Electronic Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2012

Supplementary information for: A nanoliter-scale open chemical reactor

Jean-Christophe Galas, Anne-Marie Haghiri-Gosnet and André Estévez-Torres

Laboratoire de photonique et de nanostructures, CNRS,
route de Nozay, 91460 Marcoussis, France

October 15, 2012

Contents

1 Calibration of the pH inside the μCSTR

Figure 1 shows that the fluorescence of Oregon Green depends lineraly on pH in the range $3.7 - 6.0$. Figure 2 shows the effect of increasing concentration of the Edblom oscillator species on Oregon Green fluorescence. The presence of bromate has a negligible effect. In contrast, NaSO$_3$ and K$_4$[Fe(CN)$_6$] clearly reduce the dye fluorescence at constant pH. The highest concentrations used in this work were $[\mathrm{NaSO_3}] = 100$ mM and $[\mathrm{K_4[Fe(CN)_6]}] = 65$ mM, resulting in a decrease of fluorescence of 20%, which was compatible with a correct determination of the pH inside the microreactor.

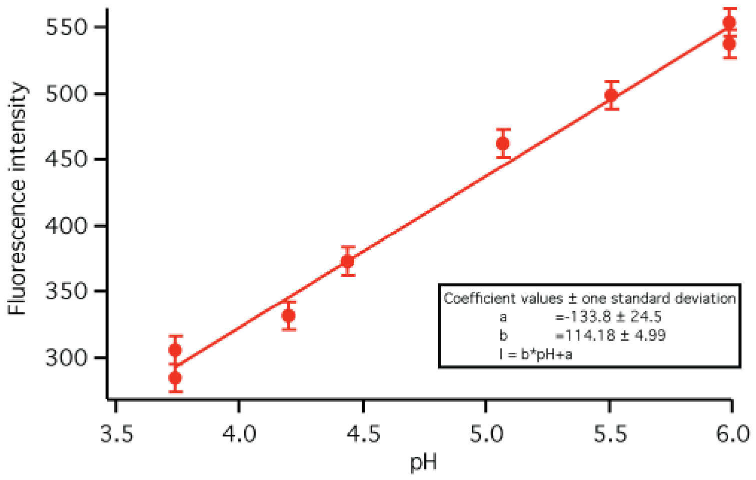

Figure S 1: Fluorescence intensity inside the μCSTR of a 10 μM solution of Oregon Green in buffers of different pH recorded with a 2.5X objective.

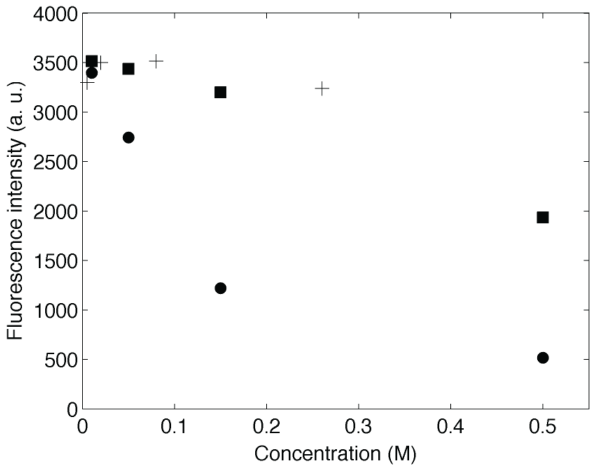

Figure S 2: Influence of reagent concentration on the maximum fluorescence intensity of Oregon Green 10 μM for KBrO$_3$ (crosses), NaSO$_3$ (squares), and K$_4$[Fe(CN)$_6$] (disks).

2 3D view of the μCSTR

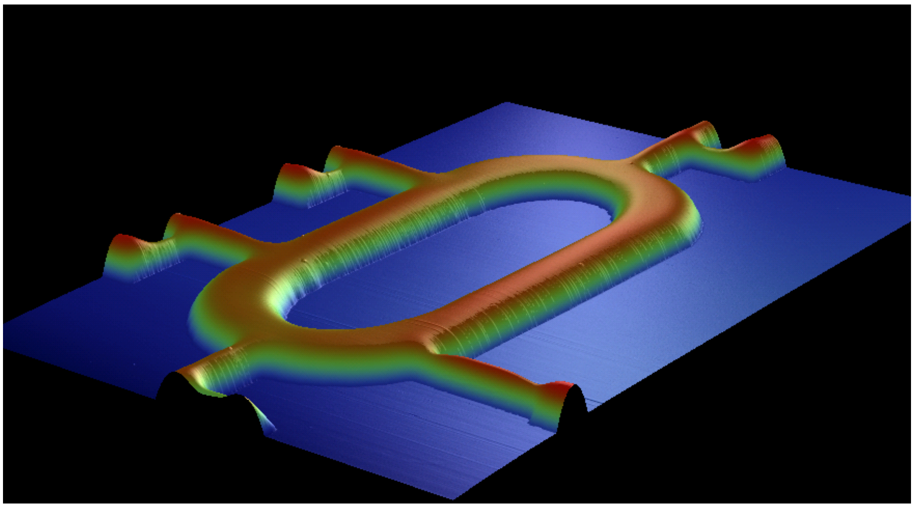

Figure S 3: 3D profile of the mould used to fabricate the μCSTR, acquired using a profilometer. The injection channels and the annular reactor are 100 and 200 μm wide, respectively. The height is color coded, the maximum height of the annular channel being 10 μm.

3 Reproducibility and influence of mixing

3.1 Determination of the mixing time

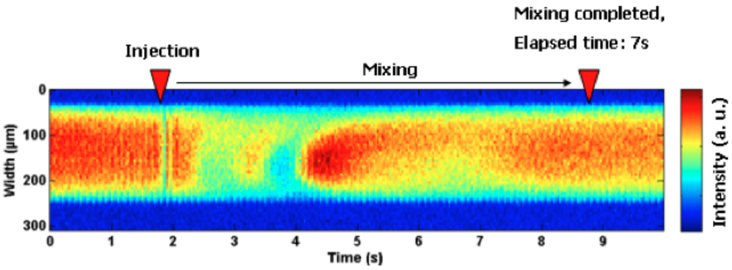

Figure S 4: Determination of the mixing time in the μCSTR. Fluorescent intensity profile across the width of the annular channel *vs* time (kymograph) during injection and mixing.

3.2 Influence of mixing on the behaviour of the Edblom oscillator in the μCSTR

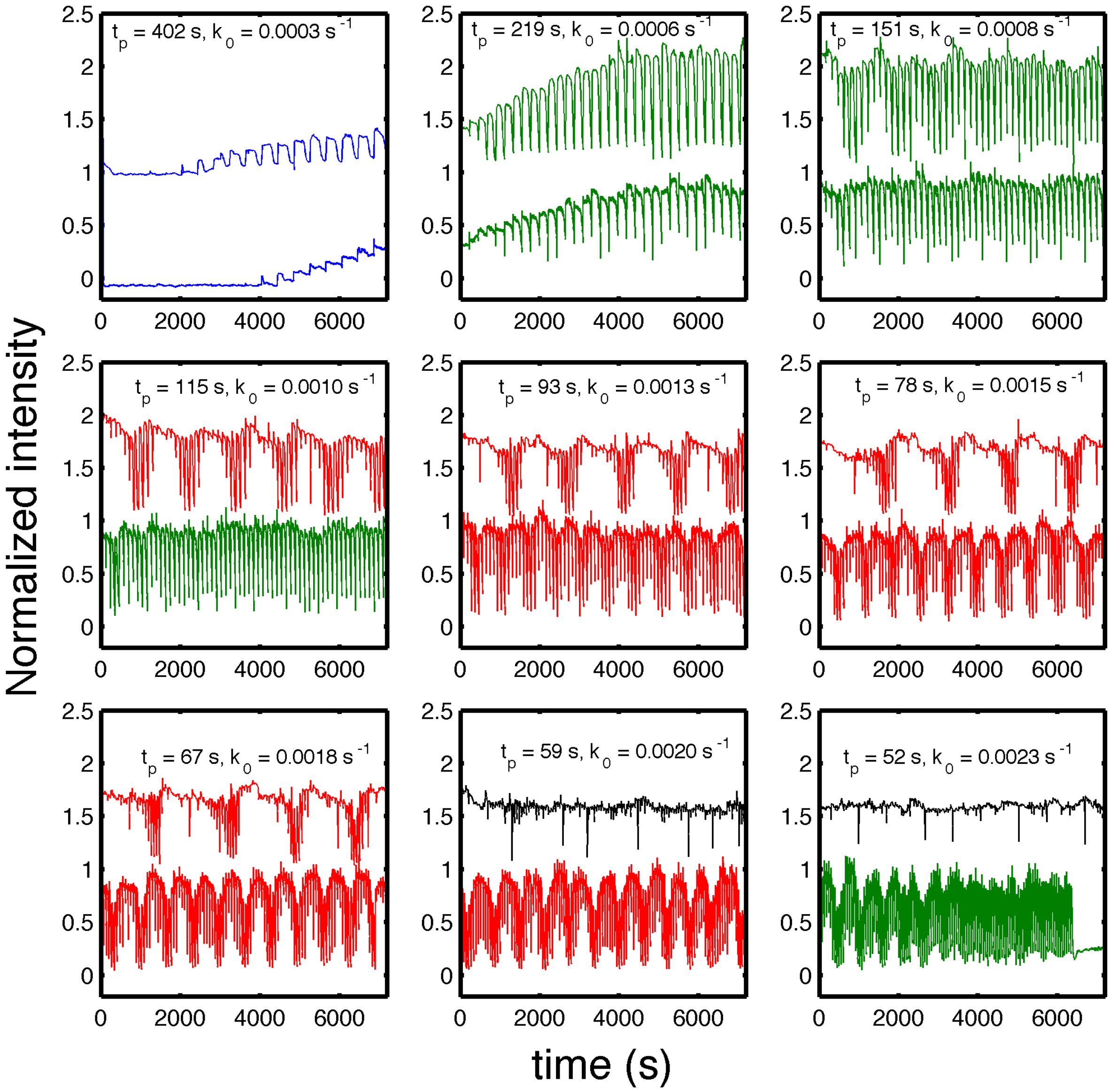

Figure S 5: Influence of mixing on the behavior of the Edblom oscillator. Normalized fluorescence intensities *vs* time for optimal mixing (lower curves) and non-optimal mixing (upper curves) at different injection periods, t_p, and their corresponding feeding rates, k_0. [Na_2SO_3] = 50 mM, [H_2SO_4] = 5 mM, [$K_4[Fe(CN)_6]$] = 20 mM and [$KBrO_3$] = 65 mM. Optimal mixing was completed in 7 s, while non-optimal mixing took 20 s. Color indicates the state of the oscillator: low pH (blue), forced oscillations (green), sustained oscillations (red), and high pH (black). Curves are shifted vertically for clarity.

4 *p*H of the bromate channel with and without purging

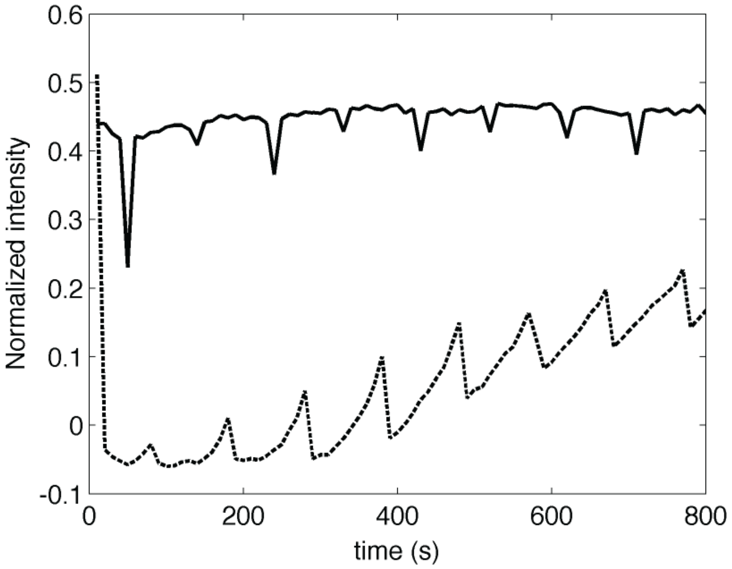

Figure S 6: Effect of the purging on the *p*H of the injected solution of $KBrO_3$ 65 mM. The *p*H is given by the fluorescence of Oregon Green 10 μM. Purging was on (solid line) or off (dashed line). The intensity was measured inside the injection channel, between valves 1 and 2.

5 Experimental *p*H *vs* time plots

In this section are displayed, in large format, the normalized fluorescence intensity *vs* time plots represented in the phase diagram in Fig. 5A in the Main Text. The following conditions are common to all graphs: 5 mM H_2SO_4, 20 mM $K_4[Fe(CN)_6]$, and 65 mM $KBrO_3$. The graphs are color-coded as in the Main Text: low *p*H (black), forced oscillations (green), sustained oscillations (red), and high *p*H (black).

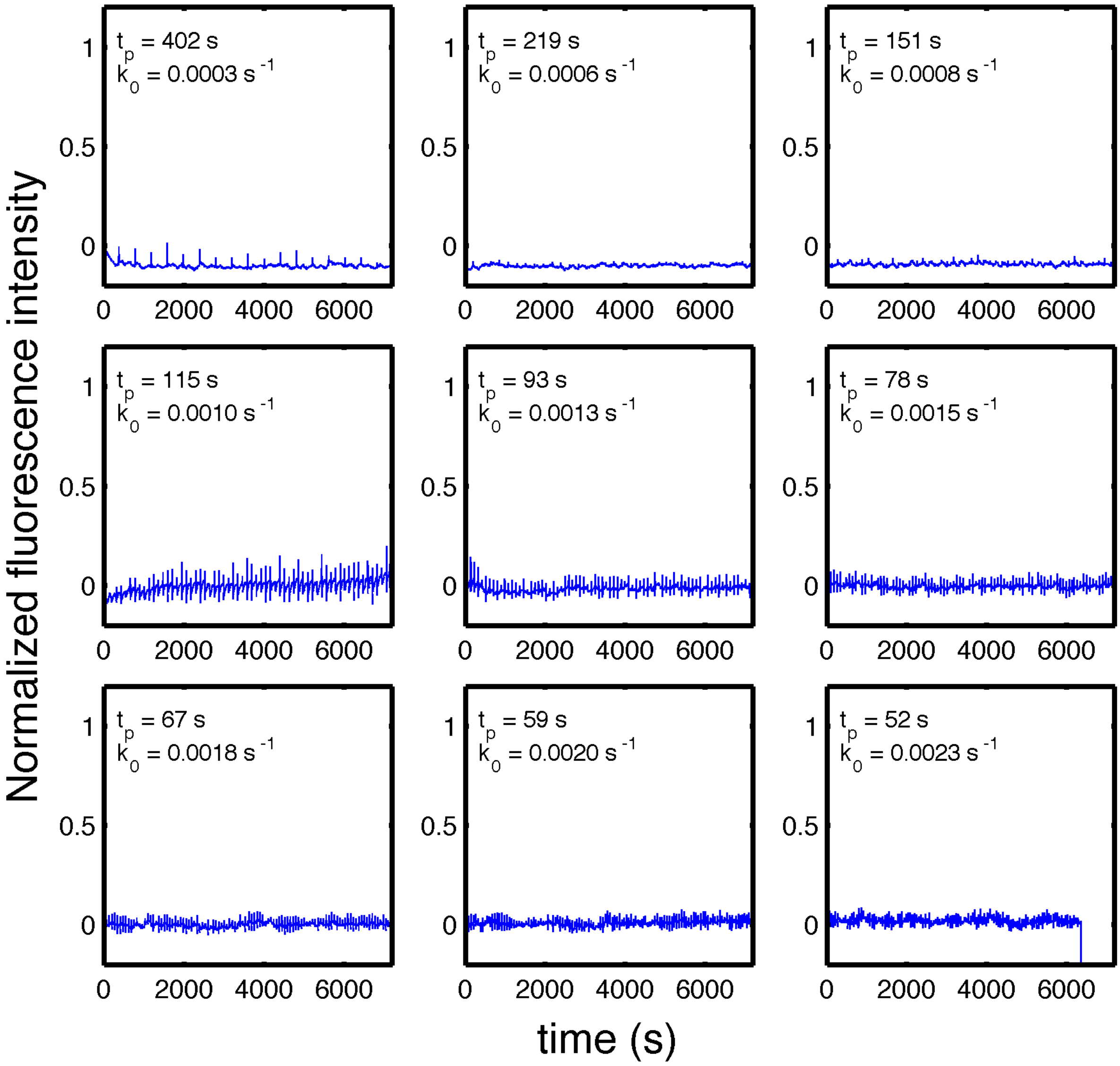

Figure S 7: Experimental normalized average fluorescence intensity for different injection periods, t_p, and their corresponding feeding rates, k_0, when the concentration of injected Na_2SO_3 was 25 mM.

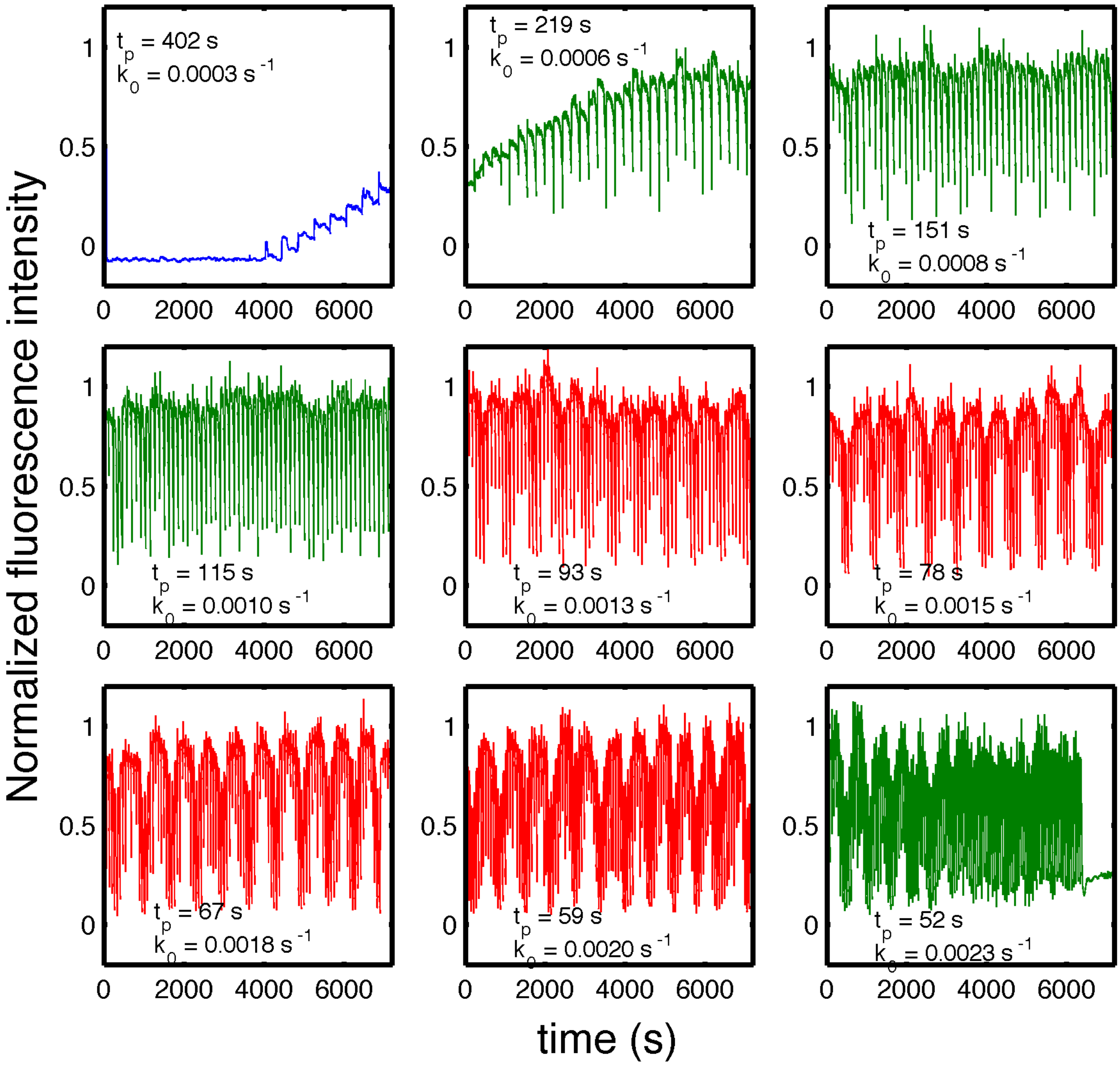

Figure S 8: Experimental normalized average fluorescence intensity for different injection periods, t_p, and their corresponding feeding rates, k_0, when the concentration of injected Na_2SO_3 was 50 mM.

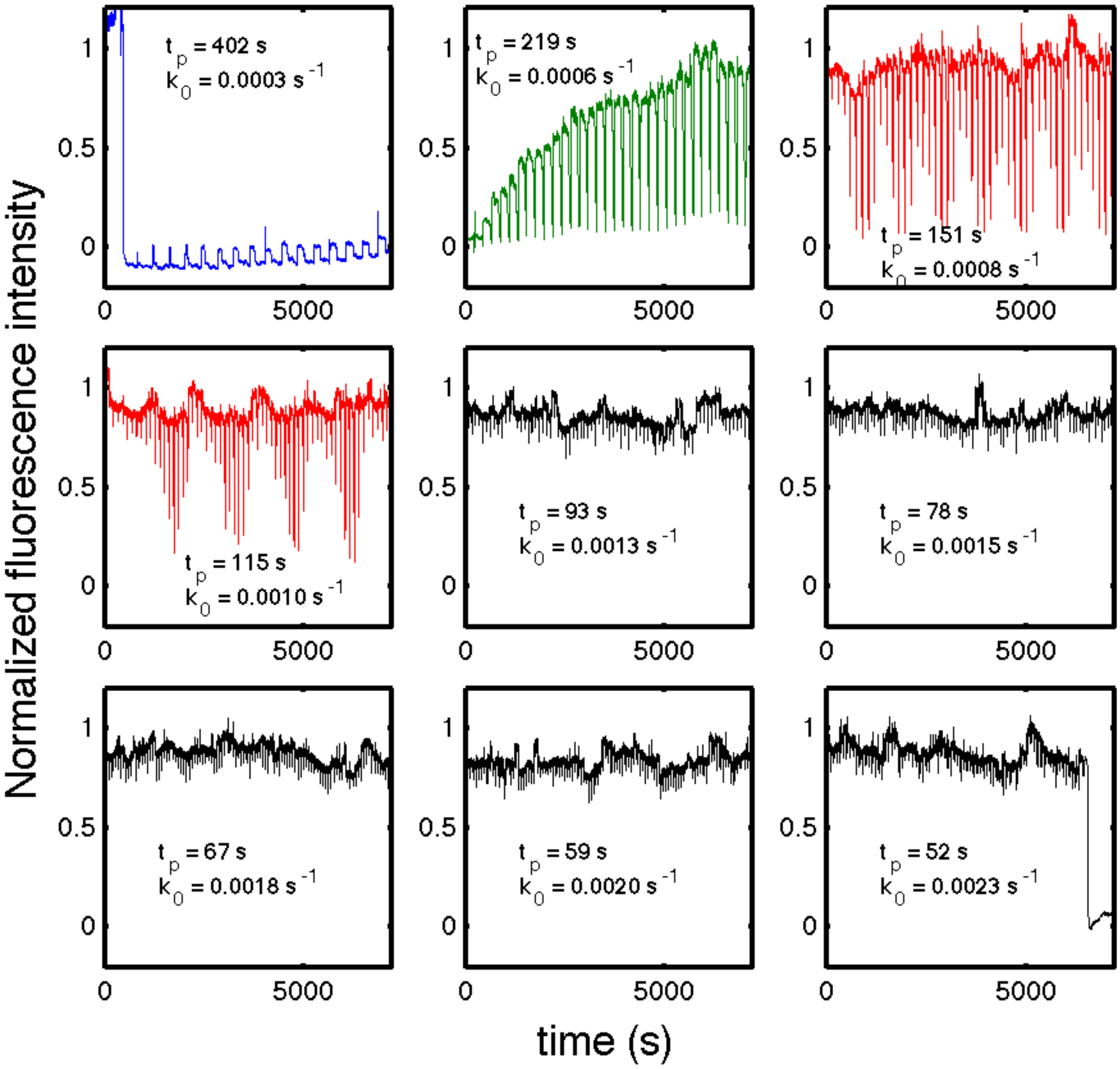

Figure S 9: Experimental normalized average fluorescence intensity for different injection periods, t_p, and their corresponding feeding rates, k_0, when the concentration of injected Na_2SO_3 was 75 mM.

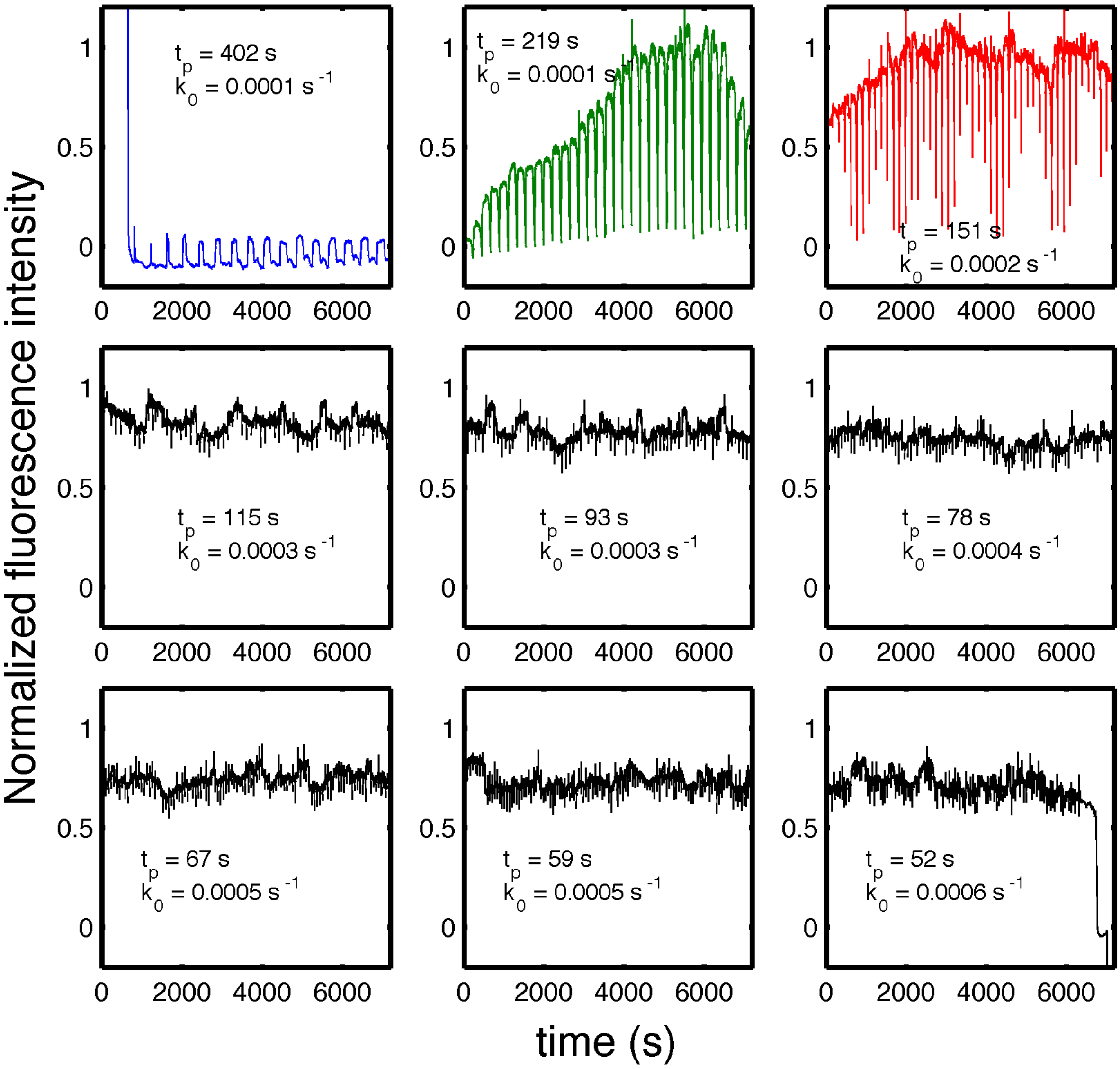

Figure S 10: Experimental normalized average fluorescence intensity for different injection periods, t_p, and their corresponding feeding rates, k_0, when the concentration of injected Na_2SO_3 was 100 mM.

6 Simulations

6.1 Model of the Edblom oscillator

Edblom et al[1] demonstrated that the main features of the oscillator studied in this work were described with the following set of reactions

$$
\begin{align}
BrO_3^- + HSO_3^- &\xrightarrow{k_1} HBrO_2 + SO_4^{2-} & k_1 = 8 \times 10^{-2} M^{-1} s^{-1} \tag{1}\\
HBrO_2 + Br^- + H^+ &\xrightarrow{k_2} 2HOBr & k_2 = 9.5 \times 10^{6} M^{-2} s^{-1} \tag{2}\\
HOBr + Br^- + H^+ &\xrightarrow{k_3} Br_2 + H_2O & k_3 = 1.6 \times 10^{1}0 M^{-2} s^{-1} \tag{3}\\
Br_2 + H_2O &\xrightarrow{k_4} HOBr + Br^- + H^+ & k_4 = 1.1 \times 10^{1} s^{-1} \tag{4}\\
2HBrO_2 &\xrightarrow{k_5} BrO_3^- + HOBr + H^+ & k_5 = 3 \times 10^{3} M^{-1} s^{-1} \tag{5}\\
Br_2 + HSO_3^- + H_2O &\xrightarrow{k_6} 2Br^- + SO_4^{2-} + 3H^+ & k_6 = 1.0 \times 10^{6} M^{-1} s^{-1} \tag{6}\\
H^+ + SO_3^{2-} &\xrightarrow{k_7} HSO_3^- & k_7 = 5.0 \times 10^{10} M^{-1} s^{-1} \tag{7}\\
HSO_3^- &\xrightarrow{k_8} H^+ + SO_3^{2-} & k_8 = 3 \times 10^{3} s^{-1} \tag{8}\\
BrO_3^- + 2Fe(CN)_6^{4-} + 3H^+ &= HBrO_2 + 2Fe(CN)_6^{3-} + H_2O & \tag{9}\\
BrO_3^- + Fe(CN)_6^{4-} + H^+ &\xrightarrow{k_9} HBrO_2 + Fe(CN)_6^{3-} + H_2O & k_9 = 3.2 \times 10^{1} M^{-2} s^{-1}, \tag{10}
\end{align}
$$

where (9) defines the stœchiometry of the oxydation of $Fe(CN)_6^{4-}$ and (10) accounts for the mechanism from which the rate is computed. The rates for reactions 1 to 9 are

$$
\begin{align}
r_1 &= k_1[BrO_3^-][HSO_3^-] \tag{11}\\
r_2 &= k_2[HBrO_2][\ Br^-][\ H^+] \tag{12}\\
r_3 &= k_3[HOBr][Br^-][H^+] \tag{13}\\
r_4 &= k_4[Br_2] \tag{14}\\
r_5 &= k_5[HBrO_2]^2 \tag{15}\\
r_6 &= k_6[Br_2][HSO_3^-] \tag{16}\\
r_7 &= k_7[H^+][\ SO_3^{2-}] \tag{17}\\
r_8 &= k_8[HSO_3^-] \tag{18}\\
r_9 &= k_9[BrO_3^-][Fe(CN)_6^{4-}][\ H^+]. \tag{19}
\end{align}
$$

The differential equations describing the temporal evolution of the species involved in the Edblom oscillator in a CSTR are thus

---

[1] E. C. Edblom, Y. Luo, M. Orban, K. Kustin and I. R. Epstein, *J. Phys. Chem.*, **1989**, 93, 2722-2727.

$$\begin{aligned}
\frac{d[\mathrm{Br}^-]}{dt} &= -r_2 - r_3 + r_4 + 2r_6 - k_0[\mathrm{Br}^-] && (20)\\
\frac{d[\mathrm{Br}_2]}{dt} &= r_3 - r_4 - r_6 - k_0[\mathrm{Br}_2] && (21)\\
\frac{d[\mathrm{HOBr}]}{dt} &= 2r_2 - r_3 + r_4 + r_5 - k_0[\mathrm{HOBr}] && (22)\\
\frac{d[\mathrm{HBrO}_2]}{dt} &= r_1 - r_2 - 2r_5 + r_9 - k_0[\mathrm{HBrO}_2] && (23)\\
\frac{d[\mathrm{BrO}_3^-]}{dt} &= -r_1 + r_5 - r_9 + k_0\left([\mathrm{KBrO}_3]^{IN} - [\ \mathrm{BrO}_3^-]\right) && (24)\\
\frac{d[\mathrm{SO}_3^{2-}]}{dt} &= -r_7 + r_8 + k_0\left([\mathrm{Na}_2\mathrm{SO}_3]^{IN} - [\ \mathrm{SO}_3^{2-}]\right) && (25)\\
\frac{d[\mathrm{HSO}_3^-]}{dt} &= -r_1 - r_6 + r_7 - r_8 - k_0[\mathrm{HSO}_3^-] && (26)\\
\frac{d[\mathrm{Fe(CN)}_6^{4-}]}{dt} &= -2r_9 + k_0\left([\mathrm{K}_4\mathrm{Fe(CN)}_6]^{IN} - [\mathrm{Fe(CN)}_6^{4-}]\right) && (27)\\
\frac{d[\mathrm{Fe(CN)}_6^{3-}]}{dt} &= 2r_9 - k_0[\mathrm{Fe(CN)}_6^{3-}] && (28)\\
\frac{d[\mathrm{H}^+]}{dt} &= -r_2 - r_3 + r_4 + r_5 + 3r_6 - r_7 + r_8 - 3r_9 + r_{10} + k_0\left([\mathrm{H}^+]^{IN} - [\mathrm{H}^+]\right), && (29)
\end{aligned}$$

where k_0 is the feeding rate, $[\mathrm{I}]^{IN}$ is the concentration of species I being injected in the CSTR. $[\mathrm{H}^+]$ is being injected as $\mathrm{H_2SO_4}$, an acid with $p\mathrm{K}_{a,1} = -3$ and $p\mathrm{K}_{a,2} = 2$. The lowest pH of the oscillator being about 2, we can consider that $\mathrm{H_2SO_4}$ exists in solution only as species $\mathrm{HSO_4^-}$ and $\mathrm{SO_4^{2-}}$. At a given time, for a proton concentration in the reactor $[\mathrm{H}^+]$, an injection of $[\mathrm{H_2SO_4}]^{IN}$ results in an injected concentration of protons given by

$$[\mathrm{H}^+]^{IN} = [\mathrm{H_2SO_4}]^{IN}\left(1 + \frac{1}{(1 + [\mathrm{H}^+]/K_{a,2})}\right). \qquad (30)$$

We consider here that the protonation equilibra of $\mathrm{H_2SO_4}$ are infinitely fast compared with reactions 1 to 9. We do neglect the contribution of protons coming from a remaining quantity of $\mathrm{HSO_4^-}$ at low pH that would liberate protons when the pH rises in the reactor.

Equations (20) to (29) take the form of Equation 1 in the Main Text, that we reproduce here for clarity,

$$\frac{dC_i(t)}{dt} = f_i\left(k_j, C_l(t)\right) + k_0\left(C_i^{IN} - C_i(t)\right). \qquad (31)$$

The system of equations (20) to (29) can be simulated in a macroscopic CSTR as is. In contrast, to simulate these equations in a μCSTR we need to take into account the digital character of the injection. For each species i, (31) was rewritten as

$$\frac{dC_i(t)}{dt} = \chi(1-H)f_i\left(k_j, C_l(t)\right) + H\frac{k_0}{t_{inj}}\left(C_i^{IN} - C_i(t)\right), \qquad (32)$$

where H is a function that takes into account the digital character of the injection. It is equal to 1 during the injection phase, that lasted $t_{inj} = 5$ s in our simulations, and equal to 0 otherwise. Note that this implementation assumes perfect mixing as soon as the injection phase ends. χ is a coupling parameter that we have added to remove a divergence due to the sharp change in the derivative introduced by the discontinuous function H. After an injection, the concentrations of the different species may change quite abruptly and make the computed derivative very large, thus introducing, for instance, negative values of the concentrations. To smooth this effect, the contribution of $f_i\left(k_j, C_l(t)\right)$ to (32) was switched on linearly after an injection, with $\chi = t/t_{swicth}$, and $t_{switch} = 10$ s.

Differential equations were solved in Matlab using `ode23s` solver for stiff problems. It is a one-step solver that uses a modified Rosenbrock formula of order 2.

6.2 Comparison experiments/simulations in a closed reactor

The experimental and simulated phase diagram of the Edblom oscillator in the same conditions (5 mM H_2SO_4, 20 mM $K_4[Fe(CN)_6]$, and 65 mM $KBrO_3$), displayed in Figure 11, are significantly different. Several reasons can be invoked to explain this discrepacy: i) mixing in the experiments takes 7 s, while in the simulations it is instantaneous, ii) injection and mixing are considered independent processes in the simulations, but they are simultaneous, and coupled to spatial transport, in the experiments, iii) the high surface to volume ratio of the μCSTR is not taken into account in the simulations, notably the evaporation of important intermediates such as Br_2 through the porous walls of PDMS, and iv) the influence of the fluorescent dye is disregarded in the simulations. Instead of studying each of these processes in detail we decided to take a phenomenological description. To do so we performed a series of experiments, and the corresponding simulations, using the μCSTR as a closed reactor for different concentrations of H_2SO_4 and Na_2SO_3. In these experiments the four reagents were injected 50 consecutive times into the annular reactor and mixed to create a reproducible initial condition corresponding to 1/4 of the volume of the reactor for each injected solution. The injection valves were closed, mixing remained active and fluorescence was recorded. The results are displayed in Figure 12. For all the experiments the fluorescence intensity (the pH) stays high and stable until it abruptly drops (within 30 s) and remains low. The time at which the fluorescence (pH) drops depends on $[Na_2SO_3]$ and $[H_2SO_4]$. The behavior of the Edblom oscillator at 5 mM H_2SO_4 in a closed reactor is significantly different for the experiments and the simulations. In contrast, results are similar when we compare simulations and experiments at 10 and 5 mM H_2SO_4 respectively. We argue that increasing the concentration of H_2SO_4 in the simulations to 10 mM phenomenologically takes into account the factors evoked above that are not incroporated in the simulations. We thus compare, in the Figure 5 of the Main Text, the experimental phase diagram at 5 mM H_2SO_4 with the simulated phase diagram at 10 mM H_2SO_4 .

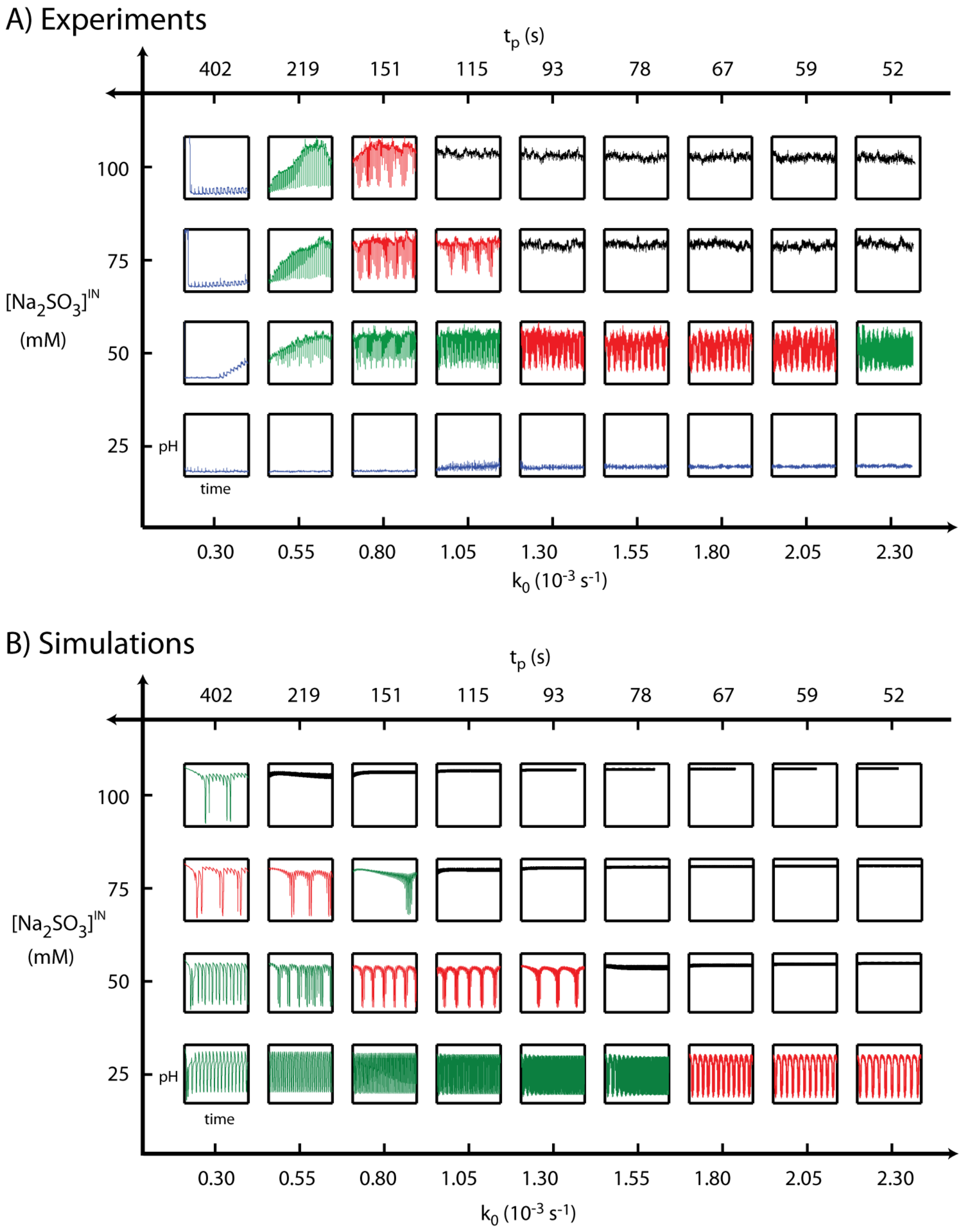

Figure S 11: Experimental (top) and simulated (bottom) phase diagram of the Edblom oscillator in the μCSTR for different Na_2SO_3 input concentrations and at different feeding rates k_0 (and their corresponding injection periods t_p). Each square represents pH *vs.* time during a 2 h experiment. Time traces are colour-coded according to the observed steady state: low pH (blue), forced oscillations (green), sustained oscillations (red), and high pH (black). In contrast with Fig. 5 in the Main Text, here [H2SO4] $=$ 5 mM both for the experiment and for the simulations. The remaining injected concentrations are $K_4[Fe(CN)_6]$ 20 mM and $KBrO_3$ 65 mM.

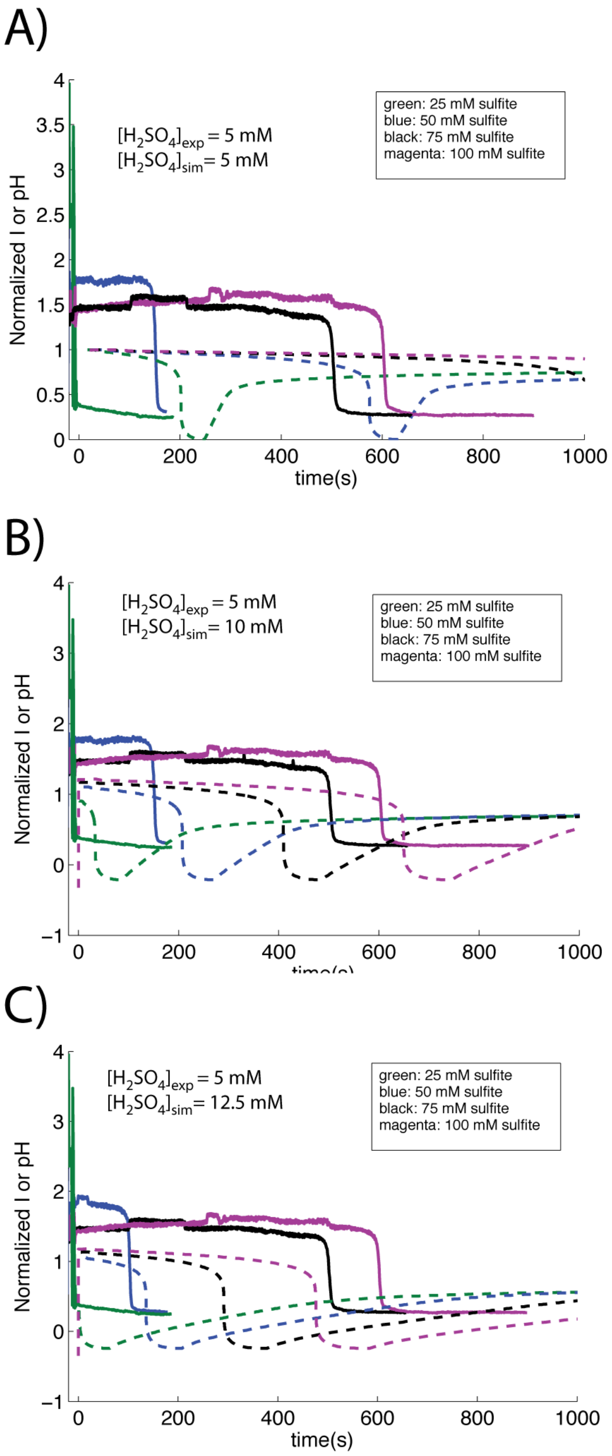

Figure S 12: Normalized intensity (experiments, solid lines) or normalized pH (simulations, dashed lines) *vs* time after mixing in a closed reactor for different concentrations of Na_2SO_3 (25 mM, green, 50 mM, blue, 75 mM, black, and 100 mM, magenta) and H_2SO, and for 20 mM $K_4[Fe(CN)_6]$ and 65 mM $KBrO_3$.

6.3 Simulated pH *vs* time plots

In this subsection are displayed, in large format, the normalized pH *vs* time plots represented in the phase diagram in Fig. 5B in the Main Text. The following conditions are common to all graphs: $[H_2SO_4] = 10$ mM, $K_4[Fe(CN)_6]$ 20 mM and $KBrO_3$. 65 mM. The graphs are color-coded as in the Main Text: low pH (black), forced oscillations (green), sustained oscillations (red), high pH (black).

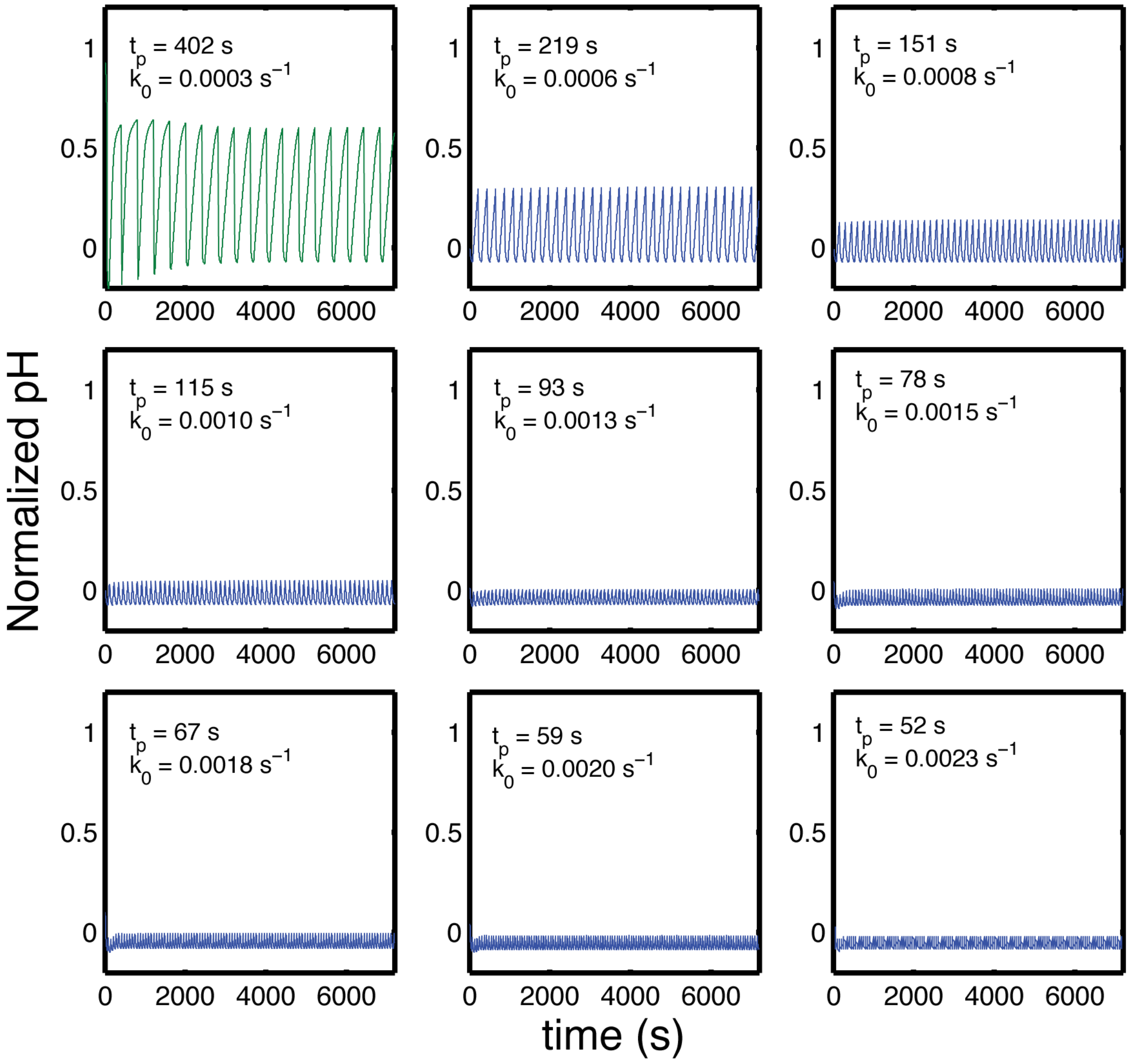

Figure S 13: Simulated normalized average fluorescence intensity for different injection periods, t_p, and their corresponding feeding rates, k_0 when the concentration of injected Na_2SO_3 was 25 mM.

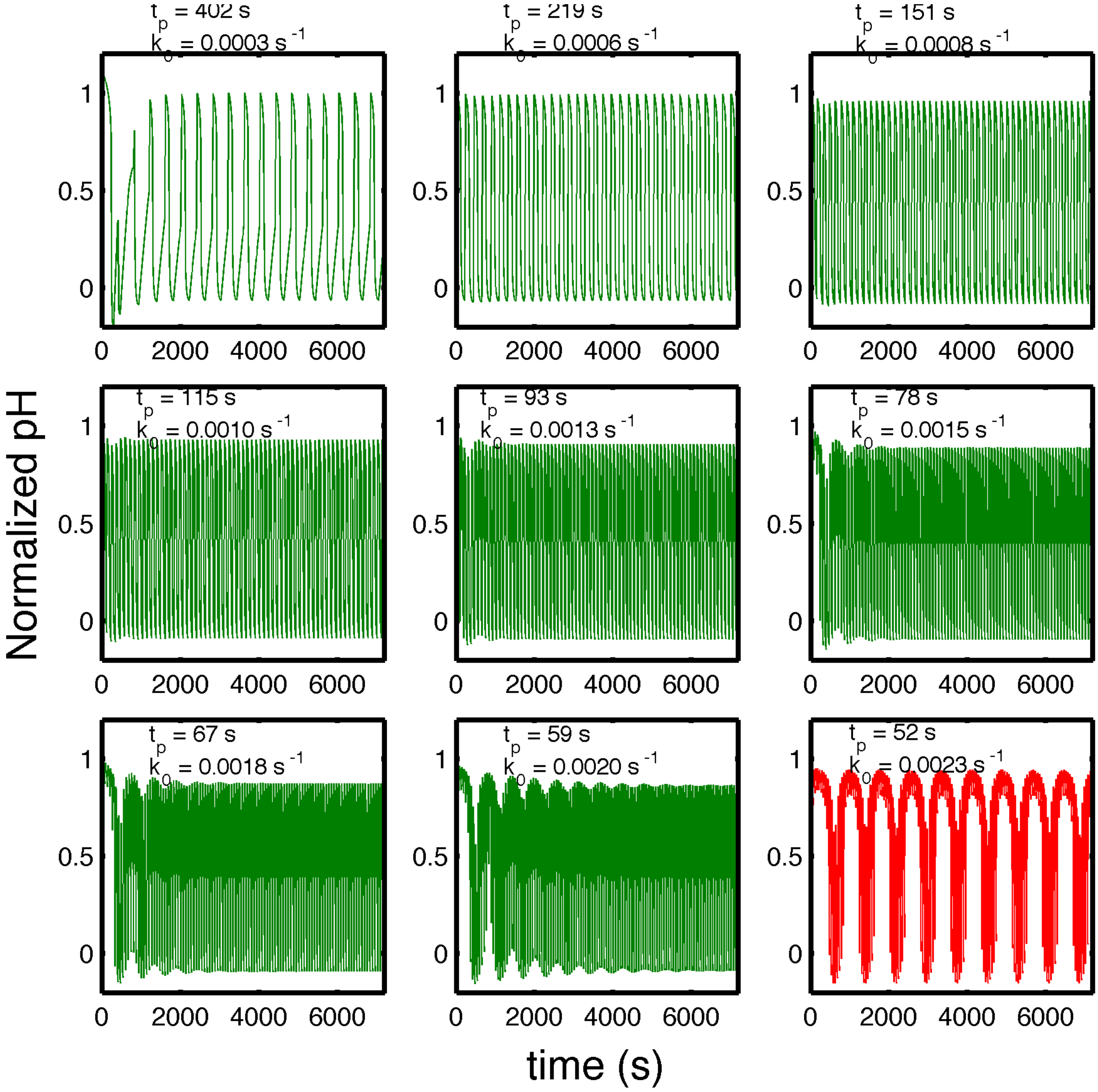

Figure S 14: Simulated normalized average fluorescence intensity for different injection periods, t_p, and their corresponding feeding rates, k_0 when the concentration of injected Na_2SO_3 was 50 mM.

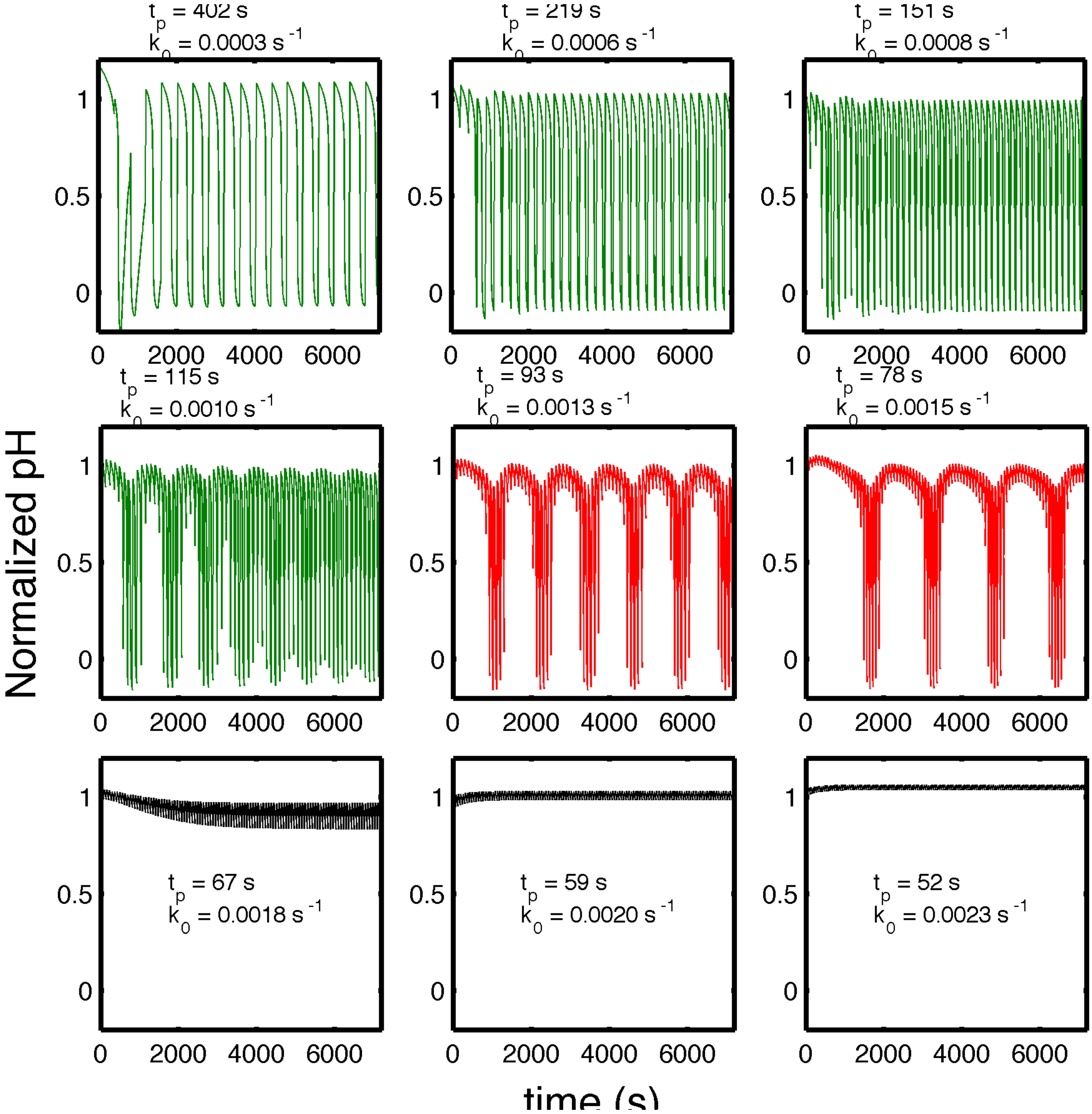

Figure S 15: Simulated normalized average fluorescence intensity for different injection periods, t_p, and their corresponding feeding rates, k_0 when the concentration of injected Na_2SO_3 was 75 mM.

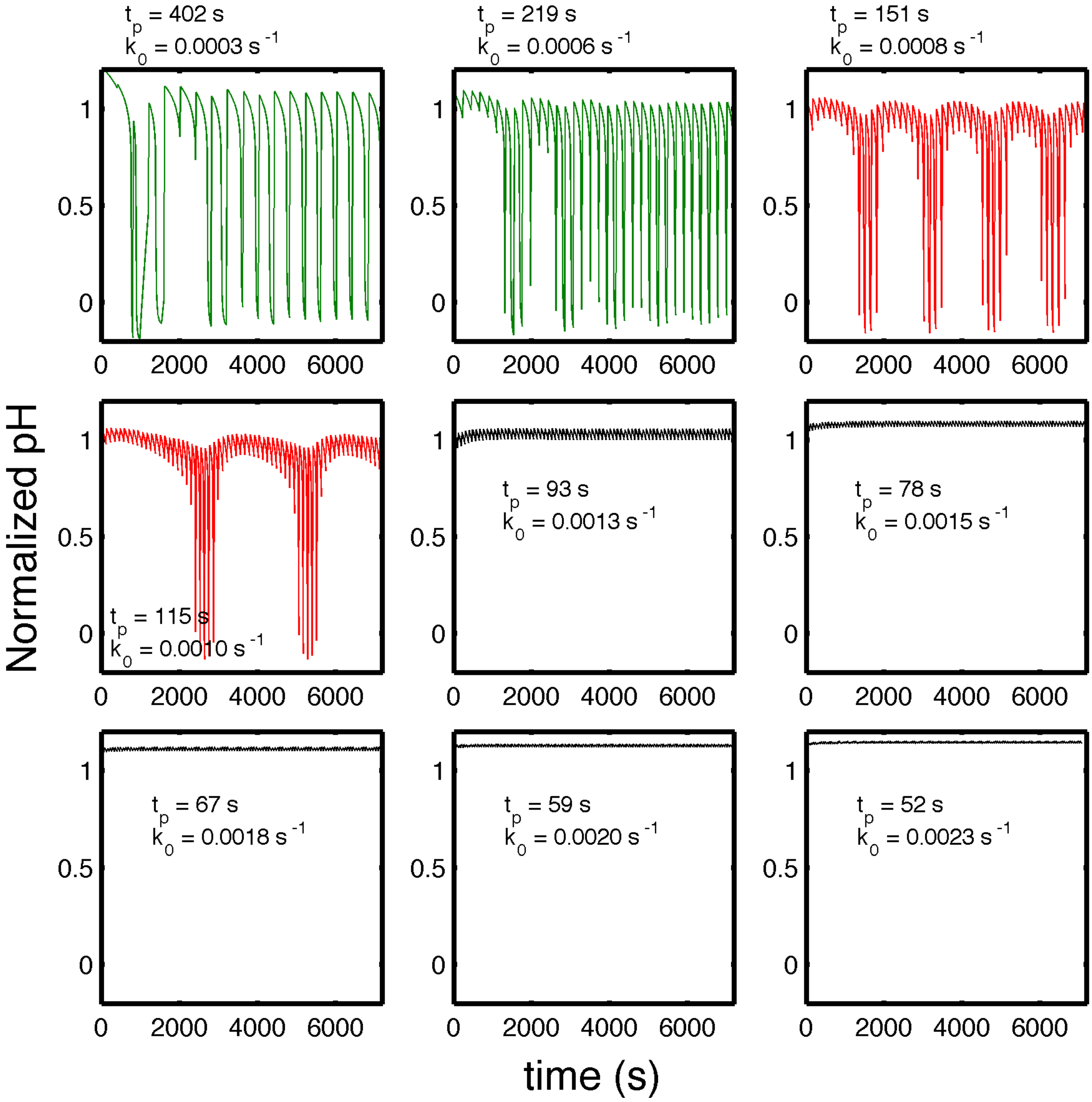

Figure S 16: Simulated normalized average fluorescence intensity for different injection periods, t_p, and their corresponding feeding rates, k_0 when the concentration of injected Na_2SO_3 was 100 mM.

7 Attached files

7.1 Video of the Edblom oscillator in the μCSTR

A video showing two periods of the Edblom oscillator for 20 mM $K_4[Fe(CN)_6]$, 65 mM $KBrO_3$, 75 mM Na_2SO_3, and 5 mM H_2SO_4 being injected, with a period $t_p = 115$ s, in the μCSTR and observed with 10μM Oregon green under a fluorescence microscope is available for download.

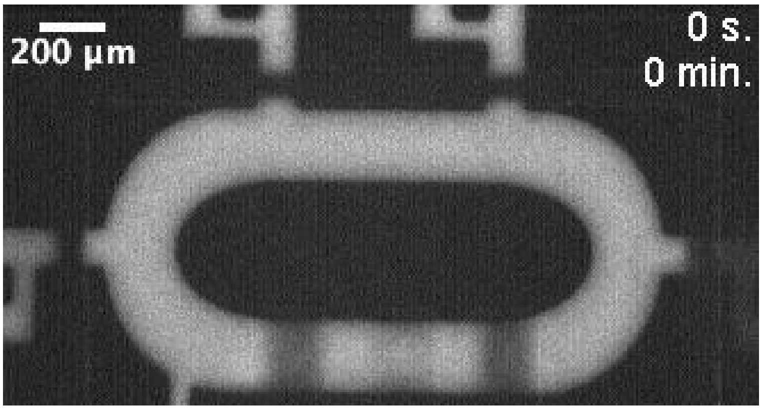

Figure S 17: First frame of the attached video.

7.2 Matlab code for simulations

The Matlab code for the simulations described in section 6 is available for download. It is constituted of the following functions:

```
function do_solve_Edblom_kinetics_for_several_input
% Calls the solver plot_solutions_parameter_Edblom_kinetics for
%different concentrations of sulfite and sulfuric acid

function plot_solutions_parameter_Edblom_kinetics(varargin)
%Calls the ODE solver function solve_kinetics_with_parameters_CSTR_Edblom( pumpingPeriod(i),
%fId ) for different values of pumpingPeriod.  Then makes plots with the
%results from that function.  'param' and 'out' are the principal
%structures, with the parameters and the output values
%If varargin > 0 the first one is the input sulfite conc and the second one
%the input sulfuric acid conc

function [t y] = solve_kinetics_with_parameters_CSTR_Edblom(param, x0, iCondition)
% This function implements the kinetic model for the pH oscillator in a CSTR described
%in Edblom, JPhysChem 1989, Table 1
%in a continuous or digital CSTR modeled through k0
%Input is a structure param with the rate constants and the file id fId to write
%results in a text file, x0 is a vector containing the initial conditions,
%'iCondition' is a counter for an experiment with different k0
%OUPUT are vectors time 't' and solution of the
%ODE 'y' as well as the structure param with normalized rates sigmai

function param = create_parameter_struct_Edblom()
% Initializes the structure 'param' which stocks all the relevant parameters
%set by the user to solve the ODEs

function out = create_output_struct_Edblom()
% Initializes the structure 'out' which stocks all the quantities
%computed during the simulation
```

```
function jacob = edblom_jacobian(x, rate, H, chemCoupling, k0Comp)
% Claculates the Jacobian matrix of the Edblom ODE system
% written in function solve_kinetics_with_parameters_CSTR_Edblom
% INPUT: 'x' vector of concentrations, 'rate' structure of rates
% 'H' equal to 0 or 1 depending on injection conditions, 'cte'
% equal to 1 or 2 for digital or continuous injection,
% 'k0Comp' computed k0 value

function plot_Edblom_data(out, param)
% Plots outputs from function plot_solutions_parameter_Edblom_kinetics
% out and param are structures defined in that function
```