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Gabriel Capson-Tojo [a, bc, *], Albie Zuo Meng Gan [a], Pablo Ledezma [a], Damien J. Batstone [a], Tim Hülsen [a, d]

[a] *Australian Centre for Water and Environmental Biotechnology, The University of Queensland, Brisbane, QLD 4072, Australia*
[b] *CRETUS Institute, Department of Chemical Engineering, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Galicia, Spain*
[c] *INRAE, Univ Montpellier, LBE, 102 Avenue des Etangs, 11100 Narbonne, France*
[d] *Fouling Doctors, Brisbane, QLD 4108, Australia*

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ABSTRACT

Purple phototrophic bacteria (PPB) can produce single-cell protein from wastewater at high yields. Growing in a biofilm *vs* suspended can improve product quality and consistency. This study compares suspended and attached growths of enriched PPB cultures in an outdoor flat plate photobioreactor treating poultry-processing wastewater. Attached growth had lower VFA removal efficiencies (95 ± 2.7 *vs* 84 ± 6.4 %) due to light limitations and low substrate diffusion rates. Nevertheless, similar overall treatment performances and productivities were achieved (16 ± 2.2 and 18 ± 2.4 $gCOD{\cdot}m^{-2}{\cdot}d^{-1}$ for attached and suspended) at loading rates of 1.2–1.5 $gCOD{\cdot}L^{-1}{\cdot}d^{-1}$. Biofilms had higher quality than suspended biomass, with lower ash contents (6.9(0.6)% *vs* 57 (16)%) and higher PPB abundances (0.45–0.67 *vs* 0.30–0.45). The biofilm (20–50 % of the total biomass) might be used as feed and the suspended fraction as fertiliser, improving the economics of the process. Semi-continuous PPB growth outdoors as biofilm is technically feasible, obtaining a superior product without jeopardising performance.

1. Introduction

Resource recovery from wastewater using purple phototrophic bacteria (PPB) is advancing rapidly. In recent years, the technology has moved from lab-scale proof-of-concept setups to pilot/demonstration scale reactors running on real wastewater (Hülsen et al., 2022c, 2016; Puyol et al., 2019). Growing photoheterotrophically, PPB grow at high COD yields (up to 1 g $COD_{biomass}{\cdot}g\ COD_{removed}^{-1}$), providing simultaneous COD and nutrients removal while generating high value-added products such as single-cell protein (SCP) (Hülsen et al., 2022a).

PPB need near-infrared (NIR) light for efficient photoheterotrophy growth (Saer and Blankenship, 2017). The economic constraints of artificial illumination impose the implementation of outdoor cultivation systems (Capson-Tojo et al., 2020). Recent articles have studied the performance of outdoor PPB flat plate photobioreactors (FPPBRs) treating real industrial wastewaters (Hülsen et al., 2022c, 2022b), confirming the technical feasibility of the approach (VFA removal efficiencies over 90 % in continuous reactors). Biomass productivities of 25–84 g $VS{\cdot}m^{-2}{\cdot}d^{-1}$ were achieved (with estimates from COD removal rates of 6.0–24 g $VS{\cdot}m^{-2}{\cdot}d^{-1}$; VS being volatile solids) at hydraulic retention times (HRTs) of 2.1–2.4 d (Hülsen et al., 2022c). The produced biomass had a crude protein (CP) content of 58 % and an amino acid profile suitable for feed applications, making it a potential source of SCP.

Most studies dealing with PPB for resource recovery have grown suspended cultures in either photobioreactors (PBRs) or open ponds (Alloul et al., 2021). A challenge that is generally overlooked in suspended cultivations systems is the accumulation of undesired components in the biomass. These include particulate inerts, heavy metals, xenobiotics, or pathogens in suspension. These contaminants are harvested with the biomass, reducing the quality of the product (Hülsen et al., 2022c). This is a crucial point, as a valuable product forms the basis of the economic feasibility of any PPB-mediated wastewater treatment process.

A possibility to overcome biomass contamination is growing PPB as biofilms in PBRs, either as granules or attached onto submerged surfaces. As contaminants tend to remain in suspension, this is a simple solution to improve biomass quality. Recent studies have proven the feasibility of forming PPB granules under artificial illumination, with set-

* Corresponding author at: Australian Centre for Water and Environmental Biotechnology, The University of Queensland, Brisbane, QLD 4072, Australia.
E-mail address: gabriel.capson-tojo@inrae.fr (G. Capson-Tojo).

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tling rates higher than 30 $m \cdot h^{-1}$ (similar to existing granular technologies) and high removal rates (*e.g.*, 1.1 kg $COD \cdot m^{-3} \cdot d^{-1}$) (Stegman et al., 2021). Hülsen et al. (2020) grew PPB biofilms onto artificially-illuminated Perspex tubes, obtaining high pollutant removal efficiencies and recovering over 60 % of the biomass as biofilm, with productivities up to 15–20 g $VS \cdot m^{-2} \cdot d^{-1}$. Biofilms were harvested at 160 g $TS \cdot L^{-1}$ (*vs* below 2–3 g $TS \cdot L^{-1}$ for suspended biomass (Hülsen et al., 2022c); TS being total solids) and had very low ash contents (4.0 % *vs* 30 % in the influent). Outdoors, a recent study using batch FPPBRs showed that PPB can be grown onto reactor walls from industrial wastewaters (Hülsen et al., 2022b). Biomass was consistently harvested at 90 g $TS \cdot L^{-1}$, with CP contents of 50–65 %, ash contents below 10 %, and productivities up to 14 g $TS \cdot m^{-2} \cdot d^{-1}$. A suspended system treating the same wastewater resulted in more diluted biomass (1.2–2.5 g $TS \cdot L^{-1}$), with much higher ash contents (~30 %) (Hülsen et al., 2022c).

This work aims at studying the differences between suspended and attached PPB growth for wastewater treatment and resource recovery under realistic conditions. For this purpose, an outdoor demonstration-scale PPB FPPBR was operated continuously, fed with real industrial wastewater. This article addresses the following questions: (i) how does biofilm formation impact FPPBR performance? (ii) are biomass productivities reduced due to biofilm formation? (iii) is biomass quality (as SCP) higher in attached systems compared to suspended? (iv) is NIR-light availability across the FPPBR affected by biofilms?

2. Materials and methods

2.1. Wastewater source, pretreatment and characteristics

Pre-fermented poultry-processing wastewater (FWW) from a facility in Brisbane (Australia) was used as influent. The FWW contained 2,501–2,926 mg $TCOD \cdot L^{-1}$, 558–851 mg $COD\text{-}VFA \cdot L^{-1}$, 137–188 mg $N \cdot L^{-1}$ and 29–36 mg $P \cdot L^{-1}$. The characteristics of the raw wastewater and the FWW can be found in the Supplementary Materials (SM).

2.2. Flat plate photobioreactor (FPPBR) set-up and operation

Raw wastewater from a grit trap was pumped into an equalization tank, allowing a consistent/continuous flow into a pre-fermentation reactor, run at an HRT of 1.0 d. The FWW was fed into the FPPBR, which had a total length of 9.8 m, a working volume of 953 L, an illuminated surface to volume ratio of 21 $m^2 \cdot m^{-3}$ and an internal thickness of 8 cm. The reactor walls and bottom were covered with an UV–VIS absorbing foil (Lee filter ND 1.2 299). The top was covered with a foam sheet. Mixing and biofilm wiping (when needed) was provided via a hydro-mechanical device (protected intellectual property). A detailed description of the treatment train can be found in Hülsen et al. (2022c).

The FPPBR was fed between 6.30 am and 6.30 pm (daytime hours), at an HRT of 2.1 d (1.0 d considering only the time when light was available). The FPPBR was run for 78 days, divided into two periods. During Period I (36 d) the FPPBR was operated in suspended growth mode, mechanically resuspending the biofilm every 30 min. During Period II (39 d), mixing was provided as in Period I, but the PPB biofilm on the reactor walls was not wiped off. Biofilm was harvested via wiping every 3–4 days (sufficient time to allow a proper biofilm development (Hülsen et al., 2022b)). Due to fluctuations in the FWW, the organic, nitrogen and phosphorus loading rates (OLR, NLR and PLR) varied. Operational conditions, average solar exposures, T, and pH are shown in Table 1.

Table 1
Operational and environmental conditions in the flat plate photobioreactor during Phase I (suspended growth) and Phase II (attached growth). Average removal efficiencies, biomass productivities and removal rates for each operational period are also shown.

Period	I	II
Duration (d)	36	39
Growth strategy	Suspended	Attached
Average daily irradiance ($MJ \cdot m^{-2}$)	23 (4.0)	20 (4.3)
T inside (°C)	31 (4.0)	29 (4.3)
pH (-)	7.3 (0.4)	7.5 (0.4)
HRT (d)	2.1 ± 0.12	2.1 ± 0.04
SRT(d)	2.1 ± 0.12	n.d. [1]
OLR ($g\ COD \cdot L^{-1} \cdot d^{-1}$)	1.5 ± 0.2	1.2 ± 0.2
NLR ($mg\ TN \cdot L^{-1} \cdot d^{-1}$)	59 ± 22	52 ± 12
PLR ($mg\ TP \cdot L^{-1} \cdot d^{-1}$)	12 ± 6.6	10 ± 2.0
TCOD removal (%)	−2.7 ± 10	29 ± 9.7
SCOD removal (%)	65 ± 7.0	74 ± 5.9
VFA removal (%)	95 ± 2.7	84 ± 6.4
TKN removal (%) [2]	56 ± 6.7	50 ± 9.6
TP removal (%) [2]	42 ± 8.8	39 ± 9.0
Biomass productivities ($g\ VS \cdot m^{-2} \cdot d^{-1}$) [3]	42 ± 8.8	39 ± 9.0
Estimated biomass productivities ($g\ COD \cdot m^{-2} \cdot d^{-1}$) [4]	18 ± 2.4	16 ± 2.2
TS content ($g \cdot L^{-1}$)	1.8 (0.6)	1.6 (0.8)
VS content ($g \cdot L^{-1}$)	1.1 (0.5)	1.1 (0.6)
SCOD removal rate ($mg \cdot L^{-1} \cdot d^{-1}$)	379 ± 85	364 ± 48
TKN removal rate ($mg\ N \cdot L^{-1} \cdot d^{-1}$) [2]	25 ± 9.9	17 ± 4.4
TP removal rate ($mg\ P \cdot L^{-1} \cdot d^{-1}$) [2]	3.1 ± 2.3	1.7 ± 1.2

T stands for temperature, HRT for hydraulic retention time, SRT for solids retention time, OLR for organic loading rate, NLR for nitrogen loading rate, PLR for phosphorus loading rate, TCOD for total COD, SCOD for soluble COD, VFA for volatile fatty acids, TKN for total Kjeldahl nitrogen, TP for total phosphorus, TS for total solids and VS for volatile solids.
Numbers in brackets are standard deviations. ± is 95 % confidence interval.
1. Not determined due to incomplete recovery of the attached biofilm.
2. Calculated considering the difference between the total nutrient concentration in the influent and the soluble nutrient concentration in the effluent.
3. Measured including solids in the influent (overestimation).
4. Values estimated from the SCOD removed, assuming PPB photoheterotrophic biomass yields of 1.0 g $COD \cdot g\ COD^{-1}$ (underestimation).

2.3. Sample collection for reactor follow-up and light attenuation assessment

Samples of raw wastewater, FWW, FPPBR effluent and attached biofilm were taken twice a week at zenith time. Biofilm samples were collected using a plastic scraper. Samples were analysed for concentrations of total COD (TCOD), soluble COD (SCOD), VFAs, NO_2^--N, NO_3^--N, NH_4^+-N, PO_4^{3-}-P, total Kjeldahl nitrogen (TKN; both soluble and total), total phosphorus (TP; both soluble and total), TS, and VS. Two daily cycle studies of 24 h were carried out at the end of each operational period (days 36–37 and 71–72) to evaluate the dynamics during day-night natural cycles, as in Hülsen et al. (2022c).

Photon flux densities (PFD) were measured during Phase II to determine the light attenuation effect of the biofilm layer. PFDs (expressed in $\mu mol \cdot m^{-2} \cdot s^{-1}$) were recorded as in Capson-Tojo et al. (2022). PFDs were measured before and after wiping the biofilm from the reactor walls (covered with UV–VIS absorbing foil). The sensor was submerged at 50 cm (around half of the reactor depth) and pointed towards the inner reactor wall. PFDs were registered, carefully placing the sensor right onto the biofilm, without wiping it, and with the sensor resting on the opposite inner wall (8 cm distance between sensor and wall).

2.4. Microbial community analysis, analytical methods and data analysis

Different samples of suspended and attached biomass were taken for analysis of the microbial communities. Samples were preserved at −80 °C and given to the Australian Centre for Ecogenomics for DNA extraction and 16S Amplicon sequencing, using Illumina Miseq Platform. See Hülsen et al. (2022c) for a precise description of the procedure followed.

Concentrations of total and soluble compounds were measured as in Hülsen et al. (2022c). CP contents were estimated as in Eding et al. (2006). Daily solar irradiances were collected from a weather station located 2.0 km away from the site (Station #40917, 27.43°S, 153.12°E).

Measured values are given as average with standard deviation ($\bar{X}(s_{X_i})$) and calculated values as average with uncertainty in mean based on a two-tailed *t*-test (5 % threshold; $\bar{X}\pm E_{\bar{X}}$, where $E_{\bar{X}}$ is the 95 % confidence interval). Statistical differences were assessed via ANOVA.

3. Results and discussion

3.1. Reactor performance: Suspended vs. attached growth

During Period I (suspended growth), no net removal of organics or nutrients occurred, as assimilation into suspended biomass dominated (Table 1). VFA removal was almost complete, with average efficiencies of 95 ± 2.7 %. As previously reported (Hülsen et al., 2022c), a residual non-degradable fraction of SCOD remained in the effluent (300–600 mg·L^{-1}), limiting the SCOD removal (65 ± 7.0 %). Nutrient removal was limited by availability of biodegradable COD (removal efficiencies of 56 % and 42 % for TN and TP). This explains the high levels of NH_4^+-N and PO_4^{3-}-P in the effluent (25–80 mg N·L^{-1} and 4–30 mg P·L^{-1}).

When attached growth was started (day 37; Period II), the levels of TCOD, TKN and TP in the effluent decreased due to biofilm growth and attachment (despite increasing input solids). This resulted in the removal of TCOD during Period II (average of 29 ± 9.7 %), opposed to a negligible removal in Period I (Fig. 1A). The almost instantaneous change between both periods suggests that operational modifications (*i.e.*, no biofilm wiping) were responsible for the observed changes (instead of biological shifts). An active biofilm formed within 3–4 d.

While N and P removal efficiencies were similar between periods, VFA removal was slightly lower in Period II (84 ± 6.4 %; p = 0.002), despite the lower OLR during this period. Time trends confirm the lower VFA removal maintained in Period II (Fig. 1B). The slightly decreased performance in Period II can be further confirmed by comparing the SCOD, TKN and TP removal rates, all lower than in Period I (Table 1). The lower OLR in Period II might partially explain the lower removal rates and productivities, but not the lower VFA removal efficiencies. Different hypotheses can explain this observation: (i) the diffusion rate of soluble substrates into the biofilm limited VFA availability and reduced overall uptake rates, which were close to optimal values due to the low HRT applied (1.0 d during daytime); (ii) the formation of biofilm reduced light availability in the bulk liquid, reducing VFA removal rates due to lack of light in the suspended phase; (iii) a combination of (i) and (ii).

The cycle studies confirmed that VFA removal was limited during attached growth (SM). While VFAs were barely detectable during nighttime in Period I, in Period II a VFA baseline remained even during daytime (50–60 mg COD·L^{-1}·d^{-1}). This resulted in higher VFA concentrations at night due to hydrolysis and fermentation of residual organics. VFA accumulation impacted the pH, up to 7.9 in Period I and below 7.7 in Period II (SM).

The measured biomass productivities between both periods were similar (Table 1 and SM). Note that during Period II the productivities were calculated by measuring the biomass concentrations in the liquid after biofilm wiping. Practical limitations (*e.g.*, biofilm not completely wiped, residual biomass remaining on the wiping system, or floating biofilm aggregates causing heterogeneities when sampling) led to underestimations of the generated biofilm. Therefore, the productivities could be expected to be higher than those reported (10–30 % according to mass balances; SM). Regardless, the measured values do not differentiate between solids corresponding to biomass and those present in the influent that remained undegraded. Thus, these values overestimate actual PPB productivities. To solve this, biomass productivities were estimated from the amounts of SCOD removed, assuming a biomass yield of 1 g $COD_{biomass}$·g $COD_{removed}^{-1}$ (Capson-Tojo et al., 2020). The estimated productivities were also statistically similar between both periods. The high values obtained (measured values of 42 ± 8.8 and 39 ± 9.0 g VS·m^{-2}·d^{-1} for each period), even for the estimated productivities (18 ± 2.4 and 16 ± 2.2 g COD·m^{-2}·d^{-1}), which are underestimations that only consider SCOD uptake, confirm that PPB can achieve high productivities in outdoor reactors (Hülsen et al., 2022c, 2022b). The relatively consistent trend in the estimated values (up to 25 g COD·m^{-2}·d^{-1}; SM) confirms the consistency and robustness of the system, keeping a stable performance despite the variable influent characteristics and environmental conditions.

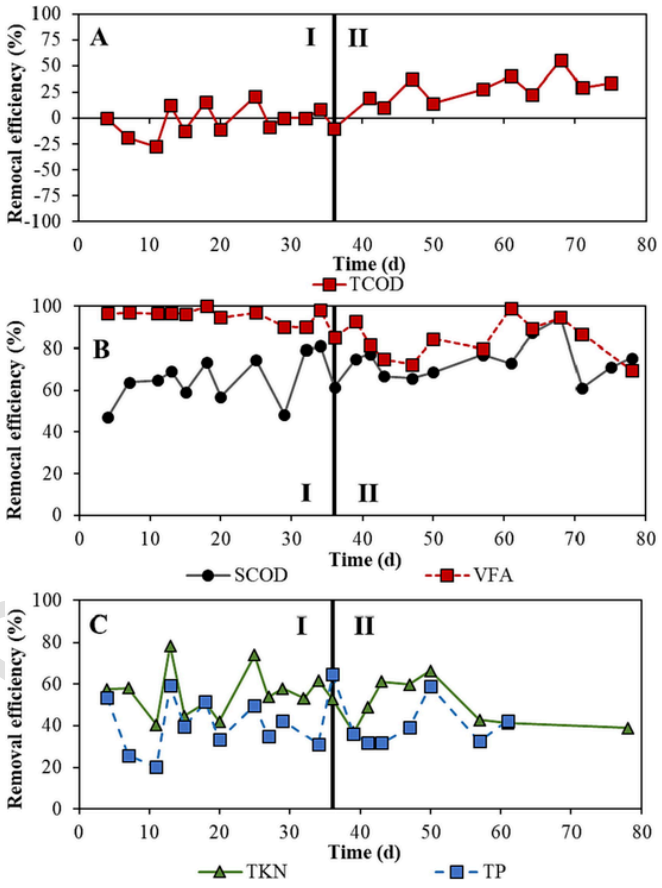

Fig. 1. Removal efficiencies over time in the flat plate photobioreactor for: (A) TCOD, (B) SCOD and VFA, and (C) TKN and TP. Roman numbers refer to the operational periods described in Table 1. TCOD stands for total COD, SCOD for soluble COD, VFA for volatile fatty acids, TKN for total Kjeldahl nitrogen and TP for total phosphorus.

3.2. Impact of biofilm on light availability through the reactor

The measured PFDs at the inner reactor wall before and after biofilm wiping show that the biofilm absorbs, on average, 61 ± 40 % of the incident NIR-light, with variations depending on the incident

PFD (SM). This increased the light attenuation throughout the reactor, and might explain the lower removal efficiencies and rates in attached growth. To confirm this, the PFDs behind the reactor inner wall (*i.e.*, right behind the biofilm) and at the opposite side were measured before and after biofilm wiping (Fig. 2).

The PFDs at the opposite side of the wall were similar before and after biofilm wiping (9.7–12 *vs* 11–12 $\mu mol \cdot m^{-2} \cdot s^{-1}$; or 1.3–1.6 *vs* 1.6–1.7 $W \cdot m^{-2}$ assuming an average NIR wavelength of 900 nm). Both light intensities would have limited effective PPB growth, reducing the growth kinetics, as PPB enriched-cultures show light half saturation constants (K_I; value at which kinetics are halved) of 4.6 $W \cdot m^{-2}$ (Capson-Tojo et al., 2022). Efficient growth at NIR levels of 1.4–3.0 $W \cdot m^{-2}$ has been reported for cultures adapted to low light intensities, but with slower growth kinetics (Dalaei et al., 2020). The main difference between Period I and II was the light intensity available right after the wall. Taking the most extreme case (day 57, Fig. 2A), the PFD before biofilm wiping was 23 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (3.8 $W \cdot m^{-2}$) and the value after wiping was 81 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (11 $W \cdot m^{-2}$). 3.8 $W \cdot m^{-2}$ is already lower than the K_I values, meaning that right behind the biofilm, the available light was already limiting the PPB uptake rates. Due to attenuation by water and suspended biomass, this effect was more pronounced at higher distances from the FPPBR wall. Thus, the proportions of dark volumes in the bulk liquid were higher during attached growth, limiting the uptake kinetics in the suspension and resulting in incomplete VFA consumptions and lower removal rates at the applied HRT.

3.3. Biomass characteristics

The biomass concentration was 50 times higher in the biofilm (90.5 (1.3) g $TS \cdot L^{-1}$ *vs* 1.80(0.60) g $TS \cdot L^{-1}$), and the biofilm also had much lower ash content (6.9 % *vs* 43 %; Table 2). The latter is crucial for feeding applications, and, in the case of fish meal substitution, it must

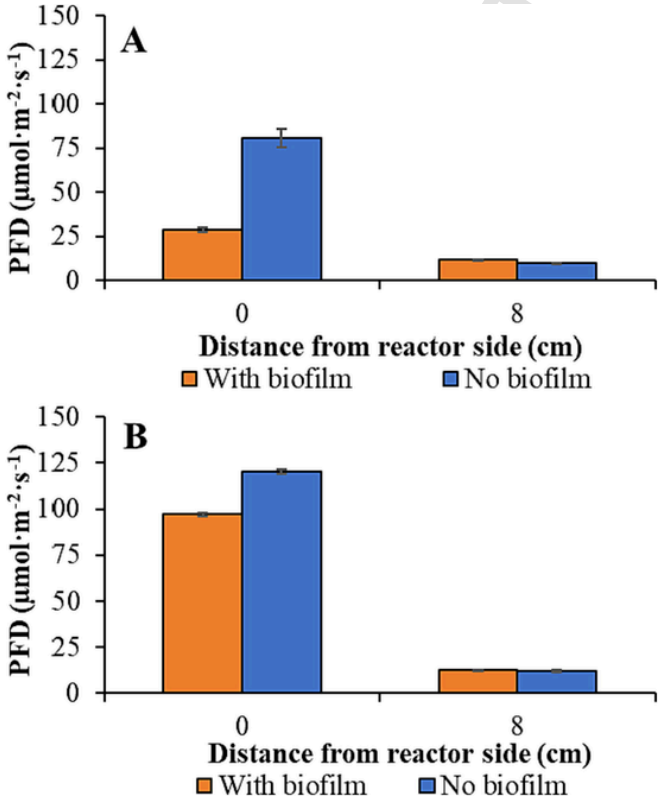

Fig. 2. Photon flux densities (PFDs) measured on the inner reactor wall and on the opposite side during Period II at days 57 (A) and 61 (B). The biofilm thickness (1–3 mm) was considered to be negligible for distance calculations.

Table 2
Average characteristics of the harvested biomass. Roman numbers refer to operational periods described in Table 1.

Period	I (suspended sample)	II (biofilm sample)
Duration (d)	36	39
Growth strategy	Suspended	Attached
TS ($g \cdot kg^{-1}$)	1.80 (0.60)	90.5 (1.3)
VS ($g \cdot kg^{-1}$)	1.57 (0.81)	84.3 (1.4)
VS/TS (%)	57 (16)	93 (1.0)
Ash (%)	43 (19)	6.9 (0.6)
TKN ($g\ N \cdot kg^{-1}$)	–	92.0 (7.9)
TP ($g\ P \cdot kg^{-1}$)	–	13.0 (0.42)
CP (% dry weight)	–	57.5 (3.95)

TS stands for total solids, VS for volatile solids, TKN for total Kjeldahl nitrogen, TP for total phosphorus and CP for crude protein.
Numbers in brackets are standard deviations.

be below 20 % (personal communication with fish feed manufacturers). Thus, while the harvested biofilm could be used directly as feed, the suspended biomass could only be used as fertiliser, a product with a much lower value (*i.e.*, 0.42–0.47 *vs* 1.5–1.6 $USD \cdot kg^{-1}$ (Capson-Tojo et al., 2020)). Results from mass balances (SM) show that the biofilm represented 20–50 % of the total COD (in agreement with previous studies (Hülsen et al., 2022b, 2020)).

PPB relative abundances were 0.30–0.45 in Period I samples and 0.48–0.68 in biofilms from Period II (SM). PPB attached preferentially, while flanking communities such as fermenters remained in suspension. Thus, biofilm growth can be used to increase PPB proportions in the product. PPB proportions in suspended samples from Period II (0.30–0.32) were lower than in Period I, due to partial growth of PPB as biofilm and due to the more significant light limitation in the bulk liquid during attached growth. Predominant PPB genera (*i.e.*, *Blastochloris* sp., *Rhodopseudomonas* sp. and *Rhodobacter* sp.) were the same in Period I and II and in the suspended and attached fractions, confirming that abiotic factors (*i.e.*, mechanical biomass wiping) were responsible for the performance differences.

3.4. Implications for practical implementation

Biofilm-based PPB outdoor reactors can be operated efficiently, with similar performances to those obtained with suspended growth. The increased biomass quality of the biofilm (20–50 % of the total biomass) resulted in a higher value-added product directly applicable, for example, as bulk fish feed (Delamare-Debouteville et al., 2019). This is enabled by the low content of ash, by a sufficient protein content (58 % of CP), and by a suitable amino acid profile (Hülsen et al., 2022c). An improved product consistency in biofilms is a main advantage, as its quality will not depend on the undesirables in the influent (suspended). Concentration steps are also avoided, as solid contents in biofilms are higher than in suspended biomass (90 *vs* 1.8 $g \cdot kg^{-1}$).

Regular biofilm wiping did not affect the treatment performance, implying that continuous operation is feasible, coupled to sequential biofilm recovery every-three-four days. This process resulted in reasonably high productivities, higher than those in indoors PPB biofilm reactors (7.0–10 g $VS \cdot m^{-2} \cdot d^{-1}$ (Hülsen et al., 2020)) or those in algal biofilm processes (up to 7.6 g $TS \cdot m^{-2} \cdot d^{-1}$ (Fica and Sims, 2016)). The formation of a light-driven biofilm is another advantage, avoiding energy for shear/mixing (Hülsen et al., 2022b). Poor light availability in the bulk liquid is a challenge, lowering removal rates and VFA removal efficiencies, which are also affected by the rates of substrate diffusion through the biofilm. Parameters such as HRT (which might be longer in biofilm systems), wiping frequency (related to biofilm thickness), reactor width (affecting light availability and land requirements), and OLR will need to be optimised to maximise treatment rates. The partial pollutant removal by biofilms is a limitation that imposes the need of harvesting

the suspended biomass (Hülsen et al., 2022c). This suspended fraction could be used as slow-release fertiliser (Sakarika et al., 2019).

4. Conclusions

Despite the lower VFA removal efficiencies during attached growth, this approach achieved similar overall treatment performances and biomass productivities compared to suspended growth. The harvested biofilm had higher quality than the suspended biomass, with much lower ash contents and higher PPB abundances. The collected biofilm (20–50 % of the total produced biomass) can be used as feed and the suspended fraction as fertiliser. Semi-continuous attached PPB growth in outdoor FPPBRs is technically feasible, generating a high quality product without jeopardising process performance.

CRediT authorship contribution statement

Gabriel Capson-Tojo : Conceptualization, Methodology, Investigation, Writing – original draft, Visualization, Supervision. **Albie Zuo Meng Gan :** Methodology, Investigation, Visualization. **Pablo Ledezma :** Resources, Writing – review & editing. **Damien J. Batstone :** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Tim Hülsen :** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: the authors declare that Tim Hülsen contributed to this study while working at Fouling Doctors, who intends to file a patent concerning the mixing/wiping technology mentioned here, with intent to commercialise.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2023.128709.

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