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Evaluation of DO and pH as key parameters for cell metabolic shift in the Wave reactor®

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I Introduction

As one of the numerous strategies employed to increase the cost-effectiveness of monoclonal antibody (MAb) production, new reactors have been designed to support high cell densities and MAb production. In this work, the Wave reactor® (Wave) - a novel reactor design - is shown to produce a higher MAb yield over cells and a more effective use of glucose and glutamine.

Moreover, since the Wave used in this work does not include pH and dissolved oxygen (DO) control, these parameters are being analyzed as possible key to the metabolic shift observed in the Wave when it is compared with a traditional Stirred Tank Reactor (STR). In this analysis, the CellFerm-Pro® (CFP) - a novel reactor design for the study of cell culture optimization and scaling up - has been used to compare the effect of different pH conditions.

II Objectives

- To analyze cell growth, MAb production, and metabolism in STR and Wave.
- To evaluate the effect of key environmental parameters, such as pH and DO, on cell metabolism in STR and Wave.

III Material and methods

Cell line: The mouse-mouse hybridoma BK-183 (produces IgG₁)

Reactors: STR: 3 L glass marine pitched impeller (Applikon)

Wave reactor®: System 20 and 2 L Cellbag (Wave Biotech LLC) (Figure 1)

CellFerm-Pro®: 1 L ball stirred vessel (DASGIP, Germany) (Figure 2)

Medium: BD Cell Mab Medium Quantum Yield (BD Biosciences, MD) (Supplement with 10% FBS, 1% Na-pyruvate (Final 1 mM), L-glutamine (2 mM) and penicillin-streptomycin (100 IU/mL))

Culture condition: Initial cell density: 2x10⁵ cells/mL

pH set point: 7.0 (STR), none (Wave), 7.0 or 6.8 (CFP)

DO set point: 50% air saturation (STR, CFP), none (Wave)

Analytical methods: Cell count: Cedex cell counter (Innovatis GmbH)
Glucose, lactate, glutamine concentration: YSI model 2700 (Yellow Spring Instruments)

MAb concentration: HPLC (1100, Agilent Technologies) with a column Poros rProtein A (Applied Bioscience)

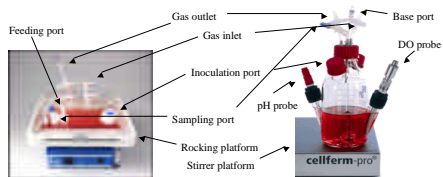


Figure 1. Picture of the a 2L Cellbag (Wave reactor®).

Figure 2. Picture of a 1L Spinner Flask (CellFerm-Pro®)

IV Wave reactor® vs. a STR

Significant differences in cell growth, MAb production, and metabolism were observed in Wave when compared with STR.

➤ **Cell Growth** - In the analysis of viable cell density (Fig. 3), the specific cell growth and death rates were found to be 1.7 and 2.3-fold lower in Wave (Table 1).

➤ **MAb Production** - In Wave, the yield of MAb over total cell density (Y_{MAb/Nt}) was 40% lower than STR at the end of growth phase but 36% higher (Fig. 4) at the end of the run (Table 1). In other words, BK-183 is shown to produce MAb mainly during the exponential growth phase in STR but during the decline and death phase in Wave.

➤ **Metabolism** - Significant differences in glucose uptake (Y_{glc/Nt}, 61% lower) and lactate production (Y_{lac/Nt}, 89% lower) were observed in Wave when compared with STR (Fig. 5). The resulting 2-fold lower yield of glucose over lactate (Y_{glc/lac}) in Wave (Table 1) was consistent with the observation of higher MAb production (>50%). The yield of glutamine over total cell concentration (Y_{gln/Nt}) was also 26% lower in Wave, suggesting a more efficient use of metabolites.

To investigate whether the above differences could be correlated with the lower pH levels (Fig. 6) in Wave, a pH analysis was conducted using the CFP.

V pH analysis in the CellFerm-Pro®

To address the effect of pH on cell metabolism, two different pH modes were analyzed in CFP, using two vessels run in parallel: constant pH 7 and a combination of pH 7 reduced to 6.8 during the exponential cell growth.

➤ A comparison of the two shows no significant differences during growth phase but a 1.3-fold lower death rate (Fig. 7) and a 1.3-fold higher Y_{MAb/Nt} (Fig. 8) during decline and death phase at acidic pH (Table 1). In addition, a 1.4 and 1.5-fold lower Y_{glc/Nt} and Y_{lac/Nt} were observed (Fig. 9). No significant differences, however, were observed in Y_{glc/lac}. While lower glucose and lactate metabolism rates were observed in lower pH, glutamine uptake remained unaffected (Fig. 10).

➤ The lower Y_{glc/lac} and Y_{gln/Nt} observed in Wave could not be correlated with lower pH.

These observations lead to the possibility of considering differences in DO^{1,2} and shear-stress³ (associated with the sparger-free environment) in Wave as possible parameters affecting Y_{glc/lac} and Y_{gln/Nt}.

Future work

- Analyze the effect of changing pH at different time of culture.
- Study the impact of DO on cell growth, MAb production, and metabolism.
- Investigate the role of aeration mode (i.e. presence of a sparger) in affecting Y_{lac/lac}.

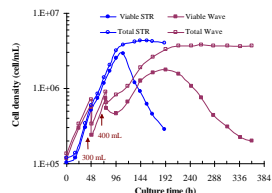


Figure 3. Viable and total cell concentrations

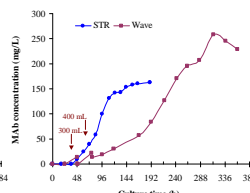


Figure 4. MAb concentration

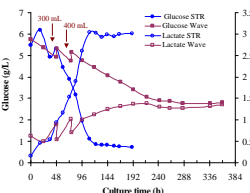


Figure 5. Glucose and lactate concentrations

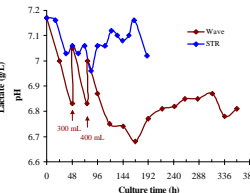


Figure 6. pH levels

Conclusions

- Lower cell growth rates (50%) and metabolite (41% glucose, 60% lactate, 20% glutamine) yields were observed in Wave in comparison with STR.
- The lower glucose over lactate yield (33%) suggests a more efficient use of metabolites in Wave.
- A higher yield of MAb over total cell concentration was observed at the end of growth phase in STR but at the end of the run in Wave.
- Lower pH levels were observed in Wave (6.8) in comparison with the STR (7.0).

Table 1. Cell growth and death rates and MAb production yields.

	Exponential phase		Death phase		Total culture		Metabolic Yields (during exp phase)			
	μ (h ⁻¹)	Total cell (10 ⁶ cells)	Y _{MAb/Nt} (pg MAb/cells)	K _d (h ⁻¹)	Total cell (10 ⁶ cells)	Y _{MAb/Nt} (pg MAb/cells)	Y _{glc/Nt} (ng glc/cell)	Y _{lac/Nt} (ng lac/cell)	Y _{glc/lac} (ng glc/cell)	Y _{gln/Nt} (pmol gln/cell)
STR	0.04	3.82	35.8	0.03	4.03	41.69	1.19	0.78	0.66	2.13
Wave	0.02	2.61	21.8	0.01	3.66	80.60	0.70	0.31	0.44	1.70
CFP pH 7	0.03	4.68	9.81	0.04	7.08	8.06	1.08	0.79	0.73	1.50
CFP pH 7/6.8	0.03	4.75	9.6	0.03	6.71	10.30	0.79	0.53	0.67	1.41

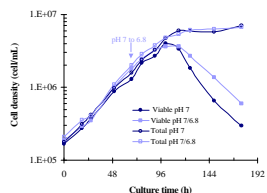


Figure 7. Viable & total cell concentrations

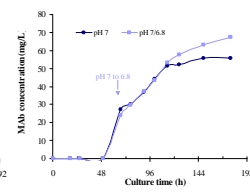


Figure 8. MAb concentration

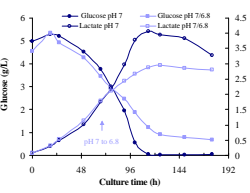


Figure 9. Glucose & lactate concentrations

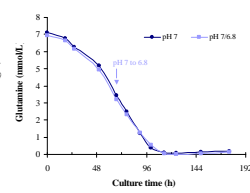


Figure 10. Glutamine concentration

Conclusions

- Lower death rate (24%) and glucose (23%), lactate (33%), and MAb (22%) yields were observed when pH was reduced from 7.0 to 6.8.
- No significant differences in cell growth and glutamine uptake were found to be associated with acidic pH conditions.

References

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