


REVIEW

Microbioreactor (micro-Matrix) potential in aerobic and anaerobic conditions with different industrially relevant microbial strains

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Abstract

Microscale fermentation systems are important high throughput tools in clone selection, and bioprocess set up and optimization, since they provide several parallel experiments in controlled conditions of pH, temperature, agitation, and gas flow rate. In this work we evaluated the performance of biotechnologically relevant strains with different respiratory requirements in the micro-Matrix microbioreactor. In particular *Escherichia coli* K4 requires well aerated fermentation conditions to improve its native production of chondroitin-like capsular polysaccharide, a biomedically attractive polymer. Results from batch and fed-batch experiments demonstrated high reproducibility with those obtained on 2 L reactors, although highlighting a pronounced volume loss for longer-term experiments. *Basfia succiniciproducens* and *Actinobacillus succinogenes* need CO₂ addition for the production of succinic acid, a building block with several industrial applications. Different CO₂ supply modes were tested for the two strains in 24 h batch experiments and results well compared with those obtained on lab-scale bioreactors. Overall, it was demonstrated that the micro-Matrix is a useful scale-down tool that is suitable for growing metabolically different strains in simple batch process, however, a series of issues should still be addressed in order to fully exploit its potential.

KEYWORDS

aerobic and anaerobic fermentations, microbioreactor, micro-Matrix, optimal growth conditions, scale down

1 | INTRODUCTION

In recent years, microbioreactor (MBR) systems demonstrated to be a helpful tool in high-throughput fermentation process design providing a large number of cultures running in limited space and with a limited amount of resources. MBR, in fact, allow to rapidly perform clonal

selection and screening for strain and culture medium improvement, and are useful in scale-down experiments to troubleshoot or debottleneck steps that are running sub-optimally,¹⁻³ thus shortening biotechnological process development.^{4,5} MBR represent the junction point between conventional stirred tank reactors (STR) and small-scale screening devices such as shake-flask cultures.⁶ In fact, STR are often

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not suitable for extensive optimization studies due to their slow turnover and high spatial demand. On the other hand, small scale cultures do not allow continuous control and monitoring of key growth parameters such as pH, dissolved oxygen (DO), and so forth.

In previous studies, three major issues were addressed: reproducibility among individual reactors (within the MBR), metabolic reproducibility of batch fermentation between scales, and process control of high-density aerobic microbial cultivations.⁷ Growth of bacteria and yeasts on these systems highlighted advantages and limitations of this approach.^{1–8} Miniaturized systems for the growth of filamentous microorganisms such as actinomycete and fungi, that take into account mass transfer, mixing, and environment homogeneity issues were also validated.⁹

Among MBR devices based on single use cassettes with pH and DO optodes and no impellers, the micro-Matrix (Applikon, Delft, The Netherlands) has recently been used in various studies for the growth of the Chinese hamster ovary (CHO) cell line in batch and fed-batch mode¹⁰ and for an *ex vivo* batch model of distal colon.¹¹ However, due to their higher growth rate compared to animal cells, microorganisms require higher gas transfer rates, monitoring of bioprocess variables at higher temporal resolution and even faster electrode response time. Since fermentations in batch and fed-batch mode on microbial strains have not been reported on the micro-Matrix MBR up to date, in the present work we aimed to assess the growth of three different microbial strains, and how those compared to results obtained on 2 L STR.

Increasing the experimental throughput to close the gap between screening and production conditions is useful for strains with industrial potential. Therefore, we tested microorganisms with different metabolic/respiratory needs, in particular (i) the facultative anaerobe *Escherichia coli* (O5:K4:H4) that produces a precursor of unsulfated chondroitin, a polymer with several potential biomedical applications, and (ii) *Actinobacillus succinogenes* and (iii) *Basfia succiniciproducens*; the latter two are anaerobic strains that naturally produce succinic acid, a precursor of many important bulk chemicals.

Fed-batch processes in DO-stat mode were also tested for *E. coli* K4 to evaluate strain and machine performance with extended process time.

2 | MATERIALS AND METHODS

2.1 | Strains and reagents

E. coli serotype (O5:K4:H4) and *B. succiniciproducens* were purchased from the Culture Collection of University of Gothenburg collection, CCUG 11307, and CCUG 57762 respectively. *A. succinogenes* 130Z was purchased from the ATCC collection as ATCC 55618D-5. All growth medium components such as glucose, antifoam agent, ammonium hydroxide, and sulfuric acid as well as salts, acid or alkali components used to prepare buffers for capillary electrophoresis and anion exchange chromatography, were purchased from Sigma-Aldrich. Agar

was furnished by Oxoid (UK). The yeast extract was bought from OrganoTechnie (La Courneuve, France).

2.2 | Media

2.2.1 | *E. coli* K4

For *E. coli* K4, a semidefined medium was used for all shake flask, MBR and fermenter experiments that consisted of a basal salt medium containing per liter: KH_2PO_4 , 2g; K_2HPO_4 , 9.7g; trisodiumcitrate dihydrate, 0.5g; $(\text{NH}_4)_2\text{SO}_4$, 1g; MgCl_2 , 0.1g supplemented with glucose 10g as the main carbon source, and yeast extract 2g as additional nitrogen source.¹²

2.2.2 | *A. succinogenes* 130Z and *B. succiniciproducens*

The strains were grown in semi-defined MH medium. The medium contained per liter: glucose, 10–14g; yeast extract, 5g; $(\text{NH}_4)_2\text{SO}_4$, 2g; $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 0.2g; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.2g; NaCl, 2g; K_2HPO_4 , 3g; MgCO_3 , 10g; $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 1mg as described in Cimini et al.^{13–15}

2.3 | Batch and fed-batch fermentation experiments

Fermentation conditions are described in Table 1.

2.3.1 | CT-plus batch and fed-batch fermentation experiments

All fermentation experiments were carried out in a Biostat CT plus reactor 3 L total volume (Sartorius Stedim; Melsungen, Germany). Automated addition of NH_4OH 25% v/v and H_2SO_4 30% v/v ensured the maintenance of a constant pH. Samples were withdrawn at regular time intervals to analyze biomass production, carbon source consumption and metabolite production (acids, polysaccharides). All experiments were carried out at least in triplicate.

For *E. coli* K4 before each experiment, cells from exponential phase glycerol 20% (v/v) stock preparations were inoculated in a pre-culture 1 L shake-flask with 0.2 L (1:5 medium/air volume ratio) of standard medium and incubated for 3 h at 37°C and 200 rpm shaking condition in a rotary shaker incubator (model Minitron, Infors, Bottmingen, Switzerland). The whole flask was used as inoculum for the main batch and fed-batch fermentations. Experiments were performed with a 1.6–1.8 L working volume, 37°C and pH was maintained constant at 7.5. For batch experiments the semidefined medium described in paragraph 2.2.1 with glucose and yeast extract was used. Moreover, 200 $\mu\text{l/L}$ of antifoam 204 (Sigma-Aldrich) were added to the medium before autoclaving. An air flow rate of 1 vvm

TABLE 1 Growth conditions applied for each strain in MBR and STR experiments

Microfermenter							
Microorganisms	Starting volume (mL)	Time (h)	Glucose (g/L)	Agitation (rpm)	Acid/base (% v/v)	Gas	PO ₂ set point (%)
<i>E. coli</i> K4 Batch	4	24	10	400	CO ₂ (gas) NH ₄ OH 12.5% (liquid)	Air	20 ^a
<i>E. coli</i> K4 Fed-batch	3	48	10 + feed	400	CO ₂ (gas) NH ₄ OH 12.5% (gas)	Air	30 ^a
<i>A. succinogenes</i>	5	24	14	130	CO ₂ (gas) NH ₄ OH 12.5% (liquid)	CO ₂	0 (discontinuous pulses) ^b
<i>B. succiniciproducens</i>	4.5	24	14	130	CO ₂ (gas) NH ₄ OH 12.5% (liquid)	CO ₂	0 (discontinuous pulses) ^b
STR							
Microorganisms	Starting volume (L)	Time (h)	Glucose (g/L)	Agitation (rpm)	Acid/base (% v/v)	Gas	PO ₂ set point (%)
<i>E. coli</i> K4 Batch	1.8	24	10	550	H ₂ SO ₄ 30%, NH ₄ OH 25%	Air	20 ^a
<i>E. coli</i> K4 Fed-batch	1.6	48	10 + feed	550	H ₂ SO ₄ 30%, NH ₄ OH 25%	Air	30 ^a
<i>A. succinogenes</i>	2.5	24	14	200	H ₂ SO ₄ 30%, NH ₄ OH 25%	CO ₂	0 (discontinuous pulses) ^b
<i>A. succinogenes</i>	2.5	24	14	200	H ₂ SO ₄ 30%, NH ₄ OH 25%	CO ₂	0 (constant sparging)
<i>B. succiniciproducens</i>	2.5	24	14	200	H ₂ SO ₄ 30%, NH ₄ OH 25%	CO ₂	0 (discontinuous pulses) ^b
<i>B. succiniciproducens</i>	2.5	24	14	200	H ₂ SO ₄ 30%, NH ₄ OH 25%	CO ₂	0 (constant sparging)

^aThe set-point was maintained by sparging air continuously and pure oxygen if necessary.

^bThe pO₂ value was set to 0% and CO₂ was automatically added if necessary by the software when the DO raised above 0%.

and a stirring rate of 550 rpm according to oxygen demand was maintained. For fed-batch experiments cells were grown in the same semidefined medium containing glucose (10.0 ± 1.5g/L) and yeast extract (2g/L). A DO-Stat controlled feeding strategy with a pO₂ set point equal to 30% of air saturation was used. After the batch phase, a concentrated nutrient solution (about 350g/L glucose and 70g/L yeast extract and inorganic salts 20-fold concentrated) was fed to the culture in the following 42 h of growth. About 120 ± 10g/L of glucose were added during the feed phase.¹⁶

A vial of working cell banks of either *A. succinogenes* 130Z or *B. succiniciproducens* were inoculated in 0.25 L bottles containing MH medium with 10g/L glucose as C-source and incubated at 37°C and 130 rpm in a rotary shaker incubator. Once the strains reached the exponential phase, about 0.2 L of pre-culture were transferred in a Biostat CT plus reactor containing MH with 14 ± 1g/L of glucose. Fermentations were carried out at a temperature of 37°C, agitation speed of 200 rpm with and pO₂ at zero % maintained either by a constant sparging of CO₂ set at 1 vvm or by automated pulsed additions of CO₂ in loop, activated when the pO₂ value was higher than the set-point. A pH of 6.5 was constantly maintained throughout the fermentation.

2.3.2 | Microbioreactor fermentations

The Applikon micro-Matrix MBR¹⁷ is composed of a built-in orbital shaker operating at shaking speeds between 0 and 400 rpm at an

orbital throw of 25 mm. The cassette is placed in a temperature-controlled chamber at a defined set point. Additionally, the temperature can be controlled individually for each well, with a maximum difference of 1°C between adjacent wells. The DO is typically controlled with nitrogen, oxygen, and air. Control of the pH is achieved through gas addition of carbon dioxide and ammonia. Furthermore, the liquid feed line can be used for automated additions of base or of a feed medium for fed-batch experiments. The DO, pH, and temperature set values are maintained through PID control loops that can be customized for each well individually. Prior to inoculation, pH offset values of the optical sensors within the micro-Matrix cassette (Applikon) were estimated by equilibrating the sensors in each well with the medium of the microorganism to use. Offline measurements of the same solution were then used to determine and correct the offset. A starting working volume of 3–5 ml was used for the fermentation processes, and specifications for each strain and process type are indicated in Table 1. Each well was then inoculated with 0.1 OD₆₀₀ of *E. coli* K4 or *B. succiniciproducens* or *A. succinogenes* and the fermentation conditions for each strain are listed in Table 1. For DO-stat experiments the pO₂ was set to 30% of air saturation; a concentrated solution (200g/L glucose and 40g/L yeast extract, inorganic salts 20-fold concentrated) was used for the feeding. About 120 ± 10g/L of glucose were added during the feed phase.

After inoculation and at the end of the experiment, the absorbance (600 nm) was measured to evaluate biomass production with a DU800 spectrophotometer (Beckman Coulter). For all fermentations the

concentration of carbon sources and produced acids present in the culture supernatant were evaluated by High Performance Liquid Chromatography (HPLC). For batch and fed-batch processes involving *E. coli* K4, production of K4 capsular polysaccharide (CPS) and/or chondroitin was evaluated by capillary electrophoresis as reported in the following paragraph. At least six replicates of each condition were performed.

2.4 | Analytical methods

2.4.1 | High-performance capillary electrophoresis (HPCE) analyses

Samples withdrawn from *E. coli* K4 supernatants were concentrated and diafiltered on 3 kDa centricon tubes (Millipore, Bedford, MA) at $\times 12,000g$ for the quantification of the CPS. The concentrations of K4 CPS were analyzed by HPCE by using a P/ACE MDQ instrument (Beckman Coulter) equipped with a diode array detector and a UV lamp, using an uncoated fused silica tube (70 cm of total length, 60 cm of effective length, 50 μm ID) at 25°C according to previously described methods.¹⁸

2.4.2 | Analysis of carbon source consumption and produced acids

The permeate obtained after the treatment of fermentation supernatants on 3 kDa centricon membranes was analyzed for the determination of glucose, xylose and acids produced during growth by an HPLC instrument (UHPLC Dionex Ultimate 3000; Thermofisher), equipped with UV-VIS and RI detectors as previously described. Briefly, runs were executed in isocratic mode on an Alltech IOA-2000 organic acids (6.5 \times 150 mm, 5 μm) column. The mobile phase consisted of 0.1% H₂SO₄ in H₂O v/v. Peak areas were evaluated through the Thermofisher Chromeleon Software.

2.4.3 | Statistical analyses

Data were compared by means of two tailed non homoscedastic t-student analyses.

3 | RESULTS AND DISCUSSION

MBR growth experiments were performed in order to compare results with those previously obtained in an STR bioreactor. One of the key points of this work was to evaluate the possible application of the micro-Matrix MBR as a rapid and robust tool for strain screening, parameters modification, and validation after a design of experiment strategy for the industrial research.

3.1 | *E. coli* K4

The wild type *E. coli* K4 was grown on a 2 L Biostat CT plus bioreactor and on the micro-Matrix in batch and fed-batch conditions to compare growth and CPS production. Glucose was chosen for fed-batch processes as it is one of the most used carbon sources on industrial scale and we previously used it to compare strain performance of several different recombinant strains to that of the wild type.^{19,20} Capillary electrophoresis analyses showed that CPS production was highly comparable between STR and micro-Matrix in both process set-ups. In fact, in batch mode 315 \pm 59 and 300 \pm 14 mg/L of K4 CPS were produced on the microfermenter and on the 2 L bioreactor, respectively. Moreover, as demonstrated by similar $Y_{p/x}$, $Y_{p/s}$, $Y_{x/s}$ and volumetric productivities the overall process performance on the two scales was considered reproducible (Table 2). A slightly higher (10%) concentration of biomass was obtained in the 2 L bioreactor, that was however not statistically significant ($p > 0.05$).

Similar final K4 CPS concentrations of about 1.41 \pm 0.20 and 1.57 \pm 0.05g/L were also obtained in fedbatch mode on the microfermenter and 2 L STR, respectively (Table 2). $Y_{p/x}$, $Y_{p/s}$, and volumetric productivities (r_p) were significantly similar on the two systems demonstrating process consistency. Only a 12% higher $Y_{x/s}$ was found in the MBR although, on the overall carbon balance, this small though significant ($p < 0.05$) change in resources distribution to biomass did not affect polysaccharide synthesis (Table 2). Differently from the batch process, in fed-batch mode the pH was controlled by addition of gaseous ammonia; in these conditions the pH was slightly higher than the set-point (7.7 vs. 7.5). Overall, the results obtained here are directly in line with previous findings in literature.^{21,22} However, one limitation of the micro-Matrix is the large loss of volume in

TABLE 2 *E. coli* K4 batch (a) and Fed-batch (b) experiments—Experiments were run on a Biostat CT plus reactor and on the Micro-Matrix

		K4-CPS (g/L)	Biomass (g _{cdw} /L)	$Y_{x/s}$ (g _{cdw} /g _{glu})	$Y_{p/s}$ (mg/g)	$Y_{p/x}$ (mg/g _{cdw})	r_p^* (mg/L-h)
Batch	MBR	0.32 \pm 0.06	3.6 \pm 0.1	0.36 \pm 0.01	31.6 \pm 5.8	86.6 \pm 13.8	13.1 \pm 2.5
	STR	0.30 \pm 0.01	4.0 \pm 0.1	0.40 \pm 0.01	30.8 \pm 0.3	75.0 \pm 0.9	12.5 \pm 0.6
Fed-batch	MBR	1.41 \pm 0.20	17.3 \pm 0.8	0.18 \pm 0.01	14.4 \pm 1.9	81.5 \pm 9.9	29.4 \pm 4.1
	STR	1.57 \pm 0.05	19.0 \pm 0.9	0.16 \pm 0.00	12.9 \pm 0.1	82.2 \pm 1.3	32.6 \pm 1.0

Note: Batch and fed-batch experiments lasted 24 and 48 h, respectively. The semidefined medium contained glucose and yeast extract as main C and N sources. The DO was maintained above the set point by sparging air and oxygen in batch and by adding a concentrated solution of glucose and YE. The production of polysaccharide (K4-CPS) and of biomass was measured. Yield of K4 CPS on glucose ($Y_{p/s}$), yield of K4 on biomass ($Y_{p/x}$) and yield of biomass produced on glucose consumed ($Y_{x/s}$). r_p^* indicates the volumetric productivity calculated in 24 and 48 h respectively for batch and fed-batch processes. K4 CPS and biomass concentrations, and all yields were recalculated for fed-batch experiments considering the volume lost during the experiment.

TABLE 3 *B. succiniciproducens* batch experiments—Experiments were run on a Biostat CT plus reactor with constant sparging of CO₂ and with discontinuous CO₂ pulses, and on the micro-Matrix with discontinuous CO₂ pulses for 24 h

	Succinic acid (g/L)	Acetic acid (g/L)	Biomass (g _{cdw} /L)	Y _{x/s} (g/g)	Y _{p/s} (g/g)	Y _{p/x} (g/g)
MBR CO ₂ pulses	8.85 ± 0.48	3.97 ± 0.20	3.42 ± 0.42	0.24 ± 0.03	0.63 ± 0.03	0.64 ± 0.07
STR CO ₂ pulses	8.08 ± 1.31	4.31 ± 0.79	3.68 ± 0.20	0.26 ± 0.03	0.59 ± 0.12	0.55 ± 0.05
STR Constant CO ₂ sparging	9.52 ± 1.33	4.31 ± 0.99	3.24 ± 0.41	0.23 ± 0.03	0.68 ± 0.09	0.73 ± 0.15

Note: In both conditions CO₂ sparging maintained the DO at 0%. Production of succinic acid (SA), acetic acid (AA) and biomass was measured. Yield of SA on glucose (Y_{p/s}), yield of SA on biomass (Y_{p/x}) and yield of biomass produced on glucose consumed (Y_{x/s}). All yields were calculated as g/g.

TABLE 4 *A. succinogenes* batch experiments—Experiments were run on a Biostat CT plus reactor with constant sparging of CO₂ and with discontinuous CO₂ pulses, and on the micro-Matrix with discontinuous CO₂ pulses for 24 h

	Succinic acid (g/L)	Acetic acid (g/L)	Biomass (g _{cdw} /L)	Y _{x/s} (g/g)	Y _{p/s} (g/g)	Y _{p/x} (g/g)
MBR CO ₂ pulses	7.80 ± 0.67	5.87 ± 0.43	3.48 ± 0.56	0.25 ± 0.04	0.56 ± 0.05	0.73 ± 0.09
STR CO ₂ pulses	7.33 ± 0.92	5.37 ± 0.47	5.15 ± 0.19	0.37 ± 0.01	0.52 ± 0.07	0.46 ± 0.04
STR constant CO ₂ sparging	8.11 ± 1.23	5.22 ± 0.95	5.55 ± 0.64	0.40 ± 0.04	0.56 ± 0.08	0.47 ± 0.06

Note: In both conditions CO₂ maintained the DO at 0%. Production of succinic acid (SA), acetic acid (AA) and biomass was measured. Yield of SA on glucose (Y_{p/s}), yield of SA on biomass (Y_{p/x}) and yield of biomass produced on glucose consumed (Y_{x/s}). All yields were calculated as g/g.

long-term experiments. In fact, in order to interpret data correctly, we considered the substantial volume reduction observed at the end of the experiment in the calculation of all yields. Wiegmann and co-workers²³ in a previous study found a liquid loss of about 33% in respect to the initial volume, when analyzing 13-days batch cultures of CHO cells. In the fed-batch experiments described here, after only 48 h of growth the final culture volume was 31% lower than expected at the end of the process. This is probably due to a less efficient condensation system compared to larger scale bioreactors with ad hoc designed cooling elements that help recovering water in the outlet gas stream. Moreover, the continuous air sparging needed for pO₂ maintenance is probably also responsible for such high evaporation rate. Therefore, in order to run fed-batch processes with microorganisms that have high oxygen demands, compensation for liquid losses, as suggested by Wiegmann et al.,²³ should be considered.

3.2 | *B. succiniciproducens* and *A. succinogenes* 130Z

Succinic acid continues to attract increasing interest due to its wide number of applications in various fields such as food, chemical and pharmaceutical, with a recent focus on its use as precursor for the production of polybutylene succinate and other biodegradable polymers.²⁴ Therefore, we evaluated the performance of two natural producers of succinic acid, *A. succinogenes* 130Z and *B. succiniciproducens*, that require CO₂ for growth, also to test the MBR performance in anaerobic conditions.

Mannheimia succiniciproducens is also used for the production of succinic acid and it was previously demonstrated that the concentration of dissolved CO₂ in the broth strongly regulates succinic acid production.²⁵ In fact, the high level of CO₂ upregulates the metabolic

carbon flux through the phosphoenolpyruvate carboxykinase pathway, addressing resources to the biosynthesis of succinic acid. Growth of *B. succiniciproducens* on the 2 L bioreactor under discontinuous automated addition of CO₂ to maintain the pO₂ set-point (zero %) resulted in a 15% lower final titer of succinic acid (Table 3, *p* > 0.05). Reducing the amount of supplied CO₂ would be more convenient from an economic and environmental point of view, therefore, experiments on the MBR were run with discontinuous CO₂ additions.

As shown in Table 3, results were comparable in terms of succinic acid, biomass and side product production, and also the yields on the two different set ups were not significantly different. Averaged data of all experiments demonstrated the production of 8.8 ± 1.0g/L of succinic acid with an Y_{p/s} of about 0.64 ± 0.08g/g.

For *A. succinogenes* the presence of a constant CO₂ flow only increased by 10% (*p* > 0.05) the final concentration of succinic acid on the Biostat CTplus bioreactor (Table 4). Similar succinic acid production titers were obtained on the two scales with an average concentration of 7.7 ± 0.7g/L and an Y_{p/s} of about 0.55 ± 0.05g/g (Table 4).

However, unexpectedly a 30% lower (*p* < 0.01) biomass concentration was observed on the micro-Matrix which resulted in a higher Y_{p/x}. In all experiments anaerobic conditions were efficiently maintained (Figure S1), moreover the addition of liquid ammonia efficiently controlled the pH, despite the constant CO₂ sparging (Figure S1).

The micro-Matrix demonstrated suitable to evaluate microbial growth and production of biotechnologically appealing metabolites such as acids and polysaccharides, in short batch processes. It is, however, also important to assess the reproducibility of the results obtained among wells. Data from all the experiments performed on each strain have shown that on average a failure rate of about 33% was obtained. This suggests that in order to obtain at least a biological triplicate an entire row of the cassette (six wells) should be dedicated

to each process set-up. Also additional experiments were considered to eventually link the specific localization of each MBR within the plate to experiment failure, and a correlation was not found (data not shown). However, a larger data set should be analyzed to support this conclusion.

A. succinogenes 130Z and *B. succiniciproducens* are promising cell factories for the production of succinic acid; *A. succinogenes* can grow on high concentrations of carbon sources and it is resistant to inhibitory molecules released during the hydrolysis of lignocellulosic materials; the latter are highly used for the production of succinic acid to increase the economic viability of biotechnological processes. In this respect there are several limitations to the use of the micro-Matrix for process optimization such as the dark color of the hydrolysed medium and the presence of eventual precipitates and solid particles that interfere with the optical sensor of pH, DO, and temperature; this feature should be improved.

4 | CONCLUSIONS

Overall the experiments performed in this work (i) highlight the scale down potential that fastens the optimization of simple fermentation experiments on semidefined media, (ii) demonstrate the possibility of growing both facultative aerobic and anaerobic bacterial strains, and (iii) show comparable results to lab scale stainless steel bioreactors with a 400-fold larger working volume. On the other hand, some issues such as the reproducibility among wells, the strong reduction of the working volume caused by gas sparging in longer (48 h) fed-batch experiments, and the less efficient control of pH with gas as compared to liquid, still limit the robustness of the applicability of these kind of MBR.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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