

A Bistable switch in urease-loaded alginate beads

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Supplementary information

Preparation of calcium alginate beads loaded with urease

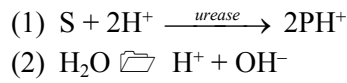
Sodium alginate solution (2.5% w/v) was prepared from sodium alginate (general purpose grade, Fisher Scientific UK) in demineralised water ($15\text{M}\Omega \cdot \text{cm}$) with cresol red indicator (general purpose grade, Fisher Scientific UK, 0.01 g/L) and stirred for a period of 5 hours at room temperature (22–24°C). The solution was loaded into 3 syringes of volume 2 ml. A fresh solution of CaCl_2 of concentration 6% w/v was prepared from calcium chloride dihydrate (meets UPS testing specifications, Sigma Aldrich, Japan). The sodium alginate solution was extruded from the syringes through a needle (18G 1½" 1.2 x 40 mm) into the CaCl_2 solution, thus creating gel beads of approximately 3 mm in diameter. The beads were gently mixed in CaCl_2 solution for 60 seconds, separated by filtration and washed with demineralised water. Subsequently, they were placed in 100 ml of urease solution prepared with urease from Jack bean (type III powder 34310 units/g solid, Sigma Aldrich USA) to give a solution activity of 20 units/ml and gently stirred for 10 minutes at room temperature (22–24°C). Then the solution was placed in the fridge and the beads soaked in enzyme solution overnight. The following day, a new solution of CaCl_2 (6% w/v) was prepared for secondary gelation. Secondary gelation was performed for precisely 10 minutes at room temperature (22–24°C). The final activity of the beads is best characterised by the clock time (time for the bead to change colour in a solution of particular urea and acid concentration). Shorter gelation times resulted in a faster clock time followed by a loss of colour of the reacted beads probably due to leaching of the enzyme from the gel matrix¹. A faster loss of enzyme was also observed in smaller beads. Therefore the gel time and bead size was optimised in order to minimise both the clock time and the diffusive loss of enzyme. The beads were separated by filtration and washed with demineralised water then used immediately in an experiment.

Experimental procedure

Stock solutions of urea in acid were prepared from sulphuric acid of 0.01M (volumetric solution, Fisher Scientific UK) and urea (Fisher Scientific UK) and cresol red indicator in demineralised water ($15\text{M}\Omega \cdot \text{cm}$). The reactor consisted of a 12×8 cm Perspex vessel split into 4 compartments and 13 ml of stock solution of different urea concentration was added to each compartment. The experiments were performed at room temperature of 24 °C. Ten beads were placed in each compartment and image capture was started. Images were obtained every 10 seconds using a CCD camera. Once the steady state profile was obtained, the beads were removed from the middle two compartments and the remaining beads distributed between the compartments. The images were processed using a threshold filter in CorelPaintshop Pro and the intensity of each pixel in the beads was obtained from the red channel of the RGB snapshot using Matlab. There was no change in the colour or pH of the solution in any of the compartments over the course of the experiment. The uncatalysed hydrolysis of urea in acid is extremely slow ($k_{\text{cat}}/k_{\text{uncat}} \sim 10^{14}$) so constant concentrations can be assumed in the surrounding solution over the course of an experiment.

Model and Simulations

For simplicity, the 8 step model of earlier work was reduced to a 2 step model that preserved the key features of the reaction²:



Where $S = \text{urea}$ and $PH^+ = NH_4^+$. The rate of the enzyme catalysed step was given by:

$$R = \frac{k_E[E][S]}{(K_M + [S]) \left(1 + \frac{K_{E2}}{[H^+]} + \frac{[H^+]}{K_{E1}} \right)}$$

where $S = \text{urea}$, $[E] = \text{total enzyme concentration in units/ml}$, $k_E = 3.7 \times 10^{-6} \text{ unit}^{-1} \text{ ml M s}^{-1}$, $K_M = 3 \times 10^{-3} \text{ M}$, $K_{E1} = 5 \times 10^{-6} \text{ M}$, $K_{E2} = 2 \times 10^{-9} \text{ M}$, $k_5 = 1 \times 10^{-3} \text{ s}^{-1}$, $k_5 = 1 \times 10^{11} \text{ M}^{-1}\text{s}^{-1}$. The reaction was considered to take place in a spherical bead in contact with a bath of solution. The total enzyme concentration was fixed in the bead and the products (including CO_2) played only a small role in the dynamics so they were not explicitly included. Spherical symmetry was assumed and the problem was transformed to a spherical coordinate system with the reaction-diffusion equations given by:

$$\frac{\partial[S]}{\partial t} = -R + D_S \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial[S]}{\partial r} \right)$$

$$\frac{\partial[H^+]}{\partial t} = k_5 - k_{5r}[H^+][OH^-] - 2R + D_H \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial[H^+]}{\partial r} \right)$$

$$\frac{\partial[OH^-]}{\partial t} = k_5 - k_{5r}[H^+][OH^-] + D_{OH} \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial[OH^-]}{\partial r} \right)$$

where the diffusion coefficients are given by: $D_H = 0.009 \text{ mm}^2 \text{ s}^{-1}$; $D_{OH} = 0.005 \text{ mm}^2 \text{ s}^{-1}$ and $D_S = 0.0014 \text{ mm}^2 \text{ s}^{-1}$, and r is the spatial coordinate (see inset of Fig. 1). To approximate the above equations by finite differences the space along a radial line within the bead was divided into 50 cells, starting from $r = 0$ at the centre of the bead (where no-flux boundary condition was imposed) proceeding outward to the last cell representing the interface between the bead and its surroundings implemented by setting the substrate and acid concentration at its outer boundary to those in the bulk solution. The above equations were then integrated for the cells as ODEs using programme XPPAUT³ with CVODE method for time. The total integration time was 30 minutes, typically a steady state pH profile was obtained after several minutes. A 2nd order finite difference formula was used for space with spatial step size of $\Delta r = 0.01 - 0.03 \text{ mm}$ depending on the radius of the bead (0.5 – 1.5 mm). The threshold values depend on the acid concentration and the size of the bead. When the length of the domain was increased, the region of bistability shifted as illustrated in Fig. S1. The simulations cannot be compared quantitatively with an experiment as a simplified version of the full model was used, however the simulations were used to guide the experiments qualitatively.

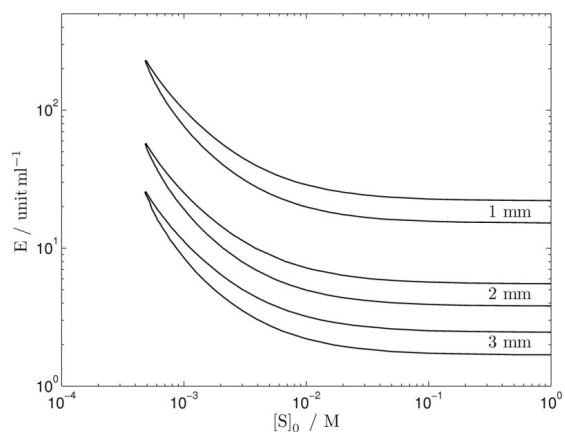


Figure S1. Regions of bistability with enzyme activity E and substrate concentration S in the surrounding solution for different sizes of the enzyme-loaded bead.

References

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