-RUIZ, S.; MÍGUEZ, D.; HOOIJMANS, C. M.; GARCÍA, H. A.; BRDJANOVIC, D. *et al.* Impact of solids retention time on the biological performance of an AnMBR treating lipid-rich synthetic dairy wastewater. **Environmental Technology,** v. 42 p. 597–608, 2021. https://doi.org/10.1080/09593330.2019.1639829

TARÓN-DUNOYER, A.; GONZÁLEZ-CUELLO, R.; COLPAS-CASTILLO, F. A predictive growth model for *Yarrowia lipolytica* ATCC 9773 in wastewater. **Revista Ambiente & Água,** v. 16, n. 1, p. 1-9, 2021. https://doi.org/10.4136/ambi-agua.2629

WANG, Y.; SERVENTI, L. Sustainability of dairy and soy processing: a review on wastewater recycling. **Journal of Cleaner Production,** v. 237, n. 117821, 2019. https://doi.org/10.1016/j.jclepro.2019.117821

WU, L.; GE, G.; WAN, J. Biodegradation of oil wastewater by free and immobilized *Yarrowia lipolytica* W29. **Journal of Environmental Sciences,** v. 21, n. 2, p. 237-242, 2009. https://doi.org/10.1016/S1001-0742(08)62257-3

YANG, L.; WANG, X. H.; CUI, S.; REN, Y. X.; YU, J.; CHEN, N. *et al.* Simultaneous removal of nitrogen and phosphorous by heterotrophic nitrification-aerobic denitrification of a metal resistant bacterium *Pseudomonas putida* strain NP5. **Bioresource Technology,** v. 285, n. 121360, 2019. https://doi.org/10.1016/j.biortech.2019.121360