A CHEMICAL OSCILLATOR IN A NANO-LITER SCALE MICROFLUIDIC OPEN REACTOR

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ABSTRACT

Well-mixed open chemical reactors, called continuous stirred tank reactors (CSTR), have been instrumental for investigating the dynamics of out-of-equilibrium chemical processes, such as oscillations, bistability, and chaos. Here, we introduce a microfuidic CSTR, called μCSTR, that reduces reagent consumption by six orders of magnitude. The effciency of the μCSTR is experimentally characterized using a bromate, sulfite, ferrocyanide pH oscillator. Simulations accounting for the digital injection process are in agreement with experimental results. The low consumption of the μCSTR will be advantageous for investigating out-of-equilibrium dynamics of chemical processes involving precious biomolecules. These studies have been scarce so far, probably for a lack of technology.

KEYWORDS: reactor, microfluidics, pH oscillator

INTRODUCTION

Living cells are highly dynamical systems involving complex network of interacting components. Among many different network behaviors, oscillations play an important role in cells: for example circadian clocks. Beyond identifying and linking together the components of those networks, a growing interest exists in engineering such complex dynamic reaction networks in vitro. Very recently, Rondelez and Winfree independently synthesized the first engineered bio-chemical oscillator in a closed reactor [1, 2]. This is the first step to the development of complex engineered bio-chemical operators.

Continuous stirred tank reactors (CSTR) i.e. perfectly mixed and continuously fed reactors have been extensively used in the 80s and 90s to study the behavior of out-of-equilibrium inorganic chemical systems, especially oscillatory ones (BZ reaction, pH oscillators…). In its standard version, the reactor has a volume of several milliliters, which precludes the study of oscillations involving precious samples, such as DNA and proteins [3]. For this reason, here we introduce the first microfluidic version of a CSTR (μCSTR), compatible with available volumes of DNA and protein solutions.

EXPERIMENTAL

The μCSTR is fabricated using the well known PDMS multilayer soft-lithography technique. Fluidic channels are 10μm high, control channels are 50μm. As depicted figure 1, the whole geometry consists of four inlets (with recirculation channels for solution renewing) and one outlet connected to the 5nL annular reactor. Three in-line valves (in red) are designed on each entrance. They are operated as peristaltic pump for digital reactor feeding. A second peristaltic pump (in blue) is used for mixing. Both are controlled via a custom-made controller. Injection of reactants into the μCSTR is digital. A single injection represents 8% of its volume and complete mixing occurs in 10s.

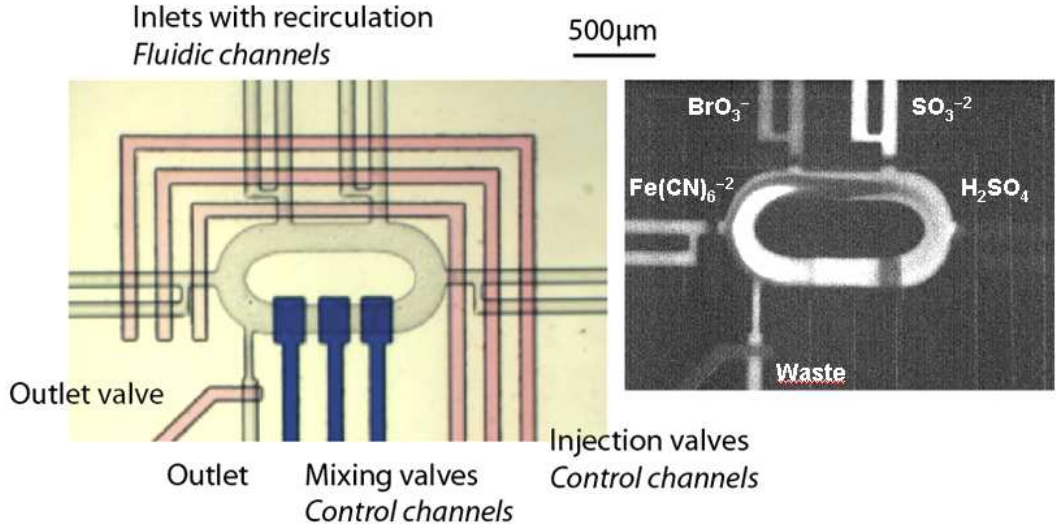

Figure 1. Pictures of microscopic CSTR, with recirculation inlets, outlet and integrated peristaltic pumps for automated digital reactor feeding (red) and mixing (blue). Right: Fluorescence image of Bromate-Sulfite-Ferrocyanide pH oscillator captured during mixing step.

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RESULTS AND DISCUSSION

We carry out in this micro-device the well described Bromate-Sulfite-Ferrocyanide pH oscillator [4]. We take advantage of the pH dependant fluorescence of Oregon Green 488 dye to visualize by microscopy the pH inside the microfluidic reactor. Varying concentrations and residence time τ =1/Ko (ko is the flow rate, it is tuned changing the frequency of repeated injections), we obtained different reaction dynamics ie low pH, oscillations, or high pH, and built the phase diagram (figure 2 left and 3 A). Note that we observed low pH, high pH but also forced oscillations (in green) due to digital injections and sustained oscillations (in red) due to chemistry. Except for forced oscillations, the results are comparable with those obtained using a macroscopic CSTR.

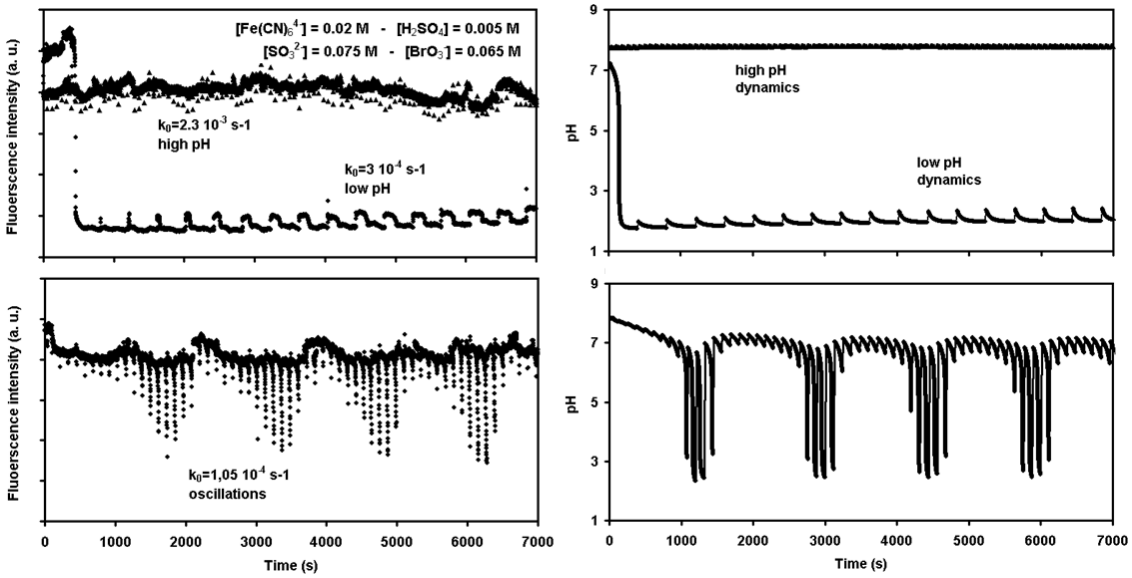
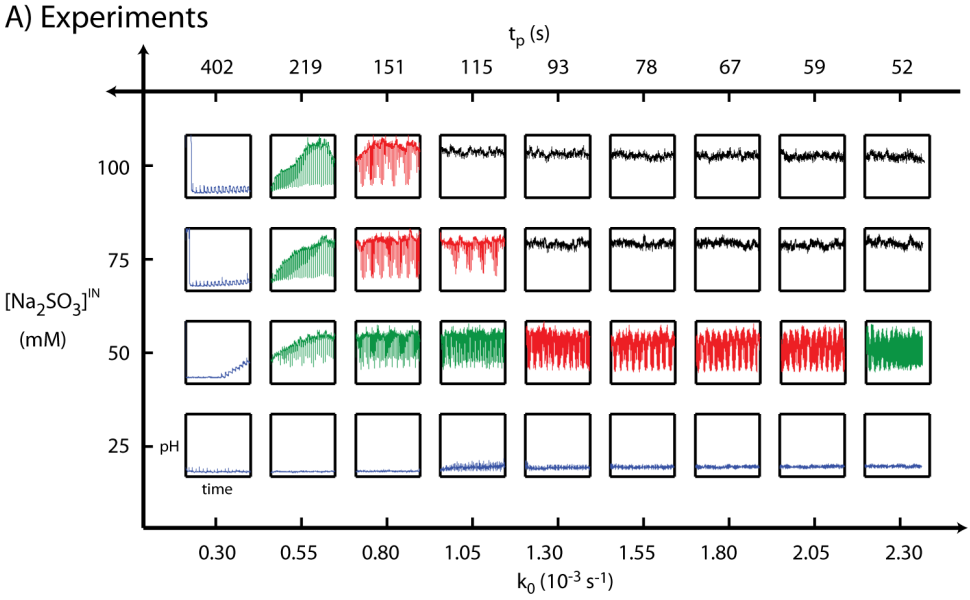


Figure 2. Left: different reaction dynamics (low pH, pH oscillations and high pH) obtained when the residence time τ =1/ko of chemicals inside the reactor is decreased. During these 3 experiments, inlet concentrations are maintained constant. Right: simulations of the dynamics of the pH oscillator in the µCSTR, including digital feeding of the reactor. They are in very good agreement with experimental results.

A simulation of the dynamics of the pH oscillator in the µCSTR has also been implemented. Key point was to take into account the digital injection, which is specific to the microscopic system. The results are in good agreement with experiments as can be seen in figure 2 right and 3 B. Indeed, simulations show the four different reaction dynamics that we obtained experimentally. Once again, this result suggests that our µCSTR could be for bio-chemical reaction networks what macroscopic CSTR was to inorganic chemical oscillators: a powerful tool to explore system dynamics.



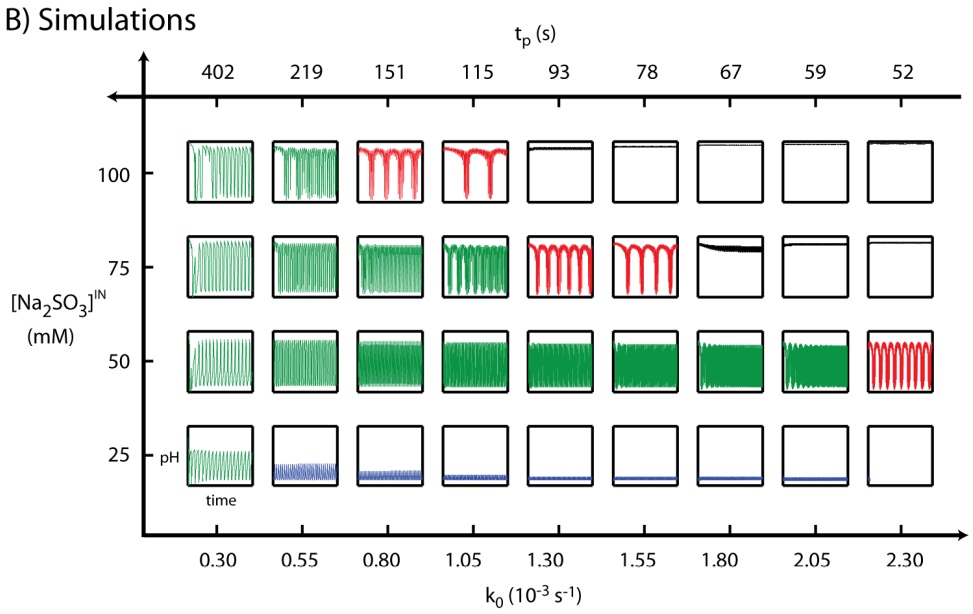


Figure 3. Experimental (A) and simulated (B) phase diagram of the Bromate-Sulfite-Ferrocyanide pH oscillator in the μCSTR for different Na2SO3 input concentrations and at different feeding rates k0 (and their corresponding injection periods tp). Each square represents pH vs. time during a 2 h experiment. 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 the experiments [H2SO4] = 5 mM while in the simulations [H2SO4] = 10 mM. The remaining injected concentrations are K4[Fe(CN)6] 20 mM and KBrO3 65 mM.

CONCLUSIONS

Finally, our μCSTR can be operated for hours with a very limited amount of reactants: 20nL/hour to be compared with 100mL/hour for the macroscopic one. Device operation is not affected by digital feeding, thus we believe our device will considerably extend the study of oscillations involving precious samples such as DNA and proteins. Moreover, this microfluidic implementation and design possibilities that follow open the way for studying interactions between temporally independent oscillators or more complex reaction networks.

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REFERENCES

[1] "Programming an in vitro DNA oscillator using a molecular networking strategy", K. Montagne, R. Plasson, Y. Sakai, T. Fujii, and Y. Rondelez, Mol Syst Biol., 7, 466 (2011)

[2] "Scaling up digital circuit computation with DNA strand displacement cascades", L. Qian, E. Winfree, Science, **332**, 6034 (2011)

[3] "Biochemical oscillators and biological rhythms", A. Goldbeter, Cambridge University Press, Cambridge (1996).

[4] "Kinetics and mechanism of the oscillatory bromate sulfite ferrocyanide reaction", E. C. Edblom, Y. Luo, M. Orban, K. Kustin, I. R. Epstein, J. Phys. Chem., **93**, 2722 (1989).

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