Complete model - iGEM INSA-UPS France 2017

1 Growth

Vibrio cholerae, *Vibrio harveyi* and *Pichia pastoris* growth depends on their growth rate and their concentration. To account for the lag phase, we assumed no growth during a first period.

1.1 *Vibrio cholerae*

$$V_{growth,Vc} = \mu_{Vc} \cdot [Vc]_W$$

$$\begin{cases} t < t_{l,Vc} \ ; \ \mu_{Vc} = 0 \ h^{-1} \\ t \geq t_{l,Vc} \ ; \ \mu_{Vc} = \mu_{MAX,Vc} \end{cases}$$

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- $[Vc]_W$: *Vibrio cholerae* concentration in water (cell/L)
- μ_{Vc}: *Vibrio cholerae* growth rate (s^{-1})
- $t_{l,Vc}$: *Vibrio cholerae* lag time (s)

1.2 *Vibrio harveyi*

$$V_{growth,Vh} = \mu_{Vh} \cdot [Vh]_D$$

$$\begin{cases} t < t_{l,Vh} \ ; \ \mu_{Vh} = 0 \ h^{-1} \\ t \geq t_{l,Vh}; \ \mu_{Vh} = \mu_{MAX,Vh} \end{cases}$$

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- $[Vh]_D$: *Vibrio harveyi* concentration in the device (cell/L)
- μ_{Vh}: *Vibrio harveyi* growth rate (s^{-1})
- $t_{l,Vh}$: *Vibrio harveyi* lag time (s)

1.3 *Pichia pastoris*

$$V_{growth,Pp} = \mu_{Pp} \cdot [Pp]_D$$

$$\begin{cases} t < t_{l,Pp} \ ; \ \mu_{Pp} = 0 \ h^{-1} \\ t \geq t_{l,Pp} \ ; \ \mu_{Pp} = \mu_{MAX,Pp} \end{cases}$$

.

- $[Pp]_D$: *Pichia pastoris* concentration in the device (cell/L)
- μ_{Pp}: *Pichia pastoris* growth rate (s^{-1})
- $t_{l,Pp}$: *Pichia pastoris* lag time (s)

2 Death

Vibrio cholerae, *Vibrio harveyi* and *Pichia pastoris* death depends on their death rate with the antimicrobial peptides (AMP) and their concentration. This process is modeled by a Michaelian model ruled by the cells IC50 with respect to AMP and on the antimicrobial peptides concentration in the compartment.

2.1 *Vibrio cholerae*

$$V_{death,Vc} = \frac{[AMP]_W}{[AMP]_W + IC50_{Vc}} \cdot k_{kill,Vc} \cdot [Vc]_W$$

- $[AMP]_W$: antimicrobial peptides concentration in water (mol/L)
- $k_{kill,Vc}$: *Vibrio cholerae* death rate with peptides (s^{-1})
- $IC50_{Vc}$: IC50 value of the antimicrobial peptides, for *Vibrio cholerae* (mol/L)

2.2 *Vibrio harveyi*

$$V_{death,Vh} = \frac{[AMP]_D}{[AMP]_D + IC50_{Vh}} \cdot k_{kill,Vh} \cdot [Vh]_D$$

- $[AMP]_D$: antimicrobial peptides concentration in the device (mol/L)
- $k_{kill,Vh}$: *Vibrio harveyi* death rate with peptides (s^{-1})
- $IC50_{Vh}$: IC50 value of the antimicrobial peptides, for *Vibrio harveyi* (mol/L)

2.3 *Pichia pastoris*

$$V_{death,Pp} = \frac{[AMP]_D}{[AMP]_D + IC50_{Pp}} \cdot k_{kill,Pp} \cdot [Pp]_D$$

- $k_{kill,Pp}$: *Pichia pastoris* death rate with peptides (s^{-1})
- $IC50_{Pp}$: IC50 value of the antimicrobial peptides, for *Pichia pastoris* (mol/L)

3 CAI-1 transfer

Initial CAI-1 concentration is high in the presence of *Vibrio cholerae*, thus there is no need to calculate the kinetics of CAI-1 production. CAI-1, initially in water, can freely diffuse through the membrane. A passive diffusion model is used for this process.

$$V_{diff,CAI\text{-}1,W\rightarrow D} = K.([CAI\text{-}1]_W - [CAI\text{-}1]_D)$$

- $[CAI\text{-}1]_W$: CAI-1 concentration in water (mol/L)
- $[CAI\text{-}1]_D$: CAI-1 concentration in the device (mol/L)
- K: transfer coefficient through the membrane (s^{-1})

4 CqsS - CAI-1 complexation

4.1 CqsS* concentration

The CqsS* concentration is obtained approximating the number of protein per cell, using the *Vibrio harveyi* concentration and the Avogadro number.

$$[\text{CqsS*}]_D = (\text{Number of CqsS*}/cell).\frac{[Vh]_D}{N_A}$$

- $[\text{CqsS*}]_D$: concentration of the CqsS* protein in the device (mol/L)
- Number of CqsS*/cell: number of CqsS* proteins per *Vibrio harveyi* cell
- N_A: Avogadro number (mol^{-1})

4.2 Complexation

$$\text{CAI-1} + \text{CqsS*} \rightleftharpoons \text{CqsS*-CAI-1}$$

Assuming kinetics of CqsS*-CAI-1 complexation is fast compared to the rest of the system, we assumed that the free and complexed forms are at equilibrum.

$$V_{complexation} = V_{dissociation}$$

$$k_1.[CAI\text{-}1]_D.[\text{CqsS*}]_D = k_2.[CqsS^*\text{-}CAI\text{-}1]_D$$

$$[CqsS^*\text{-}CAI\text{-}1]_D = \frac{[CAI\text{-}1]_D.[\text{CqsS*}]_D}{K_{eq,CqsS^*\text{-CAI-1}}}$$

$$\text{With } K_{eq,CqsS^*\text{-CAI-1}} = k_2/k_1$$

- k_1: velocity constant of dissociation ($L.mol^{-1}.s^{-1}$)
- k_2: velocity constant of complexation (s^{-1})
- $K_{eq,CqsS^*\text{-CAI-1}}$: equilibrum constant of the CqsS*-CAI-1 complexation (mol/L)

5 ALS production

The production of the ALS enzyme from the ALS gene is divided into two steps: transcription (after activation) and translation.

$$ALS_{DNA,inactivated} \longrightarrow \text{ALS}_{\text{DNA}} \longrightarrow \text{ALS}_{\text{RNA}} \longrightarrow \text{ALS}_{\text{protein}}$$

5.1 Activation

The ALS gene is activated. This is modeled using a Michaelian formalism depending on its activator (CqsS*-CAI-1 complex) concentration. The promoter strength is also taken into account.

$$ALS_{DNA/cell} = ALS_{DNA,0/cell} \cdot \frac{[CqsS^*\text{-}CAI\text{-}1]_D}{K_{a,CqsS^*\text{-CAI-1}} + [CqsS^*\text{-}CAI\text{-}1]_D} . k_{p,ALS}$$

- $ALS_{DNA,0/cell}$: total number of ALS DNA per cell
- $ALS_{DNA/cell}$: number of activated ALS DNA per cell
- $K_{a,CqsS^*\text{-CAI-1}}$: activation constant of the CqsS*-CAI-1 complex (mol/L)
- $k_{p,ALS}$: ALS promoter influence

5.2 Transcription

The ALS gene is transcribed into mRNA. The ALS transcription depends on the transcription rate of the strain and the length of the ALS gene. The Avogadro number is used to express the transcription velocity in molar concentration in one cell per time unit.

$$V_{transcription,ALS/cell} = \frac{ALS_{DNA/cell}.k_{transcript,Vh}.(\text{RNA polymerase/gene})}{\text{DNA length}.N_A.\mathcal{V}_{intracell,Vh}}$$

- ALS_{DNA}: number of ALS gene per cell
- $k_{transcript,Vh}$: *Vibrio harveyi* transcription rate (nucleotides/s)
- RNA polymerase/gene: number of RNA polymerase per gene
- DNA length (ALS): number of nucleotides on the ALS gene
- $\mathcal{V}_{intracell,Vh}$: volume of a bacterial cell (L)

5.3 Translation

The ALS mRNA is translated into protein. The ALS translation depends on the translation rate of the strain, the mRNA length and the quantity of mRNA. The translation velocity is expressed in molar concentration in one cell per time unit.

$$V_{translation,ALS/cell} = \frac{[\text{ALS mRNA}]_{Vh}.k_{translation,Vh}.(\text{Ribosomes/RNA})}{\text{RNA length}}$$

- $k_{translation,Vh}$: *Vibrio harveyi* translation rate (nucleotides/s)
- Ribosomes/RNA: number of ribosomes per mRNA
- RNA length (ALS): number of nucleotides on the ALS mRNA
- $[\text{ALS mRNA}]_{Vh}$: ALS mRNA concentration in one *Vibrio harveyi* cell (mol/L)

5.4 Degradation

Some of the ALS enzymes and mRNA are degraded. A degradation constant is used to model the degradation velocity.

$$V_{degradation,ALS} = K_{deg,ALS}.[ALS]_{Vh}$$

- $K_{deg,ALS}$: ALS degradation constant (s^{-1})

$$V_{degradation,ALSRNA} = K_{deg,ALSRNA}.[\text{ALS mRNA}]_{Vh}$$

- $K_{deg,ALSRNA}$: ALS mRNA degradation constant (s^{-1})

6 Diacetyl production

Diacetyl is produced by *Vibrio harveyi* through the reaction catalyzed by ALS and is modeled assuming a Michaelis-Menten kinetics.

$$V_{prod,dac} = k_{cat,ALS}.[ALS]_{Vh}.\frac{[S]_D}{K_M + [S]_D}.\mathcal{V}_{intracell,Vh}.[Vh]_D$$

- $[ALS]_{Vh}$: ALS enzyme concentration in one *Vibrio harveyi* cell
- $k_{cat,ALS}$: catalytic constant of the ALS enzyme (s^{-1})
- $[S]_D$: substrate concentration (mol/L)
- K_M: Michaelis constant of the ALS enzyme (mol/L)

7 Diacetyl transfer

Diacetyl produced in the device can freely diffuse through the membrane. This process is taken into account through a passive diffusion model.

$$V_{diff,dac,W\rightarrow D} = K.([dac]_W - [dac]_D)$$

- $[dac]_D$: diacetyl concentration in the device (mol/L)
- $[dac]_W$: diacetyl concentration in water (mol/L)

8 Diacetyl - Odr10 complexation

8.1 Odr10 concentration

The Odr10 concentration is obtained approximating the number of protein per cell, using the *Pichia pastoris* concentration and the Avogadro number.

$$[Odr10]_D = (\text{Number of Odr10}/cell).\frac{[Pp]_D}{N_A}$$

- $[Odr10]_D$: concentration of the Odr10 protein in the device (mol/L)
- Number of Odr10/cell: approximative number of Odr10 proteins per *Pichia pastoris* cell

8.2 Complexation

$$\text{dac} + \text{Odr10} \rightleftharpoons \text{Odr10-dac}$$

The kinetics of complexation is described assuming a first order system. The Odr10-dac complex is assumed to be at the equilibrum.

$$V_{complexation} = V_{dissociation}$$

$$k_1.[dac]_D.[Odr10]_D = k_2.[\text{Odr10-dac}]_D$$

$$[Odr10\text{-dac}]_D = \frac{[dac]_D.[Odr10]_D}{K_{eq,Odr10\text{-dac}}}$$

$$\text{With } K_{eq,Odr10\text{-dac}} = k_2/k_1$$

- k_1: velocity constant of dissociation ($L.mol^{-1}.s^{-1}$)
- k_2: velocity constant of complexation (s^{-1})
- $K_{eq,Odr10\text{-dac}}$: equilibrum constant of the Odr10-dac complexation (mol/L)

9 Antimicrobial peptide (AMP) production

The production of the antimicrobial peptides (AMP) from the AMP gene is divided into two steps: transcription (after activation) and translation.

$$\text{AMP}_{\text{DNA,inactivated}} \longrightarrow \text{AMP}_{\text{DNA}} \longrightarrow \text{AMP}_{\text{RNA}} \longrightarrow \text{AMP}_{\text{peptide}}$$

9.1 Activation

The AMP gene is activated. This is modeled using a Michaelian formalism depending on the Odr10-dac complex concentration. The promoter strength is also taken into account.

$$AMP_{DNA/cell} = AMP_{DNA,0/cell} \cdot \frac{[\text{Odr10-}dac]_D}{K_{a,\text{Odr10-dac}} + [\text{Odr10-}dac]_D} . k_{p,AMP}$$

- $AMP_{DNA,0}$: total number of AMP DNA per cell
- AMP_{DNA}: number of activated AMP DNA per cell
- $K_{a,\text{Odr10-dac}}$: activation constant of the Odr10-dac complex (mol/L)
- $k_{p,AMP}$: AMP promoter influence

9.2 Transcription

The AMP DNA is transcribed into mRNA. The AMP transcription depends on the transcription rate of the strain and the length of the AMP gene. The RNA polymerase density is also taken into account. The Avogadro number is used to express the transcription velocity in molar concentration per time unit.

$$V_{transcription,AMP/cell} = \frac{AMP_{DNA/cell}.k_{transcript,Pp}.(\text{RNA polymerase/gene})}{\text{DNA length}.N_A.\mathcal{V}_{intracell,Pp}}$$

- $k_{transcript,Pp}$: *Pichia pastoris* transcription rate (nucleotides/s)
- RNA polymerase/gene: number of RNA polymerase per gene
- DNA length (AMP): number of nucleotides on the AMP gene
- $\mathcal{V}_{intracell,Pp}$: volume of a yeast cell (L)

9.3 Translation

The AMP mRNA is translated into protein. The AMP translation depends on the translation velocity of the strain, the mRNA length and the quantity of mRNA. *Pichia pastoris* concentration is used to express the peptides concentration in the device.

$$V_{translation,AMP} = \frac{[\text{AMP mRNA}]_{Vh}.k_{translation,Pp}.(\text{Ribosomes/RNA}).\mathcal{V}_{intracell,Pp}.[Pp]_D}{\text{RNA length}}$$

- $k_{translation,Pp}$: *Pichia pastoris* translation rate (nucleotides/s)
- Ribosomes/RNA: number of ribosomes per mRNA
- RNA length (AMP): number of nucleotides on the AMP mRNA
- $[\text{AMP mRNA}]_{Pp}$: AMP mRNA concentration in one *Pichia pastoris* cell (mol/L)

9.4 Degradation

Some of the AMP and mRNA are degraded. A degradation constant is used to model the degradation velocity.

$$V_{degradation,AMP} = K_{deg,AMP}.[AMP]_D$$

- $K_{deg,AMP}$: AMP degradation constant (s^{-1})

$$V_{degradation,AMPRNA} = K_{deg,AMPRNA}.[\text{AMP mRNA}]_{Pp}$$

- $K_{deg,AMPRNA}$: AMP RNA degradation constant (s^{-1})
- $[\text{AMP mRNA}]_{Pp}$: AMP RNA concentration (mol/L)

9.5 AMP transfer

The antimicrobial peptides (AMP), initially in the device can freely diffuse through the membrane. A passive diffusion model is used for this process.

$$V_{diff,AMP,W\rightarrow D} = K([AMP_{peptide}]_W - [AMP_{peptide}]_D)$$

- $[AMP]_W$: AMP protein concentration in water (mol/L)

System of ODEs

The complete set of ODEs is detailed here:

$$\frac{d[Vc]_W}{dt} = V_{growth,Vc} - V_{death,Vc} \tag{1}$$

$$\frac{d[Vh]_D}{dt} = V_{growth,Vh} - V_{death,Vh} \tag{2}$$

$$\frac{d[Pp]_D}{dt} = V_{growth,Pp} - V_{death,Pp} \tag{3}$$

$$\frac{d[CAI\text{-}1]_D}{dt} = \frac{V_{diff,CAI\text{-}1,W\to D}}{\mathcal{V}_D} \tag{4}$$

$$\frac{d[CAI\text{-}1]_W}{dt} = -V_{diff,CAI\text{-}1,W\to D} \tag{5}$$

$$\frac{d[\text{ALS mRNA}]_{Vh}}{dt} = V_{transcription,ALS} - V_{degradation,ALSRNA} \tag{6}$$

$$\frac{d[ALS]_{Vh}}{dt} = V_{translation,ALS} - V_{degradation,ALSenzyme} \tag{7}$$

$$\frac{d[dac]_D}{dt} = V_{prod,dac} + \frac{V_{diff,dac,W\to D}}{\mathcal{V}_D} \tag{8}$$

$$\frac{d[dac]_W}{dt} = -V_{diff,dac,W\to D} \tag{9}$$

$$\frac{d[\text{AMP mRNA}]_{Pp}}{dt} = V_{transcript,AMP} - V_{degradation,AMPRNA} \tag{10}$$

$$\frac{d[AMP]_D}{dt} = V_{translation,AMP} - V_{degradation,AMP} + \frac{V_{diff,AMP,W\to D}}{\mathcal{V}_D} \tag{11}$$

$$\frac{d[AMP]_W}{dt} = -V_{diff,AMP,W\to D} \tag{12}$$

Model parameters and initial concentration

Microorganisms properties

- $\mu_{MAX,Vc}$: *Vibrio cholerae* maximum growth rate (s^{-1})
- $\mu_{MAX,Vh}$: *Vibrio harveyi* maximum growth rate (s^{-1})
- $\mu_{MAX,Pp}$: *Pichia pastoris* maximum growth rate (s^{-1})
- $t_{l,Vc}$: *Vibrio cholerae* lag time (s)
- $t_{l,Vh}$: *Vibrio harveyi* lag time (s)
- $t_{l,Pp}$: *Pichia pastoris* lag time (s)
- $IC50_{Vh}$: IC50 value of the antimicrobial peptides, for *Vibrio harveyi* (mol/L)
- $IC50_{Vc}$: IC50 value of the antimicrobial peptides, for *Vibrio cholerae* (mol/L)
- $IC50_{Pp}$: IC50 value of the antimicrobial peptides, for *Pichia pastoris* (mol/L)
- $k_{kill,Vc}$: *Vibrio cholerae* death rate with peptides (s^{-1})
- $k_{kill,Vh}$: *Vibrio harveyi* death rate with peptides (s^{-1})
- $k_{kill,Pp}$: *Pichia pastoris* death rate with peptides (s^{-1})
- $\mathcal{V}_{intracell}$: volume of a cell (respectively of a bacteria and a yeast) (L)

Technical parameters

- K: transfer coefficient through the membrane (s^{-1})
- $\mathcal{V}_B$: device volume (L), considering a water volume of 1L

Molecular and genetic properties

- $ALS_{DNA,0/cell}$: total number of ALS DNA *Vibrio harveyi* per cell
- $AMP_{DNA,0/cell}$: total number of AMP DNA per cell
- $k_{transcription,Vh}$: *Vibrio harveyi* transcription rate (nucleotides/s)
- $k_{translation,Vh}$: *Vibrio harveyi* translation rate (nucleotides/s)
- $k_{p,ALS}$: ALS promoter influence
- DNA length (ALS): number of nucleotides on the ALS gene

- Number of CqsS*/cell: approximative number of CqsS* proteins per *Vibrio harveyi* cell
- RNA length (ALS): number of translated nucleotides on the ALS mRNA
- Number of Odr10/cell: approximative number of Odr10 proteins per *Pichia pastoris* cell
- $k_{p,AMP}$: AMP promoter influence
- $k_{transcription,Pp}$: *Pichia pastoris* transcription rate (nucleotides/h)
- $k_{translation,Pp}$: *Pichia pastoris* translation rate (nucleotides/h)
- DNA length (AMP): number of nucleotides on the AMP gene
- RNA length (AMP): number of nucleotides on the AMP mRNA
- RNA polymerase/gene: number of RNA polymerase per gene, respectively for a bacteria and a yeast
- Ribosomes/RNA: number of ribosomes per mRNA, respectively for a bacteria and a yeast

Biochemical properties

- $K_{eq,CqsS^*\text{-CAI-1}}$: equilibrum constant of the CqsS*-CAI-1 complexation (mol/L)
- $K_{a,CqsS^*\text{-CAI-1}}$: activation constant of the CqsS*-CAI-1 complex (mol/L)
- $K_{deg,ALS}$: ALS degradation constant (s^{-1})
- $K_{deg,ALSRNA}$: ALS RNA degradation constant (s^{-1})
- $K_{eq,Odr10\text{-dac}}$: equilibrum constant of the Odr10-dac complexation (mol/L)
- $k_{cat,ALS}$: catalytic constant of the ALS enzyme (s^{-1})
- $K_{M,ALS}$: Michaelis constant of the ALS enzyme (mol/L)
- $K_{a,\text{Odr10-dac}}$: activation constant of the Odr10-dac complex (mol/L)
- $K_{deg,AMP}$: AMP degradation constant (s^{-1})
- $K_{deg,AMPRNA}$: AMP RNA degradation constant (s^{-1})

Initial concentration

- $[Vc]_{0,W}$: *Vibrio cholerae* initial concentration (cell/L)
- $[Vh]_{0,D}$: *Vibrio harveyi* initial concentration (cell/L)
- $[Pp]_{0,D}$: *Pichia pastoris* initial concentration (cell/L)
- $[S]_0$: initial substrate concentration (mol/L)
- $[\text{CAI-1}]_{W,0}$: CAI-1 initial concentration in water (mol/L)