Integrating Kinetic Models, Gene Circuits, and Biofilm Dynamics for Enhanced Exopolysaccharide Production in Nitrifying Bacterial Consortia

Indhuja Sakthivel[1], Boobal Rangaswamy[1]*, Bharathkumar Rajagopal[2], Lathika Shanmugam[1]

[1]*Department of Biotechnology, PSG College of Arts & Science, Coimbatore, Tamil Nadu 641014, India*

[2]*Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida 201313, India*

1 *Corresponding author. E-mail address: boobal@psgcas.ac.in (Boobal Rangaswamy)

ORCID: 0000-0002-8270-9755 (Boobal Rangaswamy)

Abstract

Bacterial consortia with extracellular polysaccharides (EPS) serves as key functional components in sustainable wastewater treatment and pollutant removal. This study integrates kinetic modelling and synthetic biology to optimize EPS production and nitrogen removal in a bacterial consortium enriched from domestic wastewater. Employing Monod and Verhulst models, consortia growth was simulated under controlled nitrogen flux (10 ppm NH_4Cl), achieving a maximum biomass concentration (OD_{590} = 5.39) and EPS yield of 2.63 g/mL by day 45. SEM analysis revealed progressive biofilm maturation, transitioning from sparse aggregates to dense EPS-embedded matrices. Nitrogen flux analysis demonstrated efficient substrate utilization, with 80% ammonia oxidized by autotrophic populations (AOB/NOB). PCR amplification confirmed the presence of the exoY gene, central to EPS biosynthesis, enabling the design of a BUFFER-gate logic circuit to regulate succinoglycan production. The consortium's dual capacity for nitrification and denitrification, coupled with high EPS yields, underscores its potential for enhancing wastewater treatment efficiency and biofilm engineering. These findings provide a framework for leveraging microbial consortia in bioremediation and industrial biotechnology through targeted genetic and kinetic optimisation.

Keywords: Bacterial-consortia, EPS, wastewater treatment, Monod model, Verhulst model, logic circuits

1. Introduction

Microbial consortia are dynamic assemblages of bacteria increasingly utilised for their pollutant-removal capabilities, particularly in biological wastewater treatment systems, where EPS play a pivotal role. Microbial consortia offer a cost-effective, eco-friendly alternative to conventional wastewater treatments, leveraging their high biodegradation efficiency to reduce pollutants. The consortium helps to enhance the wastewater quality by decreasing the odour and colour, thereby improving the value of treatment plants (Del Nery et al., 2016). Compared to traditional methods such as chemical treatments and membrane filtration, bacterial biofilms reduce sludge accumulation in large-scale wastewater treatment systems, enhancing operational sustainability (He et al., 2023). The extracellular matrix of bacterial biofilms mostly consists of proteins, nucleic acids, and dead cells, playing a key role in cell-cell communication (Heilmann & Götz, 2009). The formation of consortia is complex, as it includes bacteria from different species growing on surfaces to produce EPS. Chen et al. (2021) identified common bacteria found in biofilms such as *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Streptococcus viridans*, *Staphylococcus aureus*, and *Enterococcus faecalis*. Proteins and polysaccharides are the main components of EPS (Zhao et al., 2019). EPS also comprises uronic acids, humic acids, lipids, and inorganic elements (Guibaud et al., 2012). According to Freire-Nordi et al., (2005), research found that EPS with uronic acid and sulphates can immobilise positively charged metal ions, which is beneficial for water purification. Various factors including the source of sludge, the type of wastewater, and operational conditions influence the composition and concentrations of EPS in sludge aggregates (Yuan et al., 2017). Microbial biofilm might play role in affecting their structure, surface charge, flocculation, settling, dewatering, and adsorption properties in sewage treatment facilities (Sheng et al., 2010). Additionally, EPS has shown potential in environmental remediation by facilitating the removal of pollutants and heavy metals from contaminated areas. The consortium-associated EPS functions in cleaning up the environment (Bhattacharya et al., 2019). Moreover, EPS has exhibited potential in biotechnological research, particularly in the production of enzymes and antibiotics (Andrew & Jayaraman, 2020), and is commonly utilised in the food (Suryawanshi et al., 2022), cosmetics, and pharmaceutical (Waoo et al., 2023) sectors due to its favourable physical, rheological, and safety properties (Xiong et al., 2022). Quorum sensing (QS) regulation has been identified as the most extensively studied regulatory mechanism responsible for controlling the production of EPS, biofilm formation, and differentiation (Yadav et al., 2023; Zhou et al., 2025).

Nitrifying bacteria include ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), which are found in both autotrophs and heterotrophs. Moreover, autotrophic and heterotrophic consortia bacteria

remove heavy metals and nutrients (phosphate and nitrate) from the wastewater and stabilise the downstream treatment process. Autotrophic bacteria gain energy by oxidizing ammonia to nitrates and nitrites or utilizing organic substances as a nitrogen source (Vajda et al., 2011; Xia et al., 2019). Nitrifying consortia enhance EPS production due to their role in microbial granulation and environmental adaptability. These factors contribute to the stability and efficiency of wastewater treatment processes, making nitrifying consortia particularly effective in EPS enhancement (Li et al., 2020; Song et al., 2020).

In bioremediation, the functions of AOB and NOB have been examined using a combination of physiological and molecular in situ techniques (Cai et al., 2018). As various genes related to the synthesis of EPS (exo, epsE, eps9F, epsH, and epsF) are involved in forming the consortia structure, they play a significant role in the advancement of consortia (Janczarek, 2011). In a recent study Wang et al., (2023) reported that the EPS from *Streptococcus thermophilus* has significant applications in the dairy industry and probiotics. Gaining insights into the impact of nitrogen sources on EPS production can optimise fermentation processes, enhance the texture and health benefits of fermented dairy products, and improve the overall functionality of probiotic strains (Korcz & Varga, 2021).

Various mathematical models have been proposed to describe the changing aspects of the metabolism of compounds exposed to cultures of microbial populations of the natural environment (Smith et al., 1997). According to Hwi Jeong & Min Lee, (2013), most growth models rely on Monod kinetics and require the measurement of several parameters. The Monod model is widely used to demonstrate growth-linked substrate utilization. Additionally, Liu, (2006) highlighted the Monod equation as a highly effective model for demonstrating the relationship between specific growth rate and required substrate concentration. (Ruiz et al., 2013) simulated bacterial biomass growth rate using the Verhulst kinetic model. Dmitry Dvoretsky et al., (2015) used Verhulst logical equation for modelling biomass cultivation process. This research aims to enhance the productivity of wastewater treatment plants by integrating the Monod and Verhulst models for consortium bacterial growth. The highest biomass concentration of EPS-producing species significantly increases the consortium growth that occurred at the biofilm-liquid interface (Kreft & Wimpenny, 2001). The Monod/Verhulst model is adept at managing the complex non-linear dynamics typical of microbial EPS production processes, which traditional models with single-factor experiments may not accurately predict (Marques et al., 2017; Plattes & Lahore, 2023).

The gene circuit model using the logic gates represents high advancement in the field of synthetic biology (Lezia et al., 2022). In this study we also focused on developing a gene circuit which helps us to understand how

to regulate the production of EPS by the expression of the required gene, which enables bacteria to adapt to environmental signals and enhance EPS production by maximizing nitrogen source utilization. Overall, optimizing substrate flux kinetics and the development of gene circuits can help us to enhance EPS production efficiency in microbial consortia. Hence, this study aims to construct a logic gene circuit for the EPS pathway in consortia-forming bacteria and to assess their growth kinetics, structural properties, and wastewater treatment potential.

2. Materials and methods

2.1 Sample collection and Synthetic medium preparation

The sludge sample was collected from the domestic wastewater treatment plant in Coimbatore. The sample was transferred to the lab and stored at 4°C. Modified synthetic media was prepared following (0.3g KCl, 0.5g KH_2PO_4, NaCl 2.5, 0.02g NH_4Cl, 0.04g K_2HPO_4, 1.6g of Na_2CO_3, $CaCo_3$ 0.03, 0.5g of $CaCl_2$, and 5g starch (Youssef et al., 2016). The sample inoculated culture flasks were placed in the shaker incubator at 37°C with the revolution set at 150 rpm, an optimal temperature and pH were maintained throughout the study period.

2.2 Consortia development

The culture was enriched with NH_4Cl of 10 ppm for every two days. This addition will provide the microorganisms with a nitrogen source that influences the formation of nitrogen utilizing population. The growth of the bacterial consortia is regularly measured and growth kinetics was determined (Hamzah et al., 2013). The increase in the growth ratio correlated with the increase in the biomass of the culture.

2.3 Mathematical modeling and kinetic expression of consortia growth

This study aimed to predict the consortia development by employing the mathematical model (Sadiq et al., 2018) and focused specifically on nitrogen utilization as a substrate. The Monod model expressed growth kinetics utilizing the relationship between growth and substrate concentration.

$$\mu = \mu_{max}\left[\frac{Si}{k_i + S_i}\right] \qquad (1)$$

Where the specific growth rate is represented by μ, the substrate concentration is denoted as S_i, μ_{max} stands for the maximum specific growth rate and the saturation constant is k_i. Since focused solely on a single substrate type, the specific growth rate, μ can be expressed as

$$\mu = \mu_{max}\left[\frac{S_N}{K_N + S_N}\right] \qquad (2)$$

where S_N represents the concentration of nitrogen in the medium and k_N is the half-saturation constant for nitrogen. The specific growth rate utilised in this study is an expansion of the Monod model.

The Verhulst model is expressed as described in (Abdel-Raouf et al., 2012).

$$X(t) = \frac{X_m X_0 e^{\mu t}}{X_m - X_0 + X_{0e^{\mu t}}} \qquad (3)$$

where x represents the biomass concentration of biofilm, x_m represents the maximum cell concentration that the system can reach in batch, X_0 is initial concentration of microorganism, t represents time and μ is the specific growth rate of the biofilm. The specific growth rate in the extended Monod model (2) is substituted into the Verhulst model (3) to estimate the consortia growth.

2.4 Mathematical modeling and kinetic expression for nutrient uptake by consortia

The equation representing the Verhulst model was used to precisely explain the nutrient absorption by the consortia (Álvarez-Díaz et al., 2017).

$$S = \frac{\left(\frac{X_0}{Y} + S_0\right)(S_0 - S_{na}) - S_{na}\left(\frac{X_0}{Y} + S_0\right)}{(S_0 - S_{na}) - \left(S_0 - \left(\frac{X_0}{Y} + S_0\right)\right) e^{pt}} \qquad (4)$$

where S is the total nutrient concentration, S_0 represents the initial amount of substrate concentration in the culture medium, S_{na} is the unassimilated substrate concentration and p denotes the specific consortia growth rate and Y is the consortia yield coefficient for the ratio of biomass produced per mass of substrate incorporated as organic or structural which can be calculated using the equation as stated below

$$Y = \left[\left(\frac{X_{m-X_0}}{S_{b-}S_{na}}\right)\right] \qquad (5)$$

By some transformations to (4) as outlined in the study by Álvarez-Díaz et al., (2017), the equation can be modified to resemble equation (3)

Hence, conclude that p indicates the specific growth rate of consortia, μ and t have the same relation to estimate consortia growth and nutrient uptake by using the Verhulst model. Therefore, the specific growth rate, μ of the extended Monod model in (2) will be used in place of p for nutrient absorption by the consortium.

$$X(t) = \frac{X_m X_0 e^{\mu t}}{X_m - X_0 + X_{0e^{\mu t}}} \qquad (6)$$

2.5 EPS determination and quantification

The consortia were centrifuged at 12,000 rpm for 15 minutes (sigma 2-16KL, Germany). The supernatant with 95% cold ethanol was vigorously stirred and then kept for 24 h at 4°C to ensure complete EPS precipitation. Subsequently, the precipitates were collected by centrifugation at 12,000 rpm for 20 minutes. The pellet was dried overnight at 60°C (Padmanaban et al., 2015). The recovered EPS was figured out gravimetrically by grams per

mL of culture medium and then made to dry. The dried EPS was weighed for up to 45 days during the culture process.

2.6 Characterization of morphology

The consortium culture was incubated and the pellet was collected using an ultrafiltration after seven days of growth. The pellet was dried and then analysed using SEM to evaluate the growth of consortia and the increase in EPS production within the consortia. The same process was repeated on the 45th day of growth to evaluate any changes during the incubation period (Rangaswamy et al., 2020).

2.7 Physiochemical properties of ammonia, nitrite, nitrate, and nitrogen flux

To determine the effectiveness of the nitrification process in the consortia sample that was fed with NH_4Cl, the levels of ammonia, nitrate, and nitrite values in the samples were estimated and noted by the phenate method (Joseph et al., 2021). The values were measured before feeding, during feeding, and after feeding of NH_4Cl.

2.7.1 Estimation of ammonia

1 mL of sample was taken with 0.4 mL of sodium nitroprusside solution, 1 mL of oxidizing solution (100 mL of alkaline reagent was added to 25 mL of sodium hypochlorite and was mixed) was added, and made up to 10 mL of double-distilled water. The mixture was then incubated for 1h at room temperature. The reading was noted at an absorbance of 640 nm using a UV-vis spectrometer (Solórzano, 1969).

2.7.2 Estimation of nitrite

The 10 mL of a sample was added with 0.2 mL of sulphanilamide solution and 0.2 mL of NED. The reading was taken after 8 minutes at the absorbance of 543 nm.

2.7.3 Estimation of nitrate

A mixture was prepared by adding 10mL of the sample, 0.4 mL of buffer reagent (phenol solution and NaOH 1:1 ratio), 0.2mL of hydrazine sulphate, $CuSO_4$ in a 1:1 ratio and then incubated in the dark for 18-24 hours and again added 0.4mL of acetone, 0.2 mL sulphanilamide, and 0.2 mL NED. The reading was measured after 8 min at the absorbance of 543 nm.

2.8 Determination of inorganic nitrogen flux

The inorganic nitrogen fluxes in the consortia sample were calculated using the following equation (Yan et al., 2024).

$$F = \frac{\Delta C.V}{A.\Delta t}$$

where F (mg $m^{-2}d^{-1}$) represents inorganic nitrogen fluxes; V (m^3) is the volume of the flask; A (m^2) is the bottom area of the flask; Δt (d) denotes the incubation duration; and $\Delta C\left(\frac{mg}{l}\right)$ stands the change in the concentrations of ammonia, nitrite, and nitrate before and after incubation.

2.9 DNA extraction

Genomic DNA was isolated from the sediment using the protocol described by (Araya et al., 2003) with slight modifications. The extracted DNA was added with 10 mL of CTAB buffer and tube was vortexed and placed in a shaking incubator for 10 minutes at 60°C. Subsequently, it was centrifuged at 4000rpm for 15 minutes to separate layers. The supernatant was added with cool Isopropanol and 5M NaCl. Stored at -20°C for overnight. After centrifugation at 4000 rpm for 15 minutes, the entire solution was removed from the pellet. 2 mL of 70% ethanol was added to the tube and centrifuged at 4000 rpm for 2 minutes. The rest of the ethanol was discarded and dried for 30 minutes. 10 mM 20µl of Tris HCl was added and the collected samples were subsequently stored at -20°C.

2.10 PCR and confirmation of amplicons

PCR was used to screen the exoY gene to detect the presence of EPS in the genomic DNA sample from the consortium bacteria with the forward and reverse primers sequence, 5'-ATGCGTATCGACGGTCATC- 3' and 5'CCGAGGGGGGTGTATCTGACCC-3'. The PCR reaction was carried out at a total volume of 10µL as follows: 5µL of Amp GT PCR master mix (Takara Bio Inc), 0.4µL of both forward and reverse primers each, 3.8µL of DNA template, and made up to 10µl final volume with molecular grade water. The PCR conditions were carried out as an initial denaturation step (at 95°C for 5 min), followed by 37 cycles of repetitions of denaturation (at 95°C for 10 sec), annealing at 58°C, extension at 72°C for 1.30 sec with the final extension at 72°C for 10 minutes (Araya et al., 2003). Aliquots of PCR products were analysed by electrophoresis in 1 (w/v) molecular grade agarose (Sigma-Aldrich, USA) gels containing ethidium bromide (0.5 mg mL^{-1}).

2.11 Orthology of functional genes and Logic circuit of the pathway

The gene circuit was constructed to map regulatory interactions governing EPS biosynthesis in nitrifying consortia, using Boolean logic principles adapted from Boscaino et al., (2019). The EPS functional genes for all consortia-forming bacterial groups in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database were extracted from the datasets. KEGG programming interface information regarding the gene orthology of the bacterial organisms was obtained. The orthology content of each organism was converted into a binary logical content vector denoted by "1" (indicating the presence of the ortholog) and "0" (indicating its absence) among all orthologs found in the KEGG database. Modeling circuits of biological pathways focuses on the active events that

lead to activation. Entries are compared in GErel based on the selection of genes in the initial two consecutive reactions.

2.12 Statistical analysis

Statistical analysis was performed to assess the significance of the results. A one-way ANOVA was utilized to estimate the statistical parameters.

3. Results

3.1 Consortia development

The extended Monod and Verhulst models were used to estimate consortia growth on the 45th day in domestic wastewater **(Table 1)**. **Fig. 1** shows consortia growth in wastewater treatment plants, and the maximum cell concentration on the 45th day OD value was 5.39. The sustained increase in OD values throughout the incubation period signifies strong consortia development and maturation, highlighting the importance of long-term culture techniques in studying consortium development. The consortia development values were statistically significant ($p < 0.0005$).

3.2 Characterization of Consortia

Therefore, the SEM examination of consortia development revealed distinct differences between the initial seven days **(Fig. 2a)** and the 45th day of culture growth. During the early stages, the consortia exhibited a sparse and irregular structure with less complexity in the cell structure. In contrast to the 45th day, the consortium growth has significantly increased in complexity, displaying a dense network of clustered cells embedded with a well-developed extracellular matrix and the morphological characteristics of consortia were rod-shaped as expressed in **Fig. 2b and 2c**. SEM analysis of the consortium culture on the initial 10th day showed minimal EPS presence despite the denser EPS matrix interaction among microorganisms **(Fig. 2d)**. By the 45th day, cells exhibited a dense network and superior complexity due to increased EPS formation and well-developed EPS structures **(Fig. 2e and 2f)**.

3.3 EPS formation

The growth of EPS in the culture medium was gradually increased, enhancing the consortia growth and the maximum EPS production on the 45th day was 2.63 g/mL. These results, as given in **Fig. 3**, statistically significantly prove consortia growth, which determines the high production of EPS ($p < 0.05$).

3.4 Substrate utilization and nitrogen flux resembling autotrophic population

The consortia culture was fed with NH_4Cl at an initial concentration of 10 ppm. The amount of ammonia, nitrite, and nitrate was estimated by the phenate method. Therefore, 8 ppm ($avg.$) of ammonia has been utilised by the AOB population for ammonia oxidation and 2 ppm ($avg.$) becomes an unassimilated substrate. Further, in the next process, 5 ppm of ammonia was transferred for the conversion of nitrite formation, and 4.2 ppm ($avg.$) was utilised by the NOB population for nitrite oxidation leaving 0.8 ppm ($avg.$) of unutilised substrate. NOB further transforms 4ppm of nitrite which was effectively transferred to form nitrate. From the estimation of nitrate by phenate method, 2.4 ppm ($avg.$) was utilised by the bacteria and the remaining 1.6 ppm enters into the denitrification process for further metabolism. Therefore, the initial and final substrate concentrations of the nitrogen source were 10 ppm and 2 ppm. This shows that the nitrogen was reduced drastically, proving that the consortia in the wastewater removed the nitrogen which proves treating wastewater efficiently. This analysis highlights how autotrophic bacteria in consortia culture utilise substrates, focusing on the contributions of AOB and NOB populations **(Fig. 5)**. The nitrogen substrate was consumed by the AOB and NOB populations via the processes of nitrification to nitration. In contrast to this, the changes in the nitrogen source concentration from the nitrogen flux **(Fig. 4)** in the consortium culture were also estimated for 45 days which typically involves measuring the changes in the concentrations of nitrogen compounds (ammonia, nitrite, and nitrate) in the culture after feeding the culture with NH_4Cl at the same concentration of 10 ppm **(Fig. 5)**. The statistical analysis evidence showed moderately significant ($p>0.1$).

3.5 PCR and confirmation of amplicons

The exoY gene primer was used to identify the presence of EPS in the consortia sample and produced a DNA fragment between 200-250 bp when annealed at 55°C. Agarose gel image for the obtained PCR product (In well 1, T_a was 51°C; in well 2, T_a was 52°C; in well 3, T_a was 53°C and in well 4 is NTC followed by a 50bp molecular marker in well 5).

3.6 Construction of logic circuits from the amplified PCR product

PCR amplification and orthology data from the KEGG database identified exoY as the sole amplifiable gene linked to EPS synthesis in the consortium, prompting its selection as the circuit's central node. Binary logic gates were defined as follows: A BUFFER gate represented direct, unconditional activation, where exoY presence (input = 1) leads to the activation of ExoL which in turn triggered EPS production (output = 1). AND gates were reserved for hypothetical auxiliary interactions, but no co-activators were amplified, limiting the circuit to a simplified BUFFER architecture. OR gates were omitted due to the absence of redundant EPS genes. The logic

circuit, structured around a BUFFER gate **(Fig. 6)**, mapped exoY activation directly to exoL gene which in turn linked to succinoglycan (EPS) biosynthesis (Reuber & Walker, 1993).

4. Discussion

4.1 EPS Production Dynamics and Biofilm Structural Maturation

The synthesis of EPS by bacterial consortia is intrinsically linked to biofilm maturation, as evidenced by the progressive increase in EPS yield (2.63 g/mL on day 45) and SEM-derived morphological changes. The sparse, irregular biofilm structure observed during the initial seven days transitioned to a dense, interconnected matrix by day 45 **(Fig. 2a–f)**, corroborating the role of EPS in stabilising microbial aggregates. This aligns with studies emphasising EPS as a structural scaffold that facilitates cell adhesion and nutrient retention within biofilms (Bhawal et al., 2022; Zhao et al., 2019). The SEM-derived biofilm architecture corroborates Wang et al., (2023) who linked structural complexity to the choice of substrate. However, further studies are recommended. The rod-shaped morphology of consortia members, consistent with *Rhizobium sps.,* (Kaur et al., 2011), and *Pseudomonas sps.,* (Gangalla et al., 2021), along with the findings of exoY gene, suggests that particular rod shaped bacterial taxa dominate the consortium, potentially contributing to the observed EPS overproduction (Chen et al., 2021; Sellami et al., 2015). The temporal correlation between biofilm complexity and EPS accumulation underscores the necessity of prolonged incubation (45 days). However, achieving optimal EPS yields in a short period can be further analyzed with gene expression studies, a critical factor for industrial applications requiring high biopolymer output in a relatively short amount of time.

The gravimetric quantification of EPS revealed a gradual increase, peaking at 2.63 g/mL by day 45 **(Fig. 3)**. This trend mirrors microbial growth phases, where EPS production is elevated during exponential growth due to active nutrient assimilation and declines during stationary phases due to resource depletion. The sustained EPS synthesis in this study, however, contrasts with earlier reports of peak EPS yields of 0.042g/ml in 96 hours (Sellami et al., 2015), highlighting the advantage of consortium-based systems over monocultures in prolonging productive phases. The observed EPS yield surpasses values reported for monocultures like Pseudomonas aeruginosa, Micrococcus sps. (Yin et al., 2022) and L. plantarum (Zhang et al., 2023), underscoring the advantage of consortia in leveraging metabolic diversity. The integration of ammonium chloride as a nitrogen source likely enhanced autotrophic nitrifier activity, further driving EPS biosynthesis through metabolic synergy between ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Moreover, discrepancies in optimal pH

(Jyoti et al., 2024) and incubation duration (Sellami et al., 2015) highlight context-dependent variability in EPS production, necessitating strain-specific optimisation.

4.2 Kinetic Modelling of Consortium Growth and Substrate Utilisation

The Monod and Verhulst models provided robust frameworks for analysing consortium growth kinetics and nitrogen substrate utilisation. The Monod-derived maximum specific growth rate (μ_{max} = 5.39) and half-saturation constant (k_n = 2.69) **(Table 1)** reflect efficient nitrogen assimilation, particularly under high substrate availability (S_n = 10 ppm). The Verhulst model further shows that biomass accumulation, predicting a maximum cell concentration (X_m = 1.63) that aligns with the observed OD_{590} of 5.39 **(Fig. 1)**. The nitrogen flux analysis revealed that AOB oxidised 80% of the initial ammonium (8 ppm), while 42% of nitrite (4.2 ppm) was further metabolised by NOB. The residual nitrate (1.6 ppm) entering denitrification pathways underscores the consortium's dual role in nitrification and partial denitrification, a trait advantageous for wastewater treatment.

4.3 Gene Circuit Engineering and *exoY* Amplification in EPS Biosynthesis

The exclusive amplification of exoY gene highlights its dominant role in EPS biosynthesis within the consortium. KEGG orthology traces exoY to Rhizobiaceae, suggesting Rhizobium-related species, which is known for its symbiotic nitrogen fixation and succinoglycan production which may dominate the consortium (Jones, 2012; Ratib et al., 2018). While Rhizobium is not classically autotrophic, its presence could synergise with undetected ammonia-oxidizing bacteria (AOB), as Rhizobium-derived EPS may stabilise biofilms, enhancing retention of autotrophic nitrifiers. The observed nitrification efficiency likely arises from such metabolic partnerships, where Rhizobium's heterotrophic activity complements autotrophic ammonia oxidation by AOB. The BUFFER gate's simplicity reflects the consortium's reliance on exoY driven EPS synthesis. Moreover, the consortium's higher EPS yield (2.63 g/mL) suggests that using EPS producing bacterial consortium in water treatment plants, particularly by over expressing exoY gene is promising when compared to single strains (9.2 ± 0.66 g/L in *Rhizobium leguminosarum*) used in previous studies (Sellami et al., 2018). Future work could integrate inducible promoters to further enhance EPS titres.

4.4 Implications for Wastewater Treatment and Biotechnological Applications

The consortium's ability to reduce ammonium from 10 ppm to 2 ppm over 45 days demonstrates its efficacy in nitrogen removal, a critical metric for wastewater treatment. However, enhancing exoY gene expression might reduce this time frame further. The sequential oxidation of ammonia to nitrate, coupled with partial denitrification, could be a sustainable alternative to traditional methods. The EPS matrix's metal-binding

capacity, attributed to uronic acids and sulphates (Freire-Nordi et al., 2005), positions the consortium as a candidate for bioremediation of heavy metal-laden effluents. This opens the door for further research in the field of bioremediation.

5. Conclusion

This study demonstrates the synergistic potential of microbial consortia in wastewater treatment, driven by EPS-mediated biofilm development and nitrogen metabolism. By coupling Monod and Verhulst kinetic models, consortia growth and substrate utilisation were accurately predicted, achieving maximal EPS production (2.63 g/mL) and 80% nitrogen removal over 45 days. SEM imaging validated biofilm structural maturation, correlating EPS accumulation with microbial aggregation. The identification of the exoY gene and subsequent logic circuit design highlighted its pivotal role in regulating EPS biosynthesis, offering a genetic toolkit for enhancing biopolymer yields. Future work should explore exoY overexpression under inducible promoters to accelerate EPS production and investigate field-scale applications for heavy metal bioremediation. This integrative approach is bridging kinetic modeling, genetic circuitry, and microbial ecology and advances the rational design of consortia for environmental and industrial applications.

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Conflict of interest

The authors declare that they have no conflict of interest.

Informed consent statement

Not applicable.

Consent to publish

All authors approved the manuscript and gave their consent for submission and publication.

Data availability statement

There is no research related data stored in publicly available repositories, and the data will be made available on request.

Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

Indhuja Sakthivel: Methodology, Validation, Formal analysis, Writing - Original Draft. **Boobal Rangaswamy:** Conceptualization, Methodology, Validation, Formal analysis, Visualization, Data curation, Writing – original draft, Writing –review & editing, Supervision, Investigation. **Bharathkumar Rajagopal:** Writing - Original Draft, Writing - Review & Editing. **Lathika Shanmugam:** Data Curation, Visualization, Writing - Original Draft, Writing - Review & Editing.

Declaration of competing interest

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Table 1

Parameters derived for the kinetics of consortia growth and substrate utilisation

Parameters	Values obtained
μ_{max}	5.390
k_N	2.690
S_N	10
X_0	0.42
X_m	1.630