

### 3.3. Solving r-deFBA problems

#### 3.3.1. Implementation of Mixed Boolean Constraints

The mixed boolean constraints need to be enforced on the shifted time grid as well. That means, an inequality constraint of the form

$$\mathbf{0} \leq \mathbf{g}_{\leq}(t, \mathbf{y}(t), \mathbf{u}(t), \bar{\mathbf{x}}(t))$$

is discretized to

$$\mathbf{0} = \mathbf{g}\left(t_{m+1/2}^s, \tilde{\mathbf{y}}_{m+1}^{(1)}, \mathbf{u}_{m+1/2}, \tilde{\mathbf{x}}_{m+1}^{(1)}\right), \quad (m = 0, 1, \dots, N-1). \quad (3.1)$$

For the given linear structure of the mixed boolean constraints (3.2g), this reads

$$\mathbf{H}_{\mathbb{B},y} \cdot \tilde{\mathbf{y}}_{m+1}^{(1)} + \mathbf{H}_{\mathbb{B},u} \cdot \mathbf{u}_{m+1/2} + \mathbf{H}_{\mathbb{B},\bar{x}} \cdot \tilde{\mathbf{x}}_{m+1}^{(1)} = \mathbf{H}_{\mathbb{B},y} \cdot \frac{y_m + y_{m+1}}{2} + \mathbf{H}_{\mathbb{B},u} \cdot \mathbf{u}_{m+1/2} + \mathbf{H}_{\mathbb{B},\bar{x}} \cdot \frac{\bar{x}_m + \bar{x}_{m+1}}{2} \leq h_{\mathbb{B}}$$

and can, once again, be restated as

$$\begin{aligned} & \frac{1}{2} \cdot \begin{pmatrix} \mathbf{H}_{\mathbb{B},y} & \mathbf{H}_{\mathbb{B},y} & \mathbf{0} & \cdots & \mathbf{0} \\ \mathbf{0} & \mathbf{H}_{\mathbb{B},y} & \mathbf{H}_{\mathbb{B},y} & \cdots & \mathbf{0} \\ \vdots & & \ddots & \ddots & \vdots \\ \mathbf{0} & \cdots & \cdots & \mathbf{H}_{\mathbb{B},y} & \mathbf{H}_{\mathbb{B},y} \end{pmatrix} \cdot \begin{pmatrix} y_0 \\ y_1 \\ \vdots \\ y_N \end{pmatrix} + \begin{pmatrix} \mathbf{H}_{\mathbb{B},u} & \mathbf{0} & \cdots & \mathbf{0} \\ \mathbf{0} & \mathbf{H}_{\mathbb{B},u} & & \mathbf{0} \\ \vdots & & \ddots & \vdots \\ \mathbf{0} & \cdots & \cdots & \mathbf{H}_{\mathbb{B},u} \end{pmatrix} \cdot \begin{pmatrix} u_{1/2} \\ u_{1+1/2} \\ \vdots \\ u_{(N-1)+1/2} \end{pmatrix} + \\ & \frac{1}{2} \cdot \begin{pmatrix} \mathbf{H}_{\mathbb{B},\bar{x}} & \mathbf{H}_{\mathbb{B},\bar{x}} & \mathbf{0} & \cdots & \mathbf{0} \\ \mathbf{0} & \mathbf{H}_{\mathbb{B},\bar{x}} & \mathbf{H}_{\mathbb{B},\bar{x}} & \cdots & \mathbf{0} \\ \vdots & & \ddots & \ddots & \vdots \\ \mathbf{0} & \cdots & \cdots & \mathbf{H}_{\mathbb{B},\bar{x}} & \mathbf{H}_{\mathbb{B},\bar{x}} \end{pmatrix} \cdot \begin{pmatrix} \bar{x}_0 \\ \bar{x}_1 \\ \vdots \\ \bar{x}_N \end{pmatrix} \leq \begin{pmatrix} h_{\mathbb{B}} \\ h_{\mathbb{B}} \\ \vdots \\ h_{\mathbb{B}} \end{pmatrix} \end{aligned}$$

or in Kronecker form

$$\begin{aligned} & \underbrace{\frac{1}{2} \cdot \begin{pmatrix} 1 & 1 & 0 & \cdots & 0 \\ 0 & 1 & 1 & \cdots & 0 \\ \vdots & & \ddots & \ddots & \vdots \\ 0 & \cdots & \cdots & 1 & 1 \end{pmatrix}}_{\mathfrak{A}_{y,\text{bmix}}} \otimes \mathbf{H}_{\mathbb{B},y} \cdot \underbrace{\begin{pmatrix} y_0 \\ y_1 \\ \vdots \\ y_N \end{pmatrix}}_{\mathfrak{y}} + \underbrace{\mathbf{I}_N \otimes \mathbf{H}_{\mathbb{B},u}}_{\mathfrak{A}_{u,\text{bmix}}} \cdot \underbrace{\begin{pmatrix} u_{1/2} \\ u_{1+1/2} \\ \vdots \\ u_{(N-1)+1/2} \end{pmatrix}}_{\mathfrak{u}} + \\ & \underbrace{\frac{1}{2} \cdot \begin{pmatrix} 1 & 1 & 0 & \cdots & 0 \\ 0 & 1 & 1 & \cdots & 0 \\ \vdots & & \ddots & \ddots & \vdots \\ 0 & \cdots & \cdots & 1 & 1 \end{pmatrix}}_{\mathfrak{A}_{\bar{x},\text{bmix}}} \otimes \mathbf{H}_{\mathbb{B},\bar{x}} \cdot \underbrace{\begin{pmatrix} \bar{x}_0 \\ \bar{x}_1 \\ \vdots \\ \bar{x}_N \end{pmatrix}}_{\mathfrak{x}} \leq \underbrace{\begin{pmatrix} h_{\mathbb{B}} \\ h_{\mathbb{B}} \\ \vdots \\ h_{\mathbb{B}} \end{pmatrix}}_{\mathfrak{b}_{\text{bmix}}}. \end{aligned}$$

For the final formulation of the LP, we collect all the information like

$$\begin{aligned}
\mathbf{f} &= \begin{pmatrix} \mathbf{f}_y^\top & \mathbf{0}^\top \end{pmatrix}^\top, \\
\mathcal{A}_\leq &= (\mathcal{A}_{y,\text{mix}}, \mathcal{A}_{u,\text{mix}}), \\
\mathbf{b}_\leq &= \mathbf{b}_{\text{mix}} \\
\overline{\mathcal{A}}_\leq &= (\mathcal{A}_{y,\text{bmix}}, \mathcal{A}_{u,\text{bmix}}, \overline{\mathcal{A}}_{\bar{x},\text{bmix}}) \\
\overline{\mathbf{b}}_\leq &= \mathbf{b}_{\text{bmix}} \\
\mathcal{A}_= &= \begin{pmatrix} \mathcal{A}_{y,\text{dyn}} & \mathcal{A}_{u,\text{dyn}} \\ \mathcal{A}_{y,\text{qssa}} & \mathcal{A}_{u,\text{qssa}} \\ \mathcal{A}_{y,\text{bndry}} & \mathcal{A}_{u,\text{bndry}} \end{pmatrix} \\
\mathbf{b}_= &= \begin{pmatrix} \mathbf{0}^\top & \mathbf{0}^\top & \mathbf{b}_{\text{bndry}}^\top \end{pmatrix}^\top \\
\mathbf{lb} &= \begin{pmatrix} \mathbf{lb}_y^\top & \mathbf{lb}_u^\top \end{pmatrix}^\top \\
\mathbf{ub} &= \begin{pmatrix} \mathbf{ub}_y^\top & \mathbf{ub}_u^\top \end{pmatrix}^\top.
\end{aligned}$$

We have a linear mixed integer optimal control problem of the form

$$\min_{u(\cdot), y(\cdot), \bar{x}(\cdot)} \int_{t_0}^{t_{\text{end}}} e^{-\varphi \cdot t} \cdot \Phi_1^\top \cdot y(t) dt + \Phi_2^\top \cdot y(t_0) + \Phi_3^\top \cdot y(t_{\text{end}}) \quad (3.2a)$$

$$\text{s. t. } \dot{y}(t) = S_2 \cdot u(t) + S_4 \cdot y(t) \quad (3.2b)$$

$$0 = S_1 \cdot u(t) + S_3 \cdot y(t) \quad (3.2c)$$

$$lb \leq u(t) \leq ub \quad (3.2d)$$

$$H_y \cdot y(t) + H_u \cdot u(t) \leq h \quad (3.2e)$$

$$0 \leq y(t) \quad (3.2f)$$

$$H_{B,y} \cdot y(t) + H_{B,u} \cdot u(t) + H_{B,\bar{x}} \cdot \bar{x}(t) \leq h_B \quad (3.2g)$$

$$B_{y_0} \cdot y(t_0) + B_{y_{\text{end}}} \cdot y(t_{\text{end}}) = b_{\text{bndry}} \quad (3.2h)$$

$$y(t) \in \mathbb{R}^{n_y}, u(t) \in \mathbb{R}^{n_u} \quad (3.2i)$$

$$\bar{x}(t) \in \mathbb{B}^{n_{\bar{x}}} \quad (3.2j)$$

### 3.3.2. Example: Self-replicator

The translation of the r-deFBA problem of a regulatory self-replicator introduced in [Liu and Bockmayr \(2020\)](#) into the MILP given above will be shown in the following.

The flux vector is given by

$$u(t) = (v_{C_1}(t) \ v_{C_2}(t) \ v_Q(t) \ v_R(t) \ v_{T_1}(t) \ v_{T_2}(t) \ v_{RP}(t))^\top, \quad (3.3)$$

and the species vector reads

$$y(t) = (C_1(t) \ C_2(t) \ Q(t) \ R(t) \ T_1(t) \ T_2(t) \ RP(t))^\top, \quad (3.4)$$

and thus

$$\dot{y}(t) = (C_1\dot{(t)} \ C_2\dot{(t)} \ Q\dot{(t)} \ R\dot{(t)} \ T_1\dot{(t)} \ T_2\dot{(t)} \ RP\dot{(t)})^\top. \quad (3.5)$$

Note that the species vector does not contain species for which a quasi-steady state is assumed (QSSA) since these are by definition not dynamic ( $\dot{M} = 0$ ).

#### Objective

The objective weights of the macromolecules are usually equal to their molecular weights. Since the objective function is minimized, the entries of the vector  $\Phi_1$  needs to carry a negative sign:

$$\Phi_1 = (0 \ 0 \ -300 \ -7459 \ -400 \ -1500 \ -300)$$

$$\Phi_2 = (0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0)$$

$$\Phi_3 = (0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0)$$

How to choose  $\varphi$ ?

### Dynamics of External Species and Macromolecules

$$\begin{aligned}\dot{C}_1(t) &= -v_{C1}(t) \\ \dot{C}_2(t) &= -v_{C2}(t) \\ \dot{P}(t) &= v_P(t) - kd_p \cdot P(t)\end{aligned}$$

Using Equation 3.2b,  $S_2$  contains a row for each dynamical species and a column for each reaction:

$$S_2 = \begin{matrix} & v_{C_1} & v_{C_2} & v_Q & v_R & v_{T_1} & v_{T_2} & v_{RP} \\ \begin{matrix} C_1 \\ C_2 \\ Q \\ R \\ T_1 \\ T_2 \\ RP \end{matrix} & \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \end{matrix} \quad (3.6)$$

Degradation is enforced using  $S_4$  multiplied by the species vector  $y$ :

$$S_4 = \begin{matrix} & C_1 & C_2 & Q & R & T_1 & T_2 & RP \\ \begin{matrix} C_1 \\ C_2 \\ Q \\ R \\ T_1 \\ T_2 \\ RP \end{matrix} & \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -kd_Q & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -kd_R & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -kd_{T_1} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -kd_{T_2} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -kd_{RP} \end{pmatrix} \end{matrix} \quad (3.7)$$

### Steady-state assumption for internal metabolites

$$\dot{M}(t) = v_{C_1}(t) + v_{C_2}(t) - \sum n_p \cdot v_p(t) = 0$$

Using Equation 3.2c,  $S_1$  contains a row for each QSSA species and a column for each reaction:

$$S_1 = M \begin{pmatrix} v_{C_1} & v_{C_2} & v_Q & v_R & v_{T_1} & v_{T_2} & v_{RP} \\ 1 & 1 & -300 & -7459 & -400 & -1500 & -300 \end{pmatrix} \quad (3.8)$$

$$S_3 = M \begin{pmatrix} C_1 & C_2 & Q & R & T_1 & T_2 & RP \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \quad (3.9)$$

### Lower and Upper Flux Bounds

Lower and Upper Flux Bounds are not used in this example, and in general are optional in (r-)deFBA models (Reimers et al. (2017b); Liu and Bockmayr (2020)). Therefore, in Equation 3.2d all lower bounds are set to 0 (since all reactions in the regulatory self-replicator are irreversible), and upper bounds for fluxes are not given (achieved by a constant  $u = \text{big M}$ ).

$$\mathbf{lb}(t) = (0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0)^\top \quad (3.10)$$

$$\mathbf{ub}(t) = (u \ u \ u \ u \ u \ u \ u)^\top \quad (3.11)$$

### Quota Constraint

$$n_Q \cdot Q(t) \geq \Phi_Q \sum_{p \in \mathcal{P}} n_p \cdot P(t) \iff \Phi_Q \sum_{p \in \mathcal{P}} n_p \cdot P(t) - n_Q \cdot Q(t) \leq 0$$

The quota constraint can be implemented using Equation 3.2e. Since  $Q \in \mathcal{P}$ , its entry reads  $n_Q Q(t) - \Phi_Q n_Q Q(t) = (\Phi_Q - 1)n_Q$ . Since no fluxes are involved here,  $\mathbf{H}_{u,1}$  contains only zeros.

$$\mathbf{H}_{y,1} = Q \begin{pmatrix} C_1 & C_2 & Q & R & T_1 & T_2 & RP \\ 0 & 0 & (\Phi_Q - 1)300 & \Phi_Q \cdot 7459 & \Phi_Q \cdot 400 & \Phi_Q \cdot 1500 & \Phi_Q \cdot 300 \end{pmatrix} \quad (3.12)$$

$$\mathbf{H}_{u,1} = (0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0) \quad (3.13)$$

$$\mathbf{h}_1 = (0) \quad (3.14)$$

### Enzyme Capacity Constraint

The inequalities

$$\begin{aligned} v_{C_1}(t) &\leq k_{cat1} \cdot T_1(t) \\ v_{C_2}(t) &\leq k_{cat2} \cdot T_2(t) \\ \sum \frac{v_p(t)}{k_{cat}^{p,R}} &\leq R(t) \end{aligned}$$

can be expressed in the form  $H_C \cdot V \leq H_E \cdot P \iff -H_E \cdot P + H_C \cdot V \leq 0$  (Waldherr et al. (2015)) and thus be implemented by using Equation 3.2e.  $\mathbf{H}_{y,2}$  and  $\mathbf{H}_{u,2}$  both contain a row for each catalytic macromolecule  $p \in \mathcal{E}$ .

$$\mathbf{H}_{y,2} = \begin{matrix} & C_1 & C_2 & Q & R & T_1 & T_2 & RP \\ \begin{matrix} T_1 \\ T_2 \\ R \end{matrix} & \begin{pmatrix} 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & 0 \end{pmatrix} \end{matrix} \quad (3.15)$$

$$\mathbf{H}_{u,2} = \begin{matrix} & v_{C_1} & v_{C_2} & v_Q & v_R & v_{T_1} & v_{T_2} & v_{RP} \\ \begin{matrix} T_1 \\ T_2 \\ R \end{matrix} & \begin{pmatrix} 3000^{-1} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 2000^{-1} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 4.2^{-1} & 0.1689^{-1} & 3.15^{-1} & 0.84^{-1} & 4.2^{-1} \end{pmatrix} \end{matrix} \quad (3.16)$$

$$\mathbf{h}_2 = (0 \ 0 \ 0)^\top \quad (3.17)$$

### Control of discrete jumps

$$\begin{aligned} \overline{RP}(t) = 1 &\iff C_1(t) \geq \gamma \Rightarrow l(1 - \overline{RP}) \leq C_1 - \gamma \leq (u + \epsilon)\overline{RP} - \epsilon \\ \overline{T_2}(t) = 0 &\iff RP(t) \geq \alpha \Rightarrow l(1 - \overline{T_2}) \leq RP - \alpha \leq (u + \epsilon)\overline{T_2} - \epsilon \end{aligned}$$

where  $l = -\text{big M}$ ,  $u = \text{big M}$ ,  $\epsilon > 0$ .

These four inequalities can be reformulated as:

$$\begin{aligned} l(1 - \overline{RP}) \leq C_1 - \gamma &\iff -l \cdot \overline{RP} - C_1 \leq \gamma - l & I \\ C_1 - \gamma \leq (u + \epsilon)\overline{RP} - \epsilon &\iff -(u + \epsilon)\overline{RP} + C_1 \leq \gamma - \epsilon & II \\ l(1 - \overline{T_2}) \leq RP - \alpha &\iff -l \cdot \overline{T_2} - RP \leq \alpha - l & III \\ RP - \alpha \leq (u + \epsilon)\overline{T_2} - \epsilon &\iff -(u + \epsilon)\overline{T_2} + RP \leq \alpha - \epsilon & IV \end{aligned}$$

and enforced using [Equation 3.2g](#) such that  $\mathbf{H}_{B,y,1}$  denotes which species amounts govern the state changes of the boolean variables ( $\mathbf{H}_{B,y,1}$  analogous, if the boolean variables depend on fluxes).

$$\mathbf{H}_{B,y,1} = \begin{matrix} & C_1 & C_2 & Q & R & T_1 & T_2 & RP \\ \begin{matrix} I \\ II \\ III \\ IV \end{matrix} & \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \end{matrix} \quad (3.18)$$

$$\mathbf{H}_{B,u,1} = \begin{matrix} & v_{C_1} & v_{C_2} & v_Q & v_R & v_{T_1} & v_{T_2} & v_{RP} \\ \begin{matrix} I \\ II \\ III \\ IV \end{matrix} & \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \end{matrix} \quad (3.19)$$

$$\mathbf{H}_{B,\bar{x},1} = \begin{matrix} & \overline{RP} & \overline{T_2} \\ \begin{matrix} I \\ II \\ III \\ IV \end{matrix} & \begin{pmatrix} -l & 0 \\ \epsilon - u & 0 \\ 0 & -l \\ 0 & \epsilon - u \end{pmatrix} \end{matrix} \quad (3.20)$$

$$\mathbf{h}_{B,1} = (\gamma - l \ \gamma - \epsilon \ \alpha - l \ \alpha - \epsilon)^\top \quad (3.21)$$

### Control of continuous dynamics by discrete states

$$\begin{aligned}\overline{RP}(t) = 1 \Rightarrow v_{RP}(t) &\geq \epsilon_{RP} \wedge \overline{RP}(t) = 0 \Rightarrow v_{RP}(t) = 0 \iff \epsilon_{RP} \cdot \overline{RP} \leq v_{RP} \leq u_{RP} \cdot \overline{RP} \\ \overline{T_2}(t) = 1 \Rightarrow v_{T_2}(t) &\geq \epsilon_{T_2} \wedge \overline{T_2}(t) = 0 \Rightarrow v_{T_2}(t) = 0 \iff \epsilon_{T_2} \cdot \overline{T_2} \leq v_{T_2} \leq u_{T_2} \cdot \overline{T_2}\end{aligned}$$

These four inequalities can be reformulated as:

$$\begin{aligned}\epsilon_{RP} \cdot \overline{RP} \leq v_{RP} &\iff -v_{RP} + \epsilon_{RP} \cdot \overline{RP} \leq 0 & V \\ v_{RP} \leq u_{RP} \cdot \overline{RP} &\iff v_{RP} - u_{RP} \cdot \overline{RP} \leq 0 & VI \\ \epsilon_{T_2} \cdot \overline{T_2} \leq v_{T_2} &\iff -v_{T_2} + \epsilon_{T_2} \cdot \overline{T_2} \leq 0 & VII \\ v_{T_2} \leq u_{T_2} \cdot \overline{T_2} &\iff v_{T_2} - u_{T_2} \cdot \overline{T_2} \leq 0 & VIII\end{aligned}$$

Again, Equation 3.2g is used to implement these inequalities.  $\mathbf{H}_{B,y,2}$  describes the fluxes that are regulated by the given boolean variables.

$$\mathbf{H}_{B,y,2} = \begin{matrix} & C_1 & C_2 & Q & R & T_1 & T_2 & RP \\ \begin{matrix} V \\ VI \\ VII \\ VIII \end{matrix} & \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \end{matrix} \quad (3.22)$$

$$\mathbf{H}_{B,u,2} = \begin{matrix} & v_{C_1} & v_{C_2} & v_Q & v_R & v_{T_1} & v_{T_2} & v_{RP} \\ \begin{matrix} V \\ VI \\ VII \\ VIII \end{matrix} & \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 \end{pmatrix} \end{matrix} \quad (3.23)$$

$$\mathbf{H}_{B,\bar{x},2} = \begin{matrix} & \overline{RP} & \overline{T_2} \\ \begin{matrix} V \\ VI \\ VII \\ VIII \end{matrix} & \begin{pmatrix} \epsilon_{RP} & 0 \\ -u & 0 \\ 0 & \epsilon_{T_2} \\ 0 & -u \end{pmatrix} \end{matrix} \quad (3.24)$$

$$\mathbf{h}_{B,2} = (0 \ 0 \ 0 \ 0)^\top \quad (3.25)$$

### Initial amounts for species

Equation 3.2h can be used to set initial and final amounts for species (the latter is necessary to enforce cyclic behaviour).  $\mathbf{B}_{y_0}$  simply is an identity matrix, and  $\mathbf{b}_{\text{bndry}}$  contains the respective initial amounts.

$$\mathbf{B}_{y_0} = \begin{matrix} & C_1 & C_2 & Q & R & T_1 & T_2 & RP \\ \begin{matrix} C_1 \\ C_2 \\ Q \\ R \\ T_1 \\ T_2 \\ RP \end{matrix} & \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \end{matrix} \quad (3.26)$$

$$\mathbf{B}_{y_{\text{end}}} = \begin{matrix} & C_1 & C_2 & Q & R & T_1 & T_2 & RP \\ \begin{matrix} C_1 \\ C_2 \\ Q \\ R \\ T_1 \\ T_2 \\ RP \end{matrix} & \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \end{matrix} \quad (3.27)$$

$$\mathbf{b}_{\text{bdry}} = (1000 \quad 500 \quad 0.15 \quad 0.01 \quad 0.001 \quad 0.001 \quad 0)^\top \quad (3.28)$$

#### Initial levels for qualitative species

Since the rules are enforced at any time, initial levels don't need to be stated explicitly in the MILP.