

BIOFILM MODELING AND BACTERIAL TRACKING IN DRIP IRRIGATION LABYRINTH CHANNELS

MODÉLISATION DU BIOFILM ET SUIVI DES BACTÉRIES DANS LES CANAUX LABYRINTHIQUES D'IRRIGATION GOUTTE À GOUTTE

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ABSTRACT

Drip irrigation supplied with reclaimed wastewater (*RWW*) is an effective strategy for improving water-use efficiency and reducing freshwater demand in agriculture. However, milli-labyrinth channels in drip emitters are susceptible to biofouling and clogging due to their complex flow patterns and the microbial and organic load carried by *RWW*. This study investigates the mechanisms governing particle and bacterial attachment, and biofilm development in a square-cross-section milli-labyrinth channel (0.63 mm^2) through an integrated experimental and numerical approach. Channel hydrodynamics were characterized using micro-particle image velocimetry and computational fluid dynamics (CFD). Comparison between experimental measurements and numerical predictions showed that the $k - \varepsilon$ turbulence model accurately represented the flow field (percentage difference in range (PDR) below 8%), outperforming the $k - \omega$ model (39% PDR).

As a result, the $k - \varepsilon$ model was used for particle and bacterial tracking. Particle and bacterial transport and initial attachment were analyzed using a CFD-Discrete Phase Model, micro-particle tracking velocimetry, and green fluorescent protein-labeled *Escherichia coli* (GFP-*E. coli*). Results indicated that particles with trapping probabilities over 0.5 tended to deposit near the first inlet baffle. Experimental observations confirmed that both passive particles and bacteria accumulated in low-turbulence areas near the inlet. Additionally, passive particles attached to walls exposed to the mainstream flow, while GFP-*E. coli* attached to vortex regions behind baffles. Biofilm growth was assessed using biofilm modeling, and found that biofilm development was concentrated near the channel inlet, where nutrient availability was highest.

Simulations revealed that Chemical Oxygen Demand (limiting substrate) depletion along the channel, with concentrations in the middle baffles 5–6 orders of magnitude lower than those near the inlet. Biofilm growth was enhanced during non-irrigation periods, when residual water supported bacterial development. These findings demonstrate that biofouling results from coupled hydrodynamic and nutrient-driven processes, highlighting the importance of emitter design and drainage optimization for sustainable *RWW* irrigation systems.

Keywords: Biofilm modeling; Drip irrigation; Wastewater; Numerical approach; Fluid dynamics.

RÉSUMÉ

L'irrigation goutte à goutte alimentée par des eaux usées traitées (*RWW*) constitue une stratégie efficace pour améliorer l'efficacité d'utilisation de l'eau et réduire la demande en eau douce dans l'agriculture. Toutefois, les canaux labyrinthiques millimétriques des goutteurs sont sensibles au bio-

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encrassement et au colmatage en raison de leurs profils d'écoulement complexes et de la charge microbienne et organique transportée par les RWW. Cette étude examine les mécanismes régissant la fixation des particules et des bactéries ainsi que le développement du biofilm dans un canal labyrinthique millimétrique de section carrée ($0,63 \text{ mm}^2$) au moyen d'une approche expérimentale et numérique intégrée. L'hydrodynamique du canal a été caractérisée par micro-vélocimétrie par images de particules et par mécanique numérique des fluides (CFD). La comparaison entre les mesures expérimentales et les prédictions numériques montre que le modèle de turbulence $k - \varepsilon$ représente fidèlement le champ d'écoulement (pourcentage de différence dans la plage (PDR) inférieur à 8 %), surpassant le modèle $k - \omega$ (PDR de 39 %).

Le modèle $k - \varepsilon$ a donc été utilisé pour le suivi des particules et des bactéries. Le transport et la fixation initiale des particules et des bactéries ont été analysés à l'aide d'un modèle CFD de phase discrète, de la micro-vélocimétrie par suivi de particules et de bactéries *Escherichia coli* marquées par la protéine fluorescente verte (GFP-*E. coli*). Les résultats indiquent que les particules dont la probabilité de piégeage était supérieure à 0,5 avaient tendance à se déposer près du premier déflecteur d'entrée. Les observations expérimentales ont confirmé que les particules passives et les bactéries s'accumulaient dans les zones de faible turbulence proches de l'entrée. En outre, les particules passives se fixaient aux parois exposées à l'écoulement principal, tandis que GFP-*E. coli* se fixaient dans les zones tourbillonnaires situées derrière les déflecteurs. La croissance du biofilm a été évaluée au moyen d'une modélisation du biofilm et s'est révélée concentrée près de l'entrée du canal, où la disponibilité en nutriments était la plus élevée.

Les simulations ont mis en évidence une diminution de la demande chimique en oxygène (substrat limitant) le long du canal, avec des concentrations au niveau des déflecteurs centraux inférieures de 5 à 6 ordres de grandeur à celles observées près de l'entrée. La croissance du biofilm était favorisée pendant les périodes sans irrigation, lorsque l'eau résiduelle soutenait le développement bactérien. Ces résultats montrent que le bio-encrassement résulte de processus hydrodynamiques et nutritionnels couplés, soulignant l'importance de la conception des goutteurs et de l'optimisation du drainage pour des systèmes d'irrigation durables utilisant les RWW.

Mots clés : Modélisation du biofilm; Irrigation goutte à goutte; Eaux usées traitées; Approche numérique; Numérique des fluides.

INTRODUCTION

Effective water management is essential for addressing global water scarcity, particularly in agriculture, the largest consumer of freshwater (Hoekstra *et al.*, 2012). Drip irrigation, coupled with RWW, offers a sustainable solution by delivering water efficiently while supplying nutrient-rich irrigation, thereby reducing dependence on freshwater (Worako, 2015; Tripathi *et al.*, 2014; Wang *et al.*, 2013). Among these systems, milli-labyrinth channels in drip emitters regulate discharge through their maze-like geometry, ensuring uniform distribution and minimal water loss.

However, their narrow dimensions and complex flow pattern make them prone to clogging and biofouling, which compromise performance. Low-velocity zones, vortices, and sharp turns promote the attachment of particles and bacteria, while the organic-rich RWW accelerates biofilm growth (Hao *et al.*, 2018). Previous studies have shown that biofouling primarily occurs near the inlet and in vortex regions, where shear stress is low and nutrient availability is high (Lequette *et al.*, 2020). Understanding the interaction between flow dynamics and microbial processes is critical for identifying the mechanisms of channel clogging and biofouling (Alazzam *et al.*, 2026).

At the microscale, bacterial transport and attachment are strongly influenced by flow conditions. Microfluidic studies show that shear gradients and flow curvature drive cell accumulation and adhesion (Secchi *et al.*, 2020). Techniques such as particle tracking velocimetry (PTV) and single cell analysis further enable quantification of key physiological parameters, including lag and generation times (Schwanbeck *et al.*, 2020).

The initial attachment phase is often reversible; it marks a critical step in biofilm formation, followed by extracellular polymeric substance (EPS) production and biomass growth. EPS ensures structural cohesion within a three-dimensional matrix, while surface properties modulate attachment stability (Flemming *et al.*, 2016). Once established in hydrodynamically favorable regions, biofilms can rapidly expand and accelerate clogging (Taute *et al.*, 2015).

Biofilm modeling provides a framework to integrate these coupled processes across scales, including cell adhesion, biomass growth, substrate diffusion, and fluid–biofilm interactions (Delavar and Wang, 2022). Existing approaches span continuum and discrete formulations, differing in spatial resolution and complexity but sharing the goal of predicting biofilm dynamics and interpreting experimental observations (Wang and Zhang, 2010). In milli-labyrinth channels, continuum models are particularly suited to capture macroscale transport and growth processes, while discrete approaches offer complementary insight into microscale heterogeneity (Mattei *et al.*, 2018).

Despite these advances, the mechanisms governing the spatial localization of biofilm growth remain poorly understood. Although numerous studies report more clogging near inlet regions, the quantitative links between local hydrodynamics, bacterial attachment, and biofilm development are not yet established.

Here, we combine experiments and modeling to investigate the mechanisms driving inlet-dominated biofilm formation in milli-labyrinth channels. Channel hydrodynamics were characterized using micro-particle image velocimetry (micro-PIV), and CFD were used to analyze the channel hydrodynamics. micro-particle tracking velocimetry (micro-PTV) and CFD-discrete phase model (DPM) are used to identify particle and bacterial attachment zones, and a coupled transport–growth model is developed to describe substrate diffusion and biofilm evolution under stagnant conditions. By linking hydrodynamics, bacterial attachment, and growth dynamics, this study provides a quantitative framework for predicting spatial biofilm development and its role in clogging in drip irrigation systems.

METHODOLOGY

Geometry of the studied milli-labyrinth channel

For the present simulations and laboratory experiments, a non-pressure-compensating (NPC) labyrinth channel with fourteen baffles was used, as illustrated in **Figure 1**. To allow optical access for micro-PIV and micro-PTV experiments, the material is transparent (PMMA: poly-methyl methacrylate). All the dimensions are presented in **Table 1**. The Reynolds number (Re) of the studied emitter ranged from 173 to 693 at the channel inlet, which indicates that the flow behavior corresponds to a turbulent regime as the transition from laminar to turbulent flow in a labyrinth channel occurs at a critical Re ranging between 43 and 94 (Zhang *et al.*, 2016).

Flow Characterization

The hydrodynamics of a milli-labyrinth drip irrigation channel were investigated using a combined numerical and experimental approach. Three-dimensional CFD simulations were performed in *ANSYS Fluent 2024 R2* by solving the Reynolds-Averaged Navier–Stokes (RANS) equations under steady-state and incompressible flow conditions. Water properties at room temperature were assumed, with a density of $998.2 \text{ kg} \cdot \text{m}^{-3}$ and a dynamic viscosity of $10^{-3} \text{ Pa} \cdot \text{s}$.

A high-resolution unstructured mesh with near-wall refinement was generated to accurately resolve the complex flow structures present in the labyrinth geometry, including main flow and vortices. Mesh independence, discretization schemes, and convergence criteria were systematically evaluated. The SIMPLE algorithm was used for pressure–velocity coupling, and second-order spatial discretization schemes were adopted to improve solution accuracy. Both the standard $k - \varepsilon$ and SST $k - \omega$ RANS turbulence models were tested against experimental measurements.

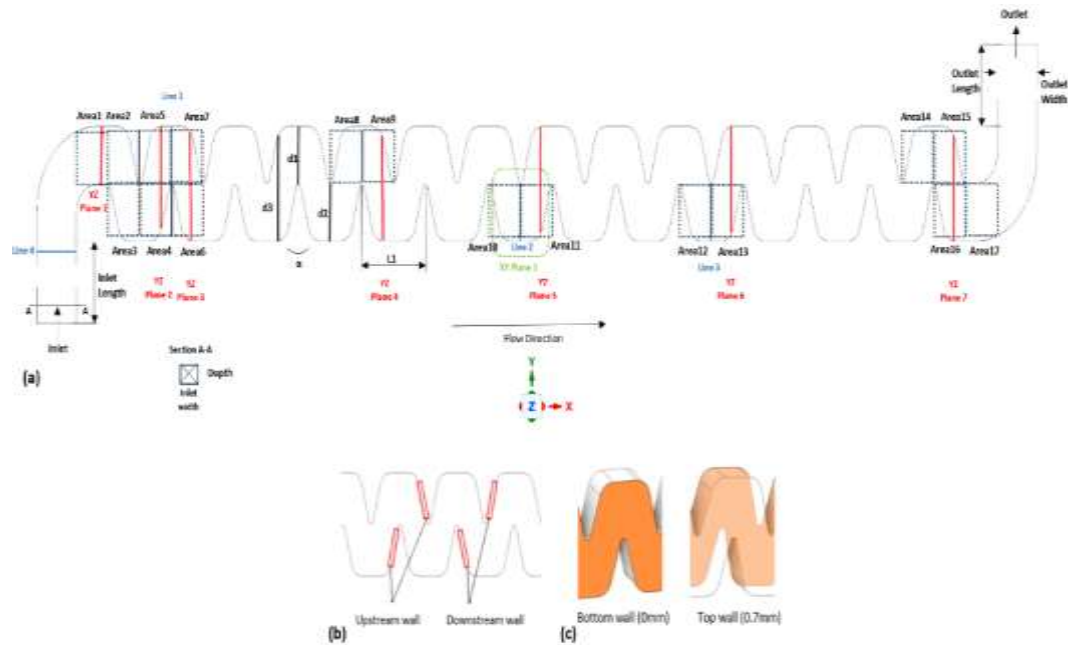


Figure 1. Current labyrinth channel geometry for CFD numerical simulations, micro-PIV, and micro-PTV experiments **(a)** locations of the red lines inside the channel indicating the zones where the results were analyzed, **(b)** and **(c)** key geometrical regions.

Table 1. Geometrical and flow parameters of the system are depicted in **Figure 1**.

Parameter Group	Parameter	Value	Unit
Inlet	Length	29	mm
Inlet	Width	0.9	mm
Outlet	Length	29	mm
Outlet	Width	0.9	mm
Channel Geometry	Depth	0.7	mm
Channel Geometry	Hydraulic diameter (D_h)	0.788	mm
Unit Geometry	d1	0.91	mm
Unit Geometry	d2	0.91	mm
Unit Geometry	d3	1.64	mm
Unit Geometry	L1	1.44	mm
Angles	α	31	°
Flow Characteristics	Re	173–693	–
Flow Characteristics	Flow rate (q)	0.5–2.0	$L \cdot h^{-1}$

Experimental validation was performed using micro-PIV. Distilled water was seeded with 1 μm (535/575nm) fluorescent polystyrene particles. Flow visualization was achieved using a double-pulsed Nd: YAG laser and an sCMOS camera mounted on an inverted microscope. Measurements were conducted at the mid-height plane of the channel. Velocity fields were calculated using ensemble cross-correlation analysis of 500 image pairs, ensuring statistical convergence. Comparison between CFD predictions and micro-PIV measurements demonstrated that the selected numerical setup accurately reproduced the velocity distribution and vortex structures within the channel.

Particle and Bacterial Transport and Initial Attachment

The transport and attachment of *E. coli*-mimicking particles (ECPs) were investigated using a coupled CFD-DPM approach and validated experimentally using micro-PTV. In the numerical simulations, particle trajectories were calculated using a Lagrangian framework under one-way coupling conditions, assuming that the dispersed phase had a negligible effect on the fluid flow. ECPs were represented as non-spherical particles with geometrical and physical properties similar to *E. coli*, including a density of $1100 \text{ kg} \cdot \text{m}^{-3}$ and dimensions representative of rod-shaped bacterial cells. Particle motion was governed by drag, rotational forces, and turbulent dispersion. Turbulent fluctuations were incorporated through the Discrete Random Walk (DRW) model, allowing stochastic representation of particle transport in turbulent eddies.

Boundary conditions consisted of reflection at the inlet, escape at the outlet, and trapping at channel walls. When a particle approached a wall within its equivalent radius, it was considered attached. Because the DRW model introduces stochastic variability, repeated simulations were conducted to estimate trapping probabilities and confidence intervals for each inlet position. Statistical convergence was evaluated using a 95% confidence level (CI).

Experimental observations were performed using micro-PTV with fluorescent $1 \mu\text{m}$ (505/515nm) tracer particles (selected due to their similarity in size to *E. coli* cells) and GFP-labeled *E. coli* (ATCC 25922GFP). An optimal particle concentration was first determined to minimize aggregation while maintaining sufficient particle counts for analysis. Images were acquired using a continuous-wave laser and fluorescence microscopy at different flow rates ranging from 0.5 to $2 \text{ L} \cdot \text{h}^{-1}$. Following image acquisition, *ImageJ* software was used for image processing, thresholding, and particle counting. The Yen thresholding algorithm was selected based on its superior detection performance.

Particle and bacterial attachment were quantified along different regions of the labyrinth channel. The experiments were designed to capture the initial attachment stage immediately after flow stopping, thereby minimizing the influence of particle aggregation and biofilm maturation. Comparisons between CFD-DPM predictions and micro-PTV observations enabled the identification of attachment regions and the influence of local hydrodynamics on particle and bacterial attachment.

Biofilm Modeling

Biofilm development was investigated through a mechanistic mathematical model that coupled nutrient transport and microbial growth under conditions representative of RWW irrigation systems. The model was based on Monod-type kinetics, which describe microbial growth as a function of substrate availability while accounting for biomass production and decay.

The analysis focused on intermittent irrigation conditions, characterized by alternating flow and no-flow periods. During irrigation, bacterial cells were assumed to remain in the lag phase because of the short hydraulic residence times. Significant biofilm growth was therefore assumed to occur mainly during the stagnant period following irrigation, when attached bacteria adapted to local conditions and entered the exponential growth phase.

Chemical Oxygen Demand (COD) was identified as the limiting substrate governing biofilm development, while nitrogen and phosphorus were considered available in excess. Under stagnant conditions, nutrient transport was assumed to be dominated by diffusion rather than convection.

A coupled one-dimensional model ($1D_{\text{vertical}} \times 1D_{\text{axial}}$) was developed to simulate substrate transport along the channel and nutrient diffusion into the biofilm layer. The model linked substrate availability to biofilm growth dynamics and allowed prediction of spatial variations in biofilm thickness. Simulations were performed over a 24-hour irrigation cycle (2 hours of irrigation/22 hours of stagnant phase). The vertical model $1D_{\text{vertical}}$ describes substrate transport through the biofilm and surrounding liquid layer, while the axial model $1D_{\text{axial}}$ accounts for variations along the channel length.

The model was used to evaluate nutrient (*COD* in the current case) depletion along the channel and its impact on biofilm distribution. Particular attention was given to the influence of diffusion-driven transport during non-irrigation periods, which was hypothesized to promote biofilm growth near the channel inlet. The modeling framework provided a mechanistic basis for interpreting the experimental observations of bacterial attachment and biofilm accumulation and for assessing the combined effects of hydrodynamics, nutrient availability, and irrigation management on biofouling development.

RESULTS AND DISCUSSIONS

Flow Analysis Using CFD and Micro-PIV

Pressure-loss predictions were compared with micro-PIV measurements to evaluate the performance of the turbulence models. The standard $k - \varepsilon$ model closely reproduced the experimental pressure losses across all investigated flow rates, whereas the SST $k - \omega$ model overpredicted the pressure drop, as shown in Figure 2.

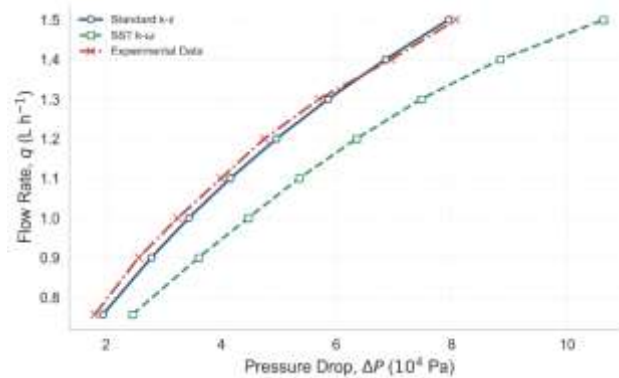


Figure 2. Pressure drop in the channel using experimental data (red), standard $k - \varepsilon$ (blue), and SST $k - \omega$ (green) for flow rates from 0.5 ($Re = 173$) to 1.5 ($Re = 520$) ($L \cdot h^{-1}$).

Velocity profiles at the inlet (Line 4) showed good agreement between CFD and micro-PIV measurements. At $Re = 262$, the measured centerline velocity was $0.636 \text{ m} \cdot \text{s}^{-1}$, matching the standard $k - \varepsilon$ prediction and remaining close to the SST $k - \omega$ result. The ratio of centerline to mean velocity indicated that the flow was still developing at the inlet. Velocity profiles measured across the baffles further confirmed the superior performance of the standard $k - \varepsilon$ model. Along Lines 1-3, the percentage difference in range (PDR) between standard $k - \varepsilon$ and micro-PIV remained below 8%, whereas the SST $k - \omega$ model exhibited deviations up to 39%. Similar agreement was observed for the secondary velocity component, demonstrating that the standard $k - \varepsilon$ model successfully reproduced the curvature-induced secondary flow structures. Mean velocity contour maps also showed strong agreement between micro-PIV and the standard $k - \varepsilon$ model, while SST $k - \omega$ overestimated velocity magnitudes throughout large portions of the channel. This behavior is attributed to the SST formulation promoting premature turbulence development in the inlet region, where the flow remains hydrodynamically developing.

Overall, the standard $k - \varepsilon$ model provided the best compromise between accuracy and computational cost and was therefore selected for subsequent particle and bacterial tracking.

Particle and Bacterial Tracking

Convergence analysis showed that particle trajectories became independent of the maximum number of tracking steps beyond 60,000 steps. However, 100,000 steps were selected for simulations. A statistical convergence study showed that 150 tracking attempts per inlet location produced stable trapping probabilities within a 95% CI. CFD-DPM simulations revealed that particles were trapped

along the upstream walls of the first baffle. High trapping probabilities (> 0.5) were concentrated near the upper and lower channel walls at the inlet, whereas particles entering through the channel centre exhibited lower trapping probabilities. Increasing the inlet sampling density confirmed that this pattern was independent of spatial resolution.

Most trapped particles accumulated near the first baffle, indicating that early trapping close to the channel inlet is a dominant mechanism controlling particle attachment. Because particle response times were shorter than the Kolmogorov time scale, particle inertia was negligible ($S_t \approx 10^{-5}$), and particle trajectories followed the local flow structures as shown in **Figure 3a**.

Micro-PTV measurements showed strong spatial variability in particle attachment. For flow rates between 0.75 and $2 \text{ L} \cdot \text{h}^{-1}$, The largest number of attached particles occurred near the first baffle, followed by a gradual decrease downstream. At the lowest flow rate ($0.5 \text{ L} \cdot \text{h}^{-1}$), attachment shifted toward downstream regions, indicating enhanced particle transport before attachment. Particle counts decreased with increasing flow rate, suggesting that higher wall shear stress promotes detachment. ANOVA confirmed significant effects of channel location, flow rate, and observation depth ($p < 0.001$), together with significant interaction effects as shown in **Figure 3b**.

Micro-PTV measurements using GFP-labelled *E. coli* showed similar spatial trends. Bacterial attachment was highest near the inlet and decreased downstream. Increasing flow rate reduced bacterial attachment, confirming the influence of hydrodynamic forces on retention. Unlike passive particles, *E. coli* attachment was greater on the top wall than on the bottom wall ($p = 0.015$). This asymmetry may result from differences in surface roughness introduced during channel fabrication, with rougher surfaces providing additional attachment sites and local protection from shear forces, as shown in **Figure 3c**.

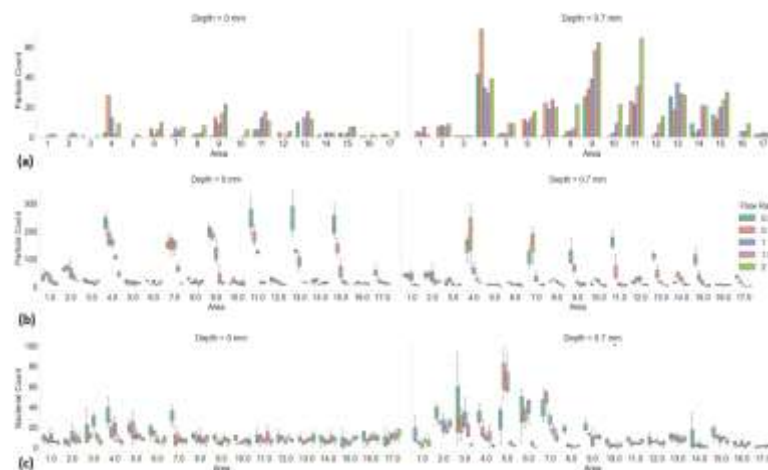


Figure 3. CFD-DPM and micro-PTV area comparison (Area1 - Area17 as presented in **Figure 1**) by counting the attached particles and bacteria in each area (image) at the bottom wall and at the top wall for different flow rates 0.5 in teal ($Re = 173$), 0.75 in orange ($Re = 262$), 1 in blue ($Re = 347$), 1.5 in pink ($Re = 520$), 2 in green ($Re = 693$) ($\text{L} \cdot \text{h}^{-1}$). **(a)** CFD-DPM with ECPs, **(b)** micro-PTV with passive particles, and **(c)** micro-PTV with GFP-labelled *E. coli*.

Both CFD-DPM and micro-PTV observations, therefore, indicate that initial attachment is concentrated near the inlet region, although subsequent biofilm development is governed by additional mechanisms beyond initial bacteria attachment alone.

Biofilm Modeling

The spatial distribution of bacterial attachment suggests that biofilm development is initiated near the inlet region of the channel. However, the observed attachment patterns alone cannot explain the biofilm distributions previously reported in labyrinth channels, where mature biofilms frequently extend

into downstream regions. Time-scale analysis demonstrated that vertical diffusion occurs much faster than both biofilm growth and axial transport. Consequently, a pseudo-steady approximation was applied in the direction normal to the wall, allowing substrate fluxes and local growth rates to be obtained from a steady-state vertical sub model and incorporated into a transient axial framework.

The model predicts that substrate availability decreases near regions of active biofilm growth due to local consumption. As biofilm thickness increases, diffusion limitations become increasingly important, reducing substrate availability within deeper biofilm layers and slowing growth near the wall. This feedback between growth and substrate transport enables the model to capture the transition from rapid initial colonization to diffusion-limited biofilm development, as illustrated in **Figure 4**.

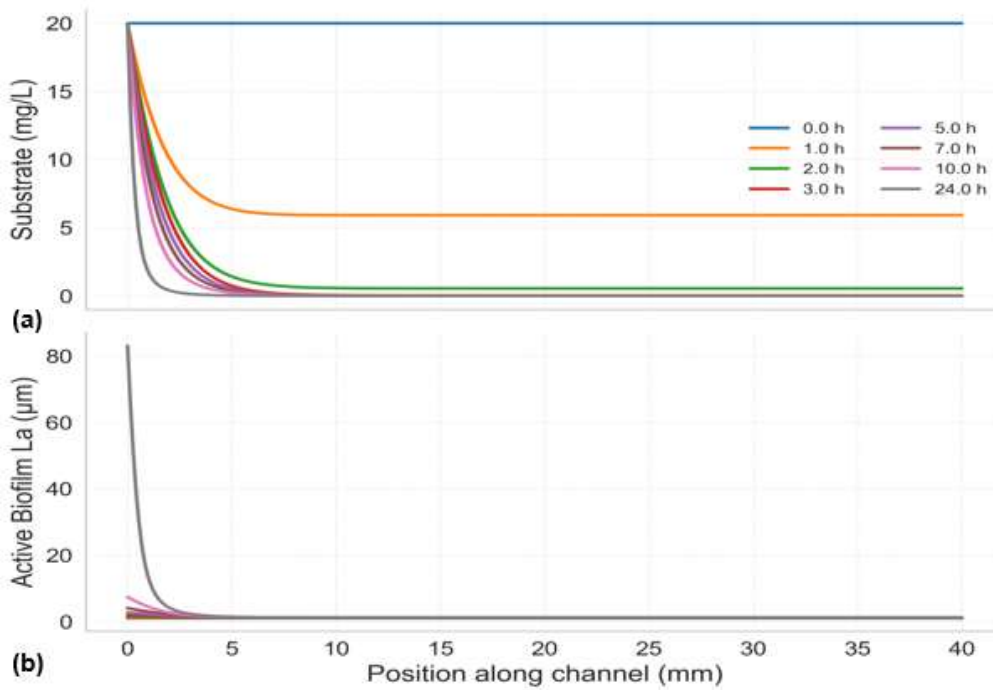


Figure 4. Over 24 hours of the ($1D_{vertical} \times 1D_{axial}$) simulation (representing 1 irrigation cycle), **(a)** substrate (COD) concentration depletion due to the biofilm consumption, the figure shows that further baffles faced a COD depletion after 2 hours, while near the inlet baffles, COD was compensated from the inlet reservoir **(b)** biofilm thickness shows that from 0 to 2 mm, the average biofilm thickness reached 83 μm . From 2 to 4 mm average thickness of 4.18 μm . From 6 to 40 mm, the biofilm thickness was constant at 1.25 μm .

Together with the particle-tracking and bacterial-attachment results, the biofilm model provides a mechanistic framework linking initial bacterial attachment, nutrient transport, and further biofilm growth in milli-labyrinth channels.

CONCLUSION

This study developed and validated a combined experimental–numerical framework for milli-labyrinth channel flow characteristics, investigating particle and bacterial transport, attachment, and early biofilm development. Numerical turbulence model assessment using micro-PIV data showed that the $k - \varepsilon$ model best reproduced the key hydraulic parameters, while the $k - \omega$ model overpredicted downstream velocities. Due to its balance of accuracy and computational efficiency, the $k - \varepsilon$ model was selected for further investigation of particle and bacterial transport and initial attachment.

Validated CFD–DPM results (against micro-PTV experiments with 1 μm passive particles) captured general attachment patterns, showing accumulation along upstream baffle walls, particularly near the inlet. Higher trapping probabilities were observed at the first baffle, while lower-probability particles exhibited more dispersed attachment. Attachment was associated with low turbulent kinetic energy regions and coherent secondary flow structures. However, discrepancies were observed between simulations and experiments: micro-PTV showed increased particle trapping at lower flow rates, whereas CFD–DPM predicted greater trapping at higher flow rates. This highlights limitations in modeling bacterial behavior using passive particle assumptions.

Flow analysis indicated that bacterial transport is strongly governed by secondary Dean vortices, which evolve along channel curvature and direct cells toward baffle walls. Differences between passive particles and bacteria were attributed to cell shape effects (rod-shaped *E. coli*), adhesion variability with PMMA surfaces, and potential surface roughness asymmetry in fabricated channels. A key outcome of the study was the development of an experimental–numerical framework for defining attachment–detachment criteria of *ECPs*, introducing quantitative hydraulic thresholds for adhesion and escape. This provides a predictive tool for fouling control and microfluidic design optimization. Micro-PTV experiments with GFP-labelled *E. coli* confirmed similar spatial trends but revealed stronger attachment near the inlet and on the top wall, with reduced attachment at higher flow rates. These differences suggest that hydrodynamic forces and surface properties influence bacterial retention.

A coupled biofilm-growth model was introduced, integrating substrate diffusion, Monod kinetics, biomass growth, and biofilm thickness evolution in both vertical and axial directions. The model shows that vertical diffusion is much faster than growth and axial transport, justifying a pseudo-steady vertical assumption. Simulations indicate that substrate depletion occurs near active biofilm regions, and increasing biofilm thickness leads to diffusion-limited growth. Nutrient transport from the inlet sustains early-stage growth near upstream regions, resulting in stronger colonization near the inlet compared to downstream locations.

Overall, biofouling in milli-labyrinth channels results from the coupled effects of hydrodynamics and nutrient-driven processes. Low-velocity and vortex regions near the inlet promote particle and bacterial attachment, while nutrient diffusion sustains upstream biofilm growth. In addition to processes occurring during irrigation, the non-irrigation phase and the residual water volume remaining in the system play a critical role in biofilm development. Therefore, optimizing dripper drainage, alongside flow and design parameters, is essential for mitigating biofouling under *RWW* conditions.

REFERENCES

- Alazzam, I, N. Ait-Mouheb, Y. Knapp, and O. Boiron. 2026 Biofouling in milli-labyrinth channels of drip irrigation systems using reclaimed wastewater: A review of optical methods and numerical modelling." *Journal of Environmental Management* 397: 128377.
- Delavar, M.A. and J. Wang. 2022. *Advanced Methods and Mathematical Modeling of Biofilms: Applications in Health Care, Medicine, Food, Aquaculture, Environment, and Industry*. Academic Press.
- Flemming, H.-C., J. Wingender, U. Szewzyk, P. Steinberg, S.A. Rice and S. Kjelleberg. 2016. Biofilms: An emergent form of bacterial life. *Nature Reviews Microbiology*. 14: 563–575.
- Hao, F., J. Li, Z. Wang and Y. Li. 2018. Effect of chlorination and acidification on clogging and biofilm formation in drip emitters applying secondary sewage effluent. *Transactions of the ASABE*. 61: 1351–1363.
- Hoekstra, A.Y., M.M. Mekonnen, A.K. Chapagain, R.E. Mathews and B.D. Richter. 2012. Global monthly water scarcity: Blue water footprints versus blue water availability. *PLOS ONE*. 7(2): e32688.
- Lequette, K., N. Ait-Mouheb and N. Wéry. 2020. Hydrodynamic effect on biofouling of milli-labyrinth channel and bacterial communities in drip irrigation systems fed with reclaimed wastewater. *Science of The Total Environment*. 738: 139778.
- Mattei, M., L. Frunzo, B. D'Acunto, Y. Pechaud, F. Pirozzi and G. Esposito. 2018. Continuum and discrete approach in modeling biofilm development and structure: A review. *Journal of Mathematical Biology*. 76: 945–1003.

- Schwanbeck, J., I. Oehmig, J. Dretzke, A.E. Zautner, U. Groß and W. Böhne. 2020. YSMR: A video tracking and analysis program for bacterial motility. *BMC Bioinformatics*. 21: 1–8.
- Secchi, E., A. Vitale, G.L. Miño, V. Kantsler, L. Eberl, R. Rusconi and R. Stocker. 2020. The effect of flow on swimming bacteria controls the initial colonization of curved surfaces. *Nature Communications*. 11: 2851.
- Taute, K., S. Gude, S. Tans and T. Shimizu. 2015. High-throughput 3D tracking of bacteria on a standard phase contrast microscope. *Nature Communications*. 6: 1–9.
- Tripathi, V.K., T. Rajput and N. Patel. 2014. Performance of different filter combinations with surface and subsurface drip irrigation systems for utilizing municipal wastewater. *Irrigation Science*. 32: 379–391.
- Wang, J., S. Gong, D. Xu, Y. Yu and Y. Zhao. 2013. Impact of drip and level-basin irrigation on growth and yield of winter wheat in the North China Plain. *Irrigation Science*. 31: 1025–1037.
- Wang, Q. and T. Zhang. 2010. Review of mathematical models for biofilms. *Solid State Communications*. 150: 1009–1022.
- Worako, A.W. 2015. Evaluation of the water quality status of Lake Hawassa by using water quality index, Southern Ethiopia. *International Journal of Water Resources and Environmental Engineering*. 7: 58–65.
- Zhang, L., P.-T. Wu, D.-L. Zhu and C. Zheng. 2016. Flow regime and head loss in a drip emitter equipped with a labyrinth channel. *Journal of Hydrodynamics, Series B*. 28: 610–616.