Supporting Information for

Observability of anammox activity in single-stage nitritation/anammox reactors using mass balances

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S1 Mass balances with catabolic equations only

The equation system for mass balances with catabolic equations only is given in equation S1.

$$\begin{pmatrix} 0 & 0 & 0 & -1 & 0 & -1 \\ 0 & -8/3 & 0 & 1 & -1 & -1 \\ 0 & 0 & -8/5 & 0 & 1 & 0 \\ -1 & -1 & -1 & 0 & 0 & 0 \\ -1 & -11/3 & -13/5 & 2 & 0 & 0 \\ -2 & 0 & 0 & -3/2 & -1/2 & 0 \\ 2 & 2 & 2 & 0 & 0 & 0 \end{pmatrix} \cdot \begin{pmatrix} r_{R,HET,O2} \\ r_{R,HET,NO2} \\ r_{R,HET,NO3} \\ r_{R,AOB} \\ r_{R,NOB} \\ r_{R,AMX} \end{pmatrix} = \begin{pmatrix} r_{NH_4^+} \\ r_{NO_2^-} \\ r_{NO_3^-} \\ r_{C_2H_3O_2^-} \\ r_{H^+} \\ r_{O_2} \\ r_{TIC} \end{pmatrix} \quad \text{(S1)}$$

S2 Mass balances based on the activated sludge model

The equation system 2, where only mass balances for dissolved compounds were considered, is given in equation S2.

$$\begin{pmatrix} -0.587 & -0.820 & 0 & 0 & 0 & 0 & -15.3 & -0.820 & -23.8 & -0.820 \\ -1.59 & 0 & -1.85 & 0 & -1.85 & 0 & 0 & 0 & 0 & 0 \\ -0.0875 & 0.0803 & -0.0875 & 0 & -0.0875 & 0.0803 & -4.85 & 0.0803 & -0.0875 & 0.0803 \\ 0 & 0 & -0.298 & -0.287 & 0 & 0 & 0 & 0 & 21.7 & 0 \\ 0 & 0 & 0 & 0 & -0.497 & -0.478 & 4.76 & 0 & -21.7 & 0 \\ -0.0186 & -0.00574 & -0.0440 & -0.0262 & -0.058 & -0.0400 & 0.687 & -0.00574 & 0.00625 & -0.00574 \\ 0.220 & 0.308 & 0.319 & 0.308 & 0.319 & 0.3082 & -0.375 & 0.308 & -0.375 & 0.308 \end{pmatrix}$$

$$\begin{pmatrix} \alpha_{R,HET,growth,O2} \\ \alpha_{R,HET,end.rep,O2} \\ \alpha_{R,HET,growth,NO3} \\ \alpha_{R,HET,end.resp,NO3} \\ \alpha_{R,HET,growth,NO2} \\ \alpha_{R,HET,end.resp,NO2} \\ \alpha_{R,AOB,growth} \\ \alpha_{R,AOB,end.resp} \\ \alpha_{R,NOB,growth} \\ \alpha_{R,NOB,end.resp} \\ \alpha_{R,AMX,growtj} \\ \alpha_{R,AMX,end.resp} \end{pmatrix} = \begin{pmatrix} r_{O_2} \\ r_{C_2H_3O_2^-} \\ r_{NH_4^+} \\ r_{NO_3^-} \\ r_{NO_2^-} \\ r_{H^+} \\ r_{TIC} \end{pmatrix} \quad \text{(S2)}$$

The equation system 3, with one type of HET and including the concentrations for the bacterial groups and inert biomass, is given in equation S3.

$$\begin{pmatrix} -0.587 & -0.820 & 0 & 0 & 0 & 0 & -15.3 & -0.820 & -23.8 & -0.820 \\ -1.59 & 0 & -1.85 & 0 & -1.85 & 0 & 0 & 0 & 0 & 0 \\ -0.0875 & 0.0803 & -0.0875 & 0 & -0.0875 & 0.0803 & -4.85 & 0.0803 & -0.0875 & 0.0803 \\ 0 & 0 & -0.298 & -0.287 & 0 & 0 & 0 & 0 & 21.7 & 0 \\ 0 & 0 & 0 & 0 & -0.497 & -0.478 & 4.76 & 0 & -21.7 & 0 \\ -0.0186 & -0.00574 & -0.0440 & -0.0262 & -0.058 & -0.0400 & 0.687 & -0.00574 & 0.00625 & -0.00574 \\ 0.220 & 0.308 & 0.319 & 0.308 & 0.319 & 0.3082 & -0.375 & 0.308 & -0.375 & 0.308 \\ 0 & 0.180 & 0 & 0.180 & 0 & 0.180 & 0 & 0.180 & 0 & 0.180 \\ 1 & -1 & 1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}.$$

$$\begin{pmatrix} \alpha_{R,HET,growth,O2} \\ \alpha_{R,HET,end.rep,O2} \\ \alpha_{R,HET,growth,NO3} \\ \alpha_{R,HET,end.resp,NO3} \\ \alpha_{R,HET,growth,NO2} \\ \alpha_{R,HET,end.resp,NO2} \\ \alpha_{R,AOB,growth} \\ \alpha_{R,AOB,end.resp} \\ \alpha_{R,NOB,growth} \\ \alpha_{R,NOB,end.resp} \\ \alpha_{R,AMX,growtj} \\ \alpha_{R,AMX,end.resp} \end{pmatrix} = \begin{matrix} r_{O_2} \\ r_{C_2H_3O_2^-} \\ r_{NH_4^+} \\ r_{NO_3^-} \\ r_{NO_2^-} \\ r_{H^+} \\ r_{TIC} \\ r_{X_I} \\ r_{X_{HET}} \\ r_{X_{AOB}} \\ r_{X_{NOB}} \\ r_{X_{AMX}} \end{matrix} \quad \text{(S3)}$$

The equation system 4, with three types of HET and including the concentrations for the bacterial groups and inert biomass, is given in equation S4.

$$\begin{pmatrix} -0.587 & -0.820 & 0 & 0 & 0 & 0 & -15.3 & -0.820 & -23.8 & -0.820 \\ -1.59 & 0 & -1.85 & 0 & -1.85 & 0 & 0 & 0 & 0 & 0 \\ -0.0875 & 0.0803 & -0.0875 & 0 & -0.0875 & 0.0803 & -4.85 & 0.0803 & -0.0875 & 0.0803 \\ 0 & 0 & -0.298 & -0.287 & 0 & 0 & 0 & 0 & 21.7 & 0 \\ 0 & 0 & 0 & 0 & -0.497 & -0.478 & 4.76 & 0 & -21.7 & 0 \\ -0.0186 & -0.00574 & -0.0440 & -0.0262 & -0.058 & -0.0400 & 0.687 & -0.00574 & 0.00625 & -0.00574 \\ 0.220 & 0.308 & 0.319 & 0.308 & 0.319 & 0.3082 & -0.375 & 0.308 & -0.375 & 0.308 \\ 0 & 0.180 & 0 & 0.180 & 0 & 0.180 & 0 & 0.180 & 0 & 0.180 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}.$$

$$\begin{pmatrix} \alpha_{R,HET,growth,O2} \\ \alpha_{R,HET,end.rep,O2} \\ \alpha_{R,HET,growth,NO3} \\ \alpha_{R,HET,end.resp,NO3} \\ \alpha_{R,HET,growth,NO2} \\ \alpha_{R,HET,end.resp,NO2} \\ \alpha_{R,AOB,growth} \\ \alpha_{R,AOB,end.resp} \\ \alpha_{R,NOB,growth} \\ \alpha_{R,NOB,end.resp} \\ \alpha_{R,AMX,growtj} \\ \alpha_{R,AMX,end.resp} \end{pmatrix} = \begin{matrix} r_{O_2} \\ r_{C_2H_3O_2^-} \\ r_{NH_4^+} \\ r_{NO_3^-} \\ r_{NO_2^-} \\ r_{H^+} \\ r_{TIC} \\ r_{X_I} \\ r_{X_{HET,O2}} \\ r_{X_{HET,NO3}} \\ r_{X_{HET,NO2}} \\ r_{X_{AOB}} \\ r_{X_{NOB}} \\ r_{X_{AMX}} \end{matrix} \quad \text{(S4)}$$

S3 Mass balances based on the activated sludge model including kinetics

The equation system 5, i.e. the mass balances to calculate the activities of the involved bacteria in a nitritation/anammox process *with* heterotrophic activity based on the activated sludge model and including kinetics, is given in equation S5.

$$\begin{pmatrix} -0.0351 & 0 & 0 & -0.189 & -0.00404 & 0 \\ -0.0490 & -0.0572 & -0.0487 & 0 & 0 & 0 \\ -0.00103 & -0.00103 & -0.000877 & -0.0592 & 0.0000664 & -0.0764 \\ 0 & -0.0152 & 0 & 0 & 0.00295 & 0.0185 \\ 0 & 0 & -0.0216 & 0.0583 & -0.00295 & -0.101 \\ -0.000691 & -0.00190 & -0.00224 & 0.00839 & -0.00000474 & -0.000412 \\ 0.0132 & 0.0163 & 0.0139 & -0.000389 & 0.000249 & -0.00340 \end{pmatrix} \cdot \begin{pmatrix} X_{HET,O2} \\ X_{HET,NO3} \\ X_{HET,NO2} \\ X_{AOB} \\ X_{NOB} \\ X_{AMX} \end{pmatrix} = \begin{pmatrix} r_{O_2} \\ r_{C_2H_3O_2^-} \\ r_{NH_4^+} \\ r_{NO_3^-} \\ r_{NO_2^-} \\ r_{H^+} \\ r_{TIC} \end{pmatrix} \quad \text{(S5)}$$

The equation system 6, i.e. the mass balances to calculate the activities of bacteria in a nitritation/anammox reactor <u>without</u> heterotrophic activity based on the activated sludge model and including kinetics, is given in equation S6.

$$\begin{pmatrix} -0.667 & -0.594 & 0 \\ -0.200 & -0.000979 & -0.104 \\ 0 & 0.531 & 0.0253 \\ 0.199 & -0.531 & -0.140 \\ 0.0285 & 0.0000699 & -0.000706 \\ -0.00588 & -0.00472 & -0.00366 \end{pmatrix} \cdot \begin{pmatrix} X_{AOB} \\ X_{NOB} \\ X_{AMX} \end{pmatrix} = \begin{pmatrix} r_{O_2} \\ r_{NH_4^+} \\ r_{NO_3^-} \\ r_{NO_2^-} \\ r_{H^+} \\ r_{TIC} \end{pmatrix} \quad \text{(S6)}$$

S4 Mass balances based on the activated sludge model including kinetics and a biomass balance

The equation system 7, for mass balances based on the activated sludge model and including both kinetics and a biomass balance, is given in equation S7.

$$\begin{pmatrix} -0.0351 & 0 & 0 & -0.189 & -0.00404 & 0 \\ -0.0490 & -0.0572 & -0.0487 & 0 & 0 & 0 \\ -0.00103 & -0.00103 & -0.000877 & -0.0592 & 0.0000664 & -0.0764 \\ 0 & -0.0152 & 0 & 0 & 0.00295 & 0.0185 \\ 0 & 0 & -0.0216 & 0.0583 & -0.00295 & -0.101 \\ -0.000691 & -0.00190 & -0.00224 & 0.00839 & -0.00000474 & -0.000412 \\ 0.0132 & 0.0163 & 0.0139 & -0.000389 & 0.000249 & -0.00340 \\ 0.0138 & 0.0138 & 0.0118 & 0.0104 & -0.000664 & 0.0103 \end{pmatrix}.$$

$$\begin{pmatrix} X_{HET,O2} \\ X_{HET,NO3} \\ X_{HET,NO2} \\ X_{AOB} \\ X_{NOB} \\ X_{AMX} \end{pmatrix} = \begin{pmatrix} r_{O_2} \\ r_{C_2H_3O_2^-} \\ r_{NH_4^+} \\ r_{NO_3^-} \\ r_{NO_2^-} \\ r_{H^+} \\ r_{TIC} \\ r_{X_{tot}} \end{pmatrix} \quad \text{(S7)}$$

S5 Initial conditions and influent concentrations

The initial conditions and the influent concentrations of the modelled compounds are given in Table S1.

Table S1 Initial conditions and influent concentrations of the modelled compounds.

	Unit	Initial condition	Influent concentration
S_H	10^3 mol·m^{-3}	$5.97 \cdot 10^{-7}$	$2.42 \cdot 10^{-9}$
S_{HCO3}	mol·m^{-3}	0.63	31.7
S_{H2CO3}	mol·m^{-3}	0.62	0.12
S_{HNO2}	g N·m^{-3}	0	0
S_{HPO4}	g P·m^{-3}	7.6	43.2
S_{H2PO4}	g P·m^{-3}	36.4	0.7
$S_{N2,AMX}$	g N·m^{-3}	0	0
$S_{N2,HET}$	g N·m^{-3}	0	0
S_{NH3}	g N·m^{-3}	0.11	132
S_{NH4}	g N·m^{-3}	8.29	566
S_{NO2}	g N·m^{-3}	0.1	0
S_{NO3}	g N·m^{-3}	6.5	0
S_{O2}	g O_2·m^{-3}	0	0
S_S	g COD·m^{-3}	0	0
S_{Sneg}	g COD·m^{-3}	0	890
$S_{S,NO2}$	g COD·m^{-3}	0	0
$S_{S,NO3}$	g COD·m^{-3}	0	0
$S_{S,O2}$	g COD·m^{-3}	0	0
X_{AMX}	g COD·m^{-3}	100	0
X_{AOB}	g COD·m^{-3}	100	0
$X_{H,NO2}$	g COD·m^{-3}	100	0
$X_{H,NO3}$	g COD·m^{-3}	100	0
$X_{H,O2}$	g COD·m^{-3}	100	0
X_I	g COD·m^{-3}	2000	0
X_{NOB}	g COD·m^{-3}	100	0

S6 Modelled compounds

The modelled compounds are listed in Table S2.

Table S2 Dissolved and particulate compounds of the dynamic model.

		unit	i_N [g N]	i_{COD} [g COD]	i_C [mol C]	i_{charge} [mol]
S_H	dissolved hydrogen proton	10^3 mol·m^{-3}	0	0	0	1000
S_{H2CO3}	dissolved carbonic acid	mol·m^{-3}	0	0	1	0
S_{H2PO4}	dissolved dihydrogen phosphate	g P·m^{-3}	0	0	0	-1/30.97
S_{HCO3}	dissolved bicarbonate	mol·m^{-3}	0	0	1	-1
S_{HNO2}	dissolved nitrous acid	g N·m^{-3}	1	-48/14	0	0
S_{HPO4}	dissolved hydrogen phosphate	g P·m^{-3}	0	0	0	-2/30.97
$S_{N2,AMX}$	dissolved dinitrogen produced by AMX	g N·m^{-3}	1	-24/14	0	0
$S_{N2,HET}$	dissolved dinitrogen produced by HET	g N·m^{-3}	1	-24/14	0	0
S_{NH3}	dissolved ammonia	g N·m^{-3}	1	0	0	0
S_{NH4}	dissolved ammonium	g N·m^{-3}	1	0	0	1/14
S_{NO2}	dissolved nitrite	g N·m^{-3}	1	-48/14	0	-1/14
S_{NO3}	dissolved nitrate	g N·m^{-3}	1	-64/14	0	-1/14
S_{O2}	dissolved oxygen	g O_2·m^{-3}	0	-1	0	0
S_S	dissolved acetic acid	g COD·m^{-3}	0	1	2/64	0
$S_{S,neg}$	dissolved acetate	g COD·m^{-3}	0	1	2/64	-1/64
$S_{S,NO2}$	dissolved acetic acid degraded by $X_{HET,NO2}$	g COD·m^{-3}	0	1	2/64	0
$S_{S,NO3}$	dissolved acetic acid degraded by $X_{HET,NO3}$	g COD·m^{-3}	0	1	2/64	0
$S_{S,O2}$	dissolved acetic acid degraded by $X_{HET,O2}$	g COD·m^{-3}	0	1	2/64	0
X_{AOB}	ammonia oxidising bacteria	g COD·m^{-3}	14/160	1	5/160	0
X_{AMX}	anammox bacteria	g COD·m^{-3}	0.15·14/36.4	1	1/36.4	0
$X_{H,NO2}$	heterotrophic bacteria using nitrite	g COD·m^{-3}	14/160	1	5/160	0

$\mathbf{X_{H,NO3}}$	heterotrophic bacteria using nitrate	g COD·m^{-3}	14/160	1	5/160	0
$\mathbf{X_{H,O2}}$	heterotrophic bacteria using oxygen	g COD·m^{-3}	14/160	1	5/160	0
$\mathbf{X_I}$	inert particulate matter	g COD·m^{-3}	0.035	1	5/160	0
$\mathbf{X_{NOB}}$	nitrite oxidising bacteria	g COD·m^{-3}	14/160	1	5/160	0

S7 Simulation of pH equilibria and aeration

Parameters and process rates for the simulation of the pH equilibria and the aeration are listed in Table S3 and Table S4, respectively.

Table S3 Parameters for the simulation of pH equilibria and aeration.

Symbol	Characterisation	Value / formula	Unit
cond	Conductivity	22	mS·cm^{-1}
$\mathbf{f_1}$	Activity coefficient for ions with charge 1	$10\text{^}\left(-0.5\cdot 1\cdot\frac{\sqrt{ionicstrength}}{\sqrt{ionicstrength}+1}-0.2\cdot io\right.$	-
$\mathbf{f_2}$	Activity coefficient for ions with charge 2	$10\text{^}\left(-0.5\cdot 4\cdot\frac{\sqrt{ionicstrength}}{\sqrt{ionicstrength}+1}-0.2\cdot io\right.$	-
$\mathbf{H_{H2CO3}}$	Henry coefficient of H_2CO_3	$\frac{1}{k_{H,T,H2CO3}\cdot 0.082057\cdot(T+273.15)}$	-
$\mathbf{H_{NH3}}$	Henry coefficient of NH_3	$\frac{1}{k_{H,T,NH3}\cdot 0.082057\cdot(T+273.15)}$	-
ionicstrength	Ionic strength	$0.01001047\cdot cond+0.0005188$	mol·L^{-1}
$\mathbf{k_{H,T,H2CO3}}$	Temperature dependency of H_{H2CO3}	$0.0345294\cdot exp\left(2428.57\cdot\left(\frac{1}{T+273.15}-\frac{1}{29}\right.\right.$	K^{-1}
$\mathbf{k_{H,T,NH3}}$	Temperature dependency of H_{NH3}	$55.8125\cdot exp\left(3708.33\cdot\left(\frac{1}{T+273.15}-\frac{1}{298.}\right.\right.$	K^{-1}
$\mathbf{K_La_{H2CO3}}$	K_La value of H_2CO_3	$0.862\cdot K_La_{O2}$	d^{-1}
$\mathbf{K_La_{O2}}$	K_La value of O_2	70	d^{-1}
$\mathbf{k_{aeq}}$	Rate constant for equilibrum processes	10^{10}	d^{-1}

$p_{CO2,air}$	Partial pressure of CO_2	0.000335	atm
pK_{H2CO3}	pK value of $H_2CO_3 \leftrightarrow HCO_3$	$-log\left(10^{-6.37} \cdot exp\left(7.6 \cdot \frac{1000}{8.314472} \cdot \left(\frac{1}{298.15}\right.\right.\right.$	-
pK_{H2PO4}	pK value of $H_2PO_4 \leftrightarrow HPO_4$	$-log\left(10^{-7.21} \cdot exp\left(4.2 \cdot \frac{1000}{8.314472} \cdot \left(\frac{1}{298.15}\right.\right.\right.$	-
pK_{HNO2}	pK value of $HNO_2 \leftrightarrow NO_2$	$-log\left(10^{-3.34} \cdot exp\left(15.2 \cdot \frac{1000}{8.314472} \cdot \left(\frac{1}{298.1}\right.\right.\right.$	-
pK_{NH4}	pK value of $NH_4 \leftrightarrow NH_3$	$-log\left(10^{-9.25} \cdot exp\left(52.21 \cdot \frac{1000}{8.314472} \cdot \left(\frac{1}{298}\right.\right.\right.$	-
pK_{SS}	pK value of S $\leftrightarrow$ S_{neg}	$-log\left(10^{-4.75} \cdot exp\left(-0.2 \cdot \frac{1000}{8.314472} \cdot \left(\frac{1}{298}\right.\right.\right.$	-
pOs	Parameter for calculation of $S_{O2,sat}$	$1.2 \cdot 10^{-6} \cdot T^4 + 0.00935467 \cdot T^2 - 0.41289$	-
pw	Parameter for calculation of $S_{O2,sat}$	$4.79 \cdot 10^{-6} \cdot T^4 + 0.00021623 \cdot T^3 + 0.0148$	-
Q_{air}	Airflow rate	$0.195 \cdot \frac{1013}{971} \cdot \frac{273.14 + T}{273.14}$	$m^3 \cdot d^{-1}$
Q_{in}	Influent flow rate	0.003	$m^3 \cdot d^{-1}$
Q_{eff}	Effluent flow rate	0.003	$m^3 \cdot d^{-1}$
SRT	Sludge retention time	50 $resp.$ 100	d
$S_{H2CO3,air}$	Concentration of H_2CO_3 in the air	$p_{CO2} \cdot 1000 \cdot \frac{1}{0.082057} \cdot \frac{1}{(T + 273.15)}$	$mol \cdot m^{-3}$
$S_{N2,tot}$	Total produced N_2	$S_{N2,AMX} + S_{N2,HET}$	$gN \cdot m^{-3}$
$S_{O2,sat}$	Saturation concentration of dissolved oxygen	$pOs \cdot \frac{975 - pw}{1013 - pw}$	$gO_2 \cdot m^{-3}$
$S_{S,tot}$	Total degraded S_S	$S_{S,O2} + S_{S,NO3} + S_{S,NO2}$	$gCOD \cdot m^{-3}$
T	Temperature	26	°C
V	Volume	0.01	m^3

Table S4 Process rates for the simulation of pH equilibria and aeration.

	Reaction	Reaction rate
aeration	H_2CO_3 strip	$\left(H_{H2CO3} \cdot S_{H2CO3} - S_{H2CO3,air}\right) \cdot \frac{Q_{air}}{V} \cdot \left(1 - exp\left(\frac{K_L a_{H2CO3} \cdot V}{H_{H2CO3} \cdot Q_{air}}\right)\right)$
	O_2 input	$K_L a_{O2} \cdot \left(S_{O2,sat} - S_{O2}\right)$
pH equilibriums	HCO_3 forward	$k_{aeq} \cdot S_{H2CO3}$
	HCO_3 backward	$k_{aeq} \cdot 10^{pK_{H2CO3}} \cdot f_1 \cdot S_H \cdot f_1 \cdot S_{HCO3}$
	HPO_4 forward	$k_{aeq} \cdot f_1 \cdot S_{H2PO4}$
	HPO_4 backward	$k_{aeq} \cdot 10^{pK_{H2PO4}} \cdot f_1 \cdot S_H \cdot f_2 \cdot S_{HPO4}$
	NH_3 forward	$k_{aeq} \cdot f_1 \cdot S_{NH4}$
	NH_3 backward	$k_{aeq} \cdot 10^{pK_{NH4}} \cdot f_1 \cdot S_H \cdot S_{NH3}$
	NO_2 forward	$k_{aeq} \cdot S_{HNO2}$
	NO_2 backward	$k_{aeq} \cdot 10^{pK_{HNO2}} \cdot f_1 \cdot S_H \cdot f_1 \cdot S_{NO2}$
	S forward	$k_{aeq} \cdot S_S$
	S backward	$k_{aeq} \cdot 10^{pK_{SS}} \cdot f_1 \cdot S_H \cdot f_1 \cdot S_{Sneg}$

S8 Structural observability with one type of HET

To test whether the lack of observability is not only practical but also structural, we performed Monte Carlo simulations: 10,000 simulations were done with uniformly and randomly distributed parameter values in a range of ± 50% of the default values. This was done with the following Matlab code:

```
clc
clear all
close all

disp('Defining default or sampled parameter values')

        a_def   =   0.630          ;    % YH,O2
        b_def   =   0.566          ;    % YH,NO3
        c_def   =   0.566          ;    % YH,NO2
        d_def   =   14/160         ;    % iN,AOB,NOB,HET
        e_def   =   0.15*14/36.4   ;    % iN,AMX
        f_def   =   0.035          ;    % iN,XI
        g_def   =   0.180          ;    % fxi
        h_def   =   0.210          ;    % YAOB
        i_def   =   0.046          ;    % YNOB
        k_def   =   0.150          ;    % YAMX

approach = 'randomized' ;
switch approach
    case 'default'
        a   =   0.630          ;    % YH,O2
        b   =   0.566          ;    % YH,NO3
        c   =   0.566          ;    % YH,NO2
        d   =   14/160         ;    % iN,AOB,NOB,HET
        e   =   0.15*14/36.4   ;    % iN,AMX
        f   =   0.035          ;    % iN,XI
        g   =   0.180          ;    % fxi
        h   =   0.210          ;    % YAOB
        i   =   0.046          ;    % YNOB
        k   =   0.150          ;    % YAMX
    case 'randomized'
        n_rand = 100 ; % number of repetitions

        a   =   rand(1,n_rand)*a_def+1/2*a_def ;
        b   =   rand(1,n_rand)*b_def+1/2*b_def ;
        c   =   rand(1,n_rand)*c_def+1/2*c_def ;
        d   =   14/160         ;    % iN,AOB,NOB,HET
        e   =   0.15*14/36.4   ;    % iN,AMX
        f   =   0.035          ;    % iN,XI
        g   =   rand(1,n_rand)*g_def+1/2*g_def ;
        h   =   rand(1,n_rand)*h_def+1/2*h_def ;
        i   =   rand(1,n_rand)*i_def+1/2*i_def ;
        k   =   rand(1,n_rand)*k_def+1/2*k_def ;

end

n_reagent = 12 ;
n_reactions = 12 ;
n_rep   = length(a) ; % length of vector a = number of repetitions
```

```
for i_rep=1:n_rep
    disp(['Repetition ' num2str(i_rep) ' of ' num2str(n_rep)])
    % ====================
    % Setup matrix:
    Matrix = nan(n_reagent,n_reactions) ;% initialize matrix

    % --------------------
    % reaction 1
    Matrix(:,1) = [     -1/(32*a(i_rep))+1/32 ;
                        -1/(64*a(i_rep)) ;
                        -d/14 ;
                        0 ;
                        0 ;
                        -1/(64*a(i_rep))+d/14 ;
                        2/(64*a(i_rep))-1/32 ;
                        0 ;
                        1/160 ;
                        0 ;
                        0 ;
                        0   ];
    % --------------------
    % reaction 2
    Matrix(:,2) = [     g(i_rep)/32-1/32    ;
                        0   ;
                        d/14-g(i_rep)*f/14  ;
                        0   ;
                        0   ;
                        (g(i_rep)*f-d)/14   ;
                        1/32-g(i_rep)/32    ;
                        g(i_rep)/160    ;
                        -1/160  ;
                        0   ;
                        0   ;
                        0   ];
    % --------------------
    % reaction 3

    Matrix(:,3) = [     0   ;
                        -1/(64*b(i_rep))  ;
                        -d/14    ;
                        1/40*(1-1/b(i_rep)) ;
                        0   ;
                        d/14-1/(64*b(i_rep))+1/40*(1-1/b(i_rep)) ;
                        1/(32*b(i_rep))-1/32    ;
                        0   ;
                        1/160   ;
                        0   ;
                        0   ;
                        0   ];
    % --------------------
    % reaction 4

    Matrix(:,4) = [     0   ;
                        0   ;
                        d/14-g(i_rep)*f/14    ;
                        (g(i_rep)-1)*1/40   ;
                        0   ;
                        (g(i_rep)*f-d)/14+(g(i_rep)-1)*1/40   ;
                        1/32-g(i_rep)/32    ;
                        g(i_rep)/160    ;
```

```
                    -1/160  ;
                    0   ;
                    0   ;
                    0   ];
% --------------------
% reaction 5

Matrix(:,5) = [     0   ;
                    -1/(64*c(i_rep))    ;
                    -d/14    ;
                    0   ;
                    1/24*(1-1/c(i_rep)) ;
                    d/14-1/(64*c(i_rep))+1/24*(1-1/c(i_rep)) ;
                    1/(32*c(i_rep))-1/32    ;
                    0   ;
                    1/160   ;
                    0   ;
                    0   ;
                    0   ];

% --------------------
% reaction 6

Matrix(:,6) = [     0   ;
                    0   ;
                    d/14-g(i_rep)*f/14    ;
                    0   ;
                    (g(i_rep)-1)*1/24   ;
                    (g(i_rep)*f-d)/14+(g(i_rep)-1)*1/24   ;
                    1/32-g(i_rep)/32    ;
                    g(i_rep)/160    ;
                    -1/160  ;
                    0   ;
                    0   ;
                    0   ];
% --------------------
% reaction 7

Matrix(:,7) = [     1/32-3/(28*h(i_rep))    ;
                    0   ;
                    -1/(14*h(i_rep))-d/14    ;
                    0   ;
                    1/(14*h(i_rep)) ;
                    1/(7*h(i_rep))+d/14  ;
                    -1/32   ;
                    0   ;
                    0   ;
                    1/160   ;
                    0   ;
                    0   ];
% --------------------
% reaction 8

Matrix(:,8) = [     g(i_rep)/32-1/32    ;
                    0   ;
                    d/14-g(i_rep)*f/14    ;
                    0   ;
                    0   ;
                    (g(i_rep)*f-d)/14 ;
                    1/32-g(i_rep)/32    ;
                    g(i_rep)/160    ;
```

```
                    0   ;
                    -1/160  ;
                    0   ;
                    0   ];
% --------------------
% reaction 9

Matrix(:,9) = [     1/32-1/(28*i(i_rep))    ;
                    0   ;
                    -d/14    ;
                    1/(14*i(i_rep)) ;
                    -1/(14*i(i_rep))    ;
                    d/14 ;
                    -1/32   ;
                    0   ;
                    0   ;
                    0   ;
                    1/160   ;
                    0   ];
% --------------------
% reaction 10

Matrix(:,10) = [    g(i_rep)/32-1/32    ;
                    0   ;
                    d/14-g(i_rep)*f/14    ;
                    0   ;
                    0   ;
                    (g(i_rep)*f-d)/14 ;
                    1/32-g(i_rep)/32    ;
                    g(i_rep)/160    ;
                    0   ;
                    0   ;
                    -1/160  ;
                    0   ];
% --------------------
% reaction 11

Matrix(:,11) = [    0   ;
                    0   ;
                    -1/(14*k(i_rep))-e/14    ;
                    0.192/(14*k(i_rep))+1/40    ;
                    -1.32/(14*k(i_rep)) ;
                    e/14+1/40-0.064/(7*k(i_rep)) ;
                    -1/36.4 ;
                    0   ;
                    0   ;
                    0   ;
                    0   ;
                    1/36.4  ];
% --------------------
% reaction 12

Matrix(:,12) = [    0   ;
                    0   ;
                    e/14-g(i_rep)*f/14    ;
                    0   ;
                    (g(i_rep)-1)*1/24   ;
                    (g(i_rep)*f-e)/14+(g(i_rep)-1)*1/24   ;
                    1/36.4-g(i_rep)/32  ;
                    g(i_rep)/160    ;
                    0   ;
```

```
                        0   ;
                        0   ;
                        -1/36.4 ];


    % ====================
    % Analyze matrix:
    rank(Matrix)
    [U,S,V]=svd(Matrix);
    BooleanInclude = diag(S)<=1e-10 ;
    disp(V(:,BooleanInclude))

end
```

S9 Structural observability with three types of HET

To test whether the lack of observability is not only practical but also structural, we performed Monte Carlo simulations: 10,000 simulations were done with uniformly and randomly distributed parameter values in a range of ± 50% of the default values. This was done with the following Matlab code:

```
clc
clear all
close all

disp('Defining default or sampled parameter values')

        a_def   =   0.630          ;    % YH,O2
        b_def   =   0.566          ;    % YH,NO3
        c_def   =   0.566          ;    % YH,NO2
        d_def   =   14/160         ;    % iN,AOB,NOB,HET
        e_def   =   0.15*14/36.4   ;    % iN,AMX
        f_def   =   0.035          ;    % iN,XI
        g_def   =   0.180          ;    % fxi
        h_def   =   0.210          ;    % YAOB
        i_def   =   0.046          ;    % YNOB
        k_def   =   0.150          ;    % YAMX

approach = 'randomized' ;
switch approach
    case 'default'
        a   =   0.630          ;    % YH,O2
        b   =   0.566          ;    % YH,NO3
        c   =   0.566          ;    % YH,NO2
        d   =   14/160         ;    % iN,AOB,NOB,HET
        e   =   0.15*14/36.4   ;    % iN,AMX
        f   =   0.035          ;    % iN,XI
        g   =   0.180          ;    % fxi
        h   =   0.210          ;    % YAOB
        i   =   0.046          ;    % YNOB
        k   =   0.150          ;    % YAMX
    case 'randomized'
        n_rand = 100 ; % number of repetitions

        a   =   rand(1,n_rand)*a_def+1/2*a_def ;
```

```
        b   =   rand(1,n_rand)*b_def+1/2*b_def ;
        c   =   rand(1,n_rand)*c_def+1/2*c_def ;
        d   =   14/160          ;    % iN,AOB,NOB,HET
        e   =   0.15*14/36.4    ;    % iN,AMX
        f   =   0.035           ;    % iN,XI
        g   =   rand(1,n_rand)*g_def+1/2*g_def ;
        h   =   rand(1,n_rand)*h_def+1/2*h_def ;
        i   =   rand(1,n_rand)*i_def+1/2*i_def ;
        k   =   rand(1,n_rand)*k_def+1/2*k_def ;

end

n_reagent = 14 ;
n_reactions = 12 ;
n_rep   = length(a) ; % length of vector a = number of repetitions

for i_rep=1:n_rep
    disp(['Repetition ' num2str(i_rep) ' of ' num2str(n_rep)])
    % ====================
    % Setup matrix:
    Matrix = nan(n_reagent,n_reactions) ;% initialize matrix

    % --------------------
    % reaction 1
    Matrix(:,1) = [     -1/(32*a(i_rep))+1/32 ;
                        -1/(64*a(i_rep)) ;
                        -d/14 ;
                        0 ;
                        0 ;
                        -1/(64*a(i_rep))+d/14 ;
                        2/(64*a(i_rep))-1/32 ;
                        0 ;
                        1/160 ;
                        0 ;
                        0 ;
                        0 ;
                        0 ;
                        0   ];
    % --------------------
    % reaction 2
    Matrix(:,2) = [     g(i_rep)/32-1/32    ;
                        0   ;
                        d/14-g(i_rep)*f/14  ;
                        0   ;
                        0   ;
                        (g(i_rep)*f-d)/14   ;
                        1/32-g(i_rep)/32    ;
                        g(i_rep)/160    ;
                        -1/160  ;
                        0   ;
                        0   ;
                        0   ;
                        0   ;
                        0   ];
    % --------------------
    % reaction 3

    Matrix(:,3) = [     0   ;
                        -1/(64*b(i_rep))  ;
                        -d/14    ;
                        1/40*(1-1/b(i_rep)) ;
```

```
                    0   ;
                    d/14-1/(64*b(i_rep))+1/40*(1-1/b(i_rep)) ;
                    1/(32*b(i_rep))-1/32    ;
                    0   ;
                    0   ;
                    1/160   ;
                    0   ;
                    0   ;
                    0   ;
                    0   ];
% --------------------
% reaction 4

Matrix(:,4) = [     0   ;
                    0   ;
                    d/14-g(i_rep)*f/14    ;
                    (g(i_rep)-1)*1/40   ;
                    0   ;
                    (g(i_rep)*f-d)/14+(g(i_rep)-1)*1/40   ;
                    1/32-g(i_rep)/32    ;
                    g(i_rep)/160    ;
                    0   ;
                    -1/160  ;
                    0   ;
                    0   ;
                    0   ;
                    0   ];
% --------------------
% reaction 5

Matrix(:,5) = [     0   ;
                    -1/(64*c(i_rep))    ;
                    -d/14    ;
                    0   ;
                    1/24*(1-1/c(i_rep)) ;
                    d/14-1/(64*c(i_rep))+1/24*(1-1/c(i_rep)) ;
                    1/(32*c(i_rep))-1/32    ;
                    0   ;
                    0   ;
                    0   ;
                    1/160   ;
                    0   ;
                    0   ;
                    0   ];

% --------------------
% reaction 6

Matrix(:,6) = [     0   ;
                    0   ;
                    d/14-g(i_rep)*f/14    ;
                    0   ;
                    (g(i_rep)-1)*1/24   ;
                    (g(i_rep)*f-d)/14+(g(i_rep)-1)*1/24   ;
                    1/32-g(i_rep)/32    ;
                    g(i_rep)/160    ;
                    0   ;
                    0   ;
                    -1/160  ;
                    0   ;
                    0   ;
```

```
                    0   ];
% --------------------
% reaction 7

Matrix(:,7) = [     1/32-3/(28*h(i_rep))    ;
                    0   ;
                    -1/(14*h(i_rep))-d/14    ;
                    0   ;
                    1/(14*h(i_rep)) ;
                    1/(7*h(i_rep))+d/14  ;
                    -1/32   ;
                    0   ;
                    0   ;
                    0   ;
                    0   ;
                    1/160   ;
                    0   ;
                    0   ];
% --------------------
% reaction 8

Matrix(:,8) = [     g(i_rep)/32-1/32    ;
                    0   ;
                    d/14-g(i_rep)*f/14    ;
                    0   ;
                    0   ;
                    (g(i_rep)*f-d)/14 ;
                    1/32-g(i_rep)/32    ;
                    g(i_rep)/160    ;
                    0   ;
                    0   ;
                    0   ;
                    -1/160  ;
                    0   ;
                    0   ];
% --------------------
% reaction 9

Matrix(:,9) = [     1/32-1/(28*i(i_rep))    ;
                    0   ;
                    -d/14    ;
                    1/(14*i(i_rep)) ;
                    -1/(14*i(i_rep))    ;
                    d/14 ;
                    -1/32   ;
                    0   ;
                    0   ;
                    0   ;
                    0   ;
                    0   ;
                    1/160   ;
                    0   ];
% --------------------
% reaction 10

Matrix(:,10) = [    g(i_rep)/32-1/32    ;
                    0   ;
                    d/14-g(i_rep)*f/14    ;
                    0   ;
                    0   ;
                    (g(i_rep)*f-d)/14 ;
```

```
                        1/32-g(i_rep)/32    ;
                        g(i_rep)/160    ;
                        0   ;
                        0   ;
                        0   ;
                        0   ;
                        -1/160  ;
                        0   ];
    % --------------------


    % reaction 11

    Matrix(:,11) = [    0   ;
                        0   ;
                        -1/(14*k(i_rep))-e/14    ;
                        0.192/(14*k(i_rep))+1/40    ;
                        -1.32/(14*k(i_rep)) ;
                        e/14+1/40-0.064/(7*k(i_rep)) ;
                        -1/36.4 ;
                        0   ;
                        0   ;
                        0   ;
                        0   ;
                        0   ;
                        0   ;
                        1/36.4  ];
    % --------------------
    % reaction 12

    Matrix(:,12) = [    0   ;
                        0   ;
                        e/14-g(i_rep)*f/14    ;
                        0   ;
                        (g(i_rep)-1)*1/24   ;
                        (g(i_rep)*f-e)/14+(g(i_rep)-1)*1/24   ;
                        1/36.4-g(i_rep)/32  ;
                        g(i_rep)/160    ;
                        0   ;
                        0   ;
                        0   ;
                        0   ;
                        0   ;
                        -1/36.4 ];


    % ====================
    % Analyze matrix:
    rank(Matrix)
    [U,S,V]=svd(Matrix);
    BooleanInclude = diag(S)<=1e-10 ;
    disp(V(:,BooleanInclude))

end
```