Simultaneous methane abatement and PHB production by *Methylocystis hirsuta* in a novel gas-recycling bubble column bioreactor

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Abstract

The limited gas-liquid mass transfer represents the main challenge in the operation of cost-effective bioreactors devoted to the treatment of poorly soluble gas pollutants such as methane (CH_4). This study evaluates the influence of internal gas-recycling strategies on the enhancement of CH_4 abatement in a bubble column bioreactor inoculated with the methanotroph *Methylocystis hirsuta*. Maximum CH_4 removal efficiencies of 72.9 ± 0.5 % (corresponding to elimination capacities of 35.2 ± 0.4 g m^{-3} h^{-1}) were recorded under process operation at an empty bed residence time of 30 min and 0.50 m^3_{gas} $m^{-3}_{reactor}$ min^{-1} of internal gas-recycling rate. The accumulation of poly-3-hydroxybutyrate (PHB) in *M. hirsuta* was evaluated batchwise under limitations of potassium, manganese, nitrogen, and nitrogen with excess of iron. Nitrogen starvation resulted in the highest PHB content (28 ± 1 %). Likewise, the implementation of sequential N starvation cycles in a continuous bubble column reactor operated at a gas residence time of 30 min and an internal gas-recycling rate of 0.50 m^3_{gas} $m^{-3}_{reactor}$ min^{-1} supported a PHB content of up to 34.6 ± 2.5 %, with a volumetric PHB productivity of 1.4 ± 0.4 kg m^{-3} d^{-1} and elimination capacities of 16.2 ± 9.5 g m^{-3} h^{-1}.

Keywords: Biological gas treatment; greenhouse gas; methanotroph; polyhydroxyalkanoate; suspended growth bioreactor.

1. Introduction

Methane (CH_4) emissions account for 20-30 % of the global warming effect worldwide based on the 25-times higher ability of this greenhouse gas (GHG) to absorb Earth's radiation compared to CO_2 [1, 2]. This GHG is mainly released to the atmosphere from cattle farming, waste management and mining at low concentrations (< 20 % *v/v*), which limits its potential energy valorization. In this context, the absence of specific regulations targeting CH_4 emissions, along with the lack of viable technical alternatives to produce energy from dilute CH_4 emissions, promote the uncontrolled release of CH_4 to the atmosphere without prior treatment. Therefore, the development of cost-efficient and environmentally-friendly technologies for the abatement of CH_4 is mandatory to achieve an effective climate change mitigation [3].

Biotechnologies, such as biofiltration, have consistently shown comparable removal efficiencies and robustness to those of physical-chemical technologies during the treatment of malodours and volatile organic pollutants [4]. Nonetheless, biofilters still present severe operational drawbacks limiting their long-term treatment performance and consequently their widespread implementation for air pollution control. These limitations include the poor mass transfer of poorly water-soluble compounds from the gas phase to the biofilm, and the occurrence of packed bed clogging and channeling as a result of biomass overgrowth [5, 6]. In this context, suspended growth bubble column bioreactors (BCBs) allow for an easy biomass control and harvesting, while they overcome mass transfer limitations due to the recent commercial availability of ultrafine bubble diffusers with micropores < 0.5 µm. In addition, the performance of BCBs can be further boosted via internal gas-recycling, which allows the decoupling of the actual gas residence time and

61 turbulence in the microbial broth from the overall empty bed residence time (EBRT).
62 However, the potential of internal gas-recycling in BCBs has been poorly explored for off-
63 gas treatment [7-9].
64
65 Methanotrophs are microorganisms capable of metabolizing CH_4 as their sole carbon and
66 energy source by using the enzyme methane monooxygenase (MMO) [10, 11].
67 Methanotrophic bacteria are typically classified into two different types based on their
68 metabolic and physiological differences: I (which belong to *γ-Proteobacteria* class) and II
69 (*α-Proteobacteria* class). Interestingly, type II methanotrophs (e.g. *Methylocysti*s,
70 *Methylosinus* and *Methylocella* genera) are able to co-produce polyhydroxyalkanoates
71 (PHAs) under nutrient-limited conditions via the so-called serine pathway [12]. In this
72 regard, CH_4 represents a low-cost substrate for the production of these high added-value
73 products (market price of 4-20 € kg^{-1}), whose competitiveness is up to date jeopardized by
74 the high cost of the carbon source employed. Commercial PHAs are nowadays produced
75 through fermentation of glucose or agricultural sugar substrates, which account for 30-40%
76 of the total production costs [13-15]. To date, CH_4-based biopolymer production has been
77 focused on the synthesis of poly-3-hydroxybutyrate (PHB), which presents similar
78 mechanical and thermal characteristics to those of conventional plastics and is
79 biodegradable, thus enabling its rapid decomposition in the environment [16,17]. Recent
80 attention has been paid to the optimization of PHB accumulation from a microbiological
81 point of view by identifying the key limiting macro/micronutrients that boost PHB
82 synthesis in methanotrophs. However, to the best of the authors' knowledge, the influence
83 of micronutrients such as Mn, Fe, and K on methanotrophic PHB synthesis has been
84 scarcely studied [18, 19]. Moreover, few studies have evaluated the simultaneous

abatement of dilute CH_4 emissions and co-production of PHB in gas-phase bioreactors under continuous operation [20].

This study aimed at optimizing the continuous abatement of diluted CH_4 emissions (4 % *v/v*) coupled to PHB accumulation at high productivities in a novel internal gas-recycling BCB using *Methylocystis hirsuta* as a model type II methanotroph. The influence of the EBRT and internal gas-recycling rates on the CH_4 removal were first investigated in a lab-scale BCB. In addition, the role of different nutrient-limiting conditions (N, K, Mn, and N with excess of Fe) on PHB accumulation in *M. hirsuta* was also assessed. Finally, the potential of the internal gas-recycling BCB for simultaneous CH_4 abatement and PHB co-production was evaluated under the optimum EBRT, internal gas-recycling rate and nutrient-limiting conditions previously identified.

2. Material and methods

2.1. Mineral salt medium, chemicals and inoculum

The mineral salt medium (MSM) used for *M. hirsuta* cultivation was modified from Mokhtari-Hosseni et al. [21]. The MSM was composed of (g L^{-1}): 2.25 $NaNO_3$, 0.1 $MgSO_4 \cdot 7H_2O$, 0.02 $CaCl_2 \cdot 2H_2O$, 0.68 KH_2PO_4, 6.14 $Na_2HPO_4 \cdot 12H_2O$, 1.3 × 10^{-3} $FeSO_4 \cdot 7H_2O$, 3.5× 10^{-3} $MnCl_2 \cdot 4H_2O$, 1.5× 10^{-3} $ZnSO_4 \cdot 7H_2O$, 0.04 × 10^{-3} $Na_2MoO_4 \cdot 2H_2O$, 0.04× 10^{-3} $CuSO_4 \cdot 5H_2O$, 0.32 × 10^{-3} $CoCl_2$, and 0.2 × 10^{-3} H_3BO_3. Unless otherwise specified, all reagents and chemicals were purchased from Panreac® (Barcelona, Spain) with a purity of at least 99 %. CH_4 (≥ 99.5 %) and O_2 (≥ 99 %) were purchased from Abelló Linde S.A. (Barcelona, Spain). Poly [(R)-3-hydroxybutyric acid-*co*-(R)-3-hydroxyvaleric

acid] (molar ratio 88/12, ≥ 99.99 %) was obtained from Sigma-Aldrich® (Sigma-Aldrich, St. Louis, MO, USA).

M. hirsuta (DSMZ 18500) inocula were initially prepared in sterile 120 mL gas-tight serum bottles containing 40 mL of sterile MSM inoculated at 1 % (*v/v*). These cultures were incubated at 25 °C and 250 rpm for 48 h under a 33:67 % (*v/v*) CH_4:O_2 headspace. The cultivation broths were finally transferred to sterile 1.25 L gas tight serum bottles made-up with sterile MSM to a final liquid volume of 200 mL, and incubated at 25º C and 600 rpm to a final optical density of the cultures at 600 nm (OD_{600}) of 1.1 (corresponding to a total suspended solid concentration – TSS – of 295 ± 16 mg L^{-1}).

2.2. Influence of the EBRT and internal gas-recycling rate in the BCB on CH_4 biodegradation

A lab-scale PVC bubble column bioreactor (0.08 m internal diameter × 0.6 m height) with a working volume of 2.5 L was used in the present study (Fig. 1). The polluted air emission, which contained CH_4 at 4 % (*v/v*), was sparged at the bottom of the bioreactor using three 0.5 µm-pore stainless steel diffusers. This synthetic emission was composed of a pure CH_4 stream supplied via a mass flow controller (Aalborg™, USA) and pressurized air. A 1-L jacketed condenser cooled with water at 20 ºC was implemented within the internal gas-recycling line. The temperature in the reactor was maintained at 25 °C. The reactor was inoculated at 194 ± 4 mg L^{-1} and initially operated for 13 days (to reach steady-state) at 60 min of EBRT without internal gas-recycling during the start-up phase. The influence of the EBRT (120, 60, 30 and 15 min) and internal gas-recycling ratio (Q_R/Q = 0, 2, 3, 6, 10 and

15, where Q_R is the recycling gas flow rate and Q the gas flow rate fed to the overall system) was investigated in order to optimize the CH_4 abatement performance (Table 1). To ensure an optimum balance of nutrients and a stable pH (7.3 ± 0.2) within the bioreactor, 500 mL of cultivation broth were drawn every 48 h, centrifuged (10000 rpm, 7 min) and the biomass pellet (resuspended in fresh 500 mL MSM) was returned to the BCB.

The inlet and outlet CH_4, O_2 and CO_2 gas concentrations were daily monitored by GC-TCD. OD_{600}, pH, TSS and total nitrogen (TN) concentrations in the cultivation broth were determined every 48 h. The elimination capacity (EC), removal efficiency (RE), CO_2 production rate (PCO_2), PHB content, PHB productivity and the maximum rate of CH_4 consumption (fitting the data to the Gompertz model) were calculated according to Zuñiga et al. [2].

2.3. Influence of micro/macro nutrient limitation on PHB accumulation

The influence of N (at low and high Fe^{2+} concentrations), K and Mn limitations on PHB accumulation and CH_4 biodegradation in *M. hirsuta* cultures were evaluated batchwise. The batch assays involved a growth phase of 15 days in MSM followed by a PHB accumulation phase of 10 days under nutrient limiting conditions according to Table 2. The assays were carried out in duplicate in 2 L gas-tight serum bottles containing 400 mL of MSM inoculated with an initial biomass concentration of 128 ± 17 mg L^{-1}. The glass bottles were sealed with butyl septa and aluminum crimp seals, and CH_4 was then added to the headspace both in the growth and accumulation stages at an initial concentration of 193 ± 7 g m^{-3} (32.5 ± 1.1 % *v/v*) in a pure O_2 atmosphere. The biomass was centrifuged at the end of the growth phase and resuspended in the corresponding nutrient-limited MSM prior to

155 the accumulation phase. Control tests with the original MSM were conducted as above
156 described. The CH_4, O_2 and CO_2 composition of the headspace, and the biomass (measured
157 through OD_{660}) in the cultivation broth were periodically monitored throughout the 25 days
158 of experiment while PHB concentrations were monitored throughout the limitation tests.
159

160 **2.4. Continuous CH_4 abatement and PHB co-production in the internal gas-recycling**
161 **BCB under optimum operational conditions**

162 The performance of the internal gas-recycling BCB was assessed under continuous mode
163 using the optimum operational conditions identified in sections 2.2 and 2.3 (EBRT = 30
164 min, internal gas-recycling rate = 0.50 m^3_{gas} $m^{-3}_{reactor}$ min^{-1} and nitrogen limitation as stress
165 to induce PHB production) at an inlet load (IL) of 49.8 ± 11.8 g CH_4 m^{-3} h^{-1}. The BCB was
166 inoculated with *M. hirsuta* at an initial biomass concentration of 152 ± 1 mg L^{-1} and
167 initially operated under nutrient-sufficient conditions and continuous CH_4 supply in order
168 to reach a biomass concentration of 4.4 ± 0.6 g TSS L^{-1}. Then, nine sequential nitrogen
169 feast-famine cycles (1 day in excess of nitrogen and 2 days under nitrogen limitation) were
170 applied to evaluate the continuous co-production of PHB during CH_4 abatement. N-
171 supplemented or N-free MSM were supplied at a dilution rate (D) of 0.1 d^{-1} during the feast
172 and famine periods, respectively. N concentration in the N-supplemented MSM was
173 adjusted to 61 ± 8 mg N L^{-1} during the feast periods to ensure a complete depletion within
174 the following 24 h. The inlet and outlet CH_4, O_2 and CO_2 gas concentrations were daily
175 monitored in the BCB. Likewise, 20 mL liquid samples were daily withdrawn to determine
176 the OD_{600}, pH, TSS concentration and PHB content.
177

2.5. Analytical methods

CH_4, O_2, and CO_2 gas concentrations were measured in a Bruker 430 GC-TCD (Palo Alto, USA) equipped with a CP-Molsieve 5A column (15 m × 0.53 µm × 15 µm) and a CP-PoraBOND Q column (25 m × 0.53 µm × 10 µm). The oven, injector, and detector temperatures were maintained at 45 °C, 150 °C and 200 °C, respectively. Helium was used as the gas carrier at 13.7 mL min^{-1}. TSS concentration was determined according to standards methods [22]. Culture absorbance was measured at 600 nm using a Shimadzu UV-2550 UV/Vis spectrophotometer (Shimadzu, Japan). TN concentration was quantified following sample filtration (0.45 µm) in a TOC-VCSH analyzer (Shimadzu, Japan) coupled with a chemiluminescence detection TN module (TNM-1) (Shimadzu, Japan). PHB accumulation was quantified in a GC-MS (Agilent Technologies: GC System 7820A MSD 5977E, Santa Clara, USA) equipped with a DB-wax column (30 m × 250 µm × 0.25 µm) according to López et al. [20].

3. Results and discussion

3.1. Influence of the EBRT and internal gas-recycling rate in the BCB on CH_4 biodegradation

Process operation at an EBRT of 120 min in the absence of internal gas-recycling allowed elimination capacities of 4.7 ± 0.48 g m^{-3} h^{-1}, corresponding to REs of 38 ± 4 %, while values ranging from 6.6 ± 0.3 to 9.8 ± 0.1 g m^{-3} h^{-1} were obtained at Q_R/Q of 2, 3, 6, 10, 15 (corresponding to internal gas-recycling rates of 0.02, 0.03, 0.05, 0.08 and 0.13 m^3 m^{-3} min^{-1}). Similarly, higher gas-recycling rates resulted in concomitant increases in EC, RE and PCO_2 (Fig. 2). Thus, the REs increased from 38 ± 4 to 54 ± 2, 60 ± 1, 69 ± 2, 73 ± 1, and

79 ± 1 % at gas-recycling ratios of 2, 3, 6, 10 and 15, respectively. Likewise, process operation at an EBRT of 60 min in the absence of internal gas-recycling supported ECs of 8.5 ± 0.3 g m^{-3} h^{-1}, PCO_2 of 12 ± 0.9 g m^{-3} h^{-1} and a RE of 35 ± 1 %. The implementation of gas-recycling ratios of 2, 3, 6, 10, and 15 resulted in REs of 50 ± 2, 56 ± 2, 67 ± 1, 71 ± 1 and 75 ± 1 %, respectively. Both EC and PCO_2 increased at increasing gas-recycling ratios, with a maximum EC of 18.7 ± 0.2 g m^{-3} h^{-1} (corresponding to a RE = 75 ± 0.6 %) at a Q_R/Q of 15. Similar removal efficiencies (70%) were reported in a biofilter treating CH_4 at an EBRT of 50 min **[**23**]**. This suggests that the turbulence (i.e. shear stress on the cells) induced by the Q_R/Q tested in the BCB did not significantly affect the microbial activity. Surprisingly, comparable CH_4 REs were obtained at EBRTs of 120 and 60 min regardless of the Q_R/Q ratio. The results here obtained were in accordance with those previously reported by Estrada et al. **[**24**]**, who recorded a 2.5 increase in CH_4 REs at a Q_R/Q ratio of 18 in a methanotrophic biotrickling filter compared to conventional operation without recycling rate.

However, despite the CH_4 abatement performance of the BCB at EBRTs of 120 and 60 min was significantly enhanced by the internal gas-recycling, the EBRTs here investigated were higher than those previously evaluated in biotrickling filters and would entail prohibitively large reactor volumes [24]. Therefore, process operation at an EBRT of 30 min was evaluated for Q_R/Q ratios of 10 and 15, and at an EBRT of 15 min for a Q_R/Q = 15. In this context, a decrease in the EBRT always promoted an increase in the EC and PCO_2, at the expense of lower CH_4 REs (Fig. 3). Thus, maximum ECs of 35.2 ± 0.4 g m^{-3} h^{-1} and REs of 72.9 ± 0.5 % were achieved at an EBRT of 30 min, while process operation at an EBRT of 15 min resulted in ECs of 54.4 ± 0.9 g m^{-3} h^{-1} and REs of 56.6 ± 1.5 %. Moreover, the

224 decrease in the EBRT at a Q_R/Q of 15 did not entail a decrease in the mineralization ratio
225 (PCO_2/EC), which remained constant at 2.0 ± 0.1, 1.9 ± 0.2, 1.7 ± 0.4 and 2.0 ± 0.1 for
226 EBRTs of 15, 30, 60 and 120 min, respectively (Table 1).
227 Unfortunately, the high shear stress caused by the high turbulence in the cultivation
228 medium prevailing during process operation at an EBRT of 15 min and a Q_R/Q ratio of 15
229 finally caused a deterioration in microbial activity, and therefore, a decrease in the EC to
230 21.1 ± 5.2 g m^{-3} h^{-1} (corresponding to a REs = 23.3 ± 4.7 %). This high turbulence caused
231 biomass aggregation and settling at the bottom of the BCB, thus reducing the concentration
232 of active biomass in the effective volume of the reactor. This deterioration in microbial
233 activity was also confirmed by the increase in the mineralization ratio (1.8 times higher
234 than that recorded at the early stages of process operation at a Q_R/Q of 15 and EBRT of 15
235 min, where no biomass aggregation was observed) mediated by the increase in the
236 endogenous cell respiration. Preliminary studies in the literature have consistently reported
237 that high turbulence in the culture broth may induce cell membrane damage and impact on
238 the off-gas treatment performance [25]. Indeed, Gram-negative bacteria such as *M. hirsuta*
239 are especially sensitive to turbulence-mediated shear stress, which can ultimately limit the
240 performance of bioreactors devoted to CH_4 treatment [26]. At this point, it should be also
241 stressed that all tests were conducted under mass transfer-limiting conditions (CH_4
242 concentration in the liquid phase ~0 g m^{-3}), which occurred at biomass concentrations > 1 g
243 L^{-1}.
244
245 The MSM dilution rate here applied (D = 0.1 d^{-1}) prevented the system from N depletion,
246 which has been shown to limit the performance of methanotrophic gas-recycling
247 bioreactors under long-term operation [24]. In fact, the lowest TN concentrations (146 ± 2

248 mg N L^{-1}) were recorded at the lowest EBRT and a Q_R/Q ratio of 15 as a result of an
249 enhanced nitrogen assimilation by *M. hirsuta*.
250
251 **3.2. Influence of micro/macro nutrient limitation on PHB accumulation**
252 CH_4 was steadily degraded by *M. hirsuta* under nutrient-sufficient conditions, resulting in a
253 biomass yield (Y_X) of 0.63 ± 0.04 gX gCH_4^{-1} and a PHB content of 7.8 ± 1.0 % (*w/w*) by
254 the end of the growth stage. A rapid accumulation of PHB and microbial growth
255 concomitant with the biodegradation of CH_4 were observed following biomass
256 resuspension in nutrient-limited MSM supplemented with CH_4 regardless of the nutrient
257 limitation tested (Fig. S1). Mn limitation did not promote PHB synthesis by *M. hirsuta*,
258 which exhibited a similar PHB content (8.1 ± 1.1 %) and Y_X (0.68 ± 0.02 gX gCH_4^{-1}) to
259 those of the control test. Interestingly, K limitation induced a slightly higher PHB content
260 of 12.5 ± 1.1 % and a Y_X of 0.38 ± 0.02 gX gCH_4^{-1} (Fig. 4A). This limitation did not affect
261 the maximum CH_4 consumption rate, similar values being recorded for K limited, Mn
262 limited and control tests (Fig. S2). The PHB contents here obtained were 2-times lower
263 than those recorded in a type II *Methylocystis* sp. consortium under K limitation, likely due
264 to the different MSM or methanotrophic species here used [27]. N limitation clearly
265 induced the highest PHB accumulation (28.0 ± 1.2 %) in *M. hirsuta* (Fig. 4A). In this
266 context, N limitation has been consistently shown to support the highest PHB content in
267 methanotrophic species belonging to the genera *Methylocystis* and *Methylosinus* [27-29].
268 Finally, the excess of Fe^{2+} under N limitation induced a PHB accumulation of up to 19.2 ±
269 1.8 % at the expense of a reduced CH_4 consumption, CO_2 production, biomass growth and
270 specific CH_4 consumption rate (Fig. 4B and S3) compared to the test conducted exclusively
271 under N limitation, where 82.5 ± 2.3 % CH_4 was consumed in the same period of time (10

272 days), with a Y_X of 0.48 ± 0.05 gX gCH_4^{-1}. These findings suggest the occurrence of a
273 microbial inhibition in *M. hirsuta* at high Fe^{2+} concentrations. In this context, previous
274 studies indicated that Fe^{2+} concentrations of 40-80 µM are required for an effective MMO
275 activity, both Fe^{2+} and Cu^{2+} being important co-factors in the metabolism of methanotrophs
276 [19]. However, the presence of high concentrations of Cu^{2+} could promote the formation of
277 hydrogen peroxide, which can react with Fe^{2+} at these high concentrations and produce
278 inhibitory free hydroxyl radicals [30].
279

280 **3.3. Continuous CH_4 abatement and PHB co-production in the internal gas-recycling**
281 **BCB under optimum operational conditions**

282 BCB operation at an EBRT of 30 min and a Q_R/Q ratio of 15 under sequential N feast-
283 famine cycles (D = 0.1 d^{-1}) was identified as the optimum operational scenario to support a
284 stable and efficient CH_4 abatement coupled to PHB production under continuous mode.
285 The system rapidly achieved steady ECs of ~ 27.9 ± 2.1 g m^{-3} h^{-1} (corresponding to REs of
286 57.8 ± 4.5 %) from day 10 onwards, while biomass concentration steadily increased up to
287 steady state values of 4.5 ± 0.6 g L^{-1} from day 20 onwards. These operational conditions
288 supported a PCO_2 and biomass productivity of 79.9 ± 8.4 g CO_2 m^{-3} h^{-1} and 26.4 ± 18.5 gX
289 m^{-3} h^{-1} (Fig. 5). These ECs and REs were slightly lower than those achieved in the mass
290 transfer optimization tests under similar operational conditions, which was attributed to the
291 gradual fouling of the fine bubble diffusers used in our study. The implementation of
292 repeated N feast-famine cycles resulted in a gradual increase in the PHB content from 0.4 ±
293 0.0 % to 25.7 ± 0.1 % during the first N limitation (which lasted 3 days instead of 2 days)
294 and up to 37.2 ± 2.0 % from the fifth cycle onwards, reaching a maximum accumulation of

40% in the fifth and eighth cycles. N addition during the N feast-famine cycles significantly improved the EC, which decreased in the absence of this macronutrient (Fig. 5). Similarly, PCO_2 concomitantly decreased with EC during the N starvation cycles, which can be attributed both to the reduced CH_4 uptake and the CO_2 requirements for PHB production within the serine pathway in type II methanotrophs [28]. Interestingly, a slight decrease in the PHB content ranging from 1.1 % to 6.8 % was consistently observed during growth cycles. This decrease in the PHB content can be explained by the fact that PHB is consumed as a readily available carbon source by type II methanotrophs following N supply to the cultivation broth [31]. PHB accumulations up to 51.6 % (*w/w*) were previously reported for *Methylocystis* species under nitrogen limitation in a forced-liquid vertical tubular loop bioreactor under a 50:50 % (*v/v*) CH_4:air feeding, though the production of this added-value product was neither maintained under continuous operation for more than 8 hours nor carried out at comparable productivities [28, 8, 32]. In our particular study, PHB productivities remained roughly constant during operation under N feast-famine cycles at 1.82-2.23 kg m^{-3} d^{-1} (which corresponded to specific PHB productivities ranging from 15.9 to 21.6 mg PHB gX^{-1} h^{-1}). These productivities ranked among the highest reported in methanotrophic cultures in continuous CH_4 abatement bioreactors, which typically remained at ~0.03 kg m^{-3} d^{-1} [15]. Further research should focus on the evaluation of shorter N-limitation periods or alternative nutrient starvation strategies aiming at co-producing PHB along with a sustained abatement of dilute CH_4 emissions.

4. Conclusions

The implementation of internal gas-recycling strategies in a BCB resulted in a superior CH_4 abatement performance under continuous operation as a result of decoupling the EBRT and the gas-liquid turbulence governing CH_4 mass transport. The increase in the gas-recycling rate during the treatment of diluted CH_4 emissions entailed a concomitant increase in both EC and PCO_2 (regardless of the EBRT tested), while the decrease in EBRT from 120 min to 30 min increased the EC without a significant deterioration in the RE. N limitation was identified as the most effective nutrient starvation to induce PHB synthesis in *M. hirsuta* (compared to K, Mn and N limitations in excess of Fe). Process operation under optimum mass transfer conditions and repeated N feast-famine cycles resulted in ECs of 16.2 ± 9.5 g m^{-3} h^{-1}, PHB contents of 34.6 ± 2.5 % and PHB productivities of 1.4 ± 0.4 kg m^{-3} d^{-1}. Therefore, this study demonstrated for the first time the potential of internal gas-recycling BCBs for the continuous bioconversion of diluted CH_4 emissions into PHB at high productivities and under long-term operation.

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Figure captions

Fig. 1. Schematic of the experimental set up. (1) BCB, (2) sampling port, (3) rotameter, (4) mass flow controller, (5) cooling water, (6) condenser, (7) internal gas-recycling peristaltic pump and (8) liquid sampling port.

Fig. 2. Influence of the internal gas-recycling rate on the EC ( ), RE ( ) and CO_2 production rate ( ) at EBRTs of 60 min (A) and 120 min (B) in the BCB.

Fig. 3. Influence of the EBRT on the EC ( ), RE ( ) and CO_2 production rate ( ) at a constant internal gas-recycling ratio of 15 in the BCB.

Fig. 4. Influence of nutrient limitation on PHB accumulation (A) and final biomass concentration (B) in the stationary phase of the batch assays.

Fig. 5. Time course of the PHB cell content (A), biomass concentration (B), CO_2 production rate (C) and eliminations capacities (D) in the BCB during the continuous abatement of CH_4 coupled to PHB production. Vertical arrows indicate nitrogen addition during each N feast-famine cycle. Dashes lines indicate the start of limitation cycles.

Table 1. Experimental conditions evaluated during the optimization of CH_4 abatement in the internal gas-recycling BCB.

Condition	EBRT (min)	Inlet load ($g\ m^{-3}\ h^{-1}$)	Q_R/Q	Recycling rate ($m^3_{gas}\ m^{-3}_{reactor}\ min^{-1}$)	Virtual residence time (min)	Mineralization ratio (PCO_2/EC)
1	120	12	0	0.000	120	2.5 ± 0.2
2			2	0.017	40	2.1 ± 0.2
3			3	0.025	30	1.8 ± 0.4
4			6	0.050	17	1.7 ± 0.2
5			10	0.083	11	1.9 ± 0.3
6			15	0.125	8	2.0 ± 0.1
7	60	24	0	0.000	60	1.4 ± 0.2
8			2	0.033	20	1.7 ± 0.5
9			3	0.050	15	1.7 ± 0.2
10			6	0.100	9	1.6 ± 0.4
11			10	0.167	5	1.7 ± 0.1
12			15	0.250	4	1.7 ± 0.4
13	30	48	10	0.333	3	1.8 ± 0.2
14			15	0.500	2	1.9 ± 0.2
15	15	96	15	1.00	0.94	2.0 ± 0.1

460

Table 2. Micro and macro-nutrients limiting conditions evaluated during batch cultivation of *M. hirsuta*.

Conditions	Nutrient limitation	Nutrient in excess	Fe Concentration (µM)
Control	-	-	4.6
1	K	-	4.6
2	Mn	-	4.6
3	N	-	4.6
4	N	Fe	60

461

Figure

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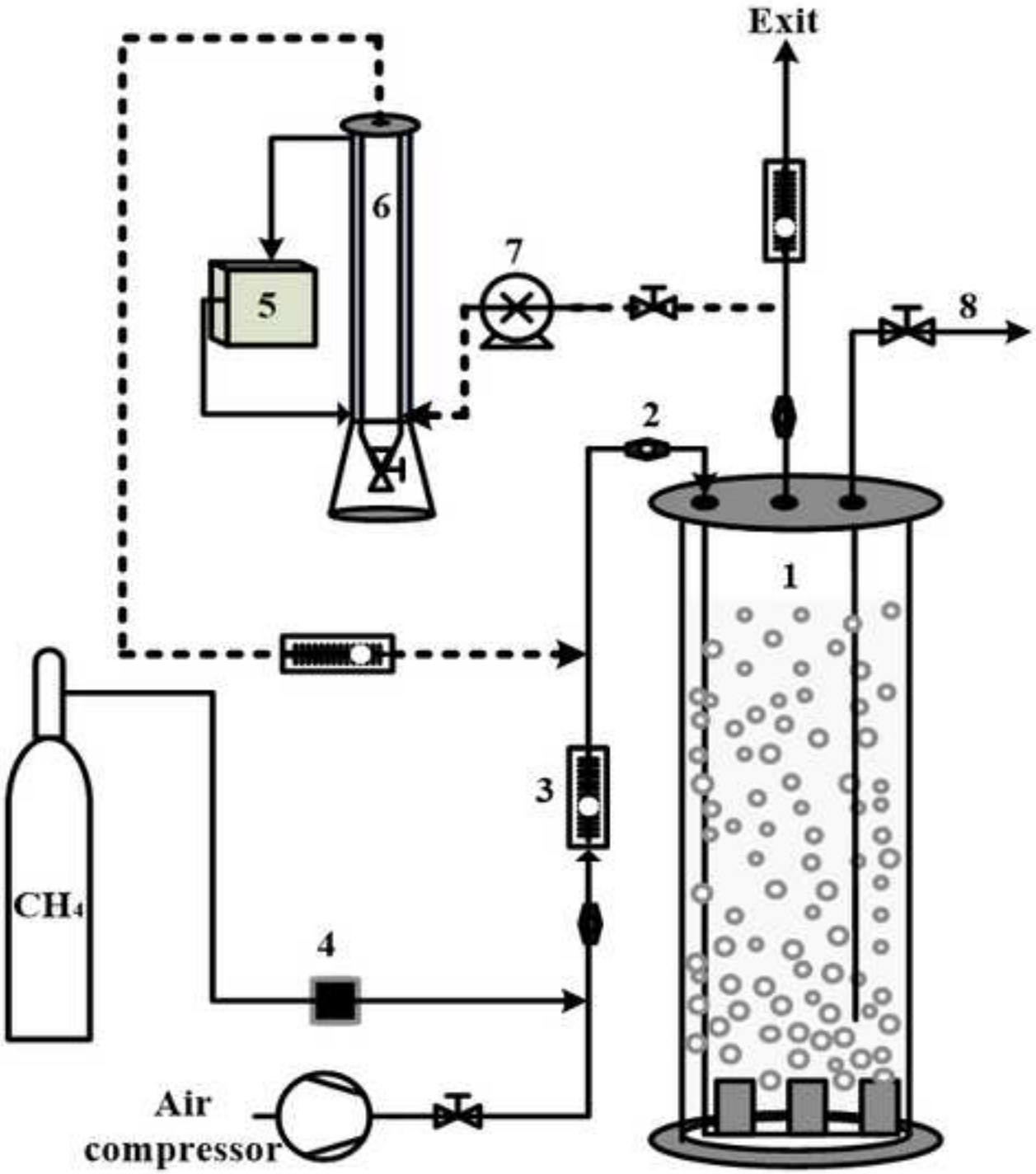

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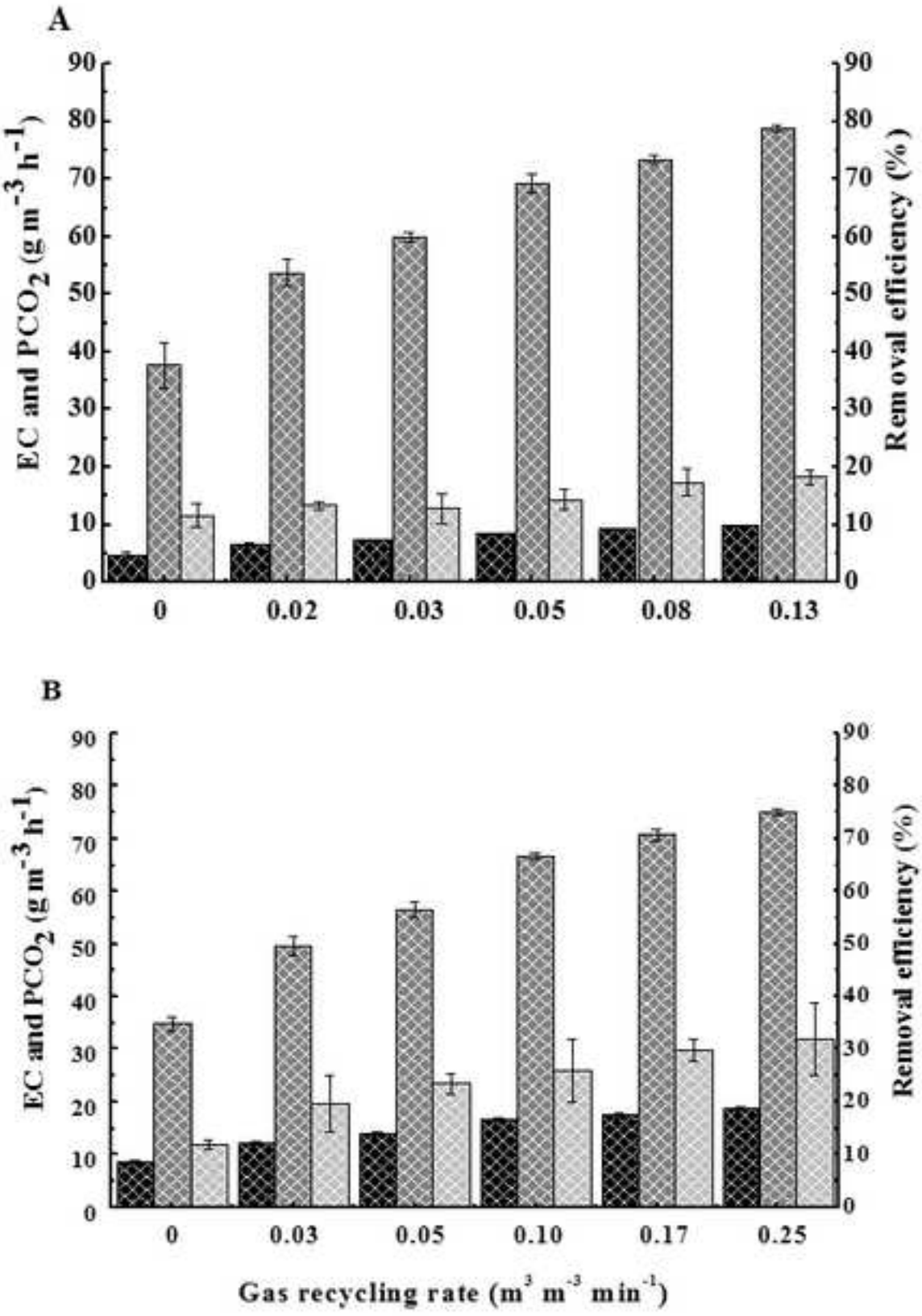

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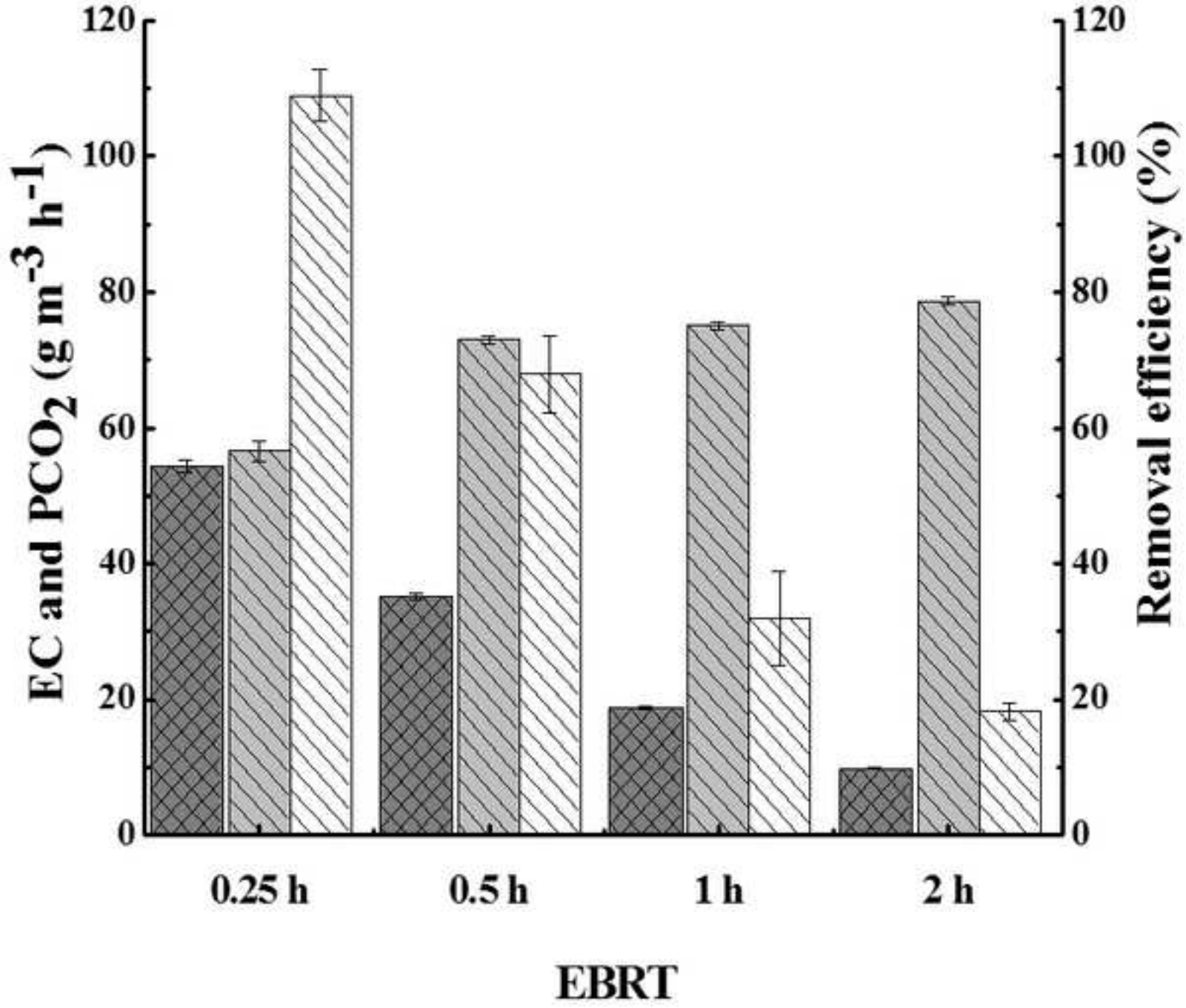

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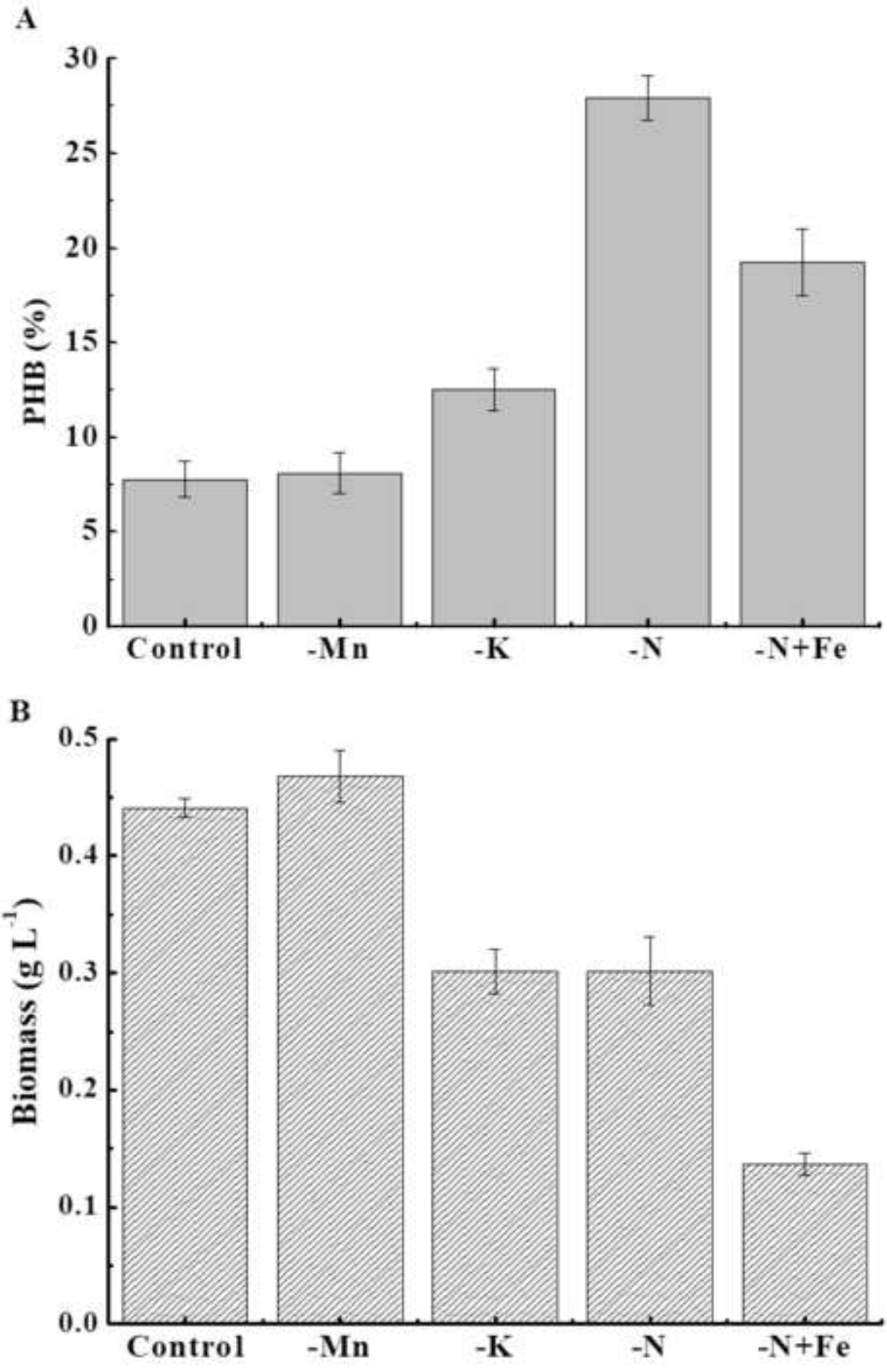

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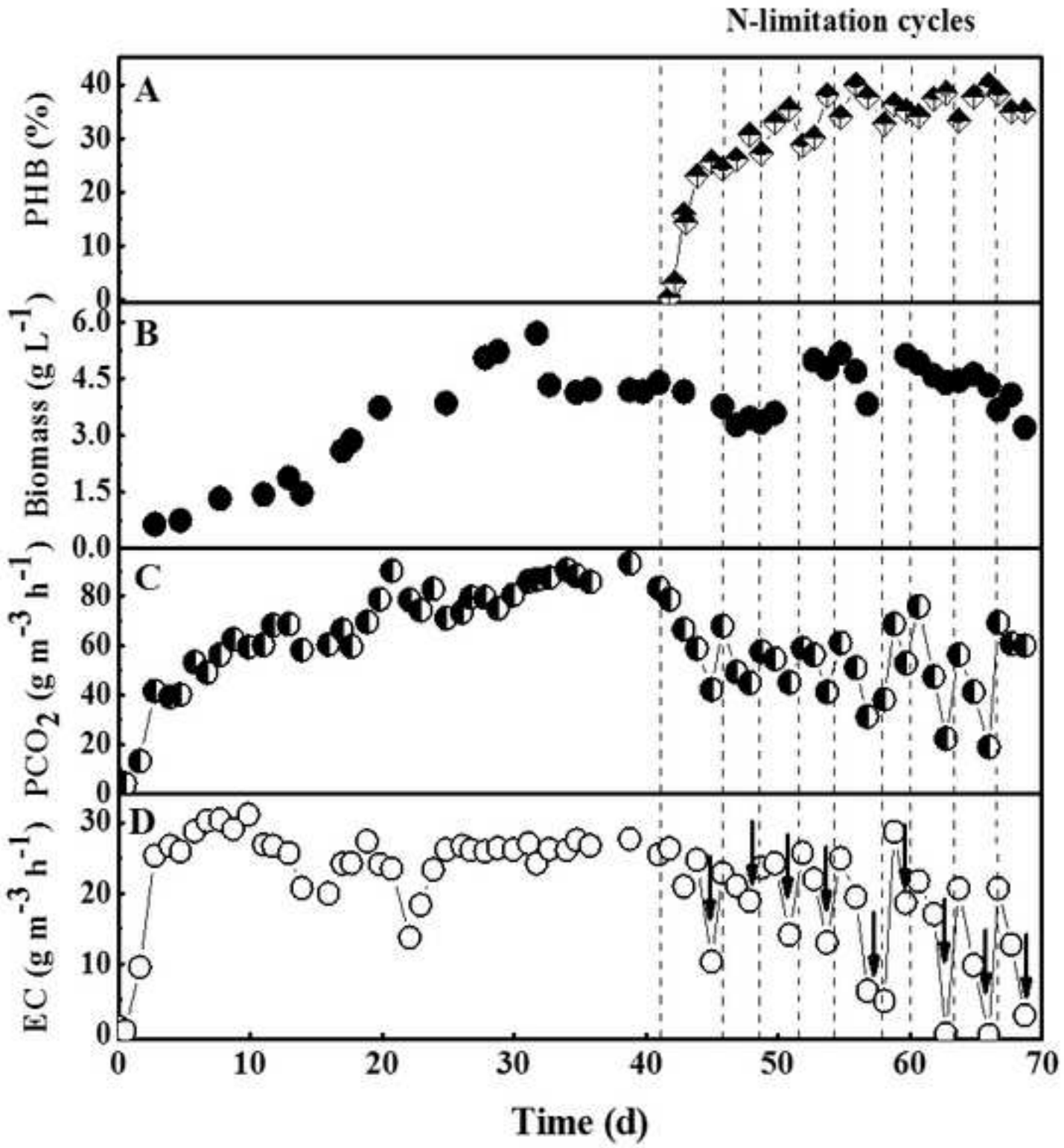

Supplementary Material

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