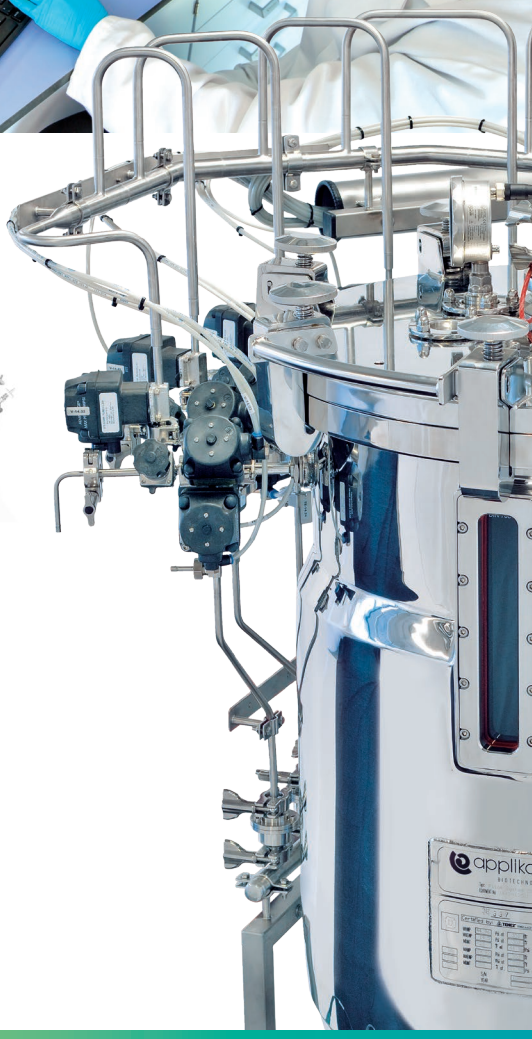


Cultivation Systems

From Discovery to Production | 2019

applikon® BIOTECHNOLOGY

Engineering for Life

Content

Applikon Biotechnology

Applikon Biotechnology is a world leader in developing and supplying advanced bioreactor systems from laboratory-scale, to pilot, and finally production. Our mission is to provide reliable solutions for the bioprocess market that will lead to an improved quality of life. We support industrial microbiology and the pharmaceutical industry in their upstream process by implementing scalable platforms from initial screening through development to full-scale production. By minimizing scale-up risks and shortening the time-to-market for our customers, we contribute to the improvement of Life Sciences. And that is our passion!

Expertise

Applikon Biotechnology is known for bringing new technologies to the market. We continuously improve and launch new bioreactor systems as well as process analytics and software tools. These new technologies enhance process efficiency and help to reduce costs. In particular our small scale designs that offer a complete solution for results generation, make us unique in the mini and micro bioreactor range.

Complete Upstream Product Portfolio

Our focus is on supplying the best product offering for our customers now and in the long-term future. Our portfolio ranges from very small miniBioreactors to large volume cultivation systems. Whether used for laboratory applications, good manufacturing practices, or the full scale-up process from lab to production, we have the right solution.

Worldwide Activities

Since we started in 1974 we have shown a healthy growth leading to a global market leader role. In fact, an expansion was necessary to keep up with this growth. Therefore, we doubled our facility in June 2017 to 5.800 m^2 total surface. All R&D, design and engineering takes place in-house in our headquarters in Delft, The Netherlands. Furthermore, we have our own sales and service organizations in the USA and UK and a network of well-trained local distributors for sales and service in over 35 countries worldwide.

Cultivation Systems | From Discovery to Production

NEW Single-Use Bioreactors for Lab-Scale

AppliFlex ST | Disposables Designed on Demand

- Fully customizable to meet your specific bioprocessing needs
- Install any number of systems to fit your workspace
- Cultivate anywhere; no laminar flowhood, water supply or drain needed
- Several perfusion options for continuous bioprocessing

The AppliFlex ST is a fully customizable stirred tank single-use bioreactor. By using 3D printing technology, we can create any head plate configuration that is optimal for your process. With this single-use system there is no risk of cross contamination between runs. The pre-sterilized, ready-to-use bioreactor makes your life in the lab a lot easier. No more assembling and sterilization before you can start your culture. No more cleaning after the culture is finished. Save time and costs, execute more runs, and reduce your time-to-market.

Features

- Integrates with software automation and automated sampling tools
- Interchangeable with multi-use systems
- Removable topplate
- Shelf life 2 years
- Up to 0.5 barg (500 mL)
- Pre-sterilized and ready-to-go

Applications

- Microbial and Cell culture
- Regenerative medicines & Stem cell cultures
- Batch, Fed-Batch, Perfusion and Continuous cultivation
- Screening studies
- Media optimization
- Process optimization

Custom Design

The AppliFlex ST differentiates from other single-use bioreactors by being a fully customizable stirred tank bioreactor. You can choose, or even design, the type and number of impellers, the number of liquid and gas additions and the type of sparger that are optimal for your process. No more unused ports with blind plugs, but an optimized bioreactor for your specific process development and R&D application.

The 3D printing production technology guarantees complete reproducibility between the different bioreactors guaranteeing exact and identical conditions between runs.

The AppliFlex ST offers the flexibility that you need for your specific bioprocess and is available for both cell culture and microbial applications.

The AppliFlex ST can be used as single unit as well as parallel processing systems. The design of the AppliFlex ST is a scale down version of our larger laboratory scale systems guaranteeing an easy scale-up of your process from the 500 mL to the larger volume cultivations. The single-use system can be exchanged with a multi-use system whenever your process or workflow needs a different approach.

NEW

Matrix | The Turnkey Multi Bioreactor Platform that Adapts to You

- Mix & Match; you decide the number of parallel bioreactor systems and relevant software, sampling tools and analytical devices that match your budget
- Do more in less time with one integrated turnkey solution
- Cultivate anywhere; no laminar flow hood, water supply or drain needed

Connect. Integrate. Accelerate.
Improve your bioprocess performance in the shortest possible time by building your optimal multi bioreactor platform. Connect and integrate our standardized software, sampling tools and analytical devices to the number of parallel bioreactor systems that fit your footprint and budget. Increase your productivity with less effort and accelerate your business.

Features

- Single-use and multi-use lab-scale bioreactors
- Integrated V-Control (DeltaV™) or Lucullus software
- Automated sampling and manual sampling options
- Integrated analytical devices and a wide range of supported online sensors

Applications

- Batch, Fed-Batch and Perfusion modes of operation
- Microbial and cell culture applications
- Continuous bioprocessing

Complete Freedom of Choice in Set-Up

Bioreactor Systems

You decide what functionality your bioreactor will have. Applikon offers tailor-made and fully reproducible solutions with 500 mL and 3 L single-use bioreactors, the AppliFlex ST. Single-use bioreactors can be exchanged with a multi-use system at any time. The complete infrastructure for both systems is identical.
Connect one of the Applikon process control systems for simple operation in combination with accurate process control and you are ready-to-go.

M A T R I X

Software Automation

Integrate powerful software packages like Lucullus and V-Control to turn data into information and to improve your decision making.
Lucullus manages your complete workflow in the lab, from planning to data analysis. It can plan and set-up parallel experiments and generate DoE recipes.
When scalability from lab to production is your most important goal, V-Control is your match. V-Control uses DeltaV Discovery to reduce time spent on technology transfer and validation by offering seamless process transfer when processes are moved to a production environment.

Sampling

Integrate an automated sampling system to minimize lab visits during evenings or weekends or to minimize deviations between different samples due to different operators sampling. Flownamics' Seg-Flow® automated online sampling system facilitates interconnectivity between your bioreactor(s), analytical instrument, process control and your data acquisition software.

Analytical Devices

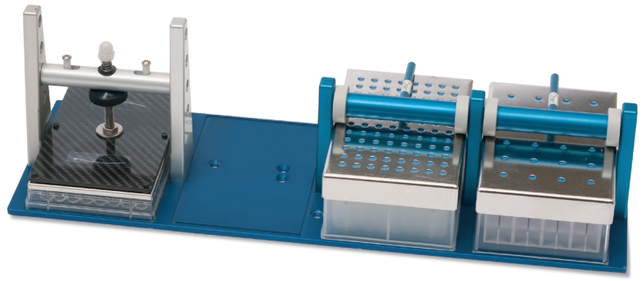
Proper control of your bioprocess requires reliable monitoring of many process parameters. Applikon offers several analytical technologies, from basic physiochemical properties as pH or dissolved oxygen to determination of biomass density or metabolite concentrations. Online analyzers can be coupled via the automated sampling platform or can be used offline with a direct connection to your data management software offering easy integration of analytical data into your process data.

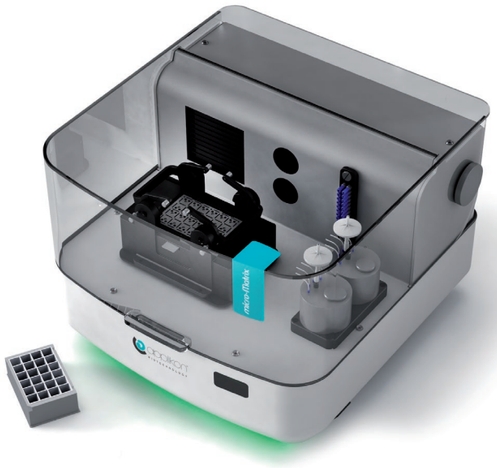
Mix & Match

Scale-Up

Micro-Flask by Duetz Cultivation in Microtiter Plates

- Conversion of 24 and 96 microtiter plates (both deep- and low-well plates) into individual microreactors
- Oxygen transfer rates similar to shake flasks in standard orbital shakers
- Simultaneous and reproducible sampling from frozen glycerol stocks
- Low and uniform evaporation rates for every well
- Sterile barrier for individual wells prevents cross contamination





micro-Matrix

The unique micro-Matrix offers total control over 24 independent bioreactors in a simple microtiter plate footprint. Each of the 24 bioreactors on a plate offers independent controls like its larger stirred-tank relatives:

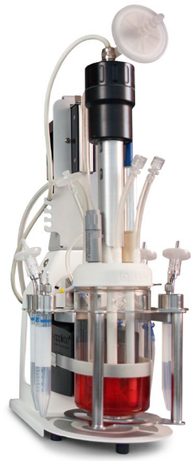
- pH (measurement and two-sided control, including plate-wide gradients)
- Temperature (measurement and two-sided control, including plate-wide gradients)
- Dissolved oxygen control (measurement and two-sided control, including plate-wide gradients)
- Individual liquid additions
- Up to 4 separate gas additions (individually controlled)

Mini Bioreactors

The MiniBio2 is an improved version of the original Applikon MiniBio bioreactors. A simplification in the process for autoclaving, installation, and connection of tubes leads to a decreased setup time for these systems. The MiniBio2 range of bioreactors (250 mL, 500 mL and 1000 mL total volume) is a true scale down of the classic laboratory scale bioreactor.

Single-Use Bioreactors

Applikon offers single-use bioreactors for lab-, pilot- and production scale.
The AppliFlex ST single-use stirred tank bioreactor is available in 500 mL and 3L. It is designed according to the high Applikon quality standard and is interchangeable with Applikon's glass vessels of same volume. The AppliFlex ST can be used for both cell culture and microbial applications.
For pilot- and production scale we offer HyPerforma Single-Use Bioreactors with Applikon SUB controller and the HyPerforma Single-Use Fermenter with Applikon SUB controller.



Autoclavable Bioreactors

Applikon offers glass autoclavable bioreactors for cell culture applications and glass autoclavable fermentors for microbial culture applications. Thanks to their modularity and flexibility, the user can always adapt the systems to changed process demands. This results in low initial investment and low running costs. Glass autoclavable bioreactors and fermentors are available in jacketed and single wall versions in 2, 3, 5, 7, 15 and 20 liter total volume (20 liter only in single wall version).

Stainless Steel Bioreactors

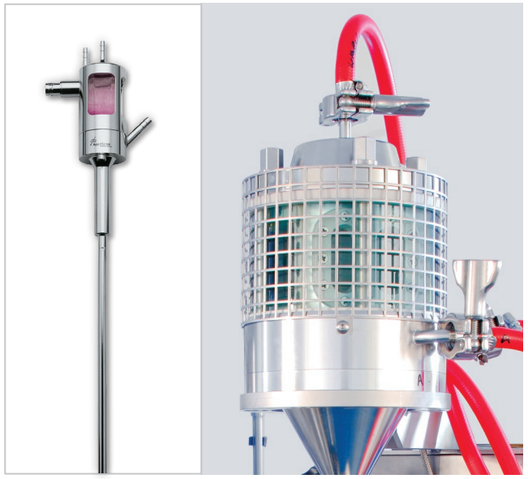
Next to a vast range of standard off-the-shelf solutions, Applikon offers customized solutions from small scale to full scale production systems. These customized solutions are tailored to the specific process and customer demands. A wide range of standard modules is available to customize the systems to your specific demands. The standard modules include pressure control, weight control, double gas inlet lines, CIP modules, biomass sensors, perfusion systems and much more.
Applikon offers a choice of ez-Control and i-Control process control systems. The i-Control can be supplied with a DeltaV controller, an Allen Bradley or a Siemens PLC. All i-Control systems support automated sterilization and cleaning procedures.

Continuous Bioprocessing

BioSep

The Applikon BioSep system is a unique, cell retention device for high-density perfusion processes. Using high frequency resonant ultrasonic waves to separate cells instead of a physical mesh or membrane, it offers all the benefits of traditional devices without their inherent problems and limitations.

- Scalable perfusion device (0,1 – 1.000 L / day)
- No fouling or blocking for long term operation
- Automatic removal of cell debris
- Up to 150 million cells/mL
- Easy to install and to operate
- Applicable on any type and brand of bioreactor



Software & Automation

Lucullus Process Information Management System

Lucullus Process Information Management System offers a new dimension in upstream bioprocess data management. Lucullus integrates functionalities for creation and planning of recipes, reactor allocation, Design of Experiments, media preparation, media component trace-ability, data analysis, data mining, automatic reporting, and modelling. The integration of all these functions into one comprehensive software solution saves the scientists time since all data is stored in one central Oracle© database. No more need for data export and import between different solutions; Lucullus integrates all functions needed for complete data management of your upstream process.

V-Control | The Scalable DeltaV™ Solution for Bioreactors in your Lab

V-Control delivers the well-known DeltaV application in a smaller footprint and at a reduced cost. With the combination of Emerson's DeltaV Discovery and the Applikon local process controllers, you can:

- Reduce your DeltaV benchtop footprint
- Control up to 32 bioreactors from one Appli-V server
- Combine DeltaV's powerful batch control and event history modules into a comprehensive lab system and easily transfer lab processes to production systems
- Deliver an open software and hardware solution
- Add this configuration to an existing system
- Customize standardized automation software to meet customer requirements
- Switch between commercial production and process development

Vero Cell Growth and EV71_C4 Replication Capacity in Small-Scale Customizable Single-Use Bioreactors

intravacc
innovating vaccines

Coolen, A[1]., Santos Fernandes, C[2]., Bakker, W[1]., van Eikenhorst, G[1]., Oosterhoff, D[1] and Walvoort, T[2].
[1] Intravacc, Antonie van Leeuwenhoeklaan 9, 3721 MA, Bilthoven, The Netherlands; [2] Applikon Biotechnology BV, Heertjeslaan 2, 2629 JG, Delft, The Netherlands

Abstract

Single-use bioreactors have been increasingly used for animal cell culture in the biopharmaceutical industry. The interest for these systems lays in the considerable reduction of cross-contamination risk, the elimination of cleaning-in-place and sterilization-in-place, no need for cleaning validation, the decrease in production turnaround times and a reduction in validation time which shortens time to market. In the present work, a Vero cell line used to produce viral vaccines was used by Intravacc to perform the cell and virus cultivations in Applikon's newly developed small-scale customizable single-use bioreactors. The growth curves of Vero cells, were compared with the growth curves of Vero cells growing in conventional autoclavable glass bioreactors under the same conditions and in the same culture volume. Subsequently, a virus for which vaccines are needed, EV71_C4, was grown on Vero cells. The single-use bioreactors are suitable for Vero cell culture and EV71_C4 virus propagation because there was no difference with respect to Vero cell culture and EV71_C4 virus culture between the glass bioreactor and disposable bioreactor. Thus the small-scale customizable single-use bioreactor holds promise for future production of viral vaccines.

Figure 1 | Single-use and autoclavable glass mini-bioreactor (Applikon Biotechnology).

Materials and Methods

- A single-use and autoclavable glass 500 mL mini-bioreactors, presented in Figure 1 (Applikon Biotechnology, Delft - The Netherlands), were used to grow Vero cells, under the same culture conditions.
- The mini-bioreactors configuration consisted of 1 marine impeller, 2 overlay inlets used for gas inlet and outlet (pipe for gas outlet had a wider diameter than the inlet), a sampling pipe, an addition pipe and a temperature pocket. The autoclavable pH and dO_2 sensors were used in both systems. In the autoclavable glass mini-bioreactor the sensors were autoclaved together with the bioreactor, whereas in the single-use, the sensors were autoclaved separately, and assembled afterwards on the mini-bioreactor inside the laminar flow cabinet.
- As with the autoclavable mini-bioreactor, the single-use unit is a cylindrical vessel with a hemispherical base reported by previous authors to provide shorter mixing times due to better circulation patterns of the liquid, than a cylindrical vessel with a flat base.
- Vero cells were cultured on microcarriers at 37 °C in VP-SFM medium supplemented with 2 mM glutamine in a culture volume of 250 mL. The initial cell concentration was $0.15*10^6$ Vero cells/mL for both bioreactors.
- Samples were taken daily to analyze the cell concentration, morphology and distribution of the Vero cells on the microcarriers.
- The specific growth rate (μ) of the cells was calculated using a standard exponential fit through the growth curve.
- For the virus culture, Vero cells were infected with the EV71-C4 virus at an MOI of 0.001 when cell concentration reached $0.8*10^6$ – $1.0*10^6$ cells/mL.
- Additional samples were taken to determine the virus titer using CCID50 assay and to monitor CPE with microscopy.

Results and Discussion

Vero cell growth and morphology

The growth of Vero cell culture in the single-use and the autoclavable glass bioreactor was monitored for 137 hours, as shown in Figure 2.

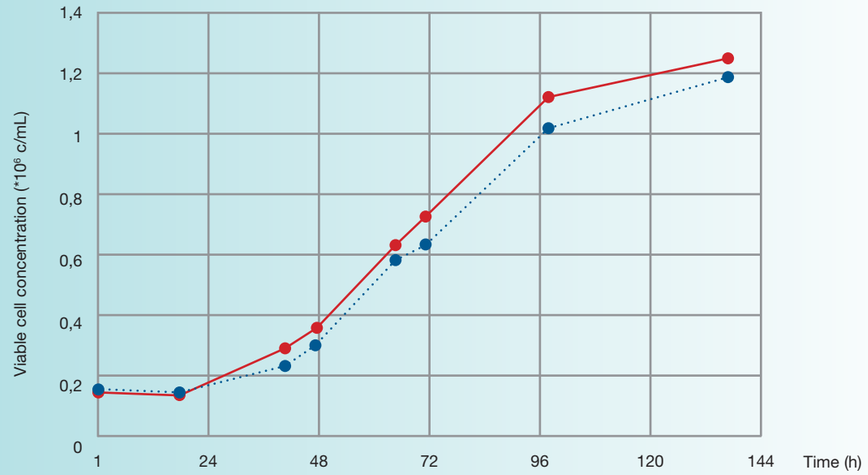


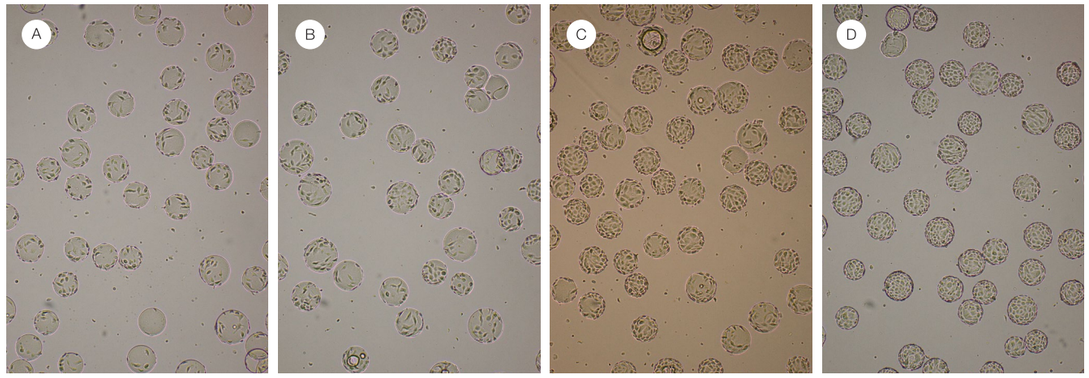
Figure 2 | Growth curves of Vero cells in the single-use bioreactor ● and in the autoclavable glass bioreactor ●

The maximal viable cell concentration and growth rate results were comparable in both bioreactors as can be seen in Table 1.

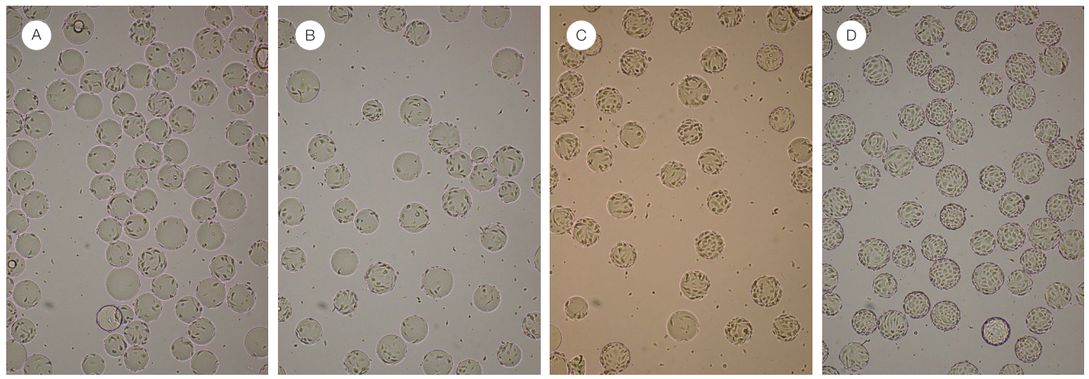
- The distribution and proliferation of the Vero cells on the micro-carriers in the single-use and the autoclavable glass bioreactors was observed by light microscopy. Pictures are shown in Figures 3 and 4.
- Figures 3 and 4 show that after the day 1 of culture, cells were attached to the microcarriers and most of the microcarriers contained cells.
  At day 4, the microcarriers were fully covered by Vero cells and little cell debris were observed. These pictures show there is no difference in distribution and proliferation of Vero cells growing on microcarriers in the two different types of bioreactors.

Table 1 | Yield and specific growth rate for Vero cell culture obtained in the two different bioreactors.

Vero cell culture	Maximal viable cell concetration (cells/mL)	μ (h^{-1})
Glass bioreactor	$1.02*10^6$	0.026
Single-use	$1.12*10^6$	0.027



3 **Figure 3** | Vero cells growing on microcarriers as observed with light microscopy during cell culture in the single-use bioreactor. Pictures were taken at day 1 (A), day 2 (B), day 3 (C) and day 4 (D).



4 **Figure 4** | Vero cells growing on microcarriers as observed with light microscopy during cell culture in the autoclavable glass bioreactor. Pictures were taken at day 1 (A), day 2 (B), day 3 (C) and day 4 (D).

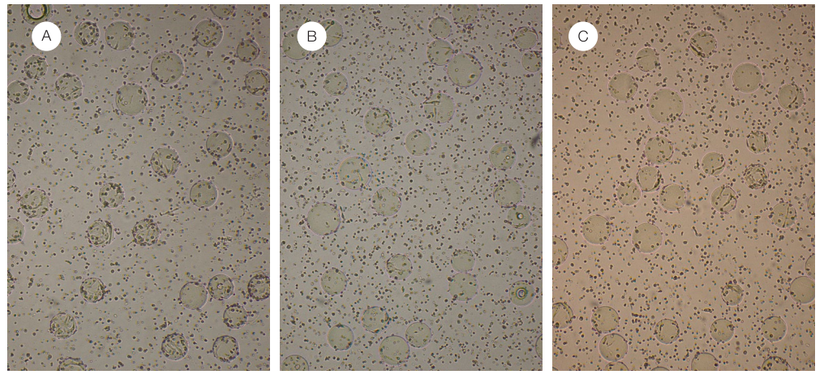
EV71_C4 infection: morphology and virus titer

In a follow-up experiment, Vero cells were cultured for 74 h to a cell concentration of $0.88*10^6$ cells/mL (μ= 0.031 h^{-1}) in the single-use and $0.93*10^6$ cells/mL (0.034 h^{-1}) in the autoclavable glass bioreactor.

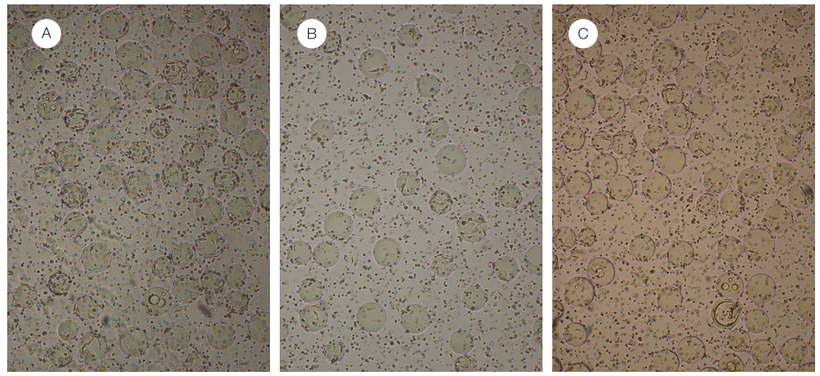
After 74 h, cells were inoculated with the virus EV71-C4 at an MOI of 0.001 and samples were taken after 3, 4 and 5 days post infection to determine the amount of infectious virus in the culture medium (Table 2).

Figures 5 and 6 show the evolution of the cytopathic effect caused by the virus in the single-use bioreactors and autoclavable glass bioreactors respectively. For both bioreactors the observed cytopathic effects are comparable.

The maximum virus titers from both bioreactors, presented in Table 2, are within the standard deviation of the in-process virus titration assay method of 0.5 LOG10, which means that the titers for both reactors are comparable. Also, no significant differences in virus titer at the different time points were observed in both types of bioreactor.



5 **Figure 5** | Pictures of Vero cells during EV71_C4 culture in the single-use bioreactor on day 3 (A), day 4 (B) and day 5 (C) post infection.



6 **Figure 6** | Pictures of Vero cells during EV71_C4 culture in the autoclavable glass bioreactor on day 3 (A), day 4 (B) and day 5 (C) post infection.

Table 2 | EV71_C4 infectious virus titer in the single-use and the autoclavable bioreactors at days 3, 4 and 5 post infection.

EV71_C4 titer (LOG_{10} $CCID_{50}$/mL)		
Day	**Single-use**	**Autoclavable**
3	7.10	7.60
4	7.30	7.10
5	7.20	6.80

Conclusions

- A new single-use bioreactor has been developed by Applikon Biotechnology and tested by Intravacc. The results showed that the single-use bioreactor is suitable for Vero cell culture and EV71-C4 virus culture.
- No differences were observed between the autoclavable glass bioreactor and single-use bioreactor when comparing the growth of Vero cells on microcarriers and the yield of EV71-C4 virus in both bioreactors.

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micro-Matrix | Screening of Feeding Strategies based on Trigger Events in a 24-micro Bioreactor Platform

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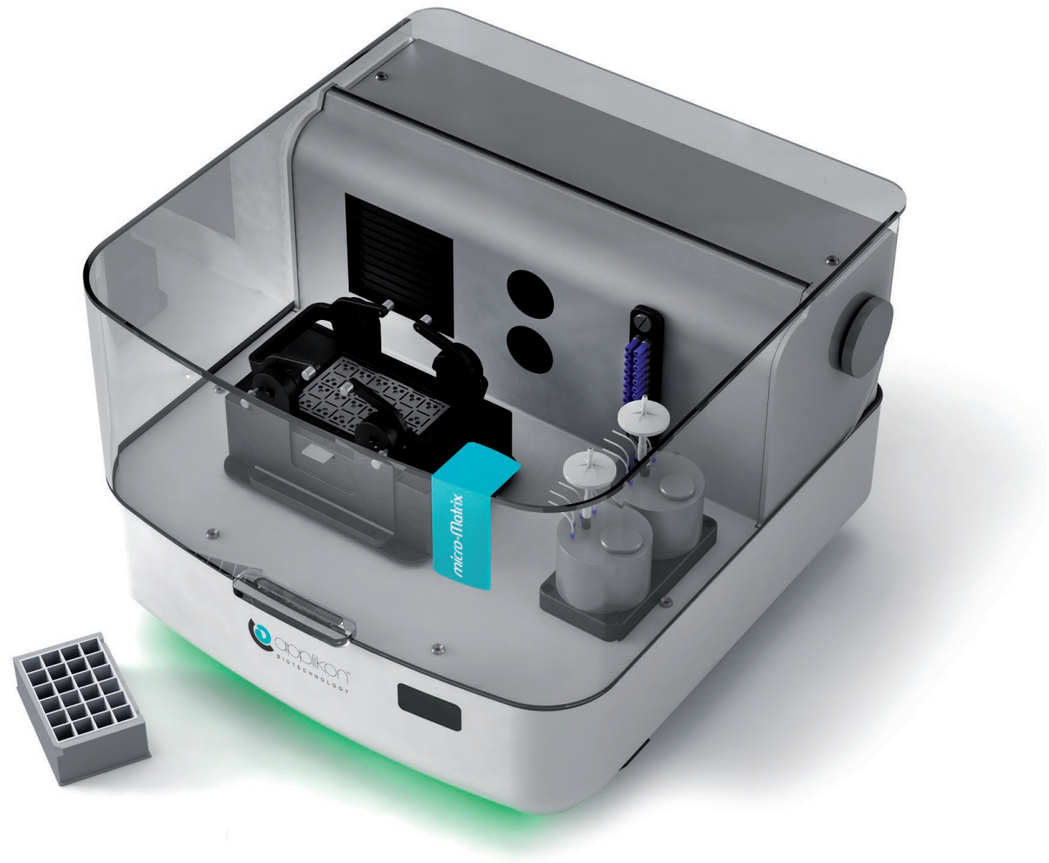


1 **Figure 1** | micro-Matrix

Abstract

Several bioprocesses exploit feeding strategies such as the minimization of acetate production in microbial cell culture. Some of these feeding strategies use values for pH or dissolved oxygen (dO_2) as a trigger point. The availability of a platform able to perform this approach on a micro scale cultivation is essential for strain optimization strategy. Applikon's micro-Matrix product is a platform that holds 24 micro bioreactors (working volume 2-5 mL) with individual control of pH, dO_2 and temperature. The micro-Matrix is able to program event-based control whereby the control strategy is based on a triggered event. In the present work, two different feeding strategies in an *E. coli* cultivation have been implemented based on i) pH above 7.1 and ii) dO_2 above 50 %, using the work of Chen *et al.,* 1997 as a reference. The results demonstrate that the micro-Matrix can be successfully used for the screening of different feeding strategies and it is able to reproduce the pH and dO_2 profiles observed for a 7 L bioreactor during a pH and DO-stat process.

Introduction

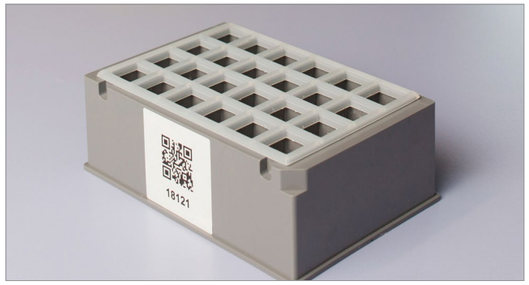
Different fed-batch strategies can be adopted in order to achieve high biomass productivity, avoid overfeeding, avoid acetate production and provide a better control over the process [1, 2]. A fed-batch based on feedback control is one of these strategies where the addition of the feed solution is linked to a dO_2 and/or pH levels.

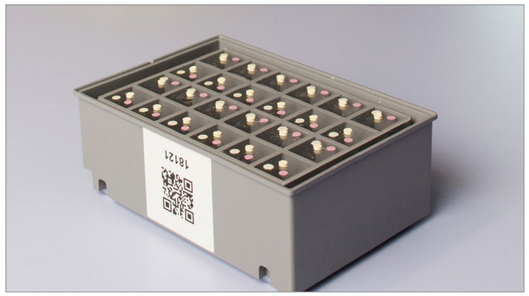
A dO_2 controlled feeding, DO-stat, is based on the fact that the dO_2 process value will increase with the declining of substrate concentration and vice-versa [1]. A pH-stat can be performed based on the principle that cellular growth using glucose as carbon source will lead to acetate production and consequently a decrease in pH levels of an *E. coli* culture [3].

The optimization and control of a fed-batch process is challenging. A well-designed *E. coli* fermentation must contemplate inhibitory acetate formation and the use of balanced medium satisfying all nutrient requirements in order to achieve a high cell density and protein yield [4], [5].

In the current work, a 24 micro-bioreactor platform has been used to study feed strategies. A pH and DO-stat have been simultaneously performed using the work of Chen and collaborators [6] as reference (where a fermentation controlled by feedback of pH and dO_2 process values, of *E. coli* in a 7 L bioreactor, was described). Liquid addition will be triggered by pH and dO_2 values in response to the conditions programmed for event-based control as implemented in the micro-Matrix software.

The aim of this work was to perform dO_2 and pH-based feeding strategies using micro scale cultivation since this approach is essential for strain and culture conditions optimization strategy. The dO_2 and pH profiles were compared to the typical profile observed during an *E. coli* fermentation controlled by dO_2 and pH-based feedback in a 7 L bioreactor, as described in [6].





Materials and methods

In the present work a pH and DO- stat were performed simultaneously during a 24 h *E. coli* fermentation using the micro-Matrix (Figure 1). The event based control feature was used whereby the event was defined as the addition of a feed solution when specific pH and dO_2 process values were detected.

The procedure followed to perform this experiment was based on the one described for the pH and DO-stat *E. coli* fermentation in a 7 L bioreactor [6].

Strain, culture media and feed solution

- Pre-culture of *E. coli* K12, free from plasmid, was cultivated in a resonance acoustic incubator (RamBio, Applikon Biotechnology) overnight at 30 °C.
- Seed medium used during cultivation and pre-culturing was prepared with 6 g/L of Na_2HPO_3, 3 g/L KH_2PO_4, 3 g/L $(NH4)_2SO_4$, 5 g/L of glucose, 5 g/L yeast extract [6], and a 2 mL/L of $MgSO_4.7H_2O$ 1M solution was added after sterilization [7].
- The feeding solution was prepared by mixing 30 g/L of glucose and 15 g/L of yeast extract after sterilization (based on the work of Chen and collaborators [6]).

Cultivation, set points and actuators

- *E. coli* cultivation started within a working volume of 2.5 mL and 1 % inoculum.
- The pH was maintained at 7 using ammonia gas, by pressurizing a NH_4OH solution 20 % (V/V) in the micro-Matrix's ammonia pressure vessel.
- The dO_2 was maintained at 30 % by using air and pure O_2 in cascade.
- Temperature had a set point of 37 °C and the orbiter shaking speed used for the experiment was 390 rpm.

Feed strategies - Event based control in the micro-Matrix

- The micro-Matrix's event-based control was programmed to detect specific dO_2 and pH values after 7 hours of run time. Once these values were detected via a programmed threshold, they triggered the addition of the nutrients feed solution through the micro valves in the micro-Matrix's liquid delivery system.
- For wells using a dO_2-based event condition, the feed solution was added at the feed rate of 2000 nL/min for 40 minutes when the process value exceeded 50 % for 10 minutes. When the dO_2 process value remained below 30 % for 10 minutes, liquid addition was stopped.
- For other wells, feed solution was added for 30 minutes (at a feed rate of 2000 nL/min) when the pH process value exceeded 7.1 for 5 minutes, and addition was interrupted when the pH value remained at 6.9 (or below) for 5 minutes.

Results and Discussion

Two different feeding strategies in an *E. coli* cultivation were applied based on i) pH exceeding 7.1 and ii) dO_2 exceeding 50 %, using the work of Chen *et al.,* 1997, as a reference.

Feeding strategy control using dO_2 as trigger point.

- The results of the process values for dO_2 and feeding medium added during a 24 h micro-Matrix run are depicted in Figure 2, as well the dO_2 profile obtained from the 7 L bioreactor [6] (green chart). The feeding medium was programmed to be added (2000 nL/min) for 40 min into the wells when the dO_2 exceeded 50 % for 10 min. It is shown how the dO_2 profile drops (red line) during the liquid addition (purple line). The system was also programmed to stop the feed if dO_2 dropped below 30 %, as occurred in the 7 L bioreactor [6]. It was also possible to verify that liquid dosage started only 7 h after the start of the run (following the end of the batch phase), as programmed. This showed that the software was effective at not dosing liquid during the batch phase when the event conditions (for pH and dO_2, but not the experiment phase) were also present.

- It was observed that every time the liquid delivery stopped the dO_2 value increased proving that the cells were growing with glucose limitation. Based on the comparison between the 7 L vessel and the micro-bioreactor it can be concluded that the micro-Matrix is a suitable system for scaling-down cultivations.

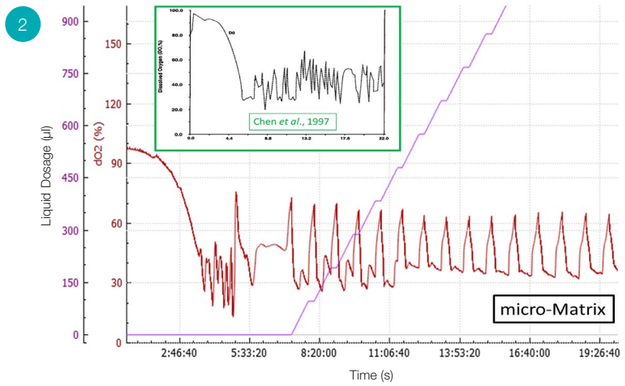


Figure 2 | Micro-Matrix liquid addition at a feed rate of 2000 nL/min triggered by a dO_2 value exceeding 50 % and stopped by a dO_2 value below 30 %, as compared to the dO_2 profile during a DO-based feedback control fermentation of *E. coli* in a 7 L bioreactor taken from [6].

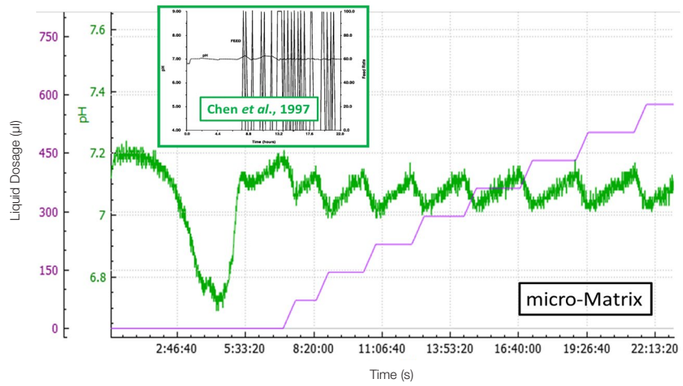


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Figure 3 | Micro-Matrix liquid addition at a feed rate of 2000 nL/min triggered by a pH value exceeding 7.1 and stopped by pH value below 6.9 compared to the pH profile during a pH-based feedback control fermentation of *E. coli* in a 7 L bioreactor taken from [6].

Feeding strategy control using pH as trigger point.

- The pH value of 7.1 was used as trigger point for the addition of feeding medium in other 8 wells. In Figure 3, the pH (green profile) and liquid dosage (purple profile) in the micro-Matrix experiment are shown as well as the profile defined in the work by Chen and collaborators [6]. The event-based control was programmed to start after 7 h of running time to ensure completion of the batch phase. The results obtained show that liquid was not added before the trigger moment. When the pH value exceeded 7.1, the feeding medium was added (at 2000 nL/min) for 30 minutes and stopped if the pH value dropped below 6.9.

- In this experiment, the pH values oscillate in response to the liquid addition as expected. The presence of glucose allows cells to grow and produce acetate which causes pH to decrease. Feeding stopped after 30 min as programmed since pH did not drop to 6.9. The feeding interruption causes the pH value to increase. As is shown in Figure 2, the pH control was similar between both bioreactors, showing that the micro-Matrix is able to reproduce a pH-stat experiment as was done for a larger bioreactor.

Conclusions

- The micro-Matrix exploits different actuators, set points, and different conditions that can be individually programmed to trigger a specific output from a given actuator in any of the 24 micro bioreactors.
- Two different event-based control approaches based on dO_2 and pH values were programmed to trigger liquid addition. The results showed that the system was efficient at controlling the liquid addition during the required time intervals.
- The micro-Matrix results obtained for the pH and dO_2 profiles were comparable to the ones previously described by Chen *et al* for a 7 L bioreactor. This shows that the micro-Matrix is suitable for scaling bioprocesses up or down, and will do so with reproducible results.
- The micro-Matrix provided the required conditions for *E. coli* to grow, as indicated by the increase in biomass.
- It was demonstrated that the micro-Matrix was efficient at screening different feeding strategies, based on different responses to a programmed condition, which was possible thanks to the individual control one can exercise on each of the 24 micro-Matrix wells.

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Scalability in Laboratory Bioreactors Based on Constant Volumetric Mass Transfer Coefficient (k_La)

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Abstract

- This work studies the scalability between 3 different volumes of lab scale bioreactors, 500 mL, 3 L and 15 L.
- The scale-up method of constant k_La is investigated.
- Impeller tip speed (Vtip) or gassed power input of the stirrer per volume of liquid in the bioreactor (Pg/VL) are used to make k_La constant between bioreactors.
- The scalability is validated by applying the findings from the k_La study, to a *K. lactis* aerobic cultivation.

Scale-Up in Bioreactors

- Oxygen plays an important role in aerobic bioprocesses and it is often the limiting factor of the bioreactor.
- k_La is a gas transfer coefficient, a measurement of the capacity of the bioreactor to transfer oxygen into the cultures.
- A common scaling-up strategy for aerobic bioprocess is keeping the k_La constant within the different scales.
- A constant k_La ensures equal oxygen transfer independent of physical differences between each scale.

Volumetric Oxygen Transfer Coefficient

- k_La is influenced by factors such as bioreactor geometry, gas flow, superficial gas velocity, impeller type and speed, and power input for mixing per unit reaction volume.
- Several empirical relationships exist to calculate the effect of these main factors in order to estimate the k_La in a bioreactor.
- Recognized degrees of freedom in the scale-up process include the impeller tip speed (Vtip), the volumetric gas flow rate (Fg) and the ratio impeller to reactor diameter (D/Tv).
- The impeller is the main gas dispersing tool in a stirred bioreactor and its configuration and tip speed have a significant effect on the k_La.
- The gas flow rate per unit of bioreactor volume (Fg) also has an important effect on the k_La, as increasing the Fg increases the gas holdup in the bioreactor which leads to a higher surface area between gas and liquid phases, which in turn increases the k_La.
- Equation 1 can be used to estimate the k_La based on the Vtip and Fg.

$$k_La = \alpha\left(\frac{Pg}{V_L}\right)^b * (vgs)^c$$

Eq. 1 (Pg gassed power input of the stirrer; V_L the liquid volume of the reactor; vgs is the superficial gas velocity and α, b and c are constants)

Materials and Methods

k_La as function of impeller tip speed and gassed power input over liquid volume

- k_La was determined for a 500 mL miniBio, 3 L and 15 L bioreactors (Applikon Biotechnology, Delft).
- The 500 mL bioreactor was assembled with an Applisens classic pH sensor, Lumisense optical DO sensor, L-type gas sparger, 2 rushton impellers, sampling pipe, 0.39 ratio impeller to reactor diameter (D/Tv) (all material from Applikon Biotechnology, Delft). Parameters were controlled with the my-Control controller (Applikon Biotechnology, Delft). The 3 L bioreactor had the same configuration as the 500 mL except 3 baffles were added and the controller used was the ez-Control (Applikon Biotechnology, Delft). The ratio of impeller to reactor diameter (D/Tv) is 0.35 for this vessel. The 15 L bioreactor used the same configuration as the 3 L. This vessel has a ratio of impeller to reactor of 0.39 (Applikon Biotechnology, Delft).
- The static method of gassing out was used to determine the k_La. Briefly, demi-water was sparged with nitrogen to purge oxygen from the bioreactors. The bioreactors were then sparged with air and the oxygen increase was registered as a function of time. When liquid and gas phase are assumed ideally mixed, the k_La is calculated by plotting the logarithmic change of oxygen concentration as a function of time.
- The k_La was determined for the three bioreactors at varying aeration and agitation ranges as described in Table 1.

Scale-up validation

- The yeast *Kluyveromyces lactis* (obtained from ATCC distributed by LGC standards, Wesel, Germany) was cultured in the 500 mL, 3 L and 15 L bioreactors.
- Yeast grew in minimal medium described by Verduyn *et al*. (1992) (nicotinic acid was increased to 5 mg/L, Kiers *et al*. (1998)).
- The cultivations were controlled at 30 °C and pH 5 by automatic addition of 4 M KOH.
- The O_2 and CO_2 in the exhaust gas were measured with BlueSense off-gas sensors (BlueSense - Applikon Biotechnology, Netherlands).
- Biomass was determined by measuring OD_{600} in a spectrophotometer and DCW at different time points during cultivation.
- The correlations found in this study were used to determine the Vtip and Pg/VL for each bioreactor (Table 2) that allows for the same k_La value in the 3 bioreactors.
- The k_La was fixed to 576 h^{-1} in the 3 bioreactors (maximum value measured for the 15 L bioreactor). To achieve this k_La value, the Vtip and Pg/VL for the 500 mL miniBio, were set to 2.6 m/s and 5.6 KW/m^3, respectively; in the 3 L and the 15 L those were set to 4.5 m/s and 9 KW/m^3.

Table 1 | Aeration and agitation range used to determine k_La.

Bioreactor	Aeration [vvm]	Agitation [m/s]
500 mL miniBio	1 – 2.0	1.5 – 2.9
3 L bioreactor	1 – 2.0	1.5 – 4.8
15 L bioreactor	1 – 1.7	1.7 – 4.5

Table 2 | The aeration and agitation settings for each bioreactor to achieve k_La of 576 h^{-1}.

Bioreactor	Aeration [L/min]	[vvm]	Agitation [rpm]	[m/s]
500 mL miniBio	0.5	1	1760	2.6
3 L bioreactor	3	1	1860	4.5
15 L bioreactor	20	1.7	1000	4.5

Results and Discussion

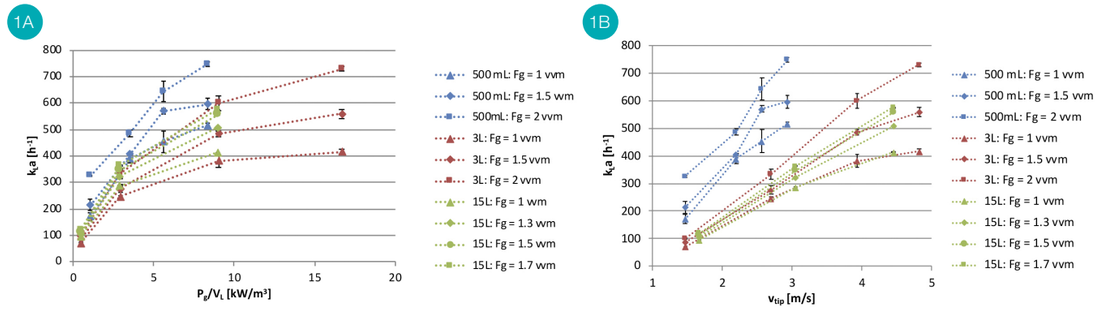
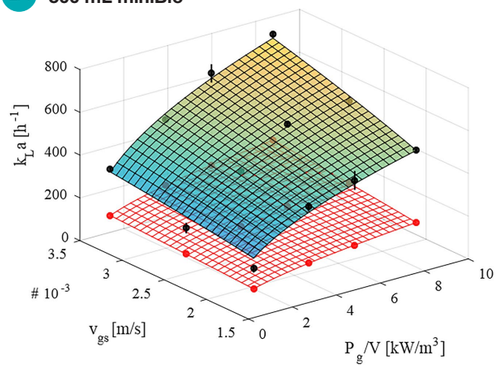
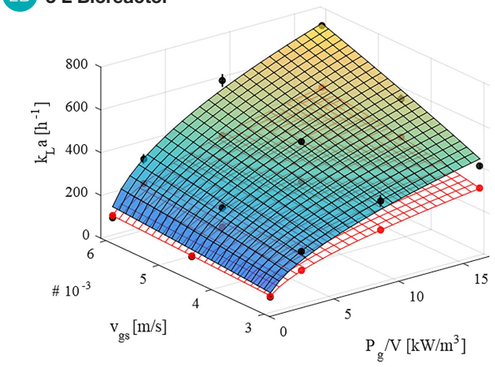


Figure 1 | (A) The k_La values for the 500 mL (blue), the 3 L (red) and the 15 L (green) bioreactors as a function of gassed power input over liquid volume at varying air flow ; (B) The k_La values for the 500 mL (blue), the 3L (red) and the 15 L (green) bioreactors as a function of the impeller tip speed at varying air flow.

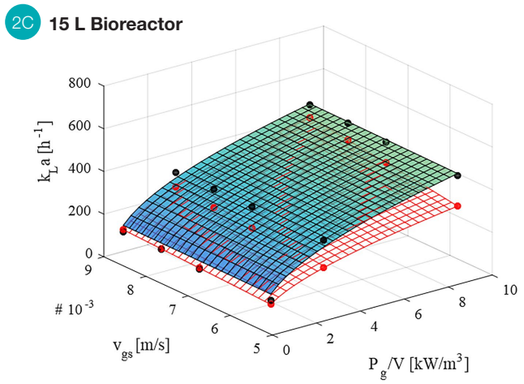
2A **500 mL miniBio**



2B **3 L Bioreactor**



2C **15 L Bioreactor**



- The results showed that the k_La values are underestimated by the theoretical correlation when the volume of the bioreactor decreases from 15 L to 500 mL.
- New k_La correlations were fitted to the experimental data, using the coefficients α, b and c as variables.
- Figure 3 (A) shows the prediction of the k_La when using the optimal correlation.
  It shows that newly calculated correlations (Figure 3 (B)) provides a more accurate prediction of the k_La.

Figure 2 | The theoretical k_La values calculated using Eq. 1, found in literature (red dots) and the experimentally determined k_La values (black dots) including standard deviation. The optimal k_La correlations (colored grid) and theoretical k_La correlation (red grid) are also indicated.

3A

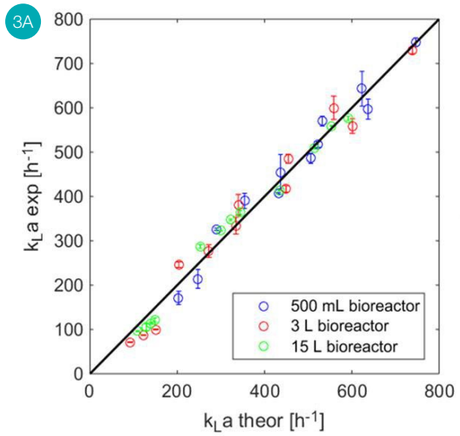


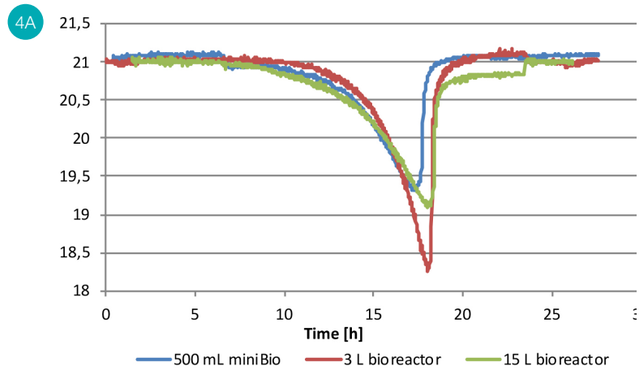
Figure 3A | Comparison of experimental k_La data with the theoretical values. The black line shows the relation between experimental and theoretical data in case of a perfect prediction.

$$k_La\ [h^{-1}] = 3600 \cdot 1.022^{10} \cdot \boldsymbol{\alpha} \cdot \left(\frac{P_g}{V_L}\right)^{\boldsymbol{b}} \cdot \left(v_{gs}\right)^{\boldsymbol{c}}$$

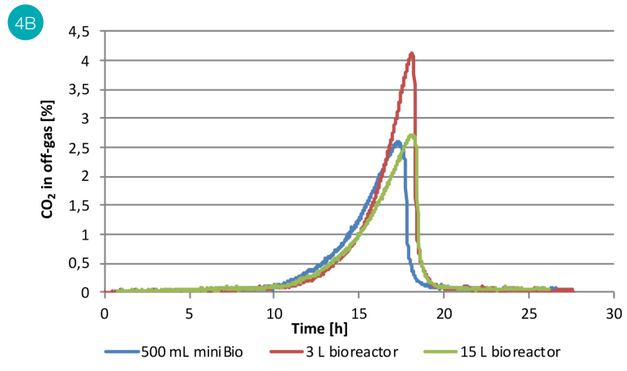
3B **Figure 3B** | The coefficients required for an optimal fit between the k_La correlations and the experimental k_La.

Bioreactor	α	b	c	adj R^2	RMSE [h^{-1}]
500 mL miniBio	0.075	0.4	0.5	0.9644	32.24
3 L bioreactor	0.079	0.45	0.72	0.9672	38.60
15 L bioreactor	0.030	0.48	0.61	0.9805	24.91

4A



4B



- The results show a high reproducibility between bioreactors, with an average biomass concentration of 10.9 ± 0.2 g DW/L (AVG ± STDEV).
- The biomass yield on glucose obtained was 0.54 ± 0.01 g DW/g glucose.
- The final OD_{660} obtained for the 500 mL, 3 L and 15 L was 69.8, 71.6 and 69.0 respectively.
- The off-gas analyses indicates (Figure 4A and 4B) similar growth profile in the three bioreactors.

Figure 4 | (A) O_2 off-gas profile during *K. lactis* cultivation; (4B) CO_2 off-gas profile during *K. lactis* cultivation.

Conclusions

- Maximal k_La values of 748 h^{-1}, 730 h^{-1} and 576 h^{-1} were obtained for 500 mL, 3 L and 15 L bioreactors respectively.
- The scaling- up method based on keeping k_La constant by varying Vtip or Pg/VL proved to be limiting in this study. Only restricted Vtip and Pg/ VL can be used to achieve a matching k_La in the 3 bioreactors.
- The coefficients α, b and c for the general k_La relation were determined and k_La correlations based on the experimental results were obtained for each of the three bioreactors. These correlations showed a better prediction of the k_La values than the theoretical correlation.
- The final biomass concentration and final yield of biomass on glucose are calculated for each bioreactor. The resulting biomass concentrations and yields are nearly identical for all three bioreactors (10.9 ± 0.2 g DW/L, and 0.54 ± 0.01 g DW/g glucose). These values agree with the values found in literature (X_t = 9.8 g DW/L and YX/S = 0.49 g DW/g glucose).
- Applikon lab-scale bioreactors 500 mL, 3 L and 15 L were successfully used for scaling-up *K. lactis* aerobic cultivation.

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Accelerating Pipeline Management in Pharmaceutical Drug Development using V-Control as a Common Process Control Platform

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Abstract

Over the past 40 years, drug development costs have been increasing exponentially and the return on investment within pharmaceutical companies has come under pressure [1]. Fast time-to-market is essential in order to be competitive and moreover to serve today's patients with the best possible medicines available. V-Control is a process control platform that has been developed as a 'one common-platform' solution from Discovery up to Production. The seamless technology transfer of modules from R&D to production systems and the scalable data transfer result in optimal bioprocess control with shorter lead times and lower development costs.

Introduction

With the inclusion of post-approval R&D, development costs have risen to 3 billion dollars according to a study by Tufts Centre for the Study of Drug Development [1]. The ever-growing body of knowledge of the underlying biological processes in (bio)pharmaceutical drug development requires investments in innovative technology and in time in order to discover and develop new medicines. These investments in technology and time drive the costs for new drug developments. Furthermore, the growing competition from biosimilars and generic drugs together with the increasingly stringent global requirements are putting more pressure on companies' pipeline management.

These pipelines are also under high pressure as different studies show that only about 1 out of 10 drugs that enter clinical trials Phase I will end up as an approved drug [2]. All these factors are driving the need for a scalable and validated process control solution from Discovery to Production. In the current market, different software platforms are scattered across different process development phases for process control and data management (Figure 1). The product life cycle as displayed in Figure 1 consists of two major technology transfer moments, going from preclinical to Phase I clinical studies and after Phase III clinical studies. This technology transfer comes with several challenges.

1

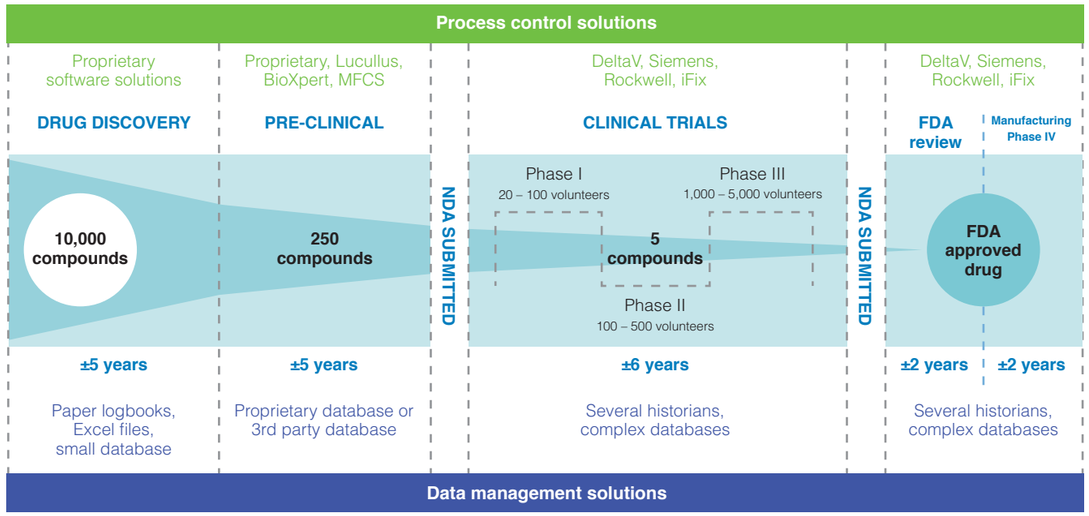


Figure 1 | Representation of the product life cycle management of pharmaceutical drug development in the current market.

2

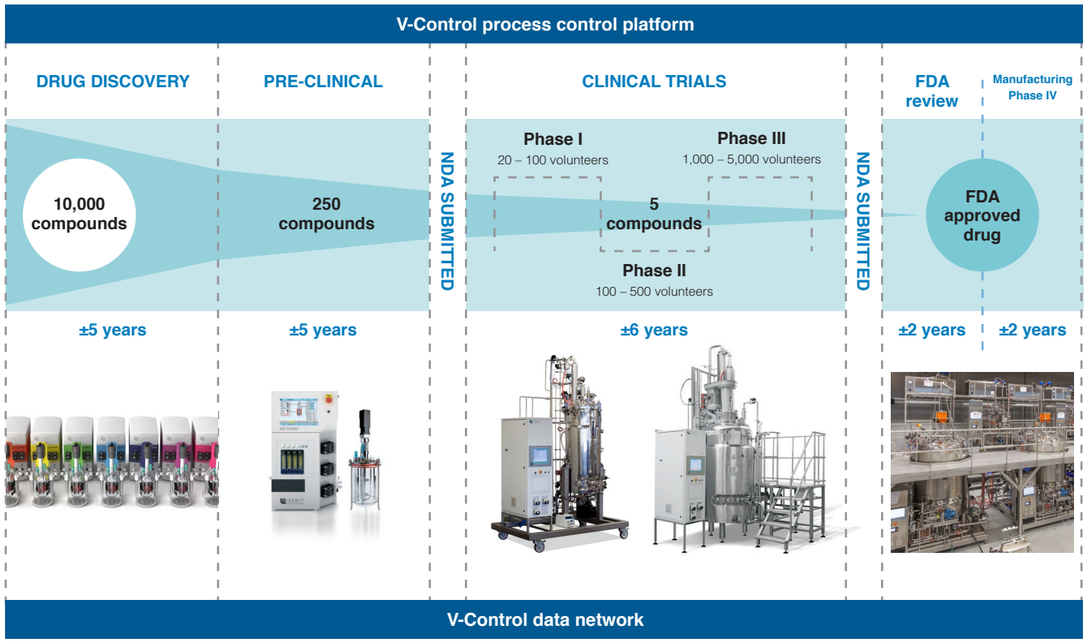


Figure 2 | Representation of the product life cycle management of pharmaceutical drug development using V-Control as the common platform from Discovery up to Production.

During the product life cycle management and the technology transfer, different departments that are often siloed from one another and focusing on their own unique needs, will need to collaborate. They need to scale-up or scale-down their bioprocesses, this becomes more challenging when the different departments end up working with different process control or data management solutions. Operators often don't speak the same 'language' while they have to compare data or translate recipes from one software package into another. Additionally, it becomes more and more challenging to find skilled operators and this will require more time if they need to be trained for different software solutions.

Furthermore, in a world of 'big data' it becomes more difficult to properly organize the increasing amount of data generated by the profusion of Process Analytical Technologies and this is aggravated by the different 'languages' that might be used by the different data management solutions. On top of that, keeping all this data secure is of utmost importance - as was shown by the Merck data breach of 2017, which cost insurers some 275 million dollars[3]. The increasing extent of the body of regulatory requirements also puts pressure on the product life cycle, since all the process and data validation can take up a large chunk of the product development timeline. It can be very time-consuming, or sometimes even impossible, to show and prove transparency and full traceability during the product life cycle as software packages used in R&D might not be developed and validated according to the industry standards.

To overcome these challenges and to improve the product life cycle management, Applikon Biotechnology and Emerson Automation Solutions developed V-Control to decrease investment costs and to reduce the time-to-market. V-Control combines scalable bioreactors with DeltaV™ technology as the sole solution from discovery up to production for process control and data integration (Figure 2).

The Product

V-Control is a process control platform consisting of 3 major parts:

- Applikon Bioreactor system
- Emerson's DeltaV™ automation platform
- V-Control DeltaV™ application

V-Control is developed as a result of a close collaboration between Applikon and Emerson whose combined knowledge and experience has led to the scalable DeltaV™ solution for bioreactors.

V-Control comes in different flavors:

- V-Control for R&D
- V-Control for Pilot & Production

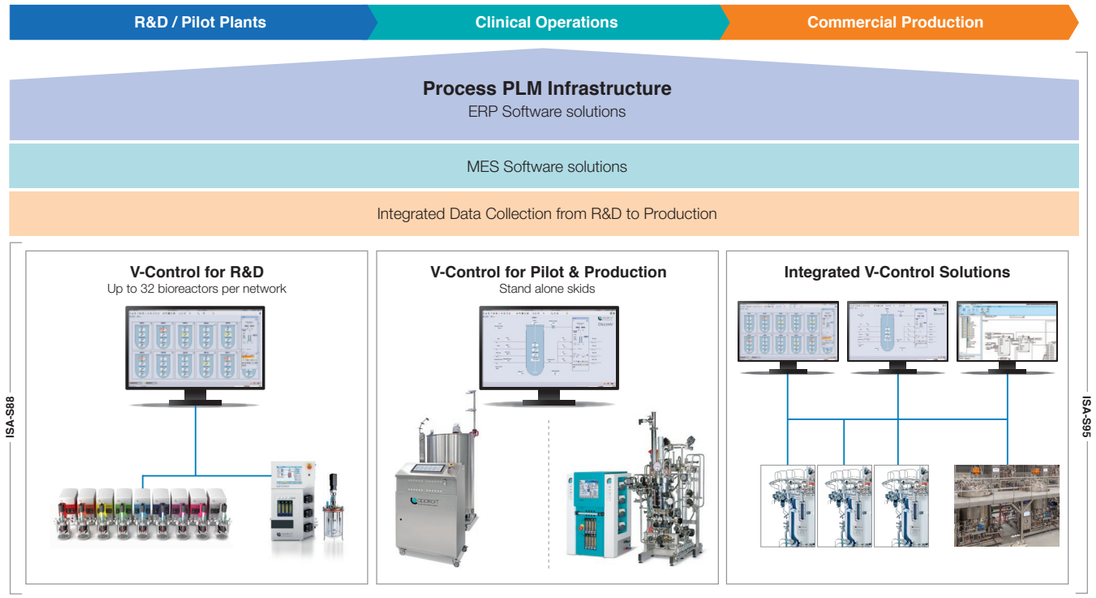
The V-Control R&D version is optimized for bioprocessing and will combine DeltaV Discovery™ with Applikon's scalable laboratory bioreactors ranging from 250 mL up to 20 L.

V-Control for Pilot & Production will combine Emerson's PK Controller with Applikon's turnkey single-use and stainless steel solutions from pilot scale up to production and can be used as powerful stand-alone solutions or as fully integrated process control solution.

Features and Benefits

- Through its **open architecture of the DeltaV™ application software** V-Control eases tech transfer and the exchange of recipes and control or equipment modules between R&D and production facilities. In addition, the customer's own libraries can be combined with the V-Control libraries.
- V-Control **Ready to GO solutions** include pre-defined and validatable control and equipment modules optimized for bioprocessing. The V-Control solutions are Ready to GO, resulting in minimal start-up time and reduced costs.
- **Optimized bioprocessing software features** include standard modules that have been built for typical bioprocessing operations such as sensor calibration, 3-point cascades, etc. This makes the application intuitive and easy-to-use and it optimizes operator time.
- **The applikon biocontroller is used as an intelligent I/O module** permitting the easy integration of novel sensor technologies and additional outputs. Up to 25 sensors such as pH, optical DO, off-gas, balance, and biomass sensors can be connected. Furthermore, external actuators such as like pumps and external stirrers can be controlled via the available 4-20 mA outputs.

Conclusions



V-Control is based on Emerson's well-known DeltaV™ platform and because of its open-source architecture it will provide scalable solutions from R&D to production. It is built according to the ISA-88 and ISA-95 architecture, and allows for integration with level 3, level 4 and level 5 data management, MES, and ERP solutions. Different departments in the development chain can be connected - from discovery all the way to production.

This integrates product and process development, enabling seamless technology transfer and data management. Using V-Control as the common process platform accelerates the product pipelines and ultimately results in a reduced time-tomarket and lower development costs. Finally, it will enable the life sciences industry to better serve the needs of patients and to improve the quality of life.

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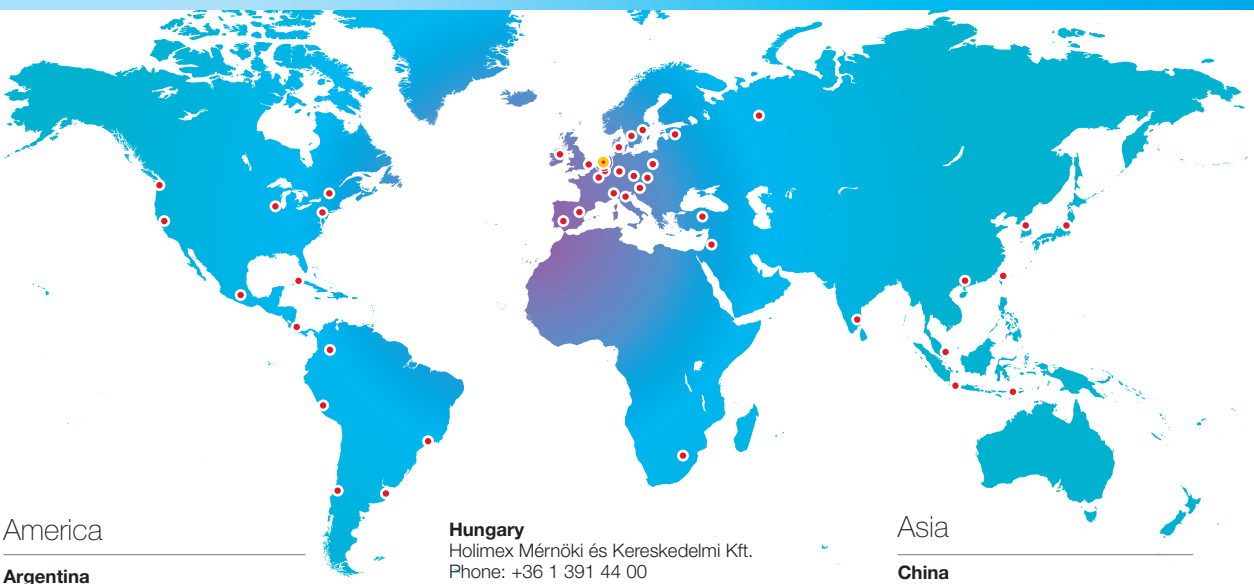

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