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Increased Throughput in Upstream Development via Automated Feed Strategy Using Qubicon®

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Introduction

Automation of manual repetitive steps combined with automatic data collection are valuable tools to overcome the increased challenges of high-throughput bioprocess development. Analyser capabilities and the number of bioreactors per unit significantly increases the amount of data generated during the past years. Consequently, more time is now needed to process the increased volume of data collected and to oversee operations of bioreactors in the lab. Ichnos was in need of a solution that enabled more efficient data management and enhanced process control. In this case study we present the implementation of Qubicon, an automated feed addition for multi parallel bioreactor systems. Based on assembled data, the system allows for real-time process insights in terms of cell culture performance and defining simple and advanced control strategies.

Objectives

Main goals were to:

- Automate the data collection and real-time monitoring of the respective culture performance using a data management system.
- Reduce the time spent in the lab to perform repetitive tasks.

Methods

- The Qubicon software was implemented as a central data management and advanced control system. Multiple bioreactors, pumps, scales as well as a substrate analyser were connected. Based on accessible data information, a mathematical script and the recipe with quantity of feeds to add was defined.
- After the release of the recipe, some initial test runs were made in order to increase accuracy of the addition. Since a two-component feeding strategy was applied, the speed of the respective pumps was adapted to ensure maximal accuracy of the media addition. As a constraint, bolus addition has to be done in the shortest time possible.
- Prior feeding tests using water were performed to determine the pump rate and volume addition, respectively, to obtain a control accuracy below the defined threshold of 5% to the targeted setpoint. As well as the calculation rate of the script.
- Finally, the automatic control strategy was applied, and the results compared to a manual addition during a fed-batch culture in bench top bioreactor.

Results
Qubicon Setup

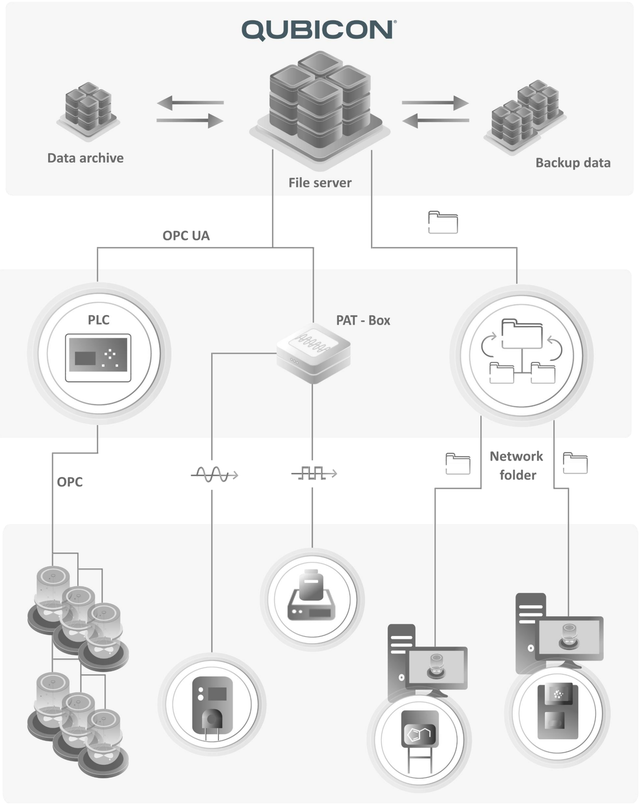

Figure 1: Schematic representation of the automation architecture at Ichnos. Devices can be connected to Qubicon either directly, through the PAT-Box or by utilizing the automated offline import.

- Access to all relevant data is the basis for real-time monitoring and advanced process control. Due to the lack of standardized equipment interfaces, the connection of devices from different vendors is the first challenge to overcome.
- Via the application of hard- and software gateways all devices could be successfully integrated. The data collection of a metabolite analyzer and a cell counter was realized via a shared folder network. Sample data assignment was automated via defined sample IDs. Translation of different in/output signals was obtained via the usage of a Pat-Box and connected to Qubicon. Monitoring and control of the multiple bioreactor set-up was realized via the direct connection to the control tower following an OPC UA protocol gateway.
- The software implementation offered the USP Team a defined feeding control strategy, while still being flexible and allowing adjustment of the feed rate if necessary.
- Online, offline and meta data were automatically contextualized and stored in one database accessible for all assigned users.

Results
Water Runs

- Water runs were performed on two different systems with various pump speeds. During the first days, the setpoint threshold of 5% was exceeded (highlighted in pink in the Table 1) with the automatic feed compared to the manual approach. This is likely to be linked to the relatively small amounts of feeds added (below 5g per feed 2 addition).
- After an initial phase, the accuracy of the automatic feed addition was within the defined acceptance range and comparable to the manual feeding. A more robust profile within the automatic pump control was observed as the variation to target value is constant and close to 0.
- The setpoint variation of feed 1 for system 1 and system 2 after 4 and 5 days, respectively, was negligible.
- A rather similar precision below the threshold for feed 2 with the automatic control compared to the manual feeding was obtained around day 7. On system 2, the automatic pump control allowed a more precise and accurate feed addition within the threshold than the manual feeding from the very beginning.

	Difference to the setpoint Feed 1 (%)				Difference to the setpoint Feed 2 (%)			
Feeding days	System 1		System 2		System 1		System 2	
	Automatic	Manual	Automatic	Manual	Automatic	Manual	Automatic	Manual
2	24.0	1.7	16.6	-0.7	35.1	19.4	4.2	15.2
3	10.7	1.5	6.6	-0.8	16.3	6.1	3.4	6.7
4	2.2	1.1	6.2	1.4	20.7	3.7	1.9	5.1
5	3.9	-0.2	1.8	-0.4	11.7	-0.2	0.4	1.5
6	1.9	0.0	3.4	0.0	9.9	0.0	1.0	3.0
7	0.9	1.6	0.6	0.0	4.7	1.3	0.2	1.3
8	0.5	0.6	1.7	0.1	5.5	1.8	0.2	1.8
9	0.6	0.8	1.6	0.3	5.2	-0.4	0.0	-0.4
10	0.1	0.1	1.8	0.5	1.1	-1.2	0.0	-1.2
11	0.3	1.3	0.5	0.8	2.5	0.8	0.0	-0.4
12	0.6	0.0	1.0	2.6	0.8	-0.3	0.0	4.7
13	0.2	0.6	0.9	0.1	1.9	-0.8	0.1	-0.8

Table 1: Automatic feeding comparison to manual feeding on two systems in terms of percentage of difference to the setpoint.

Results
Bioreactor Runs

- As with the system 1, the precision was not satisfying at this time of the development, and therefore feed 2 was added manually for the assessment in bioreactor. Available pumps were going too fast for the low volume to be added.
- During the test, two human errors led to a less precise addition of feeds (see below explanation), however this difference did not impact the performance of the culture in comparison to the manual feeding process.
- As shown on Figure 2 and 3, performances between the three bioreactors are similar.
- Both automatic feeding systems demonstrate equivalent results in comparison to the control process where addition of feeds was made manually.

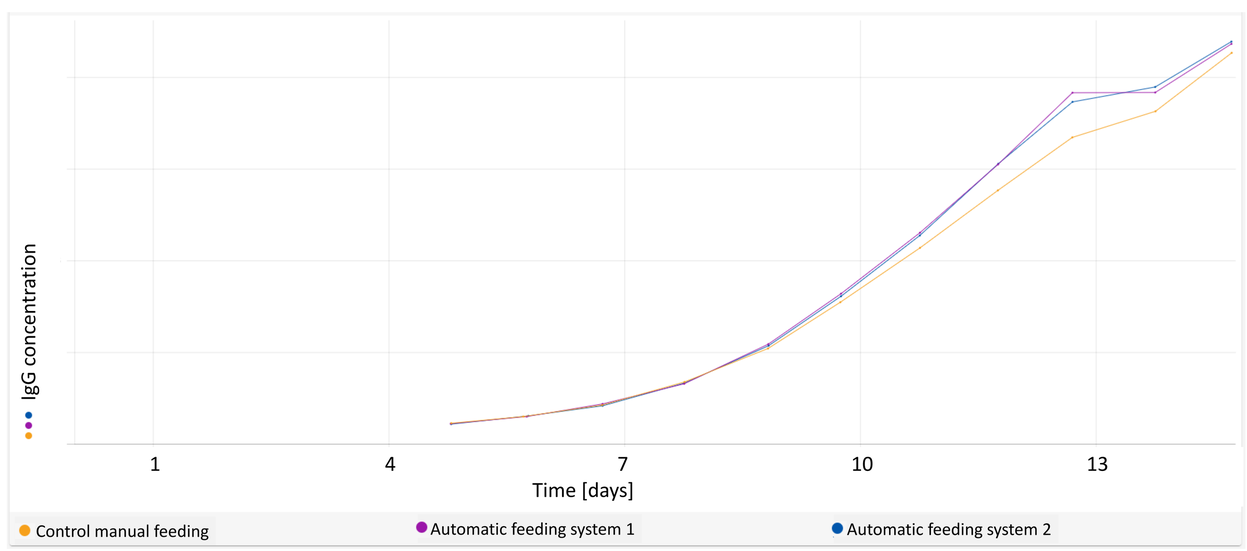

Figure 2: Titer comparison between automatic and manual feeding.

- With regards to difference between the two automatic feeding systems, the titer trends are overlapping each other.
- Manual feeding is slightly lower; however the difference is not biologically relevant.

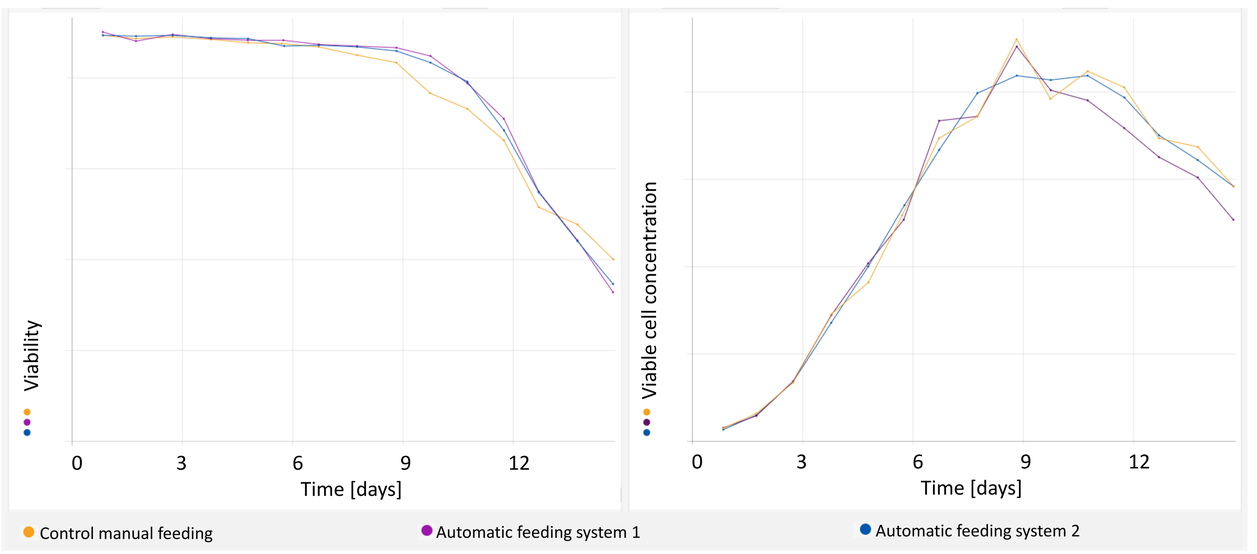

Figure 3: Viability and viable cell concentration between automatic and manual feeding.

- On Figure 3, as already observed on titer trend, the two bioreactors where the automatic feeding was used are overlapping. The bioreactor with manual feeding is slightly lower without significant difference.
- The viable cell concentration also demonstrated an equivalent trend for the entire process in all three bioreactors.

Results
Lab Set-up Issues

- Because of first implementation of this set-up, on day 6 the scale was tared by mistake which led to a second addition of feed.
- On the last day of feeds addition, the feed stock bottles were completely emptied (required volume was underestimated).
- Despite those issues, feeds addition on system 1 is matching the acceptance criteria of <5% (highlighted in pink in Table 2).
- On system 2, feed 1 is overall within the 5% of difference. On day 5 for both feeds the addition was above the target which is likely due to a move of tubings, and this impacted the weight on the scales. This would have led to a lower addition of both feeds the day after. Same is expected to be the root cause of the 13% of difference for feed 2 at day 9.

	Difference to the setpoint Feed 1 (%)		Difference to the setpoint Feed 2 (%)
Feeding days	System 1	System 2	System 2
4	2.3	5.3	5.4
5	0.5	7.3	17.0
6	56.7	-5.2	-18.2
7	0.1	0.3	-3.9
8	-0.5	5.4	2.1
9	-0.7	-1.6	13.0
10	0.4	0.8	-19.1
11	0.0	-0.9	0.7
12	0.1	1.9	2.0
13	-95.4	-9.3	-146.4
Overall	6.3	0.2	-6.2

Table 2: Difference to the setpoint during test in bioreactor.

Conclusions

- Water runs enabled the definition of the threshold below which systems are not precise enough to be used (less than 5% of difference to the setpoint). Feed 2 on the system 1 will be added manually until further development of the set-up (lower calculation rate in Qubicon or max speed of the pump).
- The confirmation run in bench top bioreactor enabled validation of the automated feeding to be implemented in routine procedure.
- Training and adaptation of the set-up to avoid movement of tubing is to be reinforced to ensure robustness of feeding process.
- Implementation of Qubicon resulted in a gain of one hour of feeding time per day and two hours of data collection at the end of the process.