Design and Engineering of Trastuzumab Emtansine production plant

Part III: Upstream, Bioreactions and Fermentation of the antitumorigenic agent Ansamitocin P3.

Bachelor's Thesis– Biotechnology, 2018

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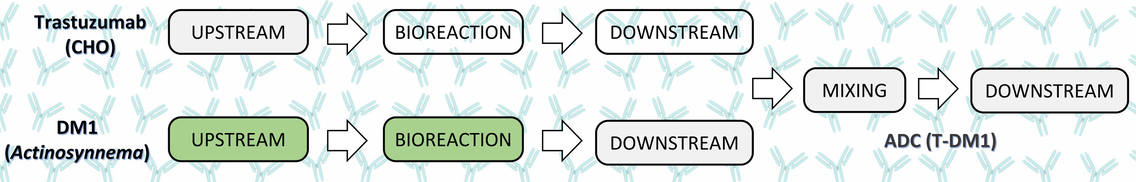
ABSTRACT

Breast cancer is the most common cancer among women following skin cancer. Specifically, HER2 overexpressing tumours reveal a particularly poor prognosis. For this reason, the aim of this project is to simulate an industrial plant for the production of the antibody drug conjugate Trastuzumab emtansine (T-DM1) which has resulted to be a good therapy for this type of breast cancer. T-DM1 is composed by a monoclonal antibody (Trastuzumab) produced in CHO DG44 cells and a cytotoxic drug (DM1) produced in *Actinosynnema pretiosum*. Therefore, two parallel processes have been designed to produce these two components followed by a mixing step. All simulations have been done using SuperPro Designer. This part, is focused on the upstream and bioreaction of the DM1 process with metabolic biosynthesis studies and fermentation simulation.



GANT CHART PROCESS

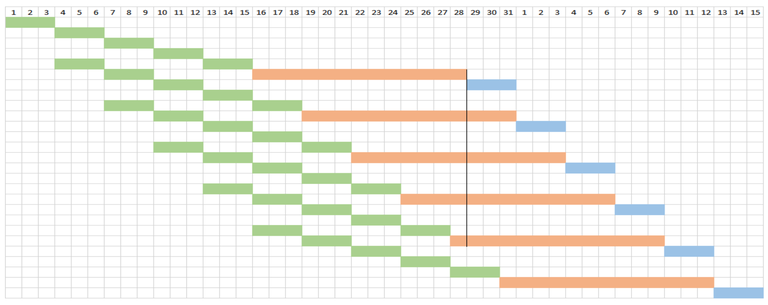


Figure 1: Gant chart process for Trastuzumab (limiting bioprocess).

- In order to optimize the process the downstream hardware has reduced the volume.
- Gant Chart development allows to design a semicontinue process in the downstream, starting new batches 3 days spaced.
- Fermentation seems to be the limiting stage as it lasts longer. 5 reactors run at the same time.
- Trastuzumab production is the slowest process. Its analysis involve DM1 production analysis.
- Both fermentations last the same (not complete processes).
- 105 batches can be done by year.
- Mixing part shared by both bioprocesses.

ANSAMITOCIN TYPES

-Ansamitocin variations depend on the residues

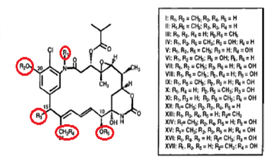


Figure 2: Ansamitocin variations depending on the residue. (Cassady et al., 2004)

-Maytansine variant.
-Antibiotic properties with effects in human organisms.
-It inhibits microtubules assembly.

Actinosynnema pretiosum

- *Actinosynnema* genre known for a "synnemata" structure - formation.
- Described in 1978 is an *Actinomycetales* type bacteria.
- Gram +, catalase positive, mesophilic and aerobic bacteria.
- Secondary metabolites produced as antibiotics.
- 8131572 bp linear genome. 2 gene clusters are found for ansamitocin P3 production, containing 51 ORFs. Clusters are spaced within 30 Kpb.
- Ribosomal subunits: 5s, 16s and 23s.
- Can be grown under submerged culture conditions.

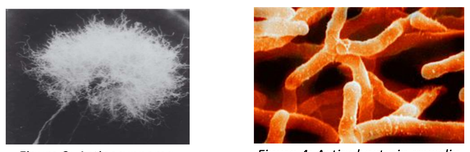


Figure 3: Actinosynnema synnemata (Labeda, 2015)

Figure 4: Actinobacteria myeclia (Wikipedia CC, Creative Commons)

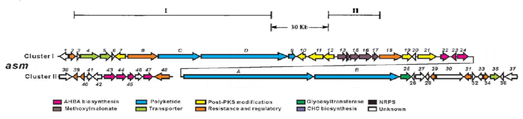


Figure 5: Ansamitocin P3 gene cluster in Actinosynnema Pretiosum (Kang, Shen and Bai, 2012)

ANSAMITOCIN BIOSYNTHESIS METABOLIC PATHWAY

1st): AHBA synthesis.

- AHBA synthesis, described in *Am. mediterranei* S699 *sare* (Rifamycin production)
- *asm23, 22, 24, 43, 44, 45 i 47* genes involved.
- PEP + C source + N source → AHBA percusor.
- Genes in clúster 1 mostly involved.

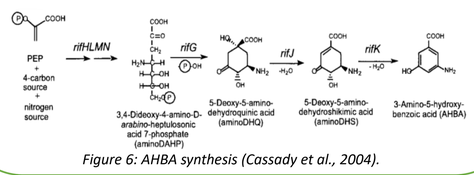


Figure 6: AHBA synthesis (Cassady et al., 2004).

2nd): Polyketide chain formation

- PKS (Polyketide synthase) adds different organic groups into the AHBA precursor.
- Complicate step (glycolate unit addition) involve other *asm* genes.
- PKS: Multienzymatic complex (1300 kDa) formed by 4 chains (*asm A, asmB, asm C* and *asmD*) with different activities involving ACP ligase, β-ketoacyl-ACP synthase, enoil reductase and others.
- Genes in clúster 2 involved.

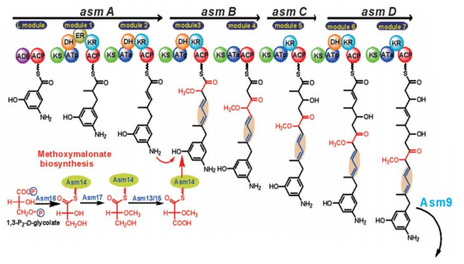


Figure 7: Polyketide chain formation, mediated by PKS (Kang, Shen and Bai, 2012)

3rd): Post polyketide modifications

- *asm 9* catalyzes the polyketide cyclization.
- They give rise to the final product ansamitocin P3.

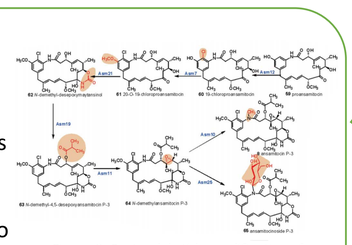


Figure 8: Post polyketide modifications for ansamitocin P3 production (Kang, Shen and Bai, 2012)

EXONENTIAL FED-BATCH SIMULATION

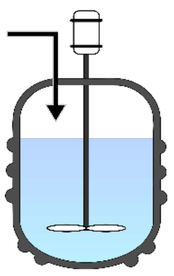


Figure 9: Fed-batch bioreactor scheme

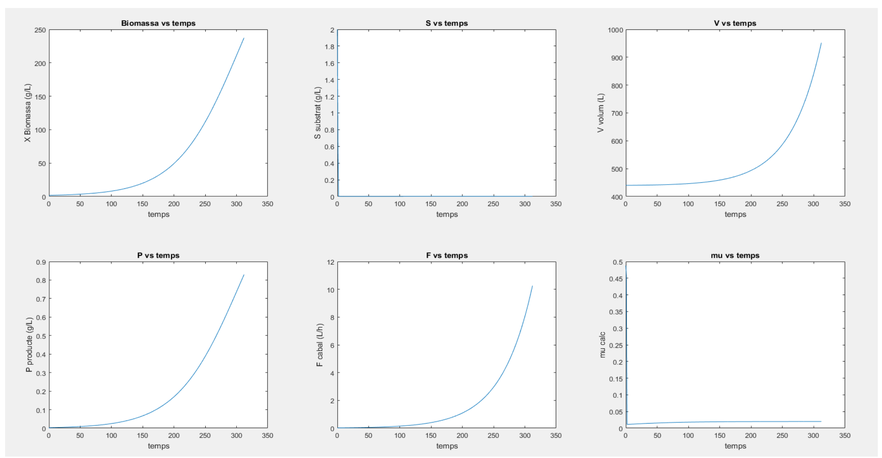
- Biomass balance → $\frac{dX}{dt} = \frac{\mu_{max} S}{K_S + S} X - \frac{F}{V} X$
- Substrate balance → $\frac{dS}{dt} = 0 = \frac{F}{V}(S_0 - S) - \frac{\mu X}{Y_{X/S}}$
- Product balance → $\frac{dP}{dt} = r_P X - \frac{F}{V} P$
- Volume change → $F(t) = \frac{dV}{dt} = \frac{\mu X_0 V_0 e^{\mu(t-t_0)}}{Y_{X/S}(S_0 - S)}$

PARAMETERS

-T = 28ºC
-pH = 6,5
-Agitation: 170 rpm
-Aeration : 1 vvm
-Dissolved oxygen: 30 %

MEDIUM

Defined medium :
- Dextrin
- Porflo
- CSP
- $CaCO_3$
- K_2HPO_4
- Isobutanol
- $CoCl_2 \cdot 6H_2O$
- SAG 471
- $FeSO_4 \cdot 7H_2O$
- Maltose



Graphic 1: Simulation variables evolution in time (Results).

- Higher final product concentrations than normal fed-batch culture (0,3 g/L)
- Specific growth rate control μ = 0,02 h^{-1}.
- Simulation can differ from the real situation: Transport phenomena limitations, metabolic variations…

SUPERPRO STOICHIOMETRIC BIOREACTION

- Stoichiometric reaction is needed for the simulation with SuperPro Designer®.
- With element balances and bibliographic data the reaction can be defined.
- Stoichiometric molar balance matrix is generated and solved.
- C source → Glucose N source → Ammonia Cl source → Sodium Chloride

$$C_6H_{12}O_3 + aNH_4^+ + bO_2 + cNaCl \rightarrow dC_{32}H_{43}ClN_2O_9 + eCO_2 + gC_{4,5}H_9NO_4$$

Element balances generated

C: 6 = 32d + e + 4,5g **H**: 12 + 4a = 43d + 2f + 9g

O: 6 + 2b = 9d + 2e + f + 4g **N**: a = 2d + g **Cl**: c = d

$g = Y_{X/S} = 0{,}51 \frac{mol\ X}{mol\ S}$ $d = Y_{P/S} = 3{,}97 \cdot 10^{-4} \frac{mol\ P}{mol\ S}$

Bibliographic data

Matrix formation and solution

$$\begin{pmatrix} 0 & 0 & 0 & 32 & 1 & 0 & 4{,}5 & 6 \\ -4 & 0 & 0 & 43 & 0 & 2 & 9 & 12 \\ 0 & -2 & 0 & 9 & 2 & 1 & 4 & 6 \\ -1 & 0 & 0 & 2 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0{,}5 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 3{,}97 \cdot 10^{-4} \\ 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \end{pmatrix}$$

$$\mathbf{C_6H_{12}O_3 + 0{,}51NH_4^+ + 4{,}07O_2 + 4 \cdot 10^{-4}NaCl \rightarrow 4 \cdot 10^{-4}C_{32}H_{43}ClN_2O_9 + 3{,}69CO_2 + 0{,}51C_{4,5}H_9NO_4}$$

Final stoichiometric reaction

BIOREACTOR AND CONTROL

Sartorius® stainless steel bioreactor (600 L) used in this process. 480 L (useful volume).
Característics:

- BioPat® controls: SIP (Sterilization In Place), agitation system, temperature control system, exhaust cooler/heater, pressure control valve, T control system, pH control system, gas inlet line, sample analysis system.
- DCU: Intelligent control unit.
- Biostat®: Support portfolio unit

13,68 kg of DM1 produced by year.

Figure 10: Sartorius® stainless steel bioreactor

Important to control the biomass evolution in order to act against external disturbances (Regulation control)

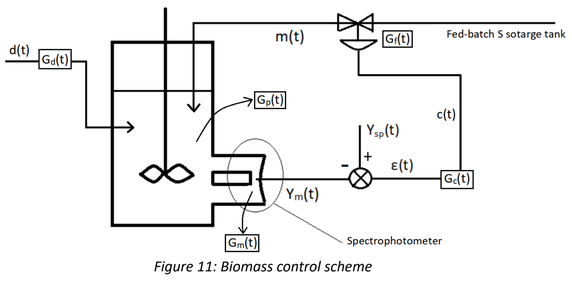


Figure 11: Biomass control scheme

SCALE-UP AND FERMENTATION

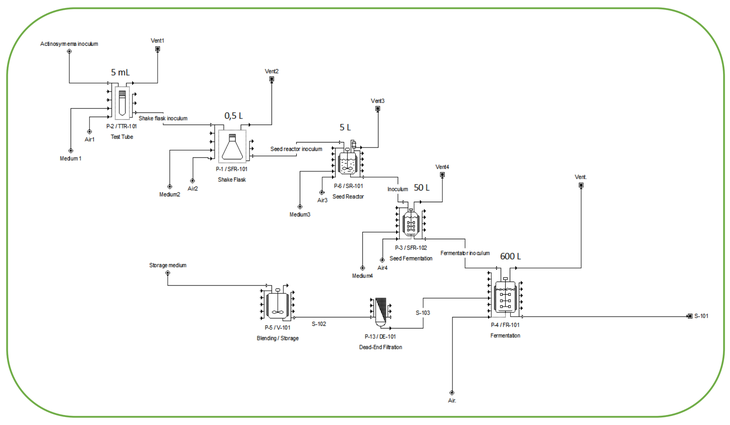


Figure 12: Upstream, scale up and fermentator (Ansamitocin process)

- Scale up process is developed in order to achieve the correct biomass concentration in the final fermentator.
- The inoculums should be 10% of the next reactor volume.
- Medium is sterilized before entering any tank.

Final biomass concentration: 105 g/L

Final ansamitocin concentration: 0,36 g/L

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