Process Development and Scale-up for *Vibrio natriegens*: From BioLector XT Microbioreactor to Pre-pilot Scale

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Abstract

Optimization, scalability and transferability represent key challenges in bioprocess development. In this study, scalable fermentation processes for *Vibrio natriegens* were explored using the BioLector XT Microbioreactor. Initial cultivation parameters, including glycerol concentration (20, 30 and 50 g/L), inoculation density (OD_{600} 0.1-0.5) and induction timing were systematically evaluated. Different feeding strategies (constant, linear, exponential) and feed medium compositions (1× and 4× VN medium) were compared to optimize mScarlet production. Two optimized process conditions were subsequently transferred to 20-L bioreactors to validate scalability. The high-performance process (4× VN feed, feed start at four hours) achieved 1.8-fold higher biomass compared to the low-performance process (1× VN feed, feed start at eight hours) in both scales. A strong correlation was observed between the BioLector XT Microbioreactor and the 20-L bioreactor performance, demonstrating successful process transferability. The data obtained in the BioLector XT instrument, regarding medium composition and process strategy, was representative of what was observed in the 20-L bioreactor, allowing strong confidence in the scalability and transferability of processes developed at microscale and providing better understanding of *V. natriegens* physiology.

Introduction

The development and optimization of microbial fermentation processes face significant challenges when transitioning from laboratory scale to industrial production scales. To optimize product yields, producer strains are genetically and metabolically engineered, and fermentation processes are optimized and scaled to technical limits to achieve the highest space-time yields. Key considerations include the solubility, instability and volatility of solid and gaseous substrates and products. Moreover, most media ingredients become inhibitory for biological systems at high concentrations. For example, in a defined medium containing the maximum non-inhibitive concentration of nutrients, Escherichia coli can be batch-cultivated only to a cell density of ~15 g(cdw)/L. Therefore, well-designed media and feeding strategies are required not only to satisfy the increasing demands for energy, carbon, nitrogen and further nutrients while preventing unwanted substrate inhibition, but also to maximize cost efficiency by minimizing expensive substrate waste and reducing downstream processing expenses.

One organism of recent interest is *Vibrio natriegens* (alternatively known as *Pseudomonas natriegens* or *Beneckea natriegens*). It is the fastest-growing, non-pathogenic organism known to date, with reported generation times of only 9.4 minutes under optimal conditions. Like E. coli, it is a Gram-negative, facultatively anaerobic bacterium, but it has higher glucose and oxygen uptake rates to support its fast-growth characteristics. It can grow on a wide variety of substrates and has been reported to secrete acetate as an overflow metabolite during aerobic cultivation on glucose (Hoffart et al. 2017; Della Valle et al. 2025).

The fast growth rates make *V. natriegens* a valuable chassis for many biotechnological applications and cloning procedures and, since 2016, accessible for genetic manipulation (Weinstock et al. 2016). A collection of genetic tools has been established and applied to genetically engineer superior production and platform strains, but further fundamental knowledge is essential for future metabolic engineering strategies, particularly regarding process scalability and transferability from laboratory to industrial scales.

The BioLector XT Microbioreactor is a powerful platform to optimize these high-oxygen-demanding cultivation strategies and processes, as it is capable of parallel fed-batch cultivations in µL scale, precisely controlling feed rates per well. The system enables high-throughput screening of up to 32 different process conditions simultaneously, providing automated bioprocess development capabilities. Real-time, non-invasive monitoring of key cultivation parameters, including biomass (via scattered light), pH, DO, and fluorescence occurs approximately every six minutes, generating comprehensive datasets for detailed process analysis. The microfluidic module enables precise pH-controlled, fed-batch cultivations with automated trigger-based feeding strategies, while the O_2 up-regulation module prevents oxygen limitation through gradual oxygen concentration increases up to 100%.

Furthermore, the sophisticated gassing lid gives superior control over gassing composition (0 to 100% oxygen) and flow rates. Oxygen-enriched air, up to 100% oxygen, can be used to provide the cultures with sufficient oxygen to enable high oxygen transfer rates, especially when FlowerPlate microtiter plates are used, supporting the high oxygen demands of fast-growing *V. natriegens*.

The objective of this study was to improve understanding of the physiology of *Vibrio natriegens* and to validate that microscale cultivation optimization correlates well with pre-pilot scale. The first step involved defining the composition of the culture medium. The initial concentration of glycerol was evaluated by comparing growth kinetics, biomass and mScarlet production when inoculated in media containing 20, 30 or 50 g/L of glycerol. The production process was then defined by comparing different feeding strategies (constant, linear or exponential flow rate), feed-medium compositions and feeding start times. Finally, to confirm the suitability of developing fermentation processes at small scale and the transferability of data and performance obtained with the BioLector XT Microbioreactor, two process strategies were transferred into a 20-L bioreactor.

Materials and Methods

Strains and Cultivation Media

A commercial chassis of *Vibrio natriegens* (strain Vmax) was engineered to produce a fluorescent protein, mScarlet—a bright, photostable, fast-maturing red fluorescent protein engineered for molecular biology applications (strain Vn_mS) under a lactose operon inducible with IPTG. Both strains were stored in 20% glycerol at −80 °C.

Seed culture medium: *Vibrio natriegens* was seed cultivated in LBVN containing 22 g L^{-1} NaCl; 10 g L^{-1} Tryptone; 10 g L^{-1} $MgCl_2 \cdot 6H_2O$; 5 g L^{-1} Yeast Extract; KCl 0.31 g L^{-1}; 0.1 g/L ampicillin.

Cultivation medium (VN_glycerol): 20 g L^{-1} glycerol; 15 g L^{-1} NaCl; 10 g L^{-1} yeast extract; 1 g/L KH_2PO_4; 1g/L K_2HPO_4; 0.5 g/L $MgSO_4 \cdot 7H_2O$; 0.1 g/L ampicillin.

Feed medium:

- 1×VN + 8% glycerol: all elements in VN_glycerol medium at the same concentration as mentioned above, except glycerol (80 g L^{-1})
- 4×VN + 8% glycerol: all elements in VN_glycerol concentrated four times including glycerol

Cultivation in BioLector XT Microbioreactor

Vibrio natriegens was cultivated in BioLector XT Microbioreactors equipped with a microfluidic module. Cultivations were performed in Gen2 Microfluidic FlowerPlate microtiter plates (part # M2P-MTP-MF32C-BOH1), sealed with gas-permeable sealing foils suited for microfluidic experiments (part # M2P-F-RSMF32-1). For real-time evaluation of the key cultivation parameters, the following filter modules were installed: Biomass (ID: 501), pH (HP8, ID: 502), DO (PSt3, ID: 503) and mScarlet (ID: 508).

The BioLector XT Microbioreactor features a gassing lid that enables precise control of gas composition—ranging from 0 to 100% oxygen or up to 12% carbon dioxide—and flow rate, while the FlowerPlate microtiter plate ensures a maximum oxygen transfer rate.

Here, experiments were conducted using normal air (21% oxygen) and with increased oxygen concentrations up to 100%, both at a fixed flow rate of 50 mL × minute^{-1}. For the experiments with increased oxygen concentrations, the O_2 up-regulation gassing mode was used, and the oxygen content was gradually ramped up during the first hour after which it was kept constant at 100%.

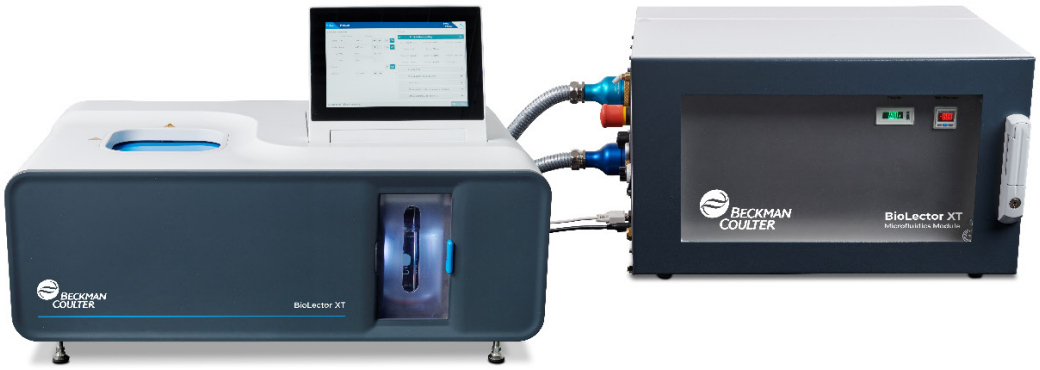


Figure 1: BioLector XT Microbioreactor equipped with a microfluidic module, featuring Gen2 Microfluidic FlowerPlate microtiter plates sealed with gas-permeable foils, and fitted with Biomass, pH, and DO filter modules for real-time monitoring of *Vibrio natriegens* cultivation parameters.

Definition of Cultivation Conditions

Batch cultures were performed in BOH1 or BOH2 FlowerPlates (part # M2P-MTP-MF32C-BOH1 and M2P-MTP-MF32C-BOH2). To determine the optimal conditions for fast and efficient strain growth and mScarlet production, several cultivation conditions were tested. The parameters investigated included the initial glycerol concentration, the timing of induction and the quantity of biomass at inoculation (quantified as initial OD_{600} nm).

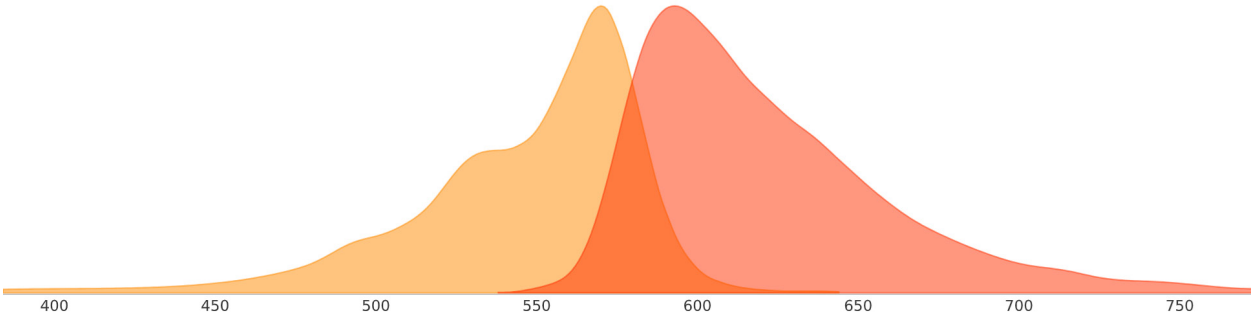
Definition of Feeding Strategy

Fed-batch cultures were conducted in BOH1 Microfluidic FlowerPlates. Cultures started with 800 µL medium, increasing to 1,300 µL after feed and base addition. Different feeding strategies were tested by varying feeding solution formulations, culture feeding rate and timing for feed start. mScarlet expression was induced by adding 1 mM IPTG to the medium one hour after inoculation.

Choice of Cultivation Plate

The Gen2 Microfluidic FlowerPlate (part # M2P-MTP-MF32C-BOH1) features 32 cultivation wells (rows C–F), each equipped with dual optodes for pH and DO monitoring, plus two reservoir rows (A and B) connected via microfluidic channels for automated feed and base addition. The plate supports culture volumes from 800 µL up to 1,400 µL (at 1,200 rpm) and reservoir volumes up to 1,800 µL, and its microfluidic architecture and gas-permeable sealing foil (M2P-F-RSMF32-1) enable precise µL-scale fed-batch experiments, pH control, and oxygen-enriched gassing suitable for a wide range of high-throughput microbial process development.

The spectral properties of mScarlet (excitation 569 nm, emission 594 nm), in combination with the measurement wavelength of the optical sensors, were considered during selection of the cultivation plates (**Figure 2**).



pH measurement	M2P-MTP-MF32C-BOH 1	M2P-MTP-MF32C-BOH 2	M2P-MTP-MF32C-BOH 3	DO measurement	M2P-MTP-MF32C-BOH 1	M2P-MTP-MF32C-BOH 2	M2P-MTP-MF32C-BOH 3
Wavelength	ex.: 470 nm: em.: 525 nm	ex.: 508 nm: em.: 550 nm	ex.: 625 nm: em.: 750 nm	Wavelength	ex.: 520 nm: em.: 600 nm	ex.: 625 nm: em.: 775 nm	ex.: 625 nm: em.: 775 nm

Figure 2: mScarlet excitation/emission spectrum (source: FPBase) and the wavelengths of the pH and DO optodes of the three available plate types.

The optimal growth pH for *V. natriegens* is 7, so plate type BOH3 (optimized for pH range 5.5–6.5) was not used. However, by using BOH2 (pH range 6.5–8.5), fast saturation of pH signal—corresponding to the overlap in the excitation and emission spectra—was noticed, so the experimental plan was developed with BOH1 plates (pH range 5.1–7.1).

Regarding the specifications of BioLector XT cultivation plates, the risk of spectral overlap with pH and DO detection is high. To check for spectral overlap and to monitor pH, DO, biomass and mScarlet signals obtained when cultivating the producing strain (Vn_mS; **Figure 3a,b**)—in induced or non-induced culture conditions—or the non-producing strain (Vmax) were compared (**Figure 3c**).

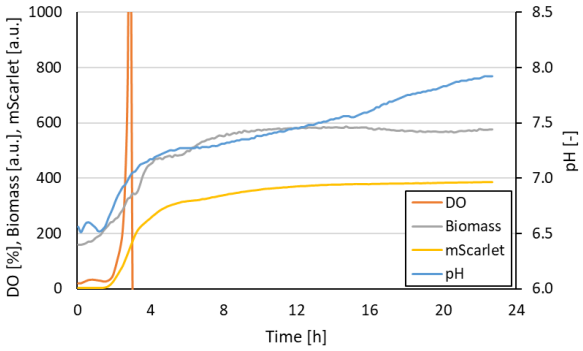


Figure 3a: Culture parameters monitoring on BOH1 plate, with mScarlet-producing *Vibrio natriegens* (Vn_mS) in VN_glycerol medium under IPTG induction.

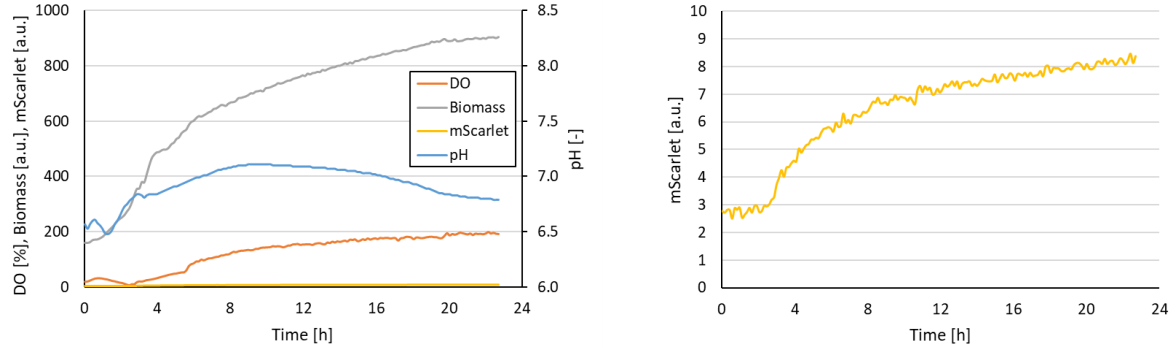


Figure 3b: Culture parameters monitoring on BOH1 plate, with mScarlet-producing *Vibrio natriegens* (Vn_mS) in VN_glycerol medium without IPTG induction (left graph). mScarlet was produced in lesser amounts due to promoter leakage (right graph).

The DO signal reacted as soon as mScarlet production began and saturated quickly. In the non-induced culture, a small production of mScarlet was visible (**Figure 3b**), probably due to promoter leakage, and this impacted the DO signal, which rose above 100%. Nevertheless, the pH signal was not impacted. Finally, in a culture of non-transformed *V. natriegens* (Vmax), the DO signal remained within the expected range between 0 and 100%, which confirms the impact on the DO monitoring of even very little production of mScarlet, in BOH1 plates (**Figure 3c**).

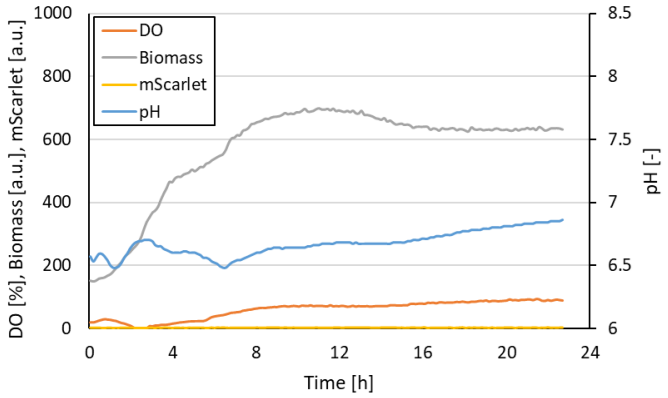


Figure 3c: Culture parameters monitoring on BOH1 plate, with commercial *Vibrio natriegens* (Vmax) in VN_glycerol medium.

20-L Bioreactor Configuration

Both bioreactors were equipped with two marine impellers and regulated at 37 °C. Seed culture was initiated in a 250-mL Erlenmeyer flask incubated for eight hours at 37 °C in LBV2 medium and transferred for four hours in the second seed culture (SC2) in 1-L bioreactor containing VN medium and incubated at 37 °C.

The initial growth medium volume of 8 L was inoculated with an initial OD_{600} nm of 0.25 using the seed culture SC2. Induction was performed one hour after inoculation by adding 1 mM IPTG in both bioreactors. Fed-batch cultivation mode was started four hours after inoculation, by adding 4×VN + 8% glycerol at a constant rate of 300 mL/h in Bioreactor S8. In Bioreactor S5, the same feed rate was applied, but the fed-batch mode was started eight hours after inoculation by adding 1×VN+8% Glycerol feed medium. Both cultivations were conducted for a total duration of 24h.

Offline Analysis

Biomass Analysis

Biomass growth kinetics were measured inline thanks to the BioLector XT Microbioreactor. The quantity of biomass produced in the culture broth was also assessed at the end of each culture using the standard method used at TWB, by measuring the optical density at 600 nm in a Thermo Cary spectrometer.

mScarlet Analysis

To compare culture parameters obtained in the BioLector XT Microbioreactor to those obtained in the bioreactor, mScarlet production was measured in the BioLector XT instrument thanks to the LED module and 10-nm passband filters (Ex 568 nm/Em 589 nm), and also using a Perkin microplate reader. In this latter case, samples were diluted 10 to 200 times in water to fit the linear range of the analyzer.

Quantification of Glycerol

Residual glycerol concentration in the culture supernatant was quantified by HPLC equipped with an H^+ column and a refractometer.

Results

Influence of Glycerol Concentration and OD at Inoculation

Batch cultures with 30 or 50 g/L glycerol starting concentration were evaluated. To assess the influence of initial optical density, 0.1, 0.25 or 0.5 initial optical density were compared in terms of growth kinetics, total biomass production and fluorescence evolution kinetics. To avoid limitation by carbon substrate, these trials were conducted with 30 or 50 g/L glycerol. In addition, the impact of induction density on the strain's growth was investigated by comparing induced and non-induced conditions with 30 or 50 g/L glycerol.

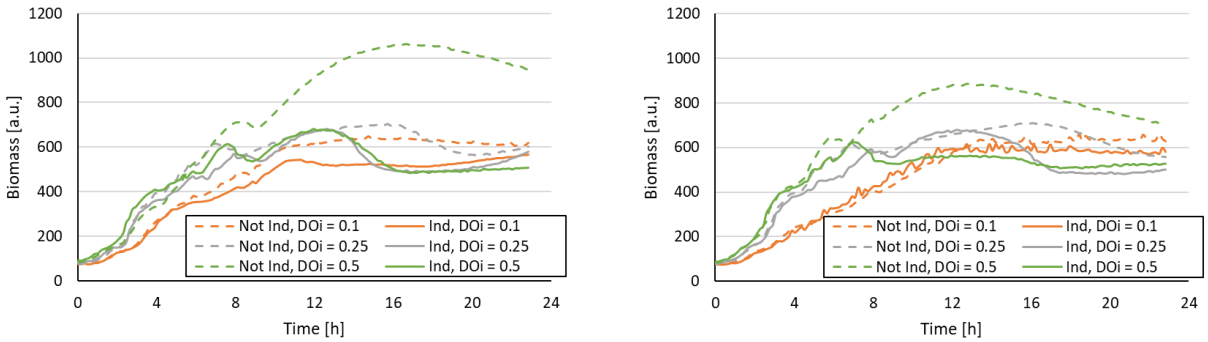


Figure 4: Impact of initial OD and induction on growth kinetics for two different initial glycerol concentrations (left graph 50 g/L; right graph 30 g/L).

The impact of induction on the total biomass was evident at a high inoculation level (starting inoculation OD at 0.5, as presented in **Figure 4**), regardless of the initial glycerol concentration. The maximal biomass measured in the BioLector XT Microbioreactor was not impacted by the initial glycerol concentration, except for the non-induced cultures at a high inoculation level. Under these conditions, the maximal biomass was nearly twice as high as in induced condition with 50g/L initial glycerol and around 20% higher with 30 g/L initial glycerol.

mScarlet production during the culture was monitored with the fluorescence signal measured through 508-dsRedII-mApple bandpass filter. Production kinetics after induction were mainly influenced by the inoculation level rather than by initial glycerol concentration (**Figure 5**). However, a higher signal intensity at lower glycerol concentration, as well as a longer maintenance of this signal over time was noticed. This could be attributed to a slower decay phase of the culture at lower glycerol concentration.

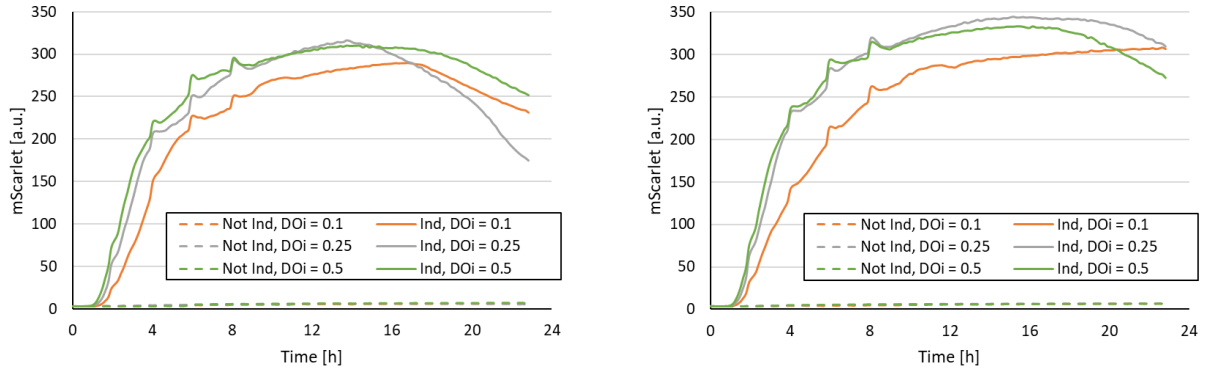


Figure 5: Impact of initial OD and induction on fluorescence production kinetics for two different initial glycerol concentrations (left graph: 50g/L; right graph: 30g/L).

The observed evolution of both OD and fluorescence was confirmed by the external measurement of optical density (**Figure 6**), whereas the total OD was significantly lower at a low level of inoculation. The offline fluorescence correlates with the online values, with low inoculation densities resulting in lower fluorescence. Also, the values with an initial 30 g/L glycerol concentration are slightly higher than those under the 50 g/L condition.

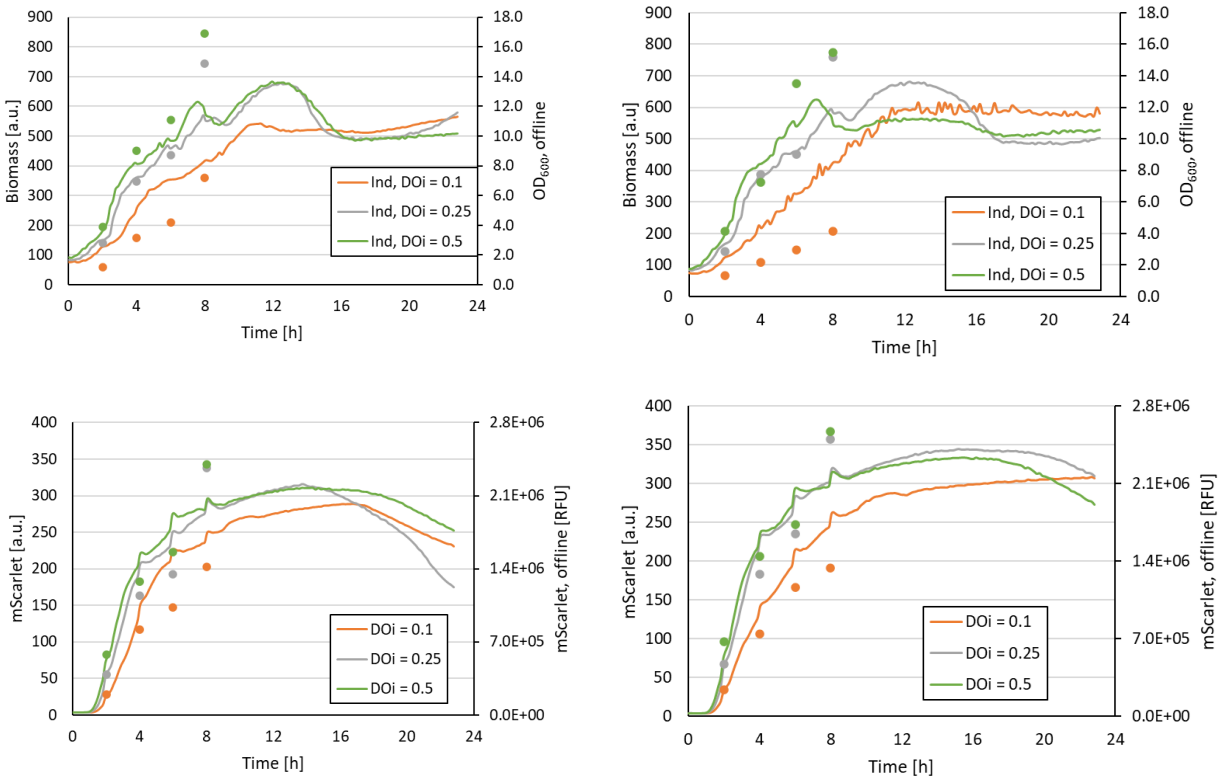


Figure 6: Offline measurements of optical density (upper graphs) and fluorescence intensity (lower graphs) at different sampling times in induced cultures of *Vibrio natriegens* with 50 g/L (left) or 30 g/L (right) glycerol.

Residual glycerol was quantified by HPLC in the culture supernatant collected at the end of the experiment. It appeared that even at low initial concentration, all cultures contained residual glycerol greater than 10 g/L at the end of the experiment. Thus, glycerol was not a limiting substrate under the tested conditions. Subsequent experiments were started with a batch phase containing 20 g/L glycerol, without any negative impact on growth kinetics (**Figure 7**).

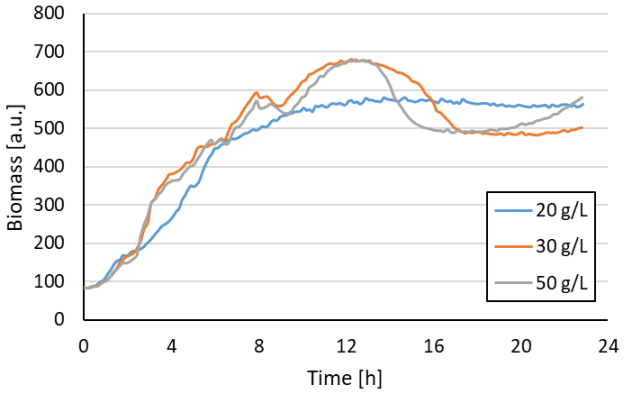
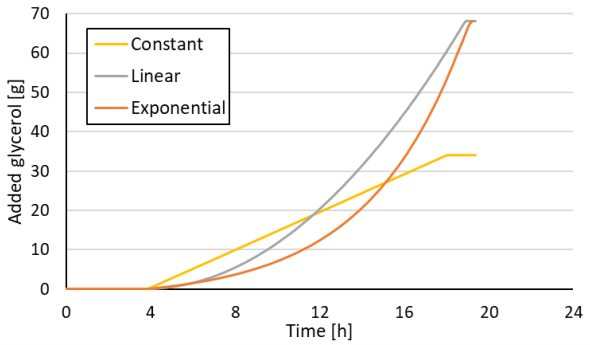


Figure 7: Comparison of growth kinetics of *V. natriegens*, cultivated in a BOH1 batch plate, in Vnat medium containing an initial glucose concentration of 20, 30 or 50 g/L. The cultures were inoculated at an OD of 0.25, and the inducer was added one hour after inoculation.

Intensification of Culture Process in Fed-batch Mode

Comparison of Fed-Batch Strategies

A fed-batch cultivation protocol was designed in order to identify the feeding and induction strategy that would maximize mScarlet production. In order to evaluate the impact of the glycerol addition rate during the production phase, feeding medium containing 8% or 16% glycerol was provided to the culture using constant, exponential or linear profile (**Figure 8**). This strategy ensured that all cultures received between 20 and 30 g/L glycerol within the first 15 hours of culture, which corresponds to the time when the growth plateau is reached in batch mode at these initial glycerol concentrations (**Figures 4 and 5**). After 15 hours, a decay in absorbance and fluorescence was observed. The objective of comparing three feeding strategies was to explore the impact of reducing glycerol feed rate on both growth and mScarlet production in the Vnat_mS strain.



Feeding strategy	Feed rate	Glycerol concentration in feed medium
Constant rate	dV/dt = 30 µL/h	8%
Linear rate	dV/dt = 3.67+0.7*t	16%
Exponential rate	dV/dt = 3.41*exp (0.22*t)	16%

Figure 8: Glucose added to the culture broth as a function of time, with constant, linear or exponential feeding strategy.

Induction was triggered by adding IPTG one hour after inoculation, as programmed. Inoculation was performed at an OD of 0.25 OD and was compared with 0.5 OD units. Ambient air was enriched with pure oxygen following a specific profile shown in (**Figure 9**)

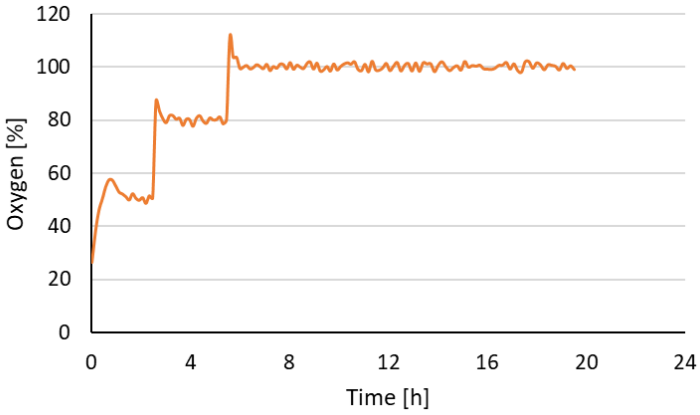


Figure 9: Oxygen enrichment profile in the BioLector XT Microbioreactor chamber.

Oxygen consumption was monitored in non-induced cultures (**Figure 10**). Fast oxygen depletion was observed between 6 and 11 hours of culture, without impacting the growth of the biomass in non-induced cultures. In this case, the biomass growth rate reduction is more likely due to nutrient limitation than to oxygen deprivation.

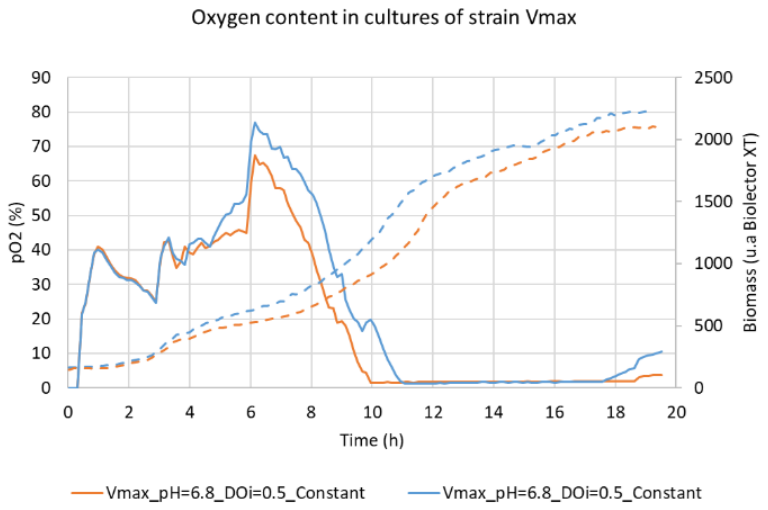


Figure 10: Evolution of oxygen content (solid lines) and biomass (dashed lines) in duplicate cultures of *Vibrio natriegens* non-mScarlet-producing strain Vmax under constant fed-batch conditions in a BOH1 plate.

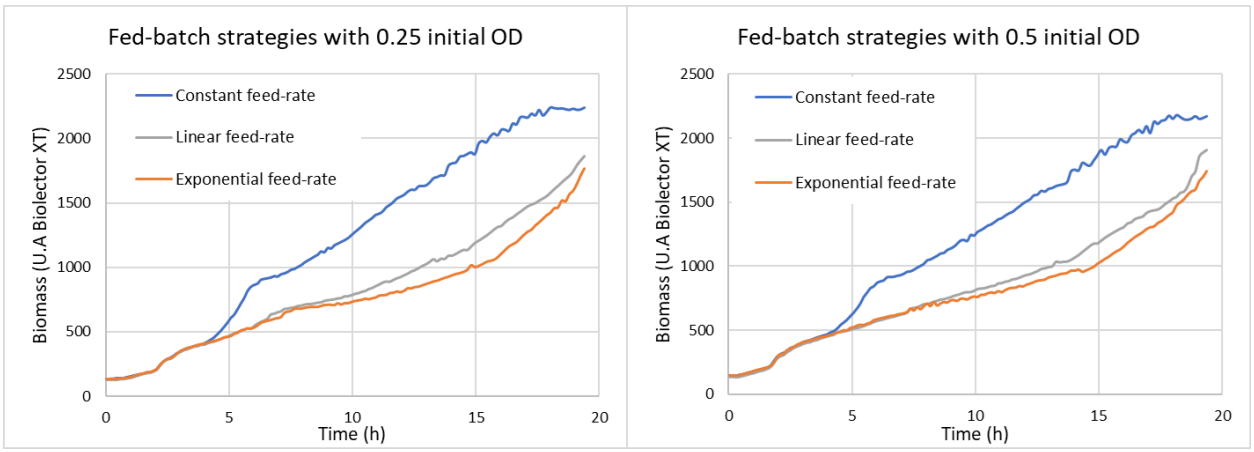


Figure 11a: Influence of feeding strategy on biomass evolution in culture, with an initial OD of 0.25 (left graph) or 0.5 (right graph). The values are corrected for volume to compensate for dilution effects from feeding.

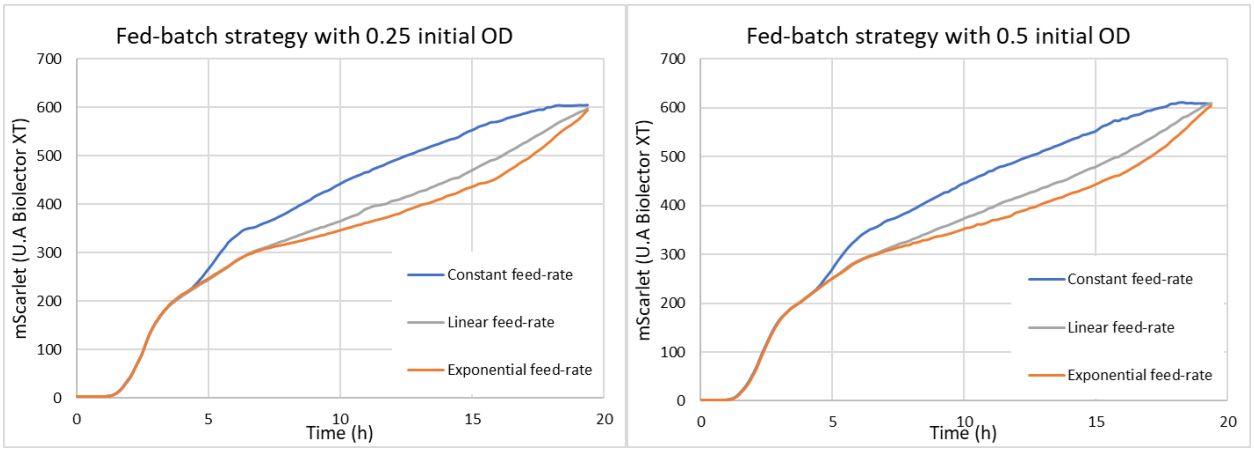


Figure 11b: Influence of feeding strategy on mScarlet production in culture, with an initial OD of 0.25 (left graph) or 0.5 (right graph). The values are corrected for volume to compensate for dilution effects from feeding.

Constant feeding was the most efficient in terms of growth rate and global productivity (**Figure 11a**). Both biomass growth and mScarlet production started slowing down after six hours of cultivation in cultures with constant feed, while with linear and exponential feed, both biomass growth and mScarlet accumulation followed the pattern of the feed medium addition. Finally, the three different strategies reached the same mScarlet concentration after 20 hours of culture, but the total added glycerol was twice as high with exponential and linear feed-rate strategies than in the constant feed-rate strategy. The constant feed-rate strategy proved more efficient in terms of mScarlet-to-glycerol yield. The fast accumulation of glycerol occurring after 10 hours with linear and exponential feed rates does not compensate for the delay in glycerol feed compared to constant feed. The inoculation level did not impact biomass growth. The production of mScarlet was also slowed down with linear and exponential feed (**Figure 11b**). We can assume that essential nutrients and oxygen were consumed faster in induced cultures than in non-induced cultures.

Influence of Feed Start Time and Feed Medium

Both feed start times (four hours and eight hours after inoculation) and feed medium (VN4× or VN1×) were compared for biomass production and mScarlet production. Each condition was replicated four times on the same plate. The feeding strategies tested were:

Feeding strategy	Feed rate	OD at inoculation	Total feed volume injected	Culture time	Total glycerol in culture
Early feed start (4h)/4x VN	dV/dt = 30 µL/h	0.25	561 µL	22.7 h	61 mg
Late feed start (8h)/4x VN	dV/dt = 30 µL/h	0.25	441 µL	22.7 h	51 mg
Early feed start (4h)/1x VN	dV/dt = 30 µL/h	0.25	561 µL	22.7 h	61 mg
Late feed start (8h)/1x VN	dV/dt = 30 µL/h	0.25	441 µL	22.7 h	51 mg

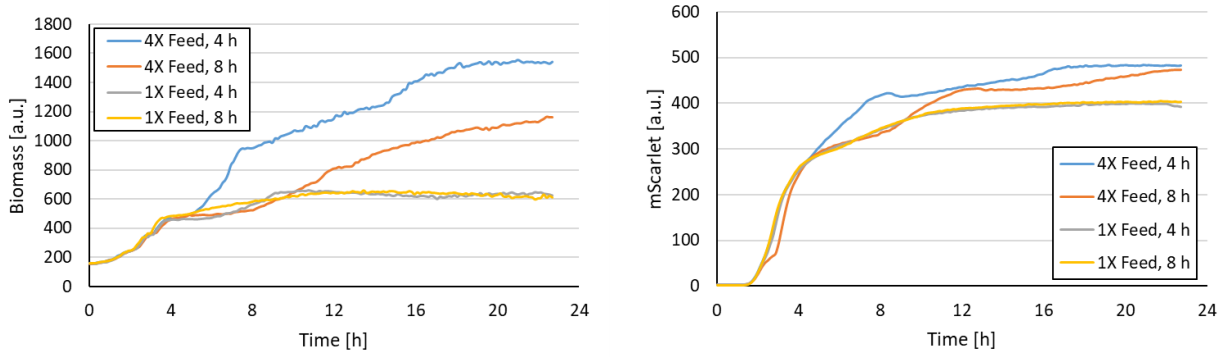


Figure 12: Influence of feed start time on biomass evolution in culture (left graph) and mScarlet production (right graph).

The main impact was due to the feeding medium composition. With 4× feed medium, the total produced biomass was twice as high as when the feed started eight hours after inoculation and 2.5 times higher when the feed started four hours after inoculation. When fed with 1× feed medium, the biomass stopped increasing eight hours after inoculation, regardless of the feed start time (**Figure 12**). These results could indicate that once the substrate was depleted in the broth—which occurred earlier in 1× feed earlier than with 4× feed—the growth capacity of the strain was not completely lost, as the OD level remained constant despite the dilution of the medium. Nevertheless, the global performance of the fermentation was impaired, likely due to rapid occurrence of cell death or at least a loss of metabolic activity. The production of mScarlet was also higher with 4× feed medium, but the delay in feed start slowed down the production without impacting visibly the total produced quantity.

Oxygen consumption rate is an indicator of metabolic activity, and its monitoring is enabled in the BioLector XT Microbioreactor for the non-producer strain Vmax. The comparison of oxygen consumption rate between the four different feeding strategies indicates that the metabolic activity is highest within the first three hours after inoculation. Then, the oxygen consumption rate returns to a high level at the feed start when the culture is fed with 4× medium, indicating a recovery of metabolic activity—even when the feed starts after eight hours of culture. On the other hand, in cultures fed with 1× medium, or when no feed is added (batch cultures), the oxygen consumption rate is lower, indicating lower metabolic activity. In both cases, the oxygen consumption rate was higher when the feed starts earlier in the culture, which confirms the former observation and the rapid occurrence of cell death.

Oxygen monitoring in cultures of Vmax strain (non-mScarlet-producing) indicated that the oxygen consumption was higher in cultures fed with VN 4x (**Figure 11**). In this case, biomass growth and mScarlet production might be limited by oxygen depletion, whereas in the cultures without feed or fed with 1× medium, depletion in nutrients might limit growth and production performance.

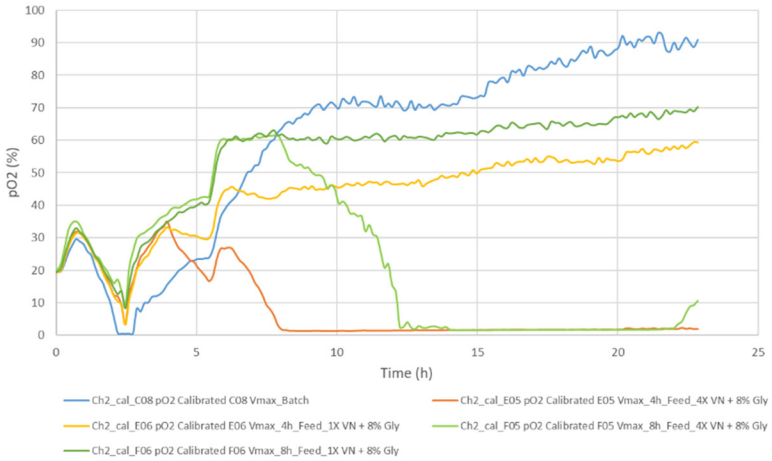


Figure 13: Evolution of oxygen content in the culture broth in cultures of Vmax strain fed 4 h after inoculation with VN 4x (orange line) or VN 1x (yellow line), or fed eight hours after inoculation with VN 4x feed medium (light green line) or VN 1x (dark green line).

Transfer to 20-L Scale Bioreactors

To assess the scalability of the data obtained in BioLector XT Microbioreactor to pre-pilot bioreactors, two cultivation protocols were reproduced in 20-L Sartorius Biostat® C bioreactor. The first cultivation protocol was considered optimal, or high-performance process (HPP), corresponding to the culture condition that led to highest accumulation of biomass and fluorescence (i.e., 4x VN + 8% Gly), and the second showed lower performance, or low-performance process (LPP), exhibiting low biomass and fluorescence accumulation (i.e., 1x VN + 8% Gly). The starting volume was 8 L. The pH remained stable between 6.6 and 6.8. The pO_2 was maintained at 20% thanks to a regulatory cascade including agitation, compressed air perfusion and oxygen enrichment of compressed air when needed.

Induction time was t0 + one hour, and the substrate was added in the same proportion as in BioLector XT Microbioreactor experiments:

	Feed medium	Feed start time	Induction time	pH	pO2	OD at inoculation
S8: High-performance process (HPP)	4x VN + 8% Gly	T0+ 4h	T0+1h	6.8	20%	0.25
S5: Low-performance process (LPP)	1x VN + 8% Gly	T0+ 8h	T0+1h	6.8	20%	0.25

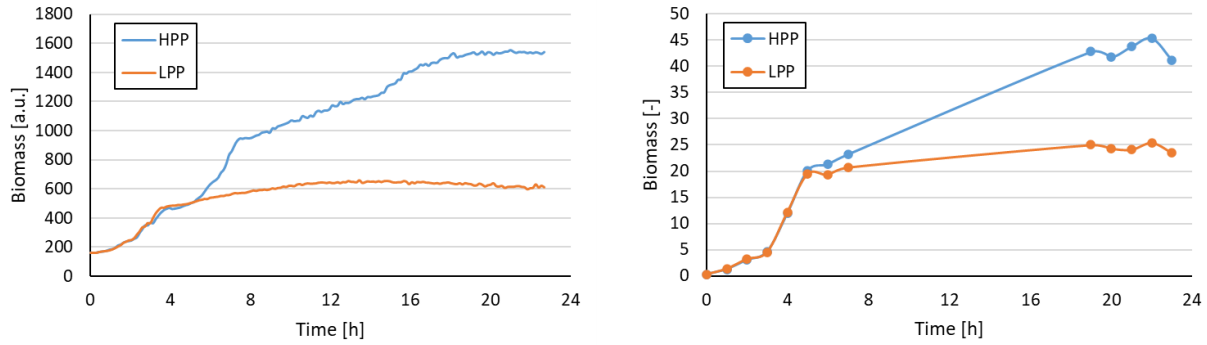


Figure 14: Kinetics of biomass growth in BioLector XT Microbioreactor (left) and 20-L bioreactor (right) in HPP cultures (blue line) compared with LPP cultures (orange line).

Comparison of Optimal and Low-Performance Processes

Both conditions tested in the BioLector XT Microbioreactor and the pre-pilot-scale 20 L bioreactor exhibited the same trend in biomass production during the initial batch phase (**Figure 14**). As observed in the BioLector XT instrument, the growth kinetics of the strain in the 20-L bioreactor was influenced by the feed medium composition. The concentration of biomass reached a plateau after the feed started in the LPP culture. As the volume increased, this plateau indicated that the growth rate of the strain remained limited and did not compensate for the dilution factor. On the other hand, in the HPP culture, the biomass continued to accumulate until approximately 19 hours after inoculation.

In the BioLector XT Microbioreactor, the final OD_{600} nm was 25 ± 4 for the HPP and 14 ± 4 for the LPP, yielding a 1.78 ratio in total biomass production, which was also found in the 20-L bioreactor (45 OD units in the HPP versus 25 in the LPP). The data from the BioLector XT Microbioreactor showed a ratio of 2.7, which may be explained by the gain that was adapted to higher values.

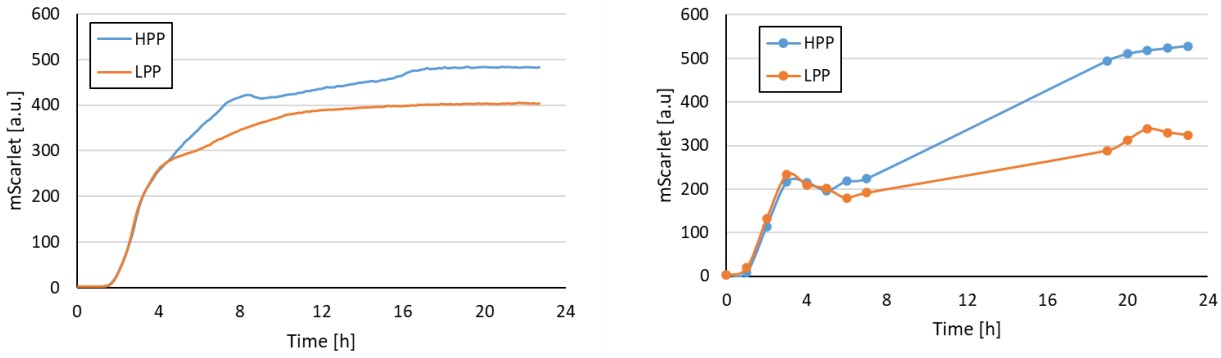


Figure 15: Kinetics of fluorescence evolution during continuous reading in the BioLector XT Microbioreactor (left) and the 20-L bioreactor (right), in HPP cultures (blue line) compared to LPP cultures (orange line). The data from the 20-L bioreactor was measured with the BioLector XT microbioreactor on samples extracted from the 20-L bioreactor.

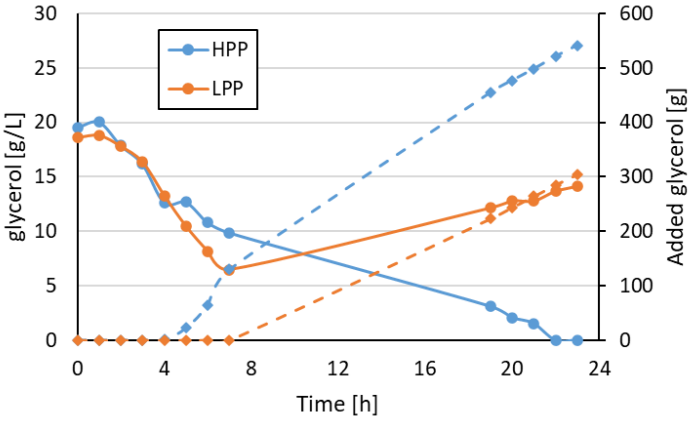


Figure 16: Kinetics of glycerol addition (expressed in grams, dotted lines) and glycerol concentration (in grams per liter, solid lines) in HPP cultures (blue line) compared to LPP cultures (orange line) in the 20-L bioreactor.

A divergence between the two compared conditions became apparent after five hours of culture, coinciding with the start of the feed phase in both systems an hour earlier. This pattern of initial similarity during batch culture followed by divergence after feed start was also observed in mScarlet fluorescence production (**Figure 15**). Mean mScarlet production rate was calculated as the total amount of mScarlet produced per time unit. During the first three hours after induction (four hours of culture), the highest mScarlet production rate was obtained in the bioreactor (**Figure 17**), identically in HPP and LPP (75 a.u/h mean productivity in the first eight hours, versus 64 a.u/h in the LPP condition). This phase also corresponded to high oxygen demand, triggering a rapid increase in stirring rate in the bioreactor (**Figure 18**).

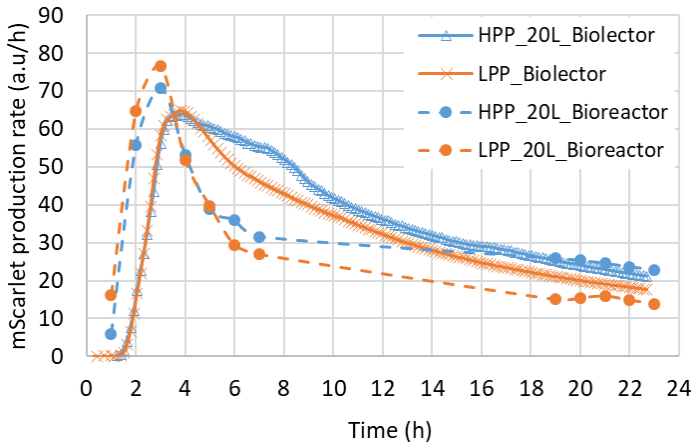


Figure 17: Evolution of mScarlet production rate over time in the BioLector XT Microbioreactor versus the 20-L bioreactor, in both HPP and LPP conditions.

The value dropped rapidly to finally stabilize around 25 a.u/h in HPP or 15 a.u/h in LPP. In the BioLector XT Microbioreactor, the mScarlet production rate decreased more smoothly to reach approximately 20 a.u/h at the end of the culture.

Finally, despite the observed difference in produced biomass, which could be mainly attributed to faster oxygen transfer in the bioreactor compared to the BioLector XT Microbioreactor, the total mScarlet production was similar. This suggests that under oxygen and nutrient limitation, mScarlet production limited biomass growth, while in the presence of oxygen, biomass growth was not limited. In the latter case, the inductor concentration might limit the ability to maintain a high mScarlet production rate after the production peak occurring two hours after induction.

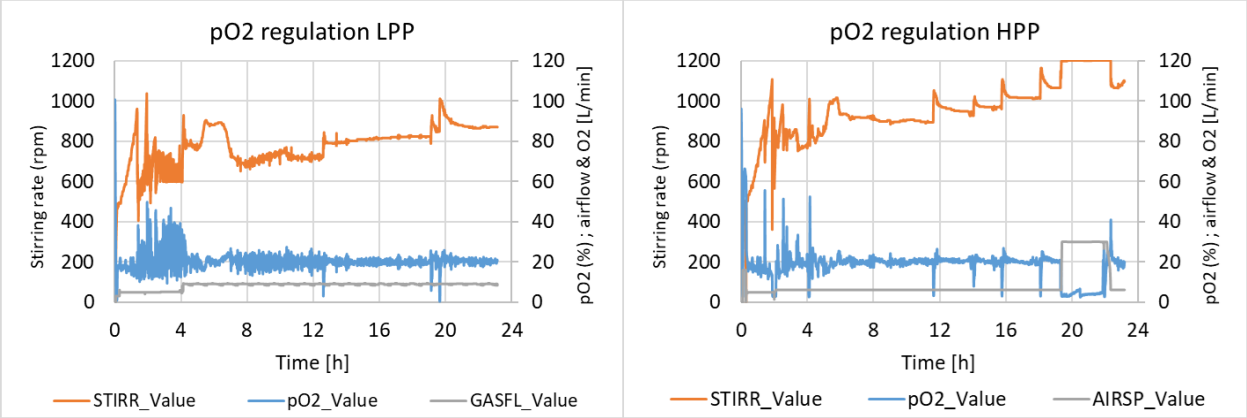


Figure 18: pO_2 regulation in 20-L bioreactor, for LPP and HPP conditions. The pO_2 setpoint was 20%, with a regulation cascade including stirring rate (up to 1,200 rpm) and airflow rate.

All cultures in the BioLector XT Microbioreactor had residual glycerol at the end of the culture, ranging from 6 g/L ± 10% in HPP cultures to 25 g/L ± 6% in LPP cultures. In 20-L bioreactors, the entire amount of added glycerol was consumed after 22 hours in HPP cultures, while it remained close to 15 g/L in LPP (**Figure 16**). The combined nutrient and oxygen deprivation in the BioLector XT instrument could explain the lower glycerol consumption observed, compared to the 20-L bioreactor, where oxygen was never limiting.

Conclusion

Scalability and transferability of *Vibrio natriegens* fermentation processes were explored using the BioLector XT Microbioreactor. The culture process for *Vibrio natriegens* developed in the BioLector XT Microbioreactor was successfully transferred to a 20-L bioreactor, with better biomass production and substrate consumption thanks to improved oxygen transfer performance. Nevertheless, the data obtained in the BioLector XT instrument, regarding feed medium composition and process strategy, was representative of what was observed in the 20-L bioreactor. This consistency in the results provides strong confidence in the scalability and transferability of processes developed at small scale in the BioLector XT Microbioreactor to 20-L bioreactors, and provides a better understanding of *V. natriegens* physiology.

The BioLector XT Microbioreactor proved to be a powerful platform for process development and optimization, enabling rapid screening of multiple cultivation parameters while maintaining precise control over critical process variables. The successful scale-up validation demonstrates the system's utility for industrial bioprocess development, particularly for fast-growing, high-oxygen-demand organisms like *V. natriegens.* The high-throughput screening capability, enabling parallel evaluation of up to 32 different conditions with automated data collection every six minutes, significantly accelerates bioprocess development timelines compared to traditional sequential optimization approaches. The combination of the microfluidic module with precise gassing control provides a powerful tool to investigate different feeding strategies while preventing harmful oxygen limitation and helps drive optimal process parameters to enable higher cell densities and productivity.

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2026-GBL-EN-109229-v1

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