CET CHEMICAL ENGINEERING TRANSACTIONS
VOL. 124, 2026
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Marco Bravi, Antonio Marzocchella, Giuseppe Caputo

ISBN 979-12-81206-25-0; **ISSN** 2283-9216
DOI: 10.3303/CET26124021

Influence of Organic Loading Rate and Feast Regime on Alginate-Like EPS Production from Aerobic Granular Sludge

Santo F. Corsino[a], Maria Castiglione[a,*], Riccardo Campo[b], Marco Capodici[a], Alida Cosenza[a], Gaetano Di Bella[b], Michele Torregrossa[a]

[a]Department of Engineering, University of Palermo, Viale delle Scienze Ed.8, 90128, Palermo, Italy
[b]Department of Engineering and Architecture, University of Enna “Kore”, Cittadella Univeresitaria, 94100, Enna, Italy
maria.castiglione01@unipa.it

This study investigated the influence of organic loading rate (OLR) and feeding regime on the production of alginate-like exopolysaccharides (ALEs) from aerobic granular sludge treating citrus-processing wastewater. Two granular sequencing batch reactors were operated under anaerobic and aerobic feast conditions at increasing OLRs (1-2.5 kgCOD/m³d). Results showed that OLR and feeding regime significantly affected extracellular polymeric substances (EPS) yield and composition. Anaerobic feast conditions promoted higher structural EPS fractions, while aerobic conditions favored higher total EPS production. The results highlight the potential of aerobic granular sludge systems for the simultaneous treatment of agro-industrial wastewater and recovery of valuable biopolymers.

1. Introduction

Recently, microbial polymer production has gained increasing interest within the scientific community, in line with the growing paradigm shift toward circular economy and resource recovery from waste streams. In particular, wastewater is no longer considered solely as a matrix to be treated for pollution control, but rather as a valuable and renewable source of energy, nutrients, and materials. In this context, the production of biopolymers by microbial consortia represents a promising strategy to replace petroleum-based polymers with more sustainable and environmentally friendly alternatives. Compared to conventional polymer production routes, microbial polymers offer advantages in terms of biodegradability, reduced carbon footprint, and compatibility with biological systems. Among the different microbial-derived materials, biopolymers extracted from aerobic granular sludge (AGS) have received growing attention in recent years. AGS is an innovative and well-established technology for the treatment of both municipal and industrial wastewater, characterized by excellent settling properties, high biomass retention, and process intensification (Di Bella and Torregrossa, 2014). Aerobic granules are dense, compact, and spherical microbial aggregates that are self-immobilized, allowing the coexistence of multiple microbial populations and the simultaneous occurrence of several biological processes within the same reactor (Campo et al., 2022). These unique features make AGS not only an efficient treatment technology, but also a highly attractive platform for the recovery of value-added products. In AGS systems, microorganisms are embedded in a complex extracellular polymeric substance (EPS) matrix, which plays a fundamental role in granule formation, stability, and long-term performance. The EPS matrix provides mechanical strength to the granules, promotes cell-to-cell adhesion, and protects the microbial community from hydraulic shear, toxic compounds, and other external stresses. From a resource recovery perspective, EPS represents the main reservoir of recoverable biopolymers within AGS. Alginate-like exopolysaccharides (ALEs) constitute the dominant fraction of EPS in aerobic granules and are commonly referred to as structural EPS (sEPS), due to their key role in maintaining the integrity and stability of the granular structure (Felz et al., 2016). ALEs produced by mixed microbial cultures are complex biopolymers composed of polysaccharides, proteins, humic-like substances, and lipids. Their composition and physicochemical properties depend on operational conditions, substrate characteristics, and microbial community structure. Owing to their strong gelling capacity, biocompatibility, and biodegradability, ALEs have attracted interest for a wide range of potential applications,

Paper Received: 5 December 2025; Revised: 28 January 2026; Accepted: 24 March 2026
Please cite this article as: Corsino S.F., Castiglione M., Campo R., Capodici M., Cosenza A., Di Bella G., Torregrossa M., 2026, Influence of Organic Loading Rate and Feast Regime on Alginate-Like EPS Production from Aerobic Granular Sludge, Chemical Engineering Transactions, 124, 121-126 DOI:10.3303/CET26124021

including in the fields of biotechnology, agriculture, packaging, and biomedical materials. To date, most studies available in the literature have focused on the production and characterization of sEPS extracted from AGS treating municipal wastewater, where relatively stable and balanced influent compositions are typically observed. Conversely, limited information is available on sEPS production from AGS systems treating industrial effluents, which are often characterized by high organic loads, unbalanced nutrient ratios, and strong seasonal variability. Moreover, the combined influence of organic loading rate and feeding regime on alginate-like EPS production has not been systematically investigated so far. Specifically, agro-industrial wastewaters represent an underexplored opportunity for the simultaneous achievement of wastewater treatment and biopolymer recovery. In this framework, the present study evaluates the production and properties of ALEs extracted from AGS treating wastewater from a citrus processing industry, a high-strength effluent with a high C/N ratio. Furthermore, this work investigates for the first time the combined effect of organic loading rate (OLR) and feeding regime on sEPS production yield under industrial wastewater conditions. The results aim to contribute to the understanding of how operational parameters influence biopolymer recovery from AGS, supporting the development of integrated wastewater treatment and resource recovery strategies.

2. Materials and methods

2.1 Plant layout

Structural EPS were extracted from two granular sequencing batch reactors (GSBR), named R1 and R2, treating wastewater from a citrus processing industry. Both GSBRs were column-type acrylic reactors with an operating volume of 4 L and were inoculated with conventional activated sludge. The reactors were operated in parallel under controlled conditions and subjected to different feeding and aeration strategies in order to evaluate their influence on aerobic granule formation and sEPS production.

Both reactors operated with a cycle duration of 4 hours, corresponding to 6 cycles per day, and were fully automated through a programmable logic controller (PLC) that managed all operational phases. Reactor R1 was operated according to a feast–famine strategy and featured a 2-minute feeding phase under complete mixing conditions (0.25 L/min), followed by 58 minutes of anaerobic mixing feast. This phase was followed by an aerobic famine period consisting of 60 minutes of aeration at an air flow rate of 2 L/min. Subsequently, 25 minutes of mixing without aeration were applied to ensure oxygen depletion, followed by a settling phase and a 5-minute effluent discharge. Reactor R2, in contrast, was operated with a rapid 2-minute aerobic feeding phase at a flow rate of 0.25 L/min and subsequently maintained under continuous aeration (2 L/min) for 145 minutes, followed by a settling phase and a 5-minute effluent discharge.

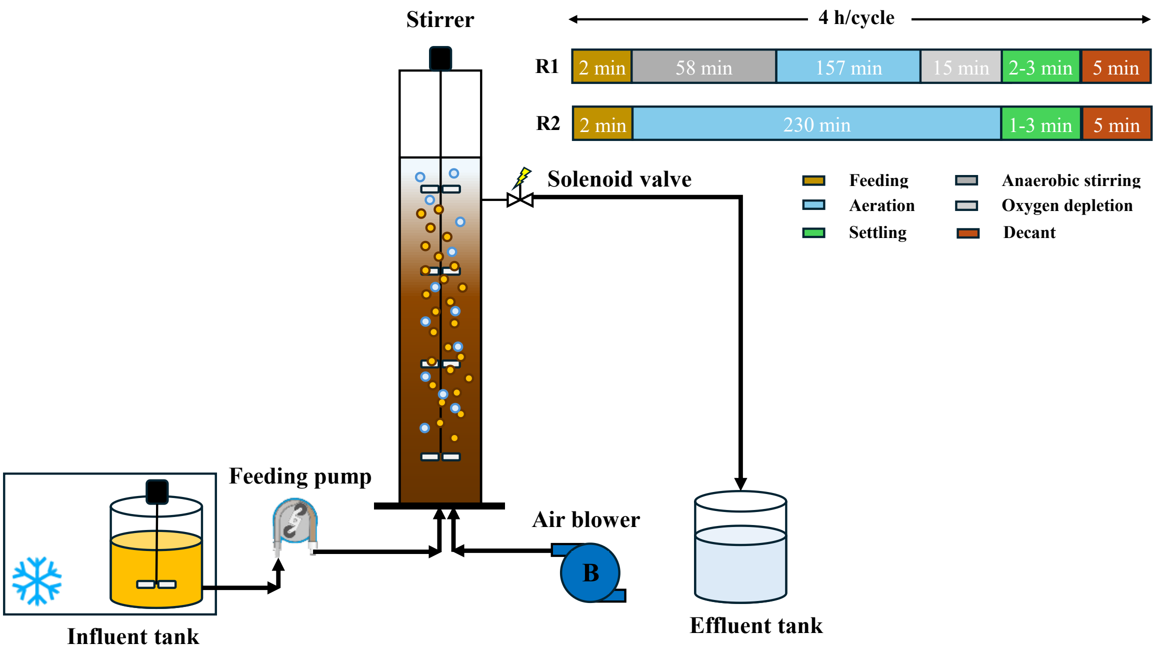

Figure 1: Layout of the experimental reactors.

2.2 Experimental campaign set-up

Granule selection pressure was progressively increased in both reactors by gradually reducing the settling time from 30 minutes to as low as 3 minutes, resulting in a corresponding increase in the minimum settling velocity from 0.18 m/h up to values ranging between 1.8 and 4.6 m/h. This operational strategy was adopted to promote the washout of poorly settling flocs and to favor the formation and retention of dense and well-structured aerobic granules. The duration of the aeration phase was adjusted accordingly to maintain a constant cycle length.
The influent soluble chemical oxygen demand (COD) ranged between 1300 and 2000 mg/L during the experimental period, reflecting the typical variability observed in citrus-processing wastewaters. The daily influent flow rate was set at 3 L/day per reactor. The reactors were operated under stepwise increasing organic loading rates (OLRs) to investigate their effect on granule stability and sEPS production. Specifically, OLRs of 1 kgCOD/m³·d (OLR1, days 0–62), 1.5 kgCOD/m³·d (OLR2, days 63–88), 2 kgCOD/m³·d (OLR3, days 89–102), and 2.5 kgCOD/m³·d (OLR4, days 103–150) were applied. The stepwise increase was designed to reproduce the seasonal variability in organic load typically observed in citrus-processing industrial effluents. The overall experimental campaign lasted approximately 150 days, and sEPS extraction was performed once mature and stable granules were obtained in both reactors.
The carbon to nitrogen and phosphorous ratio was adjusted to 100:3:1, by adding ammonium chloride and potassium di-hydrogen phosphate. This ratio was measured experimentally to avoid limitation in heterotrophic bacteria growth.

2.3 Analytical methods

Structural EPS extraction was carried out following the method described by Felz et al. (2016). Total solids (TS) and volatile solids (VS) of both aerobic granular sludge and extracted sEPS were determined according to Standard Methods (APHA, 2012). Total EPS (tEPS) and sEPS fractions were quantified using colorimetric assays, with proteins expressed as bovine serum albumin equivalents and polysaccharides expressed both as D-glucose and sodium alginate equivalents. All the physical-chemical analyses were performed once per week and in triplicates. Hereafter, the average value was calculated.
Granule size and morphology were characterized through image analysis. Particle size distribution (PSD) was obtained using a high-speed image analysis sensor (Sympatec Qicpic), while additional morphological observations were conducted using a stereomicroscope (Olympus). The percentage of granules (GR) was calculated as the cumulative particle density of particles larger than 200 μm, and the median particle diameter (D50) was assumed as the representative granule size.

3. Results and discussion

3.1 Structural EPS yield

Figure 2 shows the yields of total EPS (tEPS), structural EPS (sEPS), and the sEPS/tEPS yield ratio for reactors R1 and R2 at different OLR.
Overall, the applied OLR strongly influenced both EPS production and composition. Specifically, in the anaerobic feast reactor (R1) (Fig. 2a), the highest total EPS content was observed after 88 days, when the reactor was operating at OLR2 (1.5 kgCOD/m³·d), where EPS yields reached approximately 0.50 gTS_tEPS/gTS_AGS and 0.24 gTS_sEPS/gTS_AGS for tEPS and sEPS, respectively. At the lowest organic loading rate (OLR1), tEPS and sEPS yields were lower (≈0.33 and 0.22 gTS_EPS/gTS_AGS, respectively). When the OLR increased beyond 1.5 kgCOD/m³·d, a decline in EPS content was observed, with tEPS decreasing to about 0.40 gTS_EPS/gTS_AGS at OLR3 and further to ≈0.29 gTS_EPS/gTS_AGS at OLR4. This was accompanied by a similar reduction in sEPS yields. The sEPS/tEPS yield ratio in R1 showed a clear decreasing trend with increasing OLR, dropping from approximately 60% at OLR1 to around 50% at OLR2 and OLR3, and reaching its minimum value of about 45% at OLR4. After about 15 days of operation from the start-up with OLR1, granules' median diameter (D_{50}) grew from 0.10-0.12 mm up to 0.30-0.33 mm and it was kept until day 62. This plateau was likely due to limited substrate availability in the inner layers of microbial aggregates, linked to biofilm mass transfer diffusion-limitation, thus constraining polymer secretion. The highest EPS production at OLR2, due to both the minor effect of the mass transfer diffusion-limitation and the metabolic selection of slow-growing organic matter storing organisms, led to a fast granulation with an increase of D_{50} up to 1 mm after 25 days. Once structurally stable mature granules were formed, microorganisms produce less EPS regardless of the applied OLR. At OLR3, D_{50} reached a plateau at 1.3-1.4 mm, whereas at OLR4 a sensible drop of D_{50} down to 0.8-0.9 mm was registered. This latter occurrence was likely due to at least two reasons: the growth of flocculent fast-growing organisms over the residual particulate organic matter not hydrolysed and not stored during the anaerobic feast phase; largest granules breakage due to the formation of "dead zones" in the inner layers.

In the aerobic feast reactor (R2) (Fig. 2b), both tEPS and sEPS yields increased steadily from OLR1 to OLR3. Specifically, tEPS increased from approximately 0.28 gTS_EPS/gTS_AGS at OLR1 to about 0.47 gTS_EPS/gTS_AGS at OLR2, reaching a maximum of roughly 0.62 gTS_EPS/gTS_AGS at OLR3. Similarly, sEPS increased from ≈0.15 to ≈0.24 gTS_EPS/gTS_AGS over the same range. At OLR4, both yields decreased (tEPS ≈0.45 gTS_EPS gTS_AGS^{-1}; sEPS ≈0.19 gTS_EPS gTS_AGS^{-1}).The sEPS/tEPS yield ratio in R2 was highest at OLR1 (≈55%), decreased markedly at intermediate loadings (≈40% at OLR2 and OLR3), and slightly increased again at OLR4 (≈45%). In R2 a different microbial selection likely occurred, compared to R1, leading to a slower granulation. First granules (D_{50}≈0.2 mm) appeared after 60 days of operation (OLR1) and then they became structurally stable after about 100 days of operation (D_{50}= 0.2-0.3 mm at OLR3), later than R1. Once mature granules were formed, at OLR4 the EPS production was lower regardless of granules dimension that were maintained quite stable.
Compared to R1, R2 generally exhibited lower sEPS/tEPS ratios at comparable OLRs, particularly at OLR2 and OLR3, indicating a relatively smaller contribution of structural EPS under aerobic conditions.
Overall, the quantitative comparison between the two reactors revealed that the anaerobic feast reactor consistently exhibited a higher fraction of structural EPS, especially at low to moderate OLRs, whereas the aerobic feast reactor favored higher total EPS production but with a lower relative contribution of structural EPS.

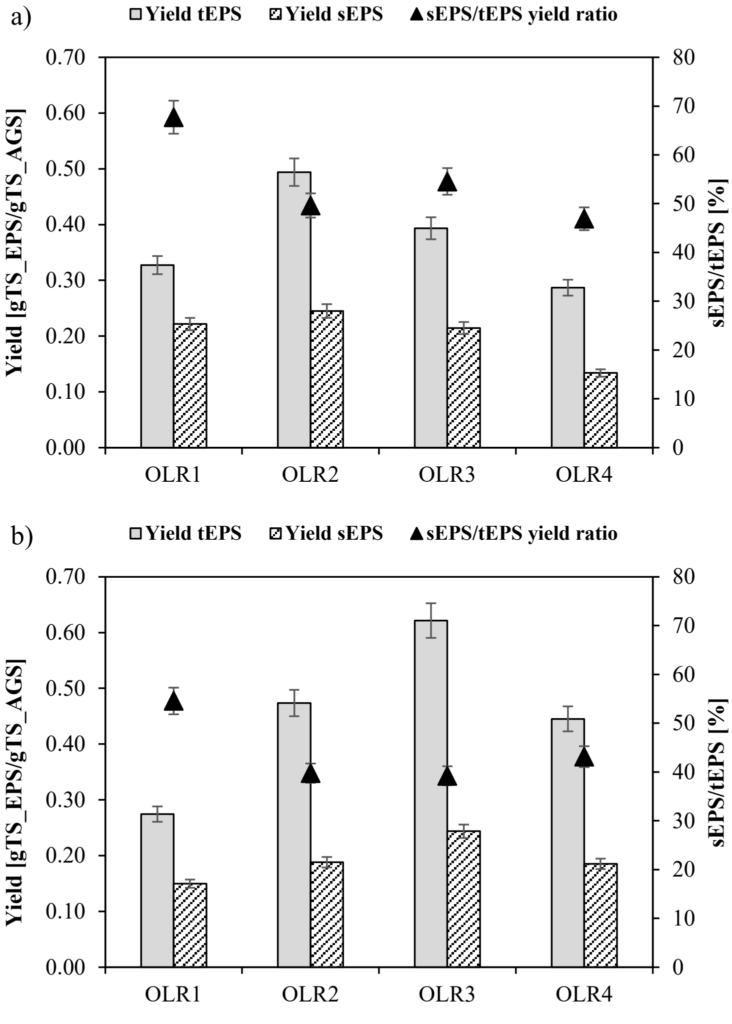

Figure 2: Total EPS (tEPS) and structural EPS (sEPS) yields, and sEPS/tEPS yield ratio (%) in the anaerobic feast reactor R1 (a) and the aerobic feast reactor R2 (b) under different OLRs.

This suggested that anaerobic feast conditions promote a more structurally oriented EPS matrix, potentially enhancing granule cohesion, while aerobic conditions primarily stimulate EPS overproduction in response to increasing organic loads. Overall, these results suggest the existence of an optimal range of organic loading that promotes structural EPS production. In addition, they demonstrate that variations in organic loading influence not only the overall EPS yield but also its composition, potentially affecting granular sludge stability and the suitability of EPS for downstream recovery and valorization.

3.2 Characterization of sEPS

Figure 3 shows the quantitative distribution of EPS components in the anaerobic (R1, Fig. 3a) and aerobic (R2, Fig. 3b) feast reactors at different OLR, with particular emphasis on the alginate-like polysaccharide fraction (PS-ALG).

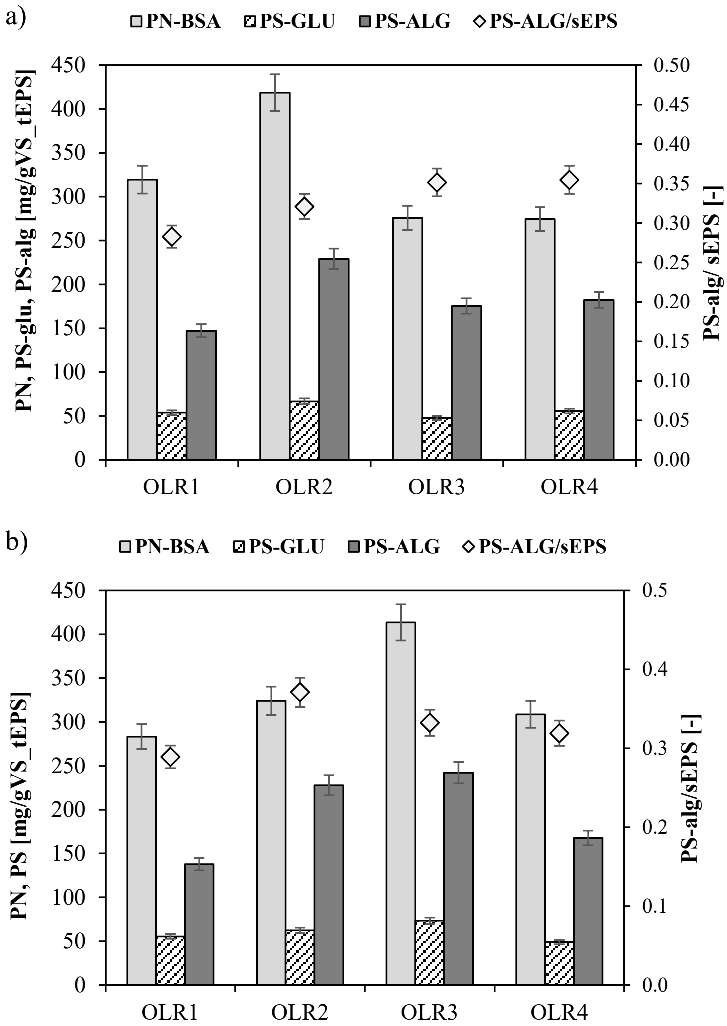

Figure 3: Composition of extracellular polymeric substances (EPS) in the anaerobic feast reactor R1 (a) and the aerobic feast reactor R2 (b) under different organic loading rates (OLRs). Protein as bovine albumin serum (PN-BSA), polysaccharides as glucose (PS-GLU), and polysaccharides as alginate-like (PS-ALG).

In the R1, PS-ALG concentrations ranged approximately from 145 to 230 mg/gVS_tEPS. The lowest value was observed at OLR1 (≈145 mg/gVS_tEPS), while the highest PS-ALG content occurred at OLR2 (≈230 mg/gVS_tEPS), when the granulation reached its peak. Once mature granules were formed, PS-ALG decreased to about 175 mg/gVS_tEPS at OLR3 and about 180 mg/gVS_tEPS at OLR4. The PS-ALG ratio increased slightly from around 0.28 at OLR1 to approximately 0.35–0.36 at OLR3 and OLR4, indicating a relatively stable but progressively higher contribution of alginate-like polysaccharides to the EPS matrix despite the reduction in absolute EPS yields. In the R2, similar PS-ALG concentrations were generally observed, varying approximately between 135 and 245 mg/gVS_tEPS. PS-ALG increased from about 135 mg/gVS_tEPS at OLR1 close to 230 mg/gVS_tEPS at OLR2 and reached a maximum of about 245 mg/gVS_tEPS at OLR3 when the granulation reached its peak. Then the PS-ALG concentration decreased markedly to 165 mg/gVS_tEPS at OLR4.

Overall, the results obtained in this study allowed to demonstrate that both OLR and feeding regime play a key role in regulating the quantity of EPS produced by aerobic granular sludge and also its structural and biochemical composition. The comparison between anaerobic and aerobic feast regimes likely involved distinct microbial

selection pathways, leading to markedly different balances between total EPS and structural EPS fractions. Specifically, anaerobic feast conditions promoted the formation of a more structurally oriented EPS matrix, characterized by higher sEPS/tEPS ratios and enhanced alginate-like polysaccharide content, especially at low to moderate organic loading rates. Conversely, aerobic feast conditions favored higher overall EPS production under increasing organic loads, although with a lower relative contribution of structural components.
The observed trends suggest the existence of an optimal operational window, in which EPS production and granule stability are maximized simultaneously, while avoiding excessive overproduction or structural weakening at high organic loads. Moreover, the evolution of granule size and stability in parallel with EPS composition confirms the tight coupling between microbial metabolism, EPS synthesis, and granular architecture. From a resource recovery perspective, these findings are particularly relevant, as they indicate that EPS quality and extractability can be tailored through operational control, enabling the production of alginate-like biopolymers with potentially adjustable properties. This opens new opportunities for integrating wastewater treatment with the recovery of value-added biopolymers from agro-industrial effluents, contributing to the development of more sustainable and circular treatment schemes. Further investigation is nevertheless required to better link operational conditions to EPS physicochemical properties, microbial selection process and downstream performance in pilot applications.

4. Conclusions

This study demonstrated that organic loading rate strongly influences both the yield and composition of EPS produced by aerobic granular sludge treating citrus-processing wastewater. Anaerobic feast conditions favored a higher relative fraction of structural EPS, contributing to a more cohesive and stable granular matrix, whereas aerobic feast conditions promoted higher total EPS. The stepwise variation of organic loading rate revealed the existence of an optimal loading range for maximizing structural EPS production before mass transfer limitations and microbial competition negatively affected both granule structure and EPS yield. These findings are particularly relevant for agro-industrial wastewaters, which are often characterized by high organic loads and strong seasonal variability, and demonstrate that AGS systems can be effectively operated as platforms for both wastewater treatment and biopolymer recovery. In this context, future studies should specifically investigate the influence of OLR on EPS production and composition under constant OLR conditions, in order to decouple loading effects from long-term adaptation and microbial community shifts. Overall, this study provides novel insights into the combined role of organic loading rate and feeding regime in controlling the production and composition of alginate-like EPS in aerobic granular sludge treating agro-industrial wastewater. By demonstrating how operational conditions can be tuned to influence both granule stability and biopolymer yield, the results contribute to the development of integrated strategies for wastewater treatment and resource recovery from high-strength industrial effluents.

Acknowledgments

This project was founded by SWRIPS (Sustainable Wastewater Re-use with Innovative Purification and Sensing system for the agrifood supply chain) project – PRIMA Section 2 -2022.

References

APHA, 2012, Standard Methods for the Examination of Water and Wastewater, Standard Methods. https://doi.org/ISBN 9780875532356

Campo, R., Carretti, E., Lubello, C., Lotti, T., 2022, Recovery of structural extracellular polymeric substances (sEPS) from aerobic granular sludge: Insights on biopolymers characterization and hydrogel properties for potential applications. J. Environ. Manage. 324, 116247. https://doi.org/10.1016/j.jenvman.2022.116247

Di Bella G, Torregrossa M (2014) Aerobic Granular Sludge for Leachate Treatment. Chemical Engineering Transaction 38:493–498. https://doi.org/10.3303/CET1438083

Felz, S., Al-Zuhairy, S., Aarstad, A.O., van Loosdrecht, M.C.M., Lin, Y.M., 2016, Extraction of structural extracellular polymeric substances from aerobic granular sludge. J Vis Exp. 2016 Sep 26:(115):54534. doi: 10.3791/54534.