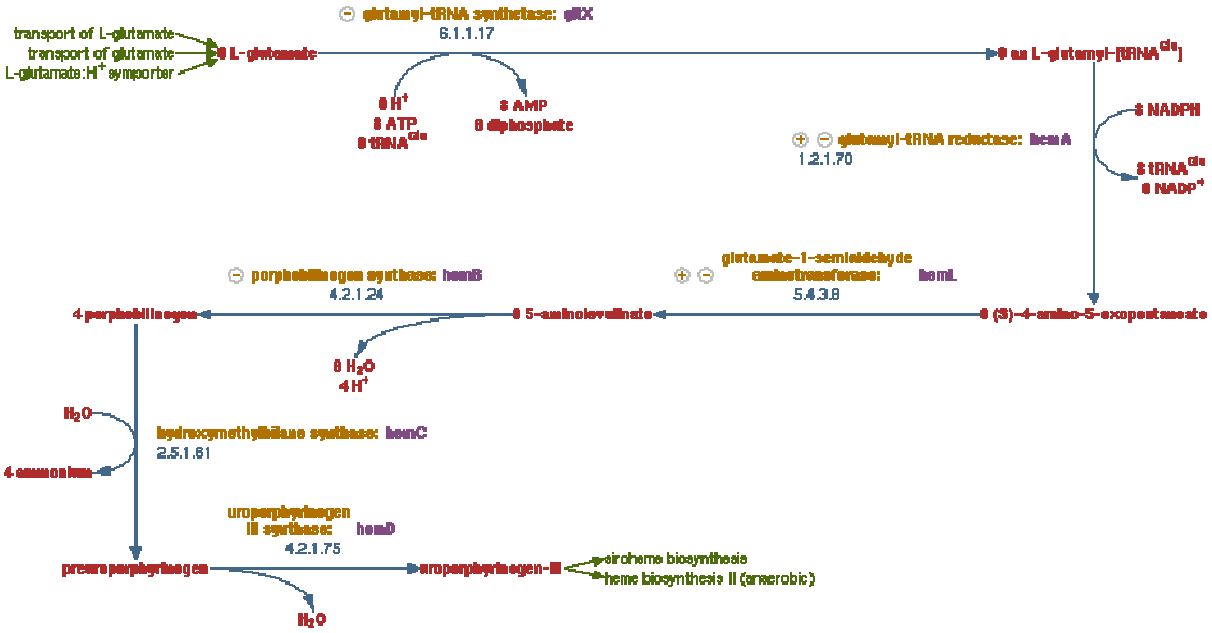
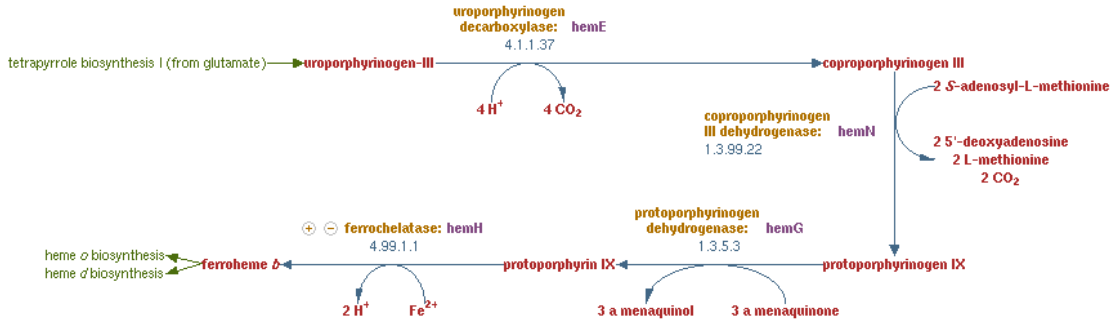
Three of the proteins required in the project are cytochrome c proteins (haem proteins). Hence, the heme biosynthesis pathway needs to be modelled.

Pathway of heme production in E. coli (C-5 pathway from glutamate)





Also,d-Aminolevulinate (ALA) produced as an intermediate in the above reactions. **δ-Aminolevulinate Synthase** (ALA Synthase) is the **committed step** of the heme synthesis pathway, and is usually rate-limiting for the overall pathway. Regulation occurs through control of gene transcription. **Heme** functions as a feedback inhibitor, **repressing transcription** of the gene for δ-Aminolevulinate Synthase.

A non-competitive irreversible feedback inhibition model is assumed for this step with a ki value of 0.02 mM. Hence, the following equation governs the conversion to ALA:

$$v = \frac{k_2[E_0][S]}{K_M\left(1+\frac{[S]}{K_M}+\frac{[I]}{K_I}+\frac{[S][I]}{K_M K_I}\right)} = \frac{v_{max}[S]}{K_M\left(1+\frac{[I]}{K_I}\right)+[S]\left(1+\frac{[I]}{K_I}\right)} = \frac{\frac{v_{max}}{\left(1+\frac{[I]}{K_I}\right)}[S]}{K_M+[S]} = \frac{v_{max}^{app}[S]}{K_M+[S]}$$

The rest of the reactions in this pathway are assumed to proceed via Henri-Michaelis-Menten kinetics:

$$v_o = \frac{(v_{max}[S])}{(k_M+[S])}$$

After putting in the rate laws and the values of various parameters in Copasi software, following graphs were obtained for the concentrations and rate of formation of various species involved:

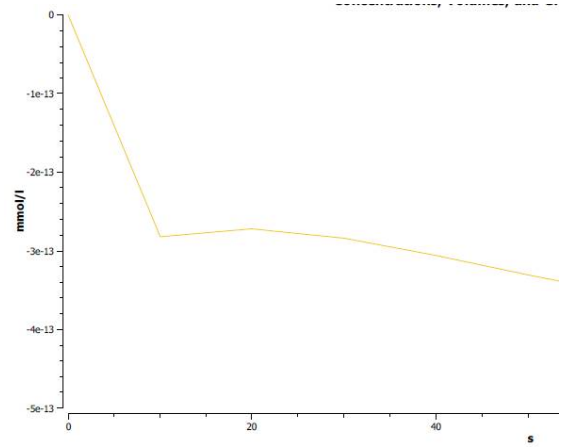


Fig 1: Amount of ferroheme b formed in the cytoplasm.

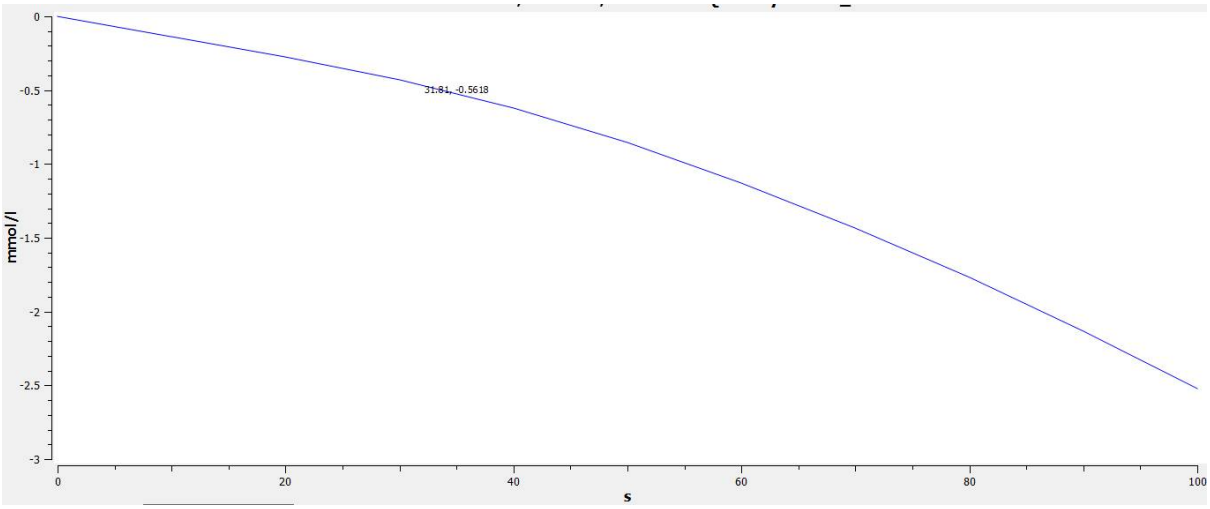


Fig 2: Amount of ferroheme transported to the periplasm

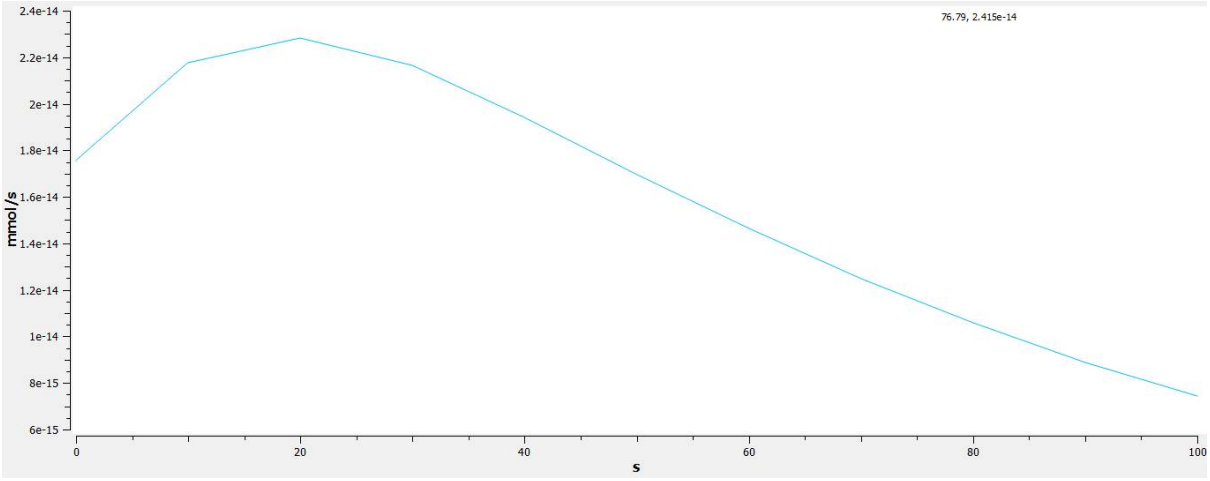


Fig 3: rate of formation of ferroheme in the cytoplasm

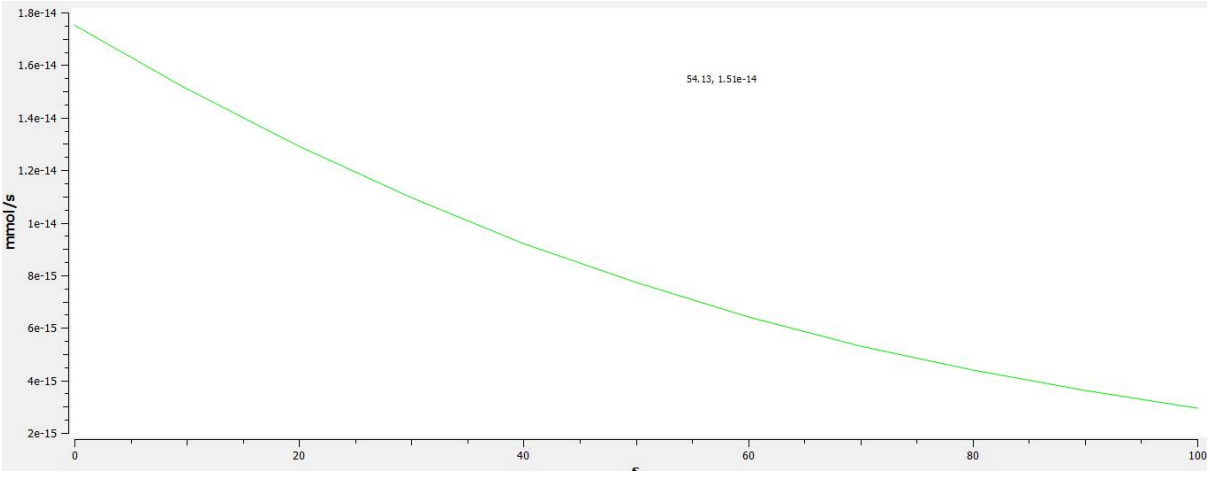


Fig 4: rate of transfer of transfer of ferroheme from the cytoplasm to the periplasm.

The following are the differential equations associated with the above processes:

$$\frac{d(["\text{ferroheme b\{CCO-CYTOSOL\}}"]\cdot V_{"\text{CCO-CYTOSOL}"})}{dt} = +V_{"\text{CCO-CYTOSOL}"}\cdot\left(\frac{0.1\cdot["\text{protoporphyrin IX\{CCO-CYTOSOL\}}"]}{4.7+["\text{protoporphyrin IX\{CCO-CYTOSOL\}}"]}\right) - V_{"\text{CCO-CYTOSOL}"}\cdot\left(\frac{0.1\cdot 0.1}{0.1+0.1}\right) - (0.1\cdot["\text{ferroheme b\{CCO-CYTOSOL\}}"]\cdot[\text{ATP}]\cdot[\text{H2O}])$$

$$\frac{d(["\text{ferroheme b\{CCO-PERI-BAC\}}"]\cdot V_{"\text{CCO-PERI-BAC}"})}{dt} = +V_{"\text{CCO-PERI-BAC}"}\cdot\left(\frac{0.1\cdot["\text{protoporphyrin IX\{CCO-PERI-BAC\}}"]}{4.7+["\text{protoporphyrin IX\{CCO-PERI-BAC\}}"]}\right) + (0.1\cdot["\text{ferroheme b\{CCO-CYTOSOL\}}"]\cdot[\text{ATP}]\cdot[\text{H2O}])$$

$$\frac{d(["\text{ferroheme o}"]\cdot V_{"\text{CCO-CYTOSOL}"})}{dt} = +V_{"\text{CCO-CYTOSOL}"}\cdot\left(\frac{0.1\cdot 0.1}{0.1+0.1}\right)$$

$$\frac{d(["\text{heme b}"]\cdot V_{"\text{CCO-CYTOSOL}"})}{dt} = -V_{"\text{CCO-CYTOSOL}"}\cdot\left(\frac{0.1\cdot["\text{heme b}"]}{0.1+["\text{heme b}"]}\right)$$

$$\frac{d(["\text{heme d}"]\cdot V_{"\text{CCO-CYTOSOL}"})}{dt} = +V_{"\text{CCO-CYTOSOL}"}\cdot\left(\frac{0.1\cdot["\text{heme b}"]}{0.1+["\text{heme b}"]}\right)$$

After production of ferroheme, the cytochrome –c protein and the ferroheme are transported to the periplasm, where they form a complex via covalent bonding.

At this point the protein becomes completely active.

After this, the following reactions take place, depending on the protein produced:

1. Nitrite reduction (nrfA protein)
   NO^{2-}----> NH_3

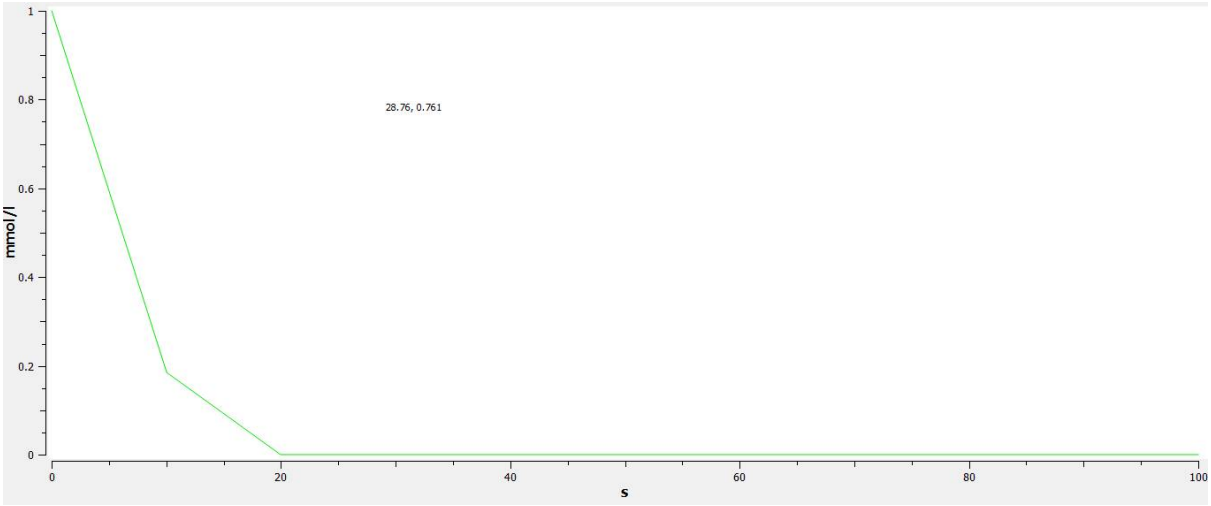


Fig : Variation in NO^{2-}concentration with time.

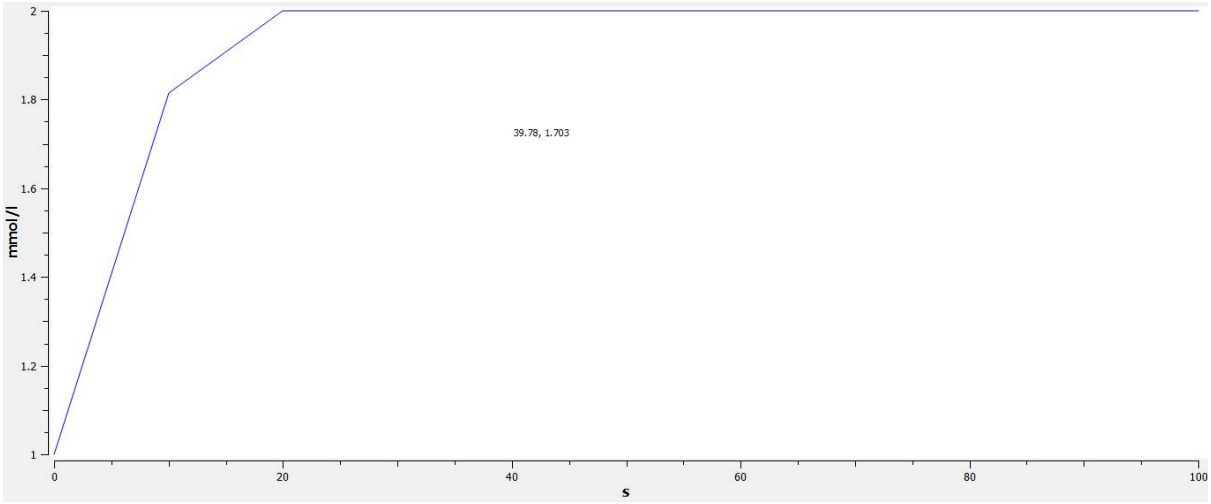


Fig : Variation in NH_3 concentration with time

Differential equations involved:

$$\frac{d([NH3]\cdot V_{"CCO\text{-}PERI\text{-}BAC"})}{dt} = +V_{"CCO\text{-}PERI\text{-}BAC"}\cdot\left(\frac{0.1\cdot["NO2\text{-}"]}{0.11+["NO2\text{-}"]}\right)$$

$$\frac{d(["NO2\text{-}"]\cdot V_{"CCO\text{-}PERI\text{-}BAC"})}{dt} = -V_{"CCO\text{-}PERI\text{-}BAC"}\cdot\left(\frac{0.1\cdot["NO2\text{-}"]}{0.11+["NO2\text{-}"]}\right)$$

2. Sulphite reduction (cys1 protein)
   SO_3^{2-}----> H_2S

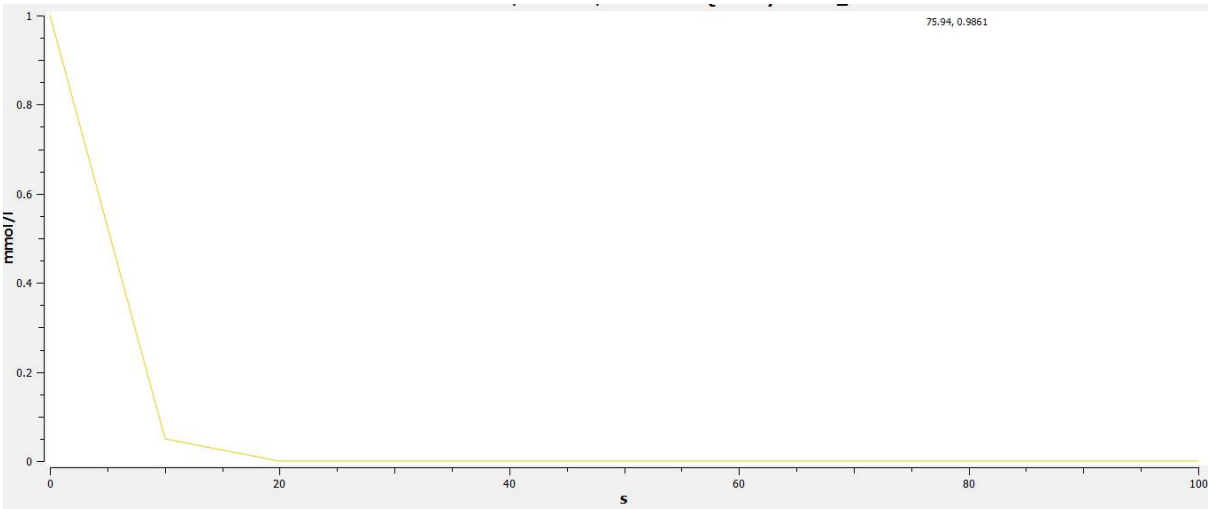


Fig: Variation of SO_3^{2-}concentration with time

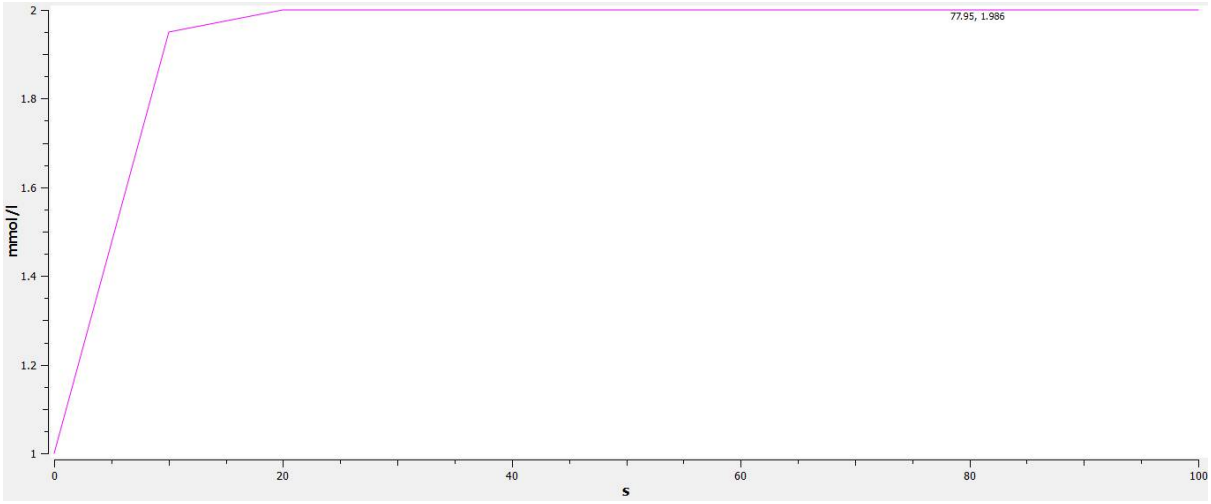


Fig: Variation in H_2S concentration with time.

$$\frac{d([H2S] \cdot V_{"CCO-PERI-BAC"})}{dt} = +V_{"CCO-PERI-BAC"} \cdot \left(\frac{0.1 \cdot ["SO3(2-)"]}{0.017 + ["SO3(2-)"]}\right)$$

$$\frac{d(["SO3(2-)"] \cdot V_{"CCO-PERI-BAC"})}{dt} = -V_{"CCO-PERI-BAC"} \cdot \left(\frac{0.1 \cdot ["SO3(2-)"]}{0.017 + ["SO3(2-)"]}\right)$$

3. Nitrous oxide reduction (NosZ protein)

   N_2O ----> N_2

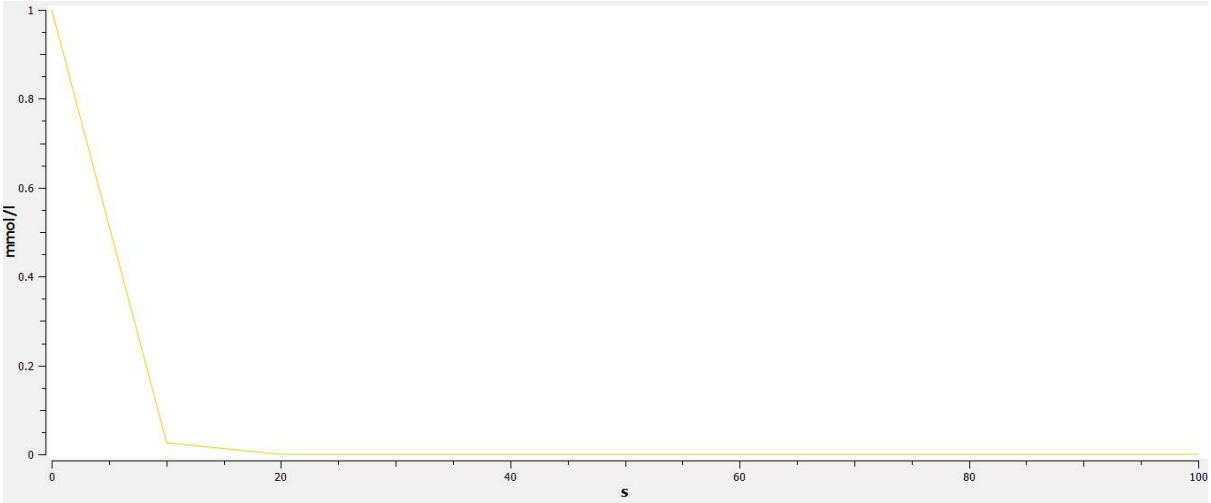


Fig: Variation of N_2O with time

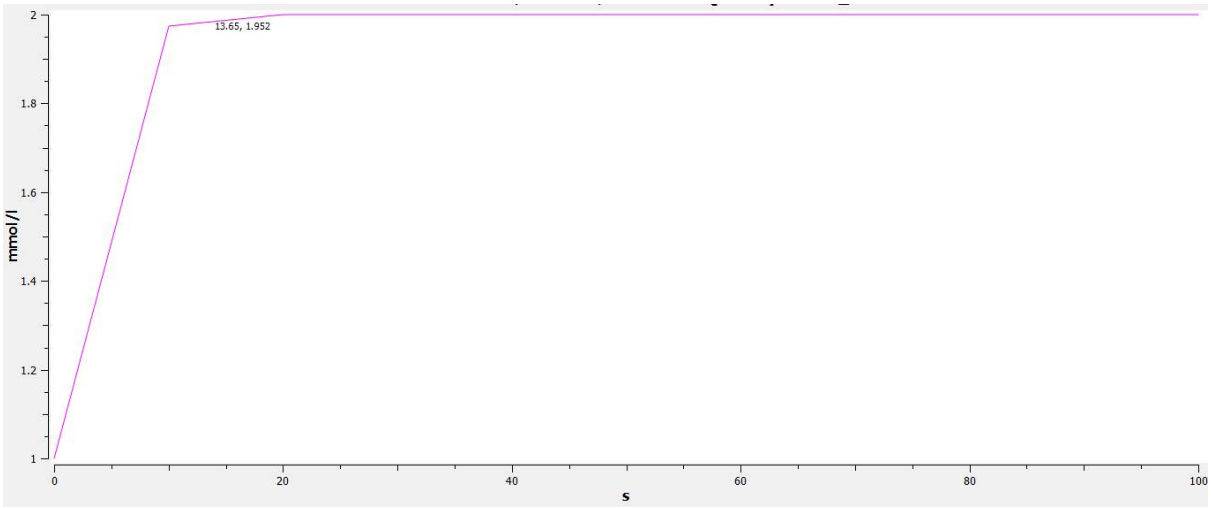


Fig: Variation of N_2 with time

$$\frac{d([N2]\cdot V_{"CCO\text{-}PERI\text{-}BAC"})}{dt} = +V_{"CCO\text{-}PERI\text{-}BAC"}\cdot\left(\frac{0.1\cdot[N2O]}{0.007+[N2O]}\right)$$

$$\frac{d([N2O]\cdot V_{"CCO\text{-}PERI\text{-}BAC"})}{dt} = -V_{"CCO\text{-}PERI\text{-}BAC"}\cdot\left(\frac{0.1\cdot[N2O]}{0.007+[N2O]}\right)$$

References:

https://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/heme.htm