Monitoring of pH and OTR using a multiple shake flask platform: a tool for metabolism and cell growth assessment in mammalian cell cultures.

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Kuhner shaker

1 BACKGROUND AND NOVELTY

PAT (Process Analytical Technology) initiative has been spreading along the growing biopharmaceutical industry[1,2] since 2004, when FDA published PAT guidance in order to encourage innovative pharmaceutical development and manufacturing[3]. PAT initiative centers the focus on process quality in order to ensure the final product quality, and thus, PAT has to be implemented from the initial steps of the bioprocess development (Quality by Design). Altogether has pushed researchers to develop new monitoring tools, mainly in the screening and scaling up processes under GMP requirements.

Adolf Kühner AG, has develop the RAMOS[4] system for bioprocess development purposes. RAMOS approach is based on single-use shakers equipped with optical probes (non-invasive), which is a useful tool for Quality by Design applications.

In this work, the combination of two monitoring systems [RAMOS and SFR (PreSens)] has been explored in order to validate an useful tool for online monitoring of cell culture. In this sense, we performed an study of different metabolic behavior of HEK293 cells triggered by means of environmental conditions manipulation. In these study cases, online measurements of oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR), dissolved oxygen (DO) and pH were registered. Then, the registered data were compared with the off-line determination of cell density and metabolites concentration measurements in order to evaluate the performance of the culturing and monitoring platform.

2 MATERIALS AND METHODS

Cell line: HEK293SF-3F6.

Culture media: SFMTransFx-293+5%FBS+10%CB5 (80g/L).

Culture conditions: A) pH_0=7 (control) ; B) pH_0=6.6 (HCl addition) ; C) pH_0=6.6 and 12mM of Na-lactate addition.

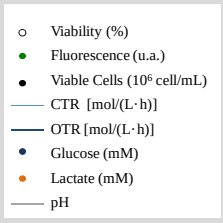
Metabolites analysis: Glucose and lactate concentrations were measured using an automatic glucose and lactate analyzer (YSI, Yellow Springs Instrument).

Culture platform: 250mL disposable shake flasks coupled to RAMOS-System[4] and SFR[5]. On-line measurements of pH, dissolved oxygen (DO), oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR) and respiratory quotient (RQ) were available.

RAMOS coupled to SFR platform (Kuhner AG)

3 RESULTS AND DISCUSSION

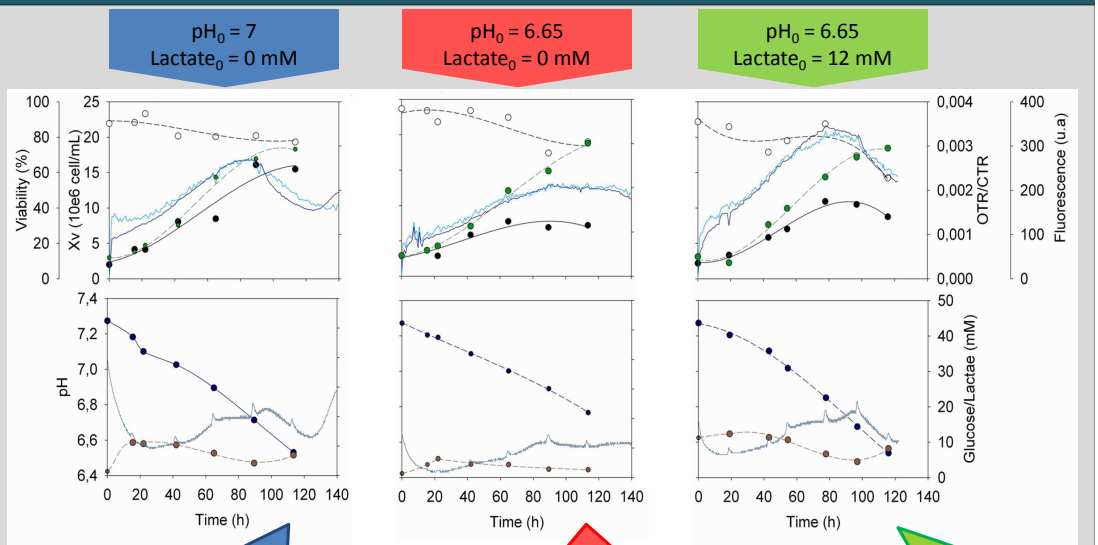
Different metabolic behaviors were observed in HEK293 cultures depending on the environmental conditions. These metabolic behaviors can be triggered at will by lowering the pH and adding lactate to the medium.



➢The pH profile fitted perfectly to the evolution of lactate concentration .

➢OTR resulted in a very good indicator of cell activity and viability in all studied cases: A and C cells grew exponentially, B cell growth inhibited.

➢The slope on OTR curve dramatically dropped approximately 24h before a decrement on viable cell density could be noticed.



- Lactate accumulation initial stages
- pH drop to 6.5
- Glucose and lactate co-consumption
- pH raise to 6.8

- Cell growth inhibition
- Small amount of lactate produced
- Slight lactate consumption and pH raise

- Lactate concentration stable at initial stages
- pH constant around 6.6
- Glucose and lactate co-consumption
- pH raise to 6.8

4 CONCLUSIONS

1. Similar to OUR (oxygen uptake rate), OTR is a good indicator of cell activity, and thus can be used to determine cell viability.
2. pH profile perfectly correlate with lactate accumulation or consumption profile, offering information about the metabolic behavior of cells.
3. An automatic sampling system would improve the platform in terms of avoiding the disturbances of the manual sampling on the measuring system.
4. Both measurements, separately or in combination, are useful monitoring tools to develop culture strategies at bench/Lab scale. Therefore, RAMOS combined to SFR platforms could match the PAT and Quality by Design requirements.

REFERENCES

[1] **Wurm, F**. Nature Biotechnology, 22 (2004).
[2] **Walsh, G**. Nature Biotechnology, 32 (2014)
[3] **Read, E K et al.** Biotechnology and Bioengineering, 105 (2010).
[4] **Anderlei, T et al.** Biochemical Engineering Journal, 17 (2004)

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