A NOVEL APPROACH TO DEVELOP CULTURED MEAT: DESIGN AND SIMULATION OF A LARGE-SCALE PRODUCTION PLANT

Part II: Upstream and Bioreaction

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INTRODUCTION

The aim of the present work is to cover a 1 % of the current market for meat substitutes in Amsterdam, so production will be 7 metric tons of cultured meat per year.

For accomplishing this goal, a large-scale process has been designed for the culture of myocytes, which comprehend two stages, proliferation and differentiation. Bioprocess is composed of:

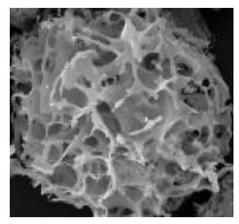
- Two 5000L industrial reactors working overlapped.
- One line semi-continuous downstream.

SCAFFOLDS

Scaffold is required because satellite stem cells need to grow under adhesion. The scaffold selected are fungal chitosan microcarriers previously described:

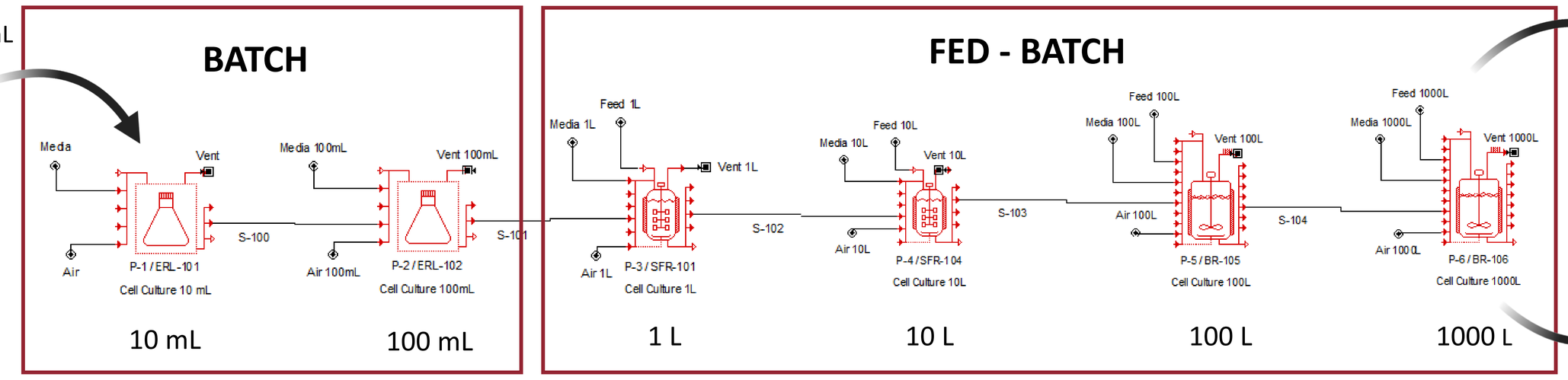
- Diameter 150 µm
- Porosity 98%
- Density 0.04g/m^3
- Specific Surface Area 30 m^2/g
- Ratio Cells-Scaffold 63.3 kg/kg

Bead-to-bead transfer & NO internal diffusional limitations



SEED TRAIN

$4 \cdot 10^6$ cells/mL of a cryo-preserved cell stock



Is used to inoculate two 5000L bioreactors with 4 hours of holding time.

BIOREACTION

FED-BATCH

PROLIFERATION

Initial values:

- [Glucose] → 1 g/L
- [Glutamine] → 0.115 g/L
- Scaffold → 1.675 kg

Cell density $4 \cdot 10^5$ cells/mL

Proliferation Kinetics Equations:

$$\frac{dXv}{dt} = (\mu - \mu d) \cdot Xv$$

$$\frac{d[GLC]}{dt} = -\left(\frac{(\mu - \mu d)}{Y_{Xv/glc}} + m_{glc}\right) \cdot Xv + feed1$$

$$feed1 = \left(\frac{(\mu - \mu d)}{Y_{Xv/glc}} + m_{glc}\right) \cdot Xv$$

$$\frac{d[GLN]}{dt} = -\left(\frac{(\mu - \mu d)}{Y_{Xv/gln}} + m_{gln}\right) \cdot Xv - d_{gln}[GLN] + feed2$$

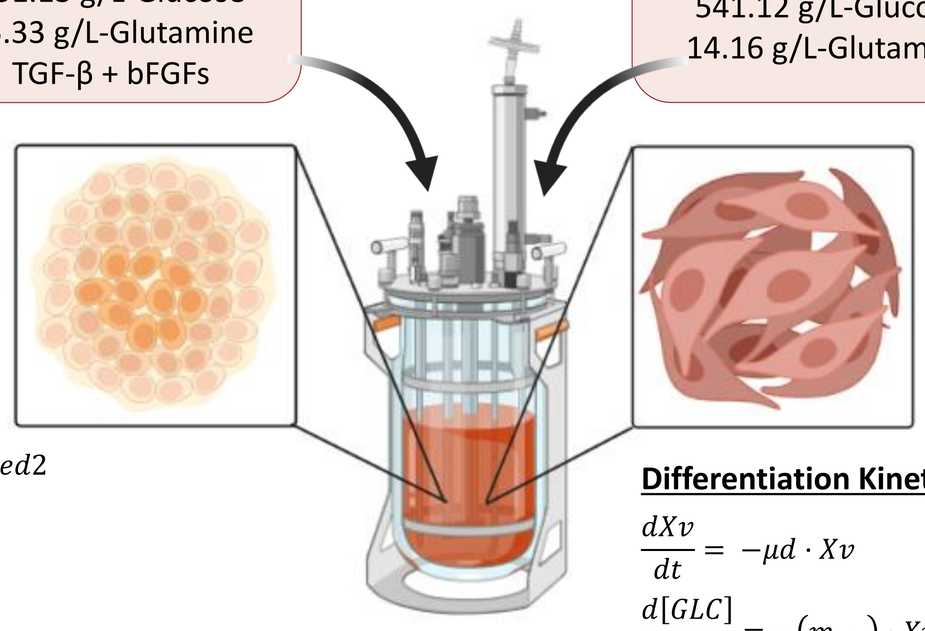
$$\frac{d[Lac]}{dt} = Y_{lac/glc}\left(\frac{(\mu - \mu d)}{Y_{Xv/glc}} + m_{glc}\right) \cdot Xv$$

$$\frac{d[Amm]}{dt} = Y_{amm/gln} \cdot \left(\frac{(\mu - \mu d)}{Y_{Xv/gln}}\right) \cdot Xv + d_{gln}[GLN]$$

$$feed2 = \left(\frac{(\mu - \mu d)}{Y_{Xv/gln}} + m_{gln}\right) \cdot Xv + d_{gln}[GLN]$$

6.08 days

PROLIFFERATION FEED
59.5 L
251.13 g/L-Glucose
25.33 g/L-Glutamine
TGF-β + bFGFs



5000L

6.25 days

DIFFERENTIATION FEED
72.5 L
541.12 g/L-Glucose
14.16 g/L-Glutamine

DIFFERENTIATION

Final values:

- [Glucose]→ 1 g/L
- [Glutamine]→ 0.115 g/L
- Scaffold → 1.675 kg

By-products:

- [Lactate] → 5.22 g/L
- [Ammonium] → 0.048 g/L

97% viability → $4 \cdot 10^6$ viable cells/mL

106 kg/bioreactor (x2) → 212 kg/bioprocess' run

70 % differentiation

Differentiation Kinetics Equations:

$$\frac{dXv}{dt} = -\mu d \cdot Xv$$

$$\frac{d[GLC]}{dt} = -(m_{glc}) \cdot Xv + feed3$$

$$\frac{d[GLN]}{dt} = -(m_{gln}) \cdot Xv - d_{gln}[GLN] + feed4$$

$$\frac{d[Lac]}{dt} = Y_{lac/glc} \cdot m_{glc} \cdot Xv$$

$$\frac{d[Amm]}{dt} = d_{gln}[GLN]$$

$$feed3 = m_{glc} \cdot Xv$$

$$feed4 = m_{gln} \cdot Xv - d_{gln}[GLN]$$

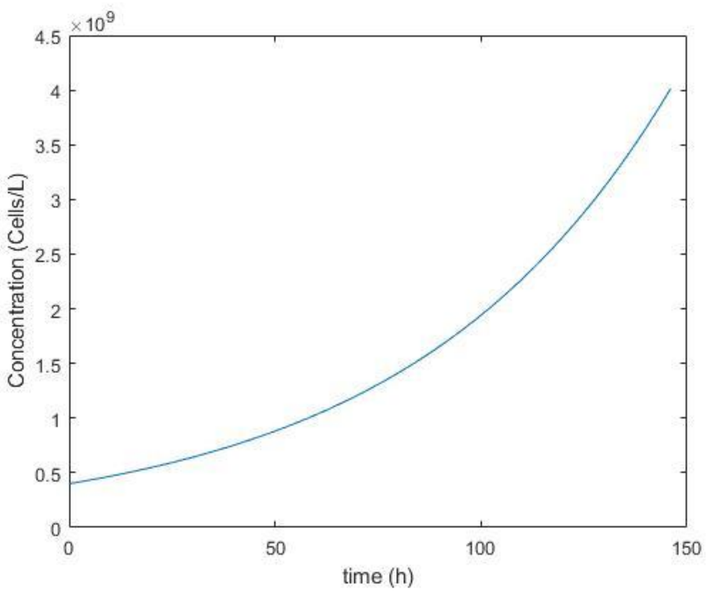


Figure 1. Representation of cell concentration in cells/L vs time in hours at the proliferation stage.

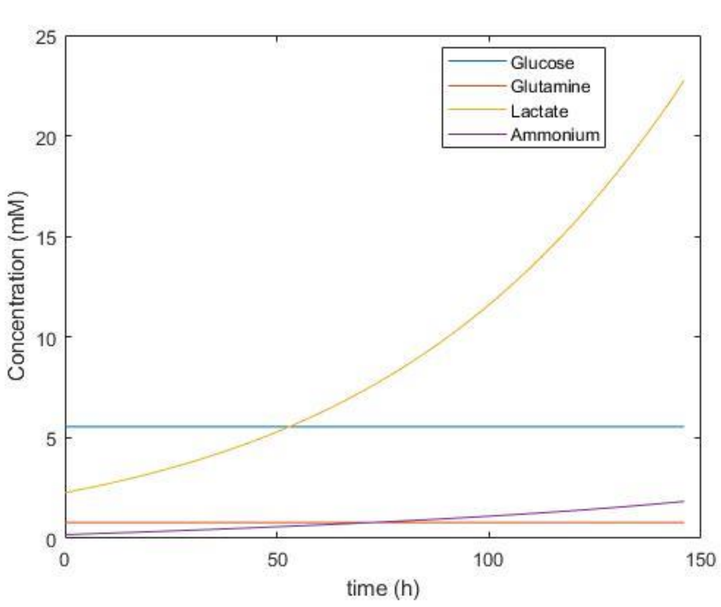


Figure 2. Evolution profile of glucose and glutamine, and of the by-products generated (lactate and ammonium) during the proliferation stage.

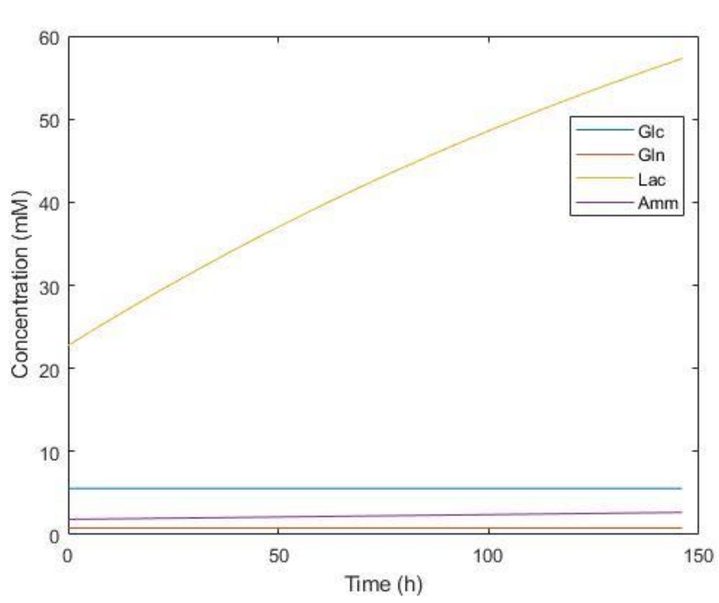


Figure 3. Evolution profile of glucose and glutamine, and of the by-products generated (lactate and ammonium) during the differentiation process.

CONCLUTIONS

In this work, we successfully carried out the simulation of a manufacturing process for the production of cell cultured meat. In summary, we started our process with a frozen vial of cells and achieved a total production of 212 kg of meat per bioprocess' run using two 5000L STR bioreactors with a process time of 12.33 days.

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