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Effect of chaotic mixing on enhanced biological growth and implications for wastewater treatment: A test case with *Saccharomyces cerevisiae*

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Abstract

Mixing patterns and modes have a great influence on the efficiency of biological treatment systems. A series of laboratory experiments was conducted with a controlled, small-scale analog of a pilot wastewater aeration tank, consisting of two eccentrically placed cylinders. By controlling the rotation direction and speed of the two cylinders, it has been possible to develop chaotic flow fields in the space between the walls of the cylinders. Our experiments utilized *Saccharomyces cerevisiae* as the biological oxidation organism and air bubbles as the mixing agent supplied by a large fine pore diffuser to the cells in their exponential growth phase. The effect of various mixing patterns on cell growth was studied at different cylinder eccentricities, rotation directions and speeds. It was found that chaotic advection flow patterns: (a) enhanced growth, and (b) sped up the onset of maximal growth of the organism by 15–18% and 14–20%, respectively.

Keywords: Chaotic advection; Wastewater treatment; Mixing; *Saccharomyces cerevisiae*

1. Introduction

Water is the most ubiquitous biological compound and is imperative to life. As the world's population continues to grow, the demand for fresh water will continue to increase. Runoff from urban settings has significant impact on the quality of the receiving waters. To improve its quality, runoff is, typically, directed into treatment facilities. In cities that have combined sewer systems, the runoff is treated with municipal wastewater in sewage treatment plants [1]. During heavy rains, the capacity of the combined sewer system may be exceeded and combined sewer overflows occur [2]. The combined sewer overflow contains many contaminants, including potentially high concentrations of suspended solids, biochemical oxygen demand, oils and grease, toxics, nutrients, floatables, pathogenic microorganisms, and other pollutants [2,3]. In the aerating phase of wastewater treatment, air is bubbled through the wastewater as it is stirred, allowing the naturally occurring bacteria to consume waste and purify the water. Keeping the water well mixed is essential to sustaining bacterial growth as it disperses air and nutrients. Violent agitation is most effective at mixing the wastewater. However, mixing at high speeds creates large shearing forces that break the bacteria apart. A method of efficient mixing at small Reynolds number (laminar flow) is necessary to sustain and possibly enhance the treatment of wastewater.

It is theorized that chaotic mixing would significantly increase the exposed surface area because contrary to conventional treatment approaches, where the flow is essentially rotated uniformly, under the proposed scheme flow changes direction continuously. This will greatly enhance biodegradation by maximizing contact between microorganisms and potential reagents. This paper presents results that demonstrate an enhancement of biological activity under chaotic mixing in a controlled, small-scale analog of a pilot wastewater aeration tank. To accomplish this, *Saccharomyces cerevisiae* is utilized as the biological oxidation organism and air bubbles as the mixing agent supplied by a large fine pore diffuser to the cells in their exponential growth phase.

2. Theory

2.1. Chaotic advection and mixing

Mixing in fluids is accomplished very slowly by molecular diffusion in laminar flows. The process is greatly accelerated

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by turbulence, which, however, is associated with large velocity gradients, especially, in the small scales (eddies), and high shear rates. For these reasons, we often cannot make use of turbulence for mixing such as in biological situations, where the high shear rates could damage cells. In other situations, such as flows of very viscous fluids the flow remains laminar. Recently, a novel and fundamental mixing process rooted in dynamical systems' theory has been proposed. It rests on the pioneering work of Aref [4] and has been termed *chaotic advection*.

Even though turbulent mixing is common in high Reynolds number flows where the large amount of energy drives particles in random directions and produces a homogeneous fluid, chaos also ensues for low Reynolds numbers when time-periodic oscillations with fixed amplitudes are imposed [5]. Chaotic advection causes simple non-turbulent flows to exhibit very complicated particle trajectories that result in enhanced mixing. In the past few decades, there have been several studies demonstrating chaotic advection in low Reynolds number flows. Previous applications of chaotic advection have been in the field of medicine where low Reynolds number mixing enabled human plasma mixing without damaging the cells [6], enhanced heat transfer for heat exchangers [7], and has been used to create long-chain polymers as low velocities prevent the viscous disunion of such molecules [8]. Chaotic advection has also been shown to contribute to shear dispersion in shallow lakes where the wind blows alternately from two prescribed directions [9] and enhanced mixing in tidal areas, termed chaotic stirring [10]. The basic theory, together with examples, has been presented in Ottino [11] and in Chevray and Mathieu [12].

Chaotic advection occurs when highly complicated particle trajectories are observed in the Lagrangian frame of reference even for simple well-behaved velocity fields. Aref [4] made the fundamental observation that the stream function ψ in 2D incompressible flows plays the role of a *Hamiltonian* in classical mechanics. If ψ were to be time dependent, it is possible for the system to exhibit chaotic particle trajectories. The required time dependence of the stream function need not be due to the effects of high Reynolds number flows in which the velocities fluctuate stochastically, but may be caused by some simple, external modulation of the flow system. Idealized models demonstrating chaotic advection include the point vortex model, tendril–whorl flow [13], and the pulsed source–sink system [14]. Finally, Zaslavsky [15] has shown that flow fields generated in a three-vortex system result in chaotic motion of particles (tracers) released within the flow field. Such a system, with well-chosen parameters, can be chaotic and consequently lead to large scale mixing. Among the physically realizable models, those most commonly considered are the periodically driven cavity flow and the journal-bearing flow. Several authors ([16–19]) have studied chaotic mixing in periodically driven 2D cavity flow. In the journal-bearing system, which consists of two eccentric cylinders, the annular region is filled with a viscous liquid and the cylinders are rotated alternately to produce chaotic advection. Chaiken et al. [20] studied this system experimentally as well as numerically. Similar numerical studies were reported by Aref and Balanchadar [21] while a detailed comparison of experimental and numerical results is reported in Swanson and Ottino [22]. Investigations of the journal-bearing system with diffusion were reported by Aref and Jones [23], and Dutta and Chevray [24]. The role of diffusion and transient velocities has been studied numerically and experimentally in the dispersal of passive scalars produced in a low Reynolds number journal-bearing flow by Dutta and Chevray [25].

It should be noted that much work has already been done at small spatial and temporal scales on chemically reacting chaotic flows. Parallel-competitive reaction systems have recently been simulated in a chaotic flow by solving the differential convection–diffusion reaction [26]. It was shown convincingly that besides the relative rates of reaction, the nature of the chaotic flow is a determining factor in the evolution of the species concentrations. The groundwork for this paper was presented in a previous investigation [27] by solving numerically the aforementioned differential equation. Here again, the product was seen to be strongly dependent on the state of the flow.

It is, therefore, clear from this literature review that application of the concept of chaotic advection to the field of wastewater treatment is a novel idea with potentially time and cost reduction benefits.

2.2. Chaos and the Lyapunov exponent

Sensitive dependence on initial conditions is an important characteristic of chaotic motion. Close neighboring trajectories are found to diverge; furthermore, for most of chaotic systems the rate of divergence is exponential. Unlike these chaotic trajectories, regular trajectories are found to separate only linearly in time. The number that distinguishes between various types of orbits is called *the Lyapunov exponent*. A positive Lyapunov exponent is the fundamental criterion for the system to be considered chaotic. It is, arguably, the most verifiable characteristic of a chaotic system and is, therefore, an important tool in engineering applications of chaotic theory [28]. The Lyapunov exponent can be defined using the following example.

Let us consider two points, x_0 and y_0 in space separated by distance ε_0 (Fig. 1). Each of these points generates an orbit in space, so if one considered an orbit as a reference orbit, the separation of two orbits is a function of time. The Lyapunov exponent measures the dependence on initial conditions, therefore, we call the separation $\varepsilon(x_0, y_0, t)$. Then, the mean exponential rate of

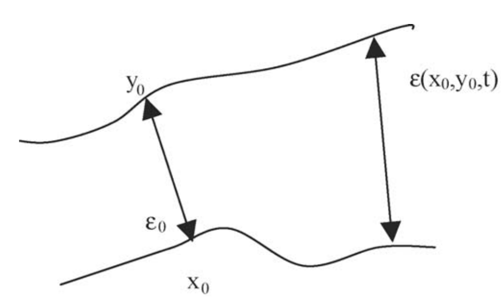

Fig. 1. Schematic used in the definition of the Lyapunov exponent.

divergence of the two initially close orbits can be calculated using:

$$\lambda = \lim_{t\to\infty} \lim_{|\varepsilon_0\to 0|} \frac{1}{t} \frac{|\varepsilon(x_0, y_0, t)|}{|\varepsilon_0|} \qquad (1)$$

where λ is the Lyapunov exponent. For a discrete-time dynamical system exhibiting chaotic advection $||\varepsilon_n|| \sim ||\varepsilon_0||e^{\lambda n}$. Such type of analysis will be utilized to demonstrate whether the flow fields in our experimental apparatus are chaotic or not.

3. Materials and methods

Two sets of experiments have been conducted, one involving dye as a tracer and another with *Saccharomyces cerevisiae* as the biological oxidation organism and air bubbles as the mixing agent.

3.1. Equipment

The apparatus employed (Fig. 2) consists of two plexiglass cylinders, a hollow outer cylinder and a solid inner cylinder, whose axes are vertical. Their eccentricity is controlled by the movement of the horizontal arm of the inner cylinder along a horizontal slot. The movement of the cylinders is computer-controlled by two stepping motors, one for each cylinder.

Before each experiment, the apparatus is leveled and 4 L of water are poured into the outer cylinder. Time is allowed to pass so that the water's motion is stopped. Dye consisting of food coloring diluted in water so that it is neutrally buoyant is then injected into the water using a hypodermic needle, through an opening cut in the lid. The rotation rate and number of revolutions performed varies; the exact program sequences can be found below for three experiments characterized by eccentricity (i.e., the inner and outer cylinders are not concentric) with different Reynolds numbers and one control experiment with the cylinders being concentric.

Program 1: Inner cylinder performs one revolution clockwise at 1 rpm, followed by one revolution counterclockwise at 1 rpm, and then followed by outer cylinder performing one revolution clockwise at 1 rpm, followed by one revolution counterclockwise at 1 rpm.

Program 2: Inner cylinder performs two revolutions clockwise at 2 rpm, followed by two revolutions counterclockwise at 2 rpm, and then followed by outer cylinder performing two revolutions clockwise at 2 rpm, followed by two revolutions counterclockwise at 2 rpm.

Program 3: Inner cylinder performs three revolutions clockwise at 3 rpm, followed by three revolutions counterclockwise at 3 rpm, and then followed by outer cylinder performing three revolutions clockwise at 3 rpm, followed by three revolutions counterclockwise at 3 rpm.

Control program (*zero eccentricity*): Inner cylinder performs three revolutions clockwise at 3 rpm, followed by three revolutions counterclockwise at 3 rpm, and then followed by outer cylinder performing three revolutions clockwise at 3 rpm, followed by three revolutions counterclockwise at 3 rpm.

For the first set of experiments, a digital camera was placed on a tripod above the apparatus and pictures were taken at regular intervals. The images were downloaded onto a computer and turned into videos for qualitative analysis, and also saved as photos for quantitative analysis. A variety of environmental parameters, such as dissolved oxygen (DO), pH, and temperature were also routinely monitored (Fig. 3).

These experiments were repeated for a second series of tests using a cell culture. Typical agitator or agitators in a stirred bioreactor are attached to a centered spinning shaft. This type of agitation is used since it yields good gas dispersion. To test the chaotic mixing pattern, the reactor needed to be modified so that air can be supplied to the cells and not disturb the slurry. A fine pore diffuser was, therefore, placed in the bottom of the outer cylinder and the inner cylinder was raised above it. The space around the cylinder was filled with silicon to maintain a flat bottom and to act as a seal. Also, a hose for the air compressor was attached to the side of the outer cylinder and into the diffuser.

Water, *Saccharomyces cerevisiae*, and sugar were placed in the cylinder and Program 3 and the control program were run. *Saccharomyces cerevisiae*, known as baker's yeast, is a carbohydrate-consuming single-celled fungus. Yeast cells decompose carbohydrates (i.e. dextrose) to obtain energy for growth. Baker's yeast cells were allowed to grow in a mixture comprising dextrose, malt extract, peptone, and distilled water

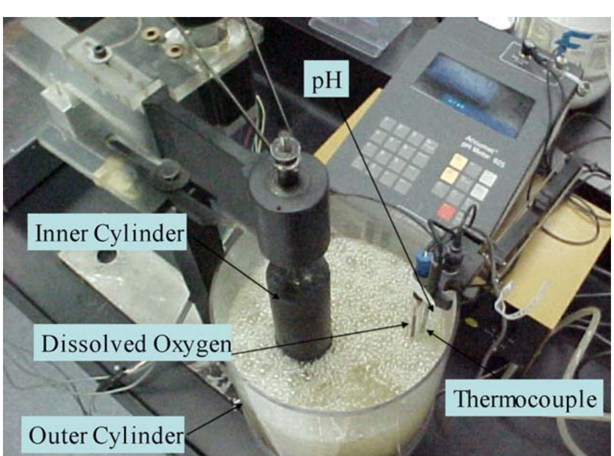

Fig. 2. Picture of experimental apparatus and associated measurement devices.

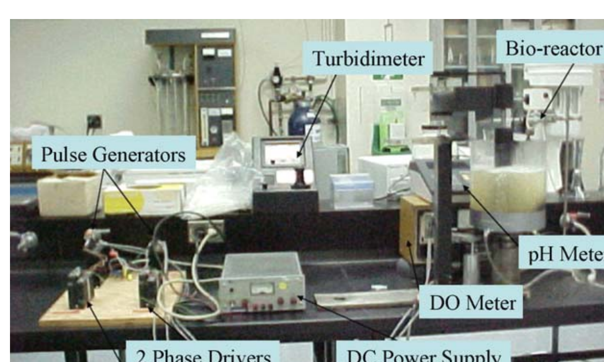

Fig. 3. Picture of experimental setup.

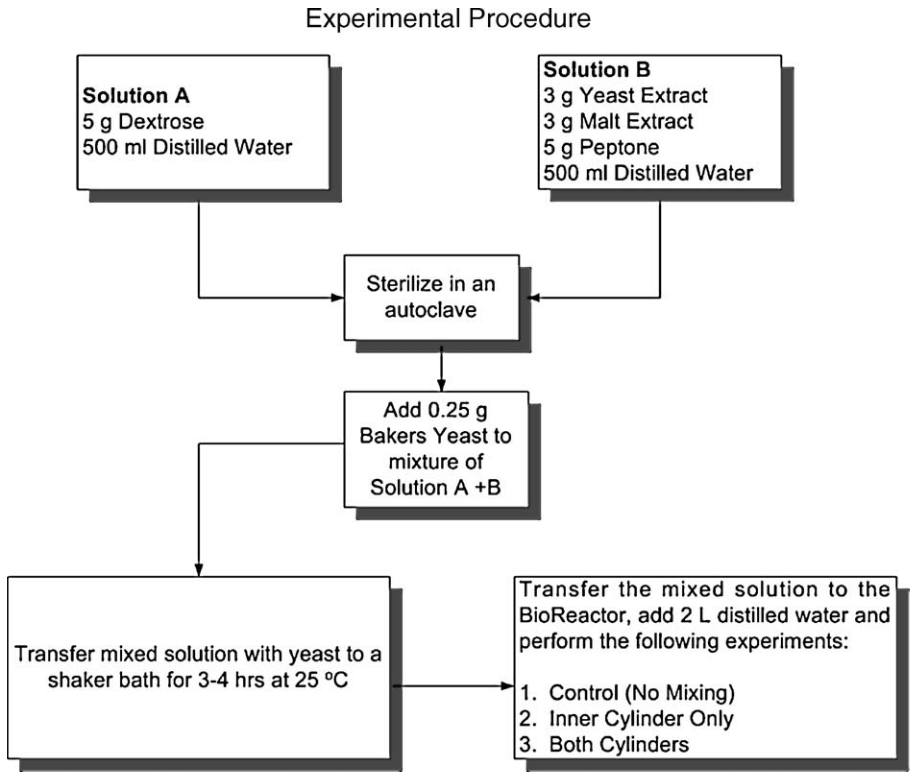

Fig. 4. Schematic representation of experimental procedure.

placed in a shaker bath at 25 °C. After 3 h, the solution was transferred to the reactor for experimentation. A schematic representation of the experimental procedure followed is shown in Fig. 4.

Various chaotic mixing patterns were tested and turbidity was monitored over time. Since turbidity and cell concentration are linearly related, one can evaluate if the chaotic mixing promoted cell growth. A complete cell growth curve was developed for each mixing regime and graphs of logarithm of cell concentration versus time were generated. Lastly, sample solutions were centrifuged, and the dextrose concentration in the cell-free supernatant was measured by high performance liquid chromatography (HPLC). To ensure that the experimental conditions were well within the linear range of the turbidity versus concentration relationship, all samples were diluted until the readings were less than 1000 NTUs (normal turbidity units).

3.2. Calculation of Lyapunov exponent, growth parameters, and Reynolds number

The procedure described in Section 2.2 was followed in order to calculate the Lyapunov exponent. The following can be said about the Lyapunov exponent λ [29]. Negative Lyapunov exponents ($\lambda < 0$) are characteristic of non-conservative systems and imply convergence of nearby trajectories. The more negative is the exponent, the greater is the stability of the system. A Lyapunov exponent of zero ($\lambda = 0$) is characteristic of a conservative system and indicates that the system is in a critical or threshold stable mode. Positive Lyapunov exponents ($\lambda > 0$) imply divergence of initially close trajectories in time, a clear indication that the orbits of the system are chaotic.

Cell growth was also analyzed by calculating the slope of the exponential growth section (steepest part) of the growth curve in a semi-logarithmic graph and inferred the specific (maximal) growth rate (1/h) and doubling time (h) as follows:

$$\mu \equiv \frac{1}{X}\frac{\mathrm{d}X}{\mathrm{d}T}, \qquad \tau_\mathrm{d} = \frac{\ln 2}{\mu_\mathrm{max}} \tag{2}$$

where X is the concentration in g/L and T is time in hours.

For each experiment, the Reynolds number was calculated in order to verify that indeed our system operated under laminar flow conditions. The Reynolds number for this system is [30]:

$$Re = R_\mathrm{i}\Omega\frac{R_\mathrm{o} - R_\mathrm{i}}{\upsilon} \tag{3}$$

where R_i and R_o, are the inner and outer radii of the apparatus, respectively, υ the kinematic viscosity of water and Ω is the angular velocity with which the cylinders rotate. For our experiments, the density of water at room temperature was 998 kg/m^3, the inner and outer radii of the apparatus, R_i and R_o, were 2.45 and 9.8 cm, respectively, and the kinematic viscosity of water was 1.01×10^{-6} m^2/s resulting in a Reynolds number in the order of 200–500, which is within the laminar flow regime.

Finally, the Reynolds number associated with the rising air bubbles was also calculated in order to ensure that the mixing observed was not dominated by this very specific secondary motion. The Reynolds number for this situation is given by:

$$Re = \frac{Ud}{v} \quad (4)$$

where U is a characteristic air bubble velocity and d is the bubble diameter. For typical conditions, the flow rate was 4.6 L/s through the whole diffuser, while U was 1.5×10^{-2} m/s and d ranged between 0.25 and 0.75 mm resulting in a Reynolds number in the order of 100, which is well within the laminar flow regime and clearly a small fraction of the Reynolds number associated with the rotation.

4. Results and discussion

4.1. Dye experiment

The role of diffusion in mixing and attenuation processes is important and as a first step, experimental observations were made during the first set of experiments by taking photographs of tracer clouds before and after stirring and analyzing the images in a well-known chaotic situation (eccentric cylinders). It is shown that the mean square separation distance of diffusing particles initialized in a chaotic region after flow reversal is a few times greater than that in a regular region, and that the separation increases exponentially (i.e. positive Lyapunov exponent) with the duration of stirring.

Laboratory experiments have also been conducted with a small-scale analog of a pilot wastewater treatment plant consisting of two eccentrically placed cylinders. By controlling the rotation direction and speed of the two cylinders, we have been able to develop chaotic flow fields in the space between the walls of the cylinders. Our experiments were conducted with *Saccharomyces cerevisiae* (baker's yeast) and air bubbles as the two mixing agents. Batch fermentation analyses allowed the comparison of the growth under control conditions with concentric cylinders, an analog of a conventional wastewater treatment facility, and under conditions of different levels of eccentricity, and rotational speeds.

Chaotic advection has been produced by alternately rotating the inner and outer cylinders consistent with the approach of Fan et al. [31] and Dutta and Chevray [30]. Changing shear directions, caused by the changing motion of the cylinders, causes a packet of water to stretch and fold. Diffusion, further, enhances the separation of particles [23]. It was observed that the mean square separation of diffusing particles increased exponentially with time (Fig. 5). The enhancement was created by a stretching of material lines during cylinder rotation, and a folding of these lines during reverse cycles.

Experiment 1 was performed at the smallest speed, the angular velocity Ω being one revolution per min or 2π radians per min for both cylinders. The Reynolds number for the first experiment is found to be in the order of 150. Similarly, the second and third experiments have Reynolds numbers of 300 and 450, respectively. These numbers are well within the laminar flow regime. For comparison, the Reynolds number used by Dutta and Chevray [25,30] in their glycerine experiments was 0.01.

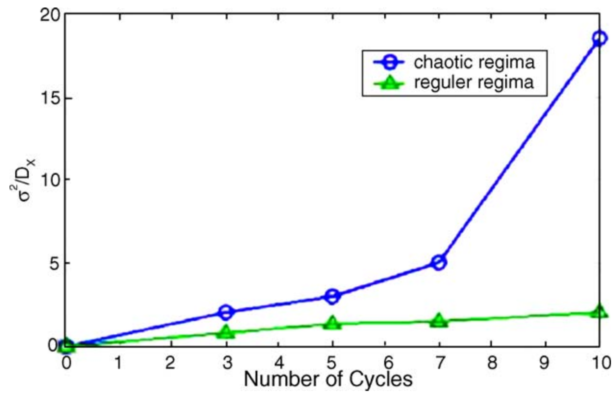

Fig. 5. Analysis of the diffusion experiment results. The spreading variance of the tracer cloud, relative to the diffusivity, clearly exhibits an exponential divergence for the mean separation with the number of stirring cycles. Note that the number of cycles has a negligible effect in the regular regime.

The eccentricity employed for our experiments is:

$$e = \frac{r_{\max} - r_{\min}}{r_{\max} + r_{\min}} \quad (5)$$

The values for $r_{\max}$ and $r_{\min}$ are 15.4 and 5.65 cm, respectively. This results in an eccentricity value of 0.46. Again, for comparison purposes, the eccentricities used by Dutta and Chevray [25,30] were 0.7 and 0.375.

Experiments 1–3 and the control were conducted and the images recorded during Experiment 1 were turned into a movie. The particles did not appear to move in a chaotic manner. The dye cloud diffused slowly from the origin but there was neither stretching nor folding. The images from Experiment 2 showed stretching and folding, but upon quantitative analysis did not reveal a statistically significant exponential separation in time.

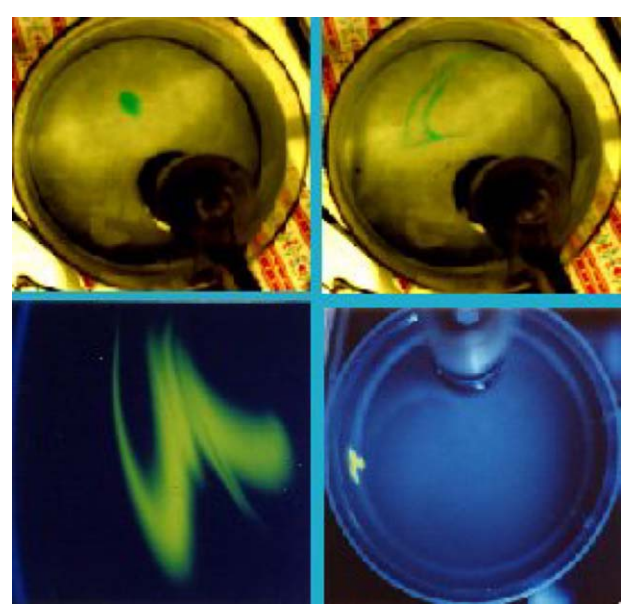

Fig. 6. Results of diffusion experiment in a chaotic region. Top two pictures depict a view of the apparatus showing the initial and final location of the green food-coloring blob in water. Bottom two pictures show top view in regular and close-up of the diffused yellow dye blob after stirring for three cycles followed by flow reversal in glycerine.

This, however, may be the result of the small number of statistical samples used. Finally, during Experiment 3 a large amount of stretching and folding occurred (Fig. 6). The greatest chaotic motion was found in this last, fastest experiment. This was verified, quantitatively, by plotting the separation distance between initially adjacent dye particles as a function of time, which revealed an exponential growth.

4.2. Yeast experiment

Results from yeast growth experiments are shown in Fig. 7 for Experiment 3 and the control. By considering each phase of program 3 (inner and outer cylinder rotation) in a sequential fashion, three different experimental configurations were investigated. The control experiment (concentric cylinders) exhibited the slowest growth (Fig. 7a). When eccentricity in the cylinder configuration was present, the growth increased even in the case, when only the inner cylinder was rotating, which resulted in a chaotic flow regime with the growth rate being increased by 18% compared to the control (Fig. 7b). Finally, when both eccentrically placed cylinders were counter-rotated the maximal specific growth rate increased by 15% compared to the control (Fig. 7c). It should also be noted that the onset of the maximal growth phase of the experiment was also enhanced significantly to 1.25 h for an average speed up of 14 and 20%, respectively. These results are summarized in Table 1.

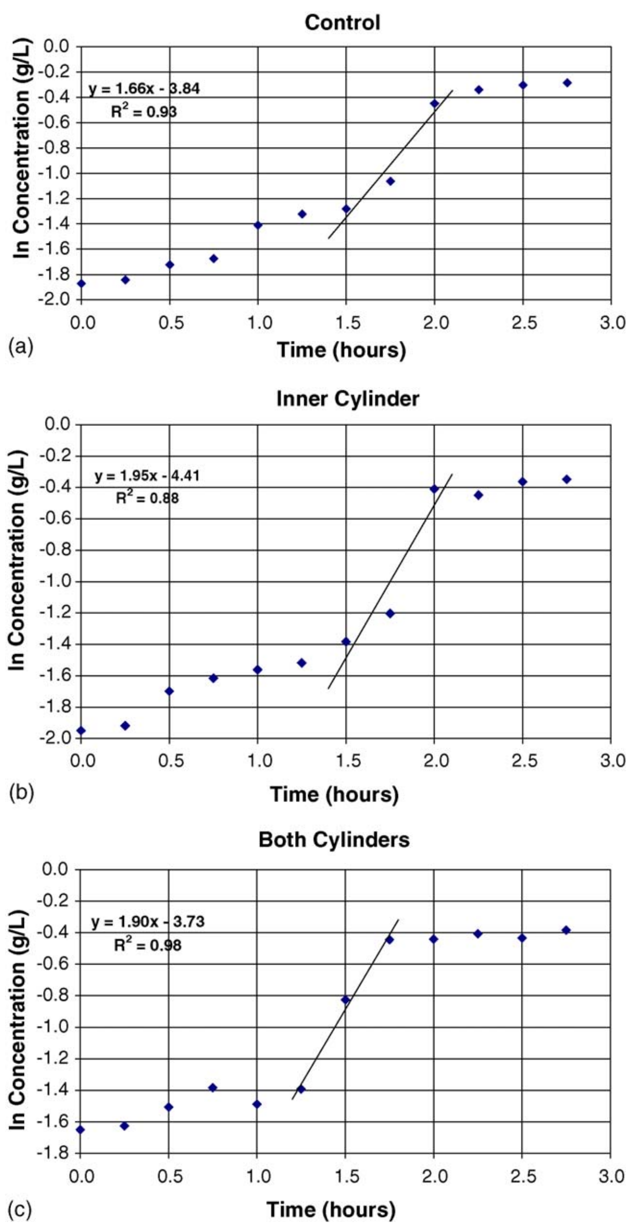

Fig. 7. Cell growth curves showing exponential phase for: (a) control experiment, (b) experiment with inner cylinder rotation only, and (c) experiment with inner and outer cylinders counter-rotating.

Table 1
Summary of cell growth results for the control and the two chaotic advection conditions

Run	μ_{max} (h^{-1})	τ_d (h)	Maximum growth period (h)
Control	1.66	0.418	1.50–2.00
Inner cylinder	1.95	0.355	1.25–2.00
Both cylinders	1.90	0.365	1.25–1.75

5. Conclusions

It has been postulated that the enhanced mixing imposed by the chaotic flow field will have a significant influence on the rate with which degradation of the wastewater constituents will take place and, therefore, decrease operating costs. This paper has presented laboratory experiments conducted with a small-scale analog of a pilot wastewater aeration tank consisting of two eccentrically placed cylinders. By controlling the rotation direction and speed of the two cylinders, the development of chaotic flow fields in the space between the walls of the cylinders was possible. Our experiments were conducted with *Saccharomyces cerevisiae* (baker's yeast) as the biological oxidation organism and air bubbles as the mixing agent. Cell growth curves were developed under control conditions with concentric cylinders, an analog of a conventional wastewater treatment facility, and under conditions of different levels of eccentricity, and rotational speeds. It was found that chaotic advection flow patterns, indeed, enhanced the biological growth observed in our experiments and the onset of the maximal growth phase.

Our findings are very encouraging and have demonstrated that application of the concept of chaotic advection to the field of wastewater treatment will have potentially time and cost reduction benefits. However, it should be noted that the results presented, herein, are preliminary and as such no generalizations of the applicability of the proposed method should be made at this time.

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