Chaos In Oscillating Chemical Reactions: The Peroxidase-Oxidase Reaction

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Abstract

The peroxidase-oxidase (PO) reaction is an important example of how oscillating reactions arise in living organisms. The reaction is important mathematically because exhibits many characteristics of chaos. Of these characteristics, sensitive dependence on initial conditions illustrates how predicting the future state of the PO reaction is next to impossible. For this reason, chaotic behavior in living organisms presents many obstacles to chemists and biologists trying to predict how systems will react to perturbations. This paper explores the chaotic behaviors of the PO reaction, using a system of four differential equations as a model. The topics analyzed are timeseries data, chaotic attractors, bifurcations, tests for sensitive dependence, Lyapunov exponents, and one-dimensional time delay embedding.

Oscillating Reactions

Less than a century ago, oscillating chemical reactions were thought to be nothing more than erroneous results caused by chemical impurities. Few believed that a reaction, in proceeding to equilibrium, could keep oscillating back and forth between two or more colors. The Lotka-Volterra Oscillator and the Belousov-Zhabotinsky reactions were two of the first oscillating reactions to be studied extensively and universally accepted to have oscillating concentrations of reactants [1]. Finding chaos in oscillating reactions is a much more recent undertaking, gaining popularity with the rise of computers. In fact there is still debate as to whether oscillating chemical reactions truly exhibit chaos, or if they are merely oscillatory with very large period [2]. However since the discovery and eventual acceptance of oscillatory reactions, many more such reactions have been designed and discovered.

One of the main areas of discovery of oscillating reactions is in living systems. The peroxidase-oxidase (PO) reaction is a prime example of such a reaction. It was one of the first reactions outside of the BZ reactions to be classified as oscillating and chaotic, and is an extensively studied example of an *in vivo* oscillating reaction (the reaction can be carried out both *in vivo* and *in vitro*). Molecular oscillating reactions are an important area of study, as they are essential to understanding the more complex oscillating systems of organism (eg. a heartbeat)[1].

The Peroxidase-Oxidase Reaction

The peroxidase-oxidase reaction is an enzyme catalyzed redox reaction. Nicotinamide adenine dinucleotide (NADH) is oxidized and molecular oxygen acts as an electron receiver. The net reaction is

$$2\mathrm{NADH} + \mathrm{O_2} + 2\mathrm{H^+} \rightarrow 2\mathrm{NAD^+} + 2\mathrm{H_2O}$$

O_2 and NADH are continuously added to and products are continuously removed from the experimental system via the use of a continuous-flow, stirred tank reactor (CSTR). Experimentation has shown that the PO reaction exhibits oscillatory behavior and a period-doubling[1] route to chaos [3]. These characteristics have been effectively modeled using a simplified eight-step

[1]Here I have adopted the terminology of [3]. This does not mean period-doubling in the traditional sense. From here on out, period-doubling refers to the maxima in the timeseries data. When the number of maxima in a period in the timeseries data doubles, we call it period-doubling.

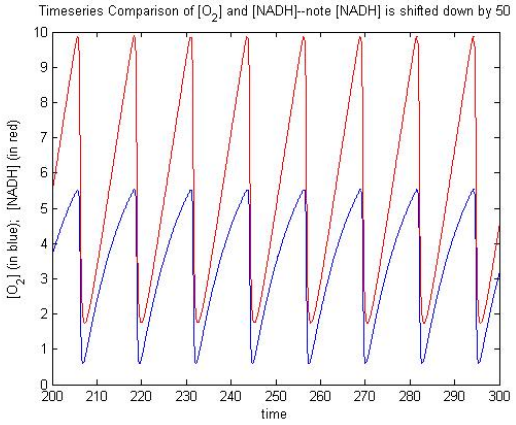

Figure 1: Timeseries plot of $[O_2]$ and [NADH] to illustrate the correlation of the concentrations between the two species. Here mechanism I and mechanism II are constantly battling to be the dominant reaction mechanism, resulting in oscillating concentrations of $[O_2]$ and [NADH].

mechanism. The Olsen model [4] is

$$B + X \xrightarrow{k_1} 2X \qquad 2X \xrightarrow{k_2} 2Y \qquad A + B + Y \xrightarrow{k_3} 3X$$
$$X \xrightarrow{k_4} P \qquad Y \xrightarrow{k_5} Q \qquad X_0 \xrightarrow{k_6} X$$
$$A_0 \xrightarrow{k_7} A \qquad B_0 \xrightarrow{k_8} B$$

where A and B are reactants (O_2 and NADH respectively), P and Q are the products, and X and Y are reaction intermediates. Experimentally, [X] corresponds with $[NAD^{\cdot}]$ and [Y] corresponds with the concentration of oxyferrous peroxidase (compound III), however it is still unknown how good the correlation is between these intermediate variables and the true intermediates. It is also important to note that $k_1 = [\text{enzyme}]$, the concentration of peroxidase enzyme, and there is also a strong correlation between k_3 and the concentration of 2,4-dichlorophenol, [DCP] [3]. Thus k_1 and k_3 are variable parameters. Oscillatory behavior arises because of competing mechanisms in the PO reaction, appearing in the Olsen model as follows: We will call mechanism I the net reaction of the autocatalytic production of X from B and X, and the production of 2Y from 2X.

Mechanism I:

$$\begin{array}{r} B + X \rightarrow 2X \\ \underline{2X \rightarrow 2Y} \\ B + X \rightarrow 2Y \end{array}$$

Mechanism I dominates the PO reaction when the concentration of X is high, but uses up X to create Y. The rate of mechanism I can be varied by changing the concentration of peroxidase enzyme, k_1. Once the concentration of X falls below some critical value, $[X]_{crit}$, another mechanism, referred to from here on as mechanism II, takes over. Mechanism II is the termolecular reaction of A, B, and Y to form X.

Mechanism II:

$$A + B + Y \rightarrow 3X$$

Mechanism II dominates when the concentration of Y is high, using up Y and turning it to X. Once the concentration of Y falls below some $[X]_{crit}$, mechanism I takes over once again. Thus the concentrations of the intermediates X and Y oscillate. Since the rate at which the reactants are converted into intermediates depends on the concentration of the intermediates, the concentrations of the reactants also oscillate and are strongly correlated. See figure 1 for an example of the correlation of $[O_2]$ and [NADH]. Also note that in mechanism II, the reaction rate is governed by k_3, the concentration of DCP. Since k_1 and k_3 are simple to control experimentally, there is an easy way to change the rate constants that govern mechanisms I and II. In fact k_1 and k_3 determine the characteristics of how the concentrations of A, B, X, and Y vary with time, and if chaos arises. In fact, either decreasing k_1 or increasing k_3 results in a period doubling route to chaos [3].

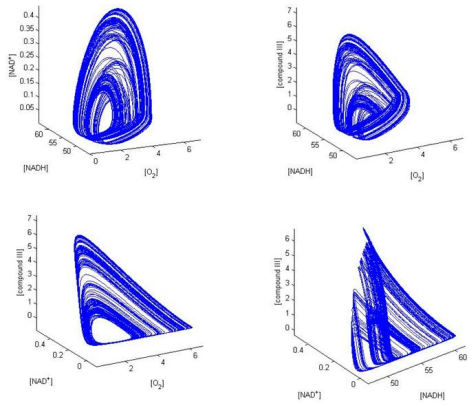

Figure 2: Projections of the four dimensional phase space ($[O_2]$, [NADH], $[NAD^{\cdot}]$, [Co III]) of a chaotic attractor for the PO reaction with $k_1 = 0.35$, and $k_3 = 0.035$.

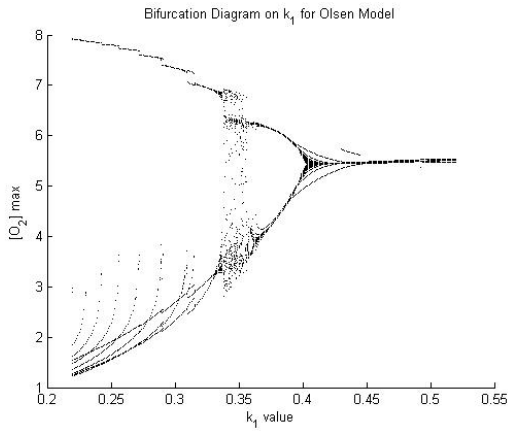

Figure 3: Bifurcation diagram of $[O_2]_{max}$ on k_1 (here $k_3 = 0.035$). Decreasing k_1 leads to period-doubling and chaos. Decreasing k_1 past the chaotic range once again yields periodic oscillations in $[O]_2$]. Bifurcations on k_1 all have a similar structure no matter what value of k_3 is used. However, the values of k_1 that show chaotic behavior change depending on k_3.

Mathematically Approximating the Olsen Model

Methods All numerical analysis was carried out using Matlab. Systems of differential equations where solved using ode45 (Runge-Kutta numerical approximation method). Timeseries data was numerically compared by fitting a cubic spline (using the Matlab 'fit' toolbox) to the data from ode45 and then interpolating values from this fit. Linear regressions were also carried out using the 'fit' toolbox.

The Olsen model can be approximated by the following system of four first-order differential equations:

$$\dot{A} = k_7(A_0 - A) - k_3ABY$$
$$\dot{B} = k_8 - k_1BX - k_3ABY$$
$$\dot{X} = k_1BX - 2k_2X^2 + 3k_3ABY - k_4X + k_6$$
$$\dot{Y} = 2k_2X^2 - k_5Y - k_3ABY$$

The following parameters were used in all of the calculations in this paper: $k_2 = 250$, $k_4 = 20$, $k_5 = 5.35$, $k_6 = 10^{-5}$, $k_7 = 0.1$, $k_8 = 0.825$, and $A_0 = 8$. Values of k_1 and k_3 are indicated in the diagrams. Initial conditions, except where indicated, are $[O_2] = A = 6$, [NADH] $= B = 58$, $[NAD^{\cdot}] = X = 0$, and [Co III] $= Y = 0$.

Phase space diagrams were computed to compare $[O_2]$, [NADH], $[NAD^{\cdot}]$, and [Co III]. No matter what values of k_1 and k_3 were used to compute the phase space, they all resulted in attractors. Chaotic attractors (as in figure 2) resulted from values of k_1 and k_3 corresponding with values of k_1 and k_3 that were found to yield chaos in figure 6. Similarly, periodic attractors resulted from values of k_1 and k_3 that were found to yield periodicity in figure 6.

Bifurcations of the concentration of reactants over k_1 (see figure 3) or k_3 (see figure 4) both illustrate successive doubling in the number of different maxima in the timeseries data, leading to chaos. This period-doubling route to chaos appears independently from either decreasing k_1 or increasing k_3. The behavior of the bifurcation diagram on k_1 is similar for any choice of k_3, as is the behavior of the bifurcation on k_3 for any choice of k_1. Furthermore, experimentation has verified the predicted behavior of the bifurcations [3],[4].

Although the bifurcation diagrams display areas indicative of chaos, further testing is required to determine whether the disordered regions do indeed give rise to chaos. Among the methods available to test this is to check for sensitive dependence on initial conditions. This was done by calculating the timeseries data from the Olsen model differential equations. Timeseries data was calculated two times from initial conditions differing in [NADH] concentration by $1e-10$. Then the natural log of the magnitude of the difference of $[O_2]$ concentration was calculated, and a line was fitted to the section with positive overall slope if it existed (see figure 5). The slope of the regression line is therefore the Lyapunov exponent corresponding to the orbit of the oxygen concentration in the system.

Tests for sensitive dependence were carried out by monitoring $[O_2]$, [NADH], $[NAD^{\cdot}]$, and [Co III], while changing the difference in initial conditions between all four variables. Also, different values of k_1 and k_3 were used. The Lyapunov exponents resulting from the sensitive dependence tests verify the data in the bifurcation diagrams: no sensitive dependence (slope ≤ 0) was found where the bifurcation diagram shows periodic behavior of $[O_2]$, while sensitive dependence was found (slope > 0) where the bifurcation diagrams predict chaos. As a tolerance for these tests, 0 was approximated to be anything less than $1e-4$.

Testing for sensitive dependence in the above manner proved to be very tedious, and is impractical for understanding how k_1 and k_3 interact to either give rise to a steady state, periodic oscillations, or chaos. In order to character-

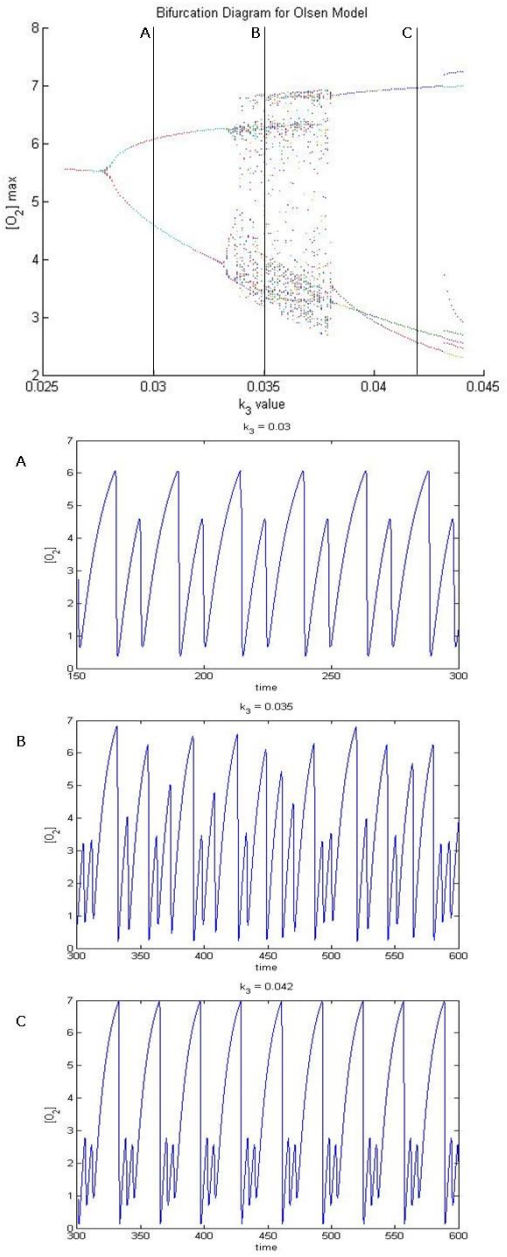

Figure 4: Bifurcation diagram of $[O_2]_{max}$ on k_3 (here $k_1 = 0.35$). The points on the bifurcation diagram correspond to the local maxima of the timeseries data. In (A), where $k_3 = 0.3$, there are two alternating peaks, corresponding to the two points above $k_3 = 0.3$ on the bifurcation diagram. In (B), there is no easily discernable pattern to the peaks in the timeseries data, corresponding to the chaotic region in the bifurcation diagram at $k_3 = 0.035$. In (C) there are three alternating peaks. Note the doubling route to chaos as k_3 increases.

ize these interactions, a plot over k_1 and k_3 was constructed, displaying Lyapunov exponents for the different k values (figure 6). The black area in figure 6 represents values of k_1 and k_3 for which the concentration of oxygen is in a steady

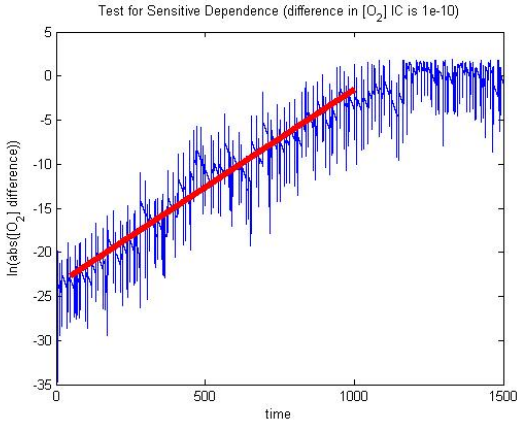

Figure 5: A plot of time versus the natural log of the absolute value of the difference in $[O_2]$ concentration when initial conditions differ by $1e-10$ in [NADH]. The slope of regression line (in red), the Lyapunov exponent, is 0.022, indicating chaotic behavior of $[O_2]$. $k_1 = 0.35$ and $k_3 = 0.035$

state. Note that when k_3 is increased just past the steady state area, the behavior of oxygen concentration becomes chaotic (light grey/white). Then as k_1 decreases and k_3 increases, there is a large area of periodic behavior (grey), eventually getting to a region of chaos denoted by white and light shades of grey in the figure. As k_1 continues to decrease and k_3 continues to increase, there is once again a large area of well behaved, periodic oscillations in $[O_2]$. Figure 6 supports the general trend seen in the bifurcation diagrams (figures 3 and 4): decreasing k_1 leads from periodic to chaotic to periodic behavior, as does increasing k_3. Furthermore figure 6 illustrates that this trend is relatively universal across values of k_1 and k_3. Lyapunov exponents were calculated as the slope of a linear regression of the natural log of the difference in $[O_2]$ (as described above for sensitive dependence test). The Lyapunov exponents are a conservative approximation: the linear regression was taken from time 50 to time 250 in all cases, so in a case of extreme sensitivity to initial conditions, the Lyapunov exponent in figure 6 is too small (but still larger than 0.005 and therefore indicative of chaos).

Finally, in an attempt to verify the chaotic nature of the timeseries data, a one-dimensional map was constructed (figure 7). The map is constructed by plotting the preceding amplitude of the $[O_2]$ timeseries data against the following amplitude. The map appears to be a fractal since zooming in on an area of the map yields an ordered structure. Furthermore, in preliminary calculations (due to time constraints) with mediocre

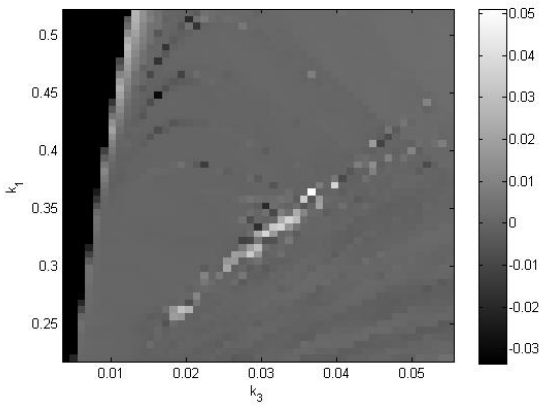

Figure 6: k_1 and k_3 phase plane illustrating combinations of k_1 and k_3 that exhibit chaotic behavior. The color bar indicates colors corresponding to the Lyapunov exponent. Areas where the Lyapunov exponent is greater than 0.005 are thought to be chaotic. Note that the Lyapunov exponents in this plot are conservative estimates due to the sampling method, ie for an exponent greater than 0.005, true value of the Lyapunov exponent may be larger than stated. The area in black represents where the concentration of oxygen is in a steady state.

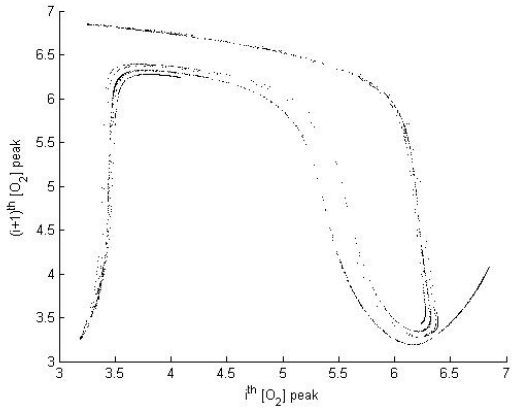

Figure 7: One-dimensional time delay embedding for $[O_2]$. This figure graphs the maxima versus the successive maxima in the $[O_2]$ timeseries data. Zooming in on the figure reveals a fractal structure. The structure supports the claim that $[O_2]$ exhibits chaotic behavior. $k_1 = 0.35$ and $k_3 = 0.035$.

resolution, the box counting dimension of the fractal appeared to be around 1.1 or 1.2. This indicates that indeed the map is a fractal.

The fact that the time-delay embedding of the successive peaks in the $[O_2]$ time series is a fractal supports the claim that the PO reaction indeed exhibits true chaos. If the orbit of $[O_2]$ was merely periodic with a very long time scale, the fractal dimension of figure 7 would be zero (just a finite collection of points). Showing that the fractal dimension of the map is greater than zero would provide strong evidence for $[O_2]$ exhibiting chaos.

Conclusions

There is significant and conclusive evidence that the PO reaction displays true chaotic behavior. Obviously this statement holds true only for certain choices of k_1 and k_3, however that there can be chaos in molecular reactions taking place inside an organism is important.

We have shown that the attractors are bounded for the Olsen model. Furthermore, for a given chaotic orbit we have seen that there exists a corresponding positive Lyapunov exponent. These two facts by themselves justify calling the orbit chaotic (this is the definition of chaos). Additionally we have shown that there indeed exists sensitive dependence on initial conditions, and that a one-dimensional map illustrating the successive change in maxima of the timeseries data for a reactant is a fractal. Finally, there is a period-doubling route to chaos. Thus it is clear that the PO reaction can exhibit chaos.

One issue that needs to be discussed is exactly how good of a model the Olsen model is. Experimentation has proven that the model describes periodic oscillations correctly: the number of different peaks in the experimental timeseries data corresponds with the predictions from the bifurcation diagrams. Similarly, experimental results mimic the chaotic behavior where predicted on the bifurcation diagrams. However since the Olsen model displays sensitive dependence for certain choices of k_1 and k_3 (presumably the PO reaction does also), it is impractical to expect the model to properly predict the chaotic timeseries data for an experimental procedure. Indeed that is the nature of chaos: it is extremely difficult to predict. The most important tests of how good the Olsen model is are those that test how well the model predicts periodic behavior, and how well the model predicts when chaos will arise.

Demonstration: The BZ reaction The demonstration shown was the Belousov-Zhabotinsky reaction. It is given by

$$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2 + 3H_2O.$$

To reproduce the demonstration, contact Charles (Charlie) Ciambra in the chemistry department. He was extremely helpful in preparing the necessary chemicals and setting up the reaction. The

procedure for the demonstration is found in [5].

References

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