Evaluating strategies for integrating bacterial cells into a biosensor designed to detect electrophilic toxins

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Abstract

To improve the process stability of wastewater treatment plants, the construction of a whole-cell bacterial biosensor is explored to harness the natural stress response of the bacterial cells. The stress response selected in this work is the glutathione-gated potassium efflux (GGKE) system, which responds to electrophilic stress by effluxing potassium from the interior to the exterior of the cell. Thus, the bulk potassium in solution can be monitored as an indicator of bacterial stress. By utilizing this stress response in a biosensor, the efflux of potassium can be correlated to the stress response of the immobilized culture, providing an early warning system for electrophilic shock. This type of shock is a causative factor in many process upset events in wastewater treatment plants, so the application of the sensor would be an early warning device for such plants.

The research conducted here focused on the biological element of the biosensor under development. Three immobilization matrices were explored to determine the cell viability and potassium efflux potential from immobilized cells: a calcium alginate, a photopolymer, and a thermally reversible gel. The calcium alginate was unstable, and dissolved after five days, such that the long-term impact of immobilization on the cells could not be determined in the matrix. The photopolymer resulted in very low actvity and viability of immobilized cellsOf the three matrices tested, indicating that the composition of the polymer was toxic to the cells. Of the matrices tested, the thermally-reversible gel showed the best response for further study, in that the matrix did not inhibit cell activity or potassium efflux.

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Introduction

Process upset in wastewater treatment plants results in permit violations, low quality effluent, and detrimental impacts on receiving waters. Even the most well-designed plant may experiment occasional process upset due to shock loads of toxic and/or inhibitory compounds. Unfortunately, the precise causes of these upset events are often unclear, and due to the lack of real-time monitoring, operators are typically unaware that upset is occurring until the biomass is already damaged. The implementation of a real-time sensing system would provide operators with warning that a toxin is approaching and give them time to proactively respond to reduce the impact of the shock, instead of merely reacting to an upset event already underway.

In a survey performed by Love and Bott in 2000, operators reported that the most common modes of process upset are ineffective biological oxygen demand (BOD) removal, ineffective nitrification, deflocculation, and sludge bulking. Although the causes of these upsets have been speculated, operators generally do not have sufficient information to identify the source of the problem. However, when asked, operators suggested that heavy metals and toxic organic compounds (which include electrophilic chemicals) are among the most common sources of these upsets (Love and Bott, 2000). Thus, the major types of upset events are thought to be due in part to electrophilic shock loads. Creating a sensor for electrophilic compounds would provide warning of these types of upset events, although it would not be useful for upsets due to other classes of compounds, such as oil and grease.

Because electrophiles elicit a natural stress response from bacterial cells, this stress response can be harnessed to create a biosensor for electrophilic toxins. During an electrophilic toxin upset event, the bacterial biomass initiates the glutathione-gated potassium

efflux (GGKE) system (Bott and Love, 2002), in which potassium ions are effluxed from within the cell (Apontoweil and Berends, 1975 a and b). By creating a biosensor with bacterial cells containing the GGKE response, the intrinsic stress response of the bacterial culture is harnessed and genetic engineering of the bacteria is not required. Many existing biosensors have been slow to commercialize, mostly due to the limitations on genetically engineered organisms. The commercial Microtox® assay, which uses naturally luminescent *Vibrio fischerii* bacteria, is widely used as an indicator of toxicity, whereas many promising biosensors with better response times and repeatability that use genetically modified bacteria remain limited to laboratory use (for example, Philp *et al.*, 2003; Gu and Choi, 2002). Because the sensor developed in this work sensor does not require genetic modification, it has an advantage in terms of eventual commercialization and field application.

By monitoring bulk potassium as an indicator of cellular stress, the biosensor will monitor for electrophilic compounds that have been shown to cause upset in the activated sludge process. The microscale biosensor under development includes a whole cell biological element, potassium detection membranes, and microfluidic componentry that compose a lab-on-a-chip device. The biological element consists of bacterial cells immobilized in a hydrogel polymer matrix. Immobilization of the bacterial cells is important because it keeps the cell concentration constant by maintaining the viability of the immobilized cells while reducing cell division. Additionally, immobilized cells cannot slough off as a biofilm layer could and clog the microfluidic channels in the device.

A series of screening experiments were conducted in this work to select an appropriate polymer matrix for bacterial cell immobilization within the biosensor under development, and are discussed in Chapter 2. The ideal matrix is physically and chemically

stable over several weeks, non-toxic to the cells, and does not limit the diffusion of oxygen, compounds that are necessary for cell growth, the electrophiles that would trigger a stress response, or the potassium that would indicate that a stress response has been initiated. Three matrices were examined here, including calcium alginate, several photopolymerizable hydrogels, and a thermal polymer. The alginate showed good viability, but poor mechanical stability, while the photopolymerizable polymers showed poor viability. The thermal polymers were the best choice for continued study, in that the bacterial culture showed extended viability after immobilization and the matrix did not inhibit the efflux response.

Experimental Objectives

The overall objectives of this research were:

- Elucidate potassium efflux in response to a model electrophile from planktonic cultures of *P. aeruginosa* and *E. coli* at various growth states
- Select the growth state and the strain with maximal potassium efflux per cell for further study
- Screen a series of immobilization hydrogels for cell viability within the matrix
- Quantify potassium efflux from immobilized bacterial culture selected

The specific results and methods used to meet these objectives will be discussed within the following chapters of the thesis.

References

Apontoweil, P., Berends, W. (1975a). Glutathione biosythesis in *Escherichia coli* K-12: properties of the enzymes and regulation. *Biochimica et Biophysica Acta,* **399** (1), 1-9.

Apontoweil, P., Berends, W. (1975b). Isolation and initial characterization of glutathione-deficient mutants of *Escherichia coli* K-12. *Biochimica et Biophysica Acta*. **399**(1), 10-22.

Bott, C. B. and Love, N. G. (2002) Investigating a mechanistic cause for activated sludge deflocculation in response to shock loads of toxic electrophilic chemicals. *Water Environment Research*. **74**:306-315.

Gu, Man Bock, and Sue Hyung Choi. A portable toxicity biosensor using freeze-dried recombinant bioluminescent bacteria. *Biosensor and Bioelectronics.* **17** (2002) 433-440

Love, N. G., Bott, C.B. (2000). WERF Project 99-WWF-2 Report: A review of and needs survey of upset early warning devices. *Water Environment Research Foundation*.

Philp, Jim C., Severine Balmand, Eva Hajto, Mark J. Bailey, Siouxsi Wiles, Andrew S. Whiteley, Andrew K. Lilley, Janos Hajto, and Sandra A. Dunbar. (2003). Whole cell immobilized biosensor for toxicity assessment of a wastewater treatment plant treating phenolics-containing waste. *Analytica Chimica Acta*.

Chapter 1: Literature Review

Introduction

Process upset events in wastewater treatment plants result in high nutrient and pathogen loading to receiving waters, endangering public heath and stability of waterways. Electrophilic compounds are one cause of upset events, typically resulting in deflocculation, which is thought to be due to potassium efflux through the glutathione-gated potassium efflux (GGKE) mechanism (Bott and Love, In Press). Controls to eliminate such upset events are limited by the scarcity of real-time toxicity monitoring devices. Biosensors show promise in the field and have been developed to respond to specific compounds (for example, Strachan *et al*., 2001 and Reid *et al*., 1998) as well as general stress and oxidative damage (for example, Belkin *et al*., 1997 and Elasri *et al*., 2000). Most biosensors employ genetically modified bacteria, which limits their potential for commercialization. Non-engineered strains have been used to some degree, but more work is needed in this area. To create a real-time live cell sensor, a flow-through system is required. Microfluidic technology can be applied to create micro-scale sensors containing bacteria immobilized in hydrogels (for example, Heo *et al.*, 2003), because these sensors provide rapid response and require low sample volumes. Incorporating these microfluidic sensors into a real-time monitoring system can provide forewarning of toxic shock loads to treatment plants, allowing operators time to proactively mitigate the threat.

Toxic shocks cause treatment upset

Wastewater treatment plants employing biological treatment schemes to reduce or remove organic and inorganic pollutants play a pivotal role in pollution reduction. The

optimal functioning of these plants is critical for receiving stream health and downstream water reuse capacity. Despite the dependence on these plants for pollutant removal, even the most well designed plant may experience occasional process upset events due to changes in flow pattern or waste composition, or due to toxic loads of inhibitory chemicals.

A survey of Water Environment Research Foundation subscribers revealed that the common modes of process upset are ineffective BOD removal, deflocculation, ineffective nitrification, and sludge bulking (Love and Bott, 2000). Some survey responders reported that the most common causes of upsets they experienced were high flow, toxic organics, oil and grease, and heavy metals (Love and Bott, 2000). Unfortunately, in most cases, the cause of the upset is commonly not discovered until well after the event, if at all. Interestingly, several of the purported causative factors can be grouped as electrophiles, such as the heavy metals and selected toxic organics.

During process upset from a shock load of an electrophilic compound, the treatment plant loses treatment efficiency because the chemical stressors cause deflocculation of the activated sludge flocs. This loss of biomass from the system is believed to occur because stress response mechanisms cause bacteria to efflux potassium (Bott and Love, 2002). The additional monovalent cations in the floc matrix is believed to increase the intrafloc divalent to monovalent cation ratio (Bott and Love, 2002), which is an important predictor of floc strength (Higgins and Novak, 1997a and 1997b). Disrupting this balance causes the flocs to lose stability and results in loss of biomass from the system (Murthy *et al*., 1998).

Bacterial stress responses

Oxidative stress mechanisms in bacteria

Damaging oxidative (electrophilic) chemicals enter cells either from the outer environment or are generated internally as part of cellular metabolism. The types of oxidative stressors generated within the cell (reviewed in Storz and Imlay, 1999) are superoxide anions, hydrogen peroxide, and hydroxyl radicals, as well as methylglyoxal (Kalapos, 1999). Superoxide anions, $O_2^{\bullet-}$, are reduced to O_2 and H_2O_2 by superoxide dismutase. Hydrogen peroxide originates from the reduction of superoxide by its dismutase or by reduction of oxygen. Hydroxyl radicals ($HO^{\bullet}$) are produced from superoxide and H_2O_2, and are very reactive with and damaging to DNA. Example equations illustrating these reactions (reviewed in Storz and Imlay, 1999) are provided below. Equation 1 shows the complete stepwise reduction of the superoxide radial through peroxide and hydroxyl radical to water. Equation 2 shows the reduction of the oxygen radial to peroxide, a reaction catalyzed by superoxide dismutase. Equation 3 shows the reduction of peroxide to water, a reaction catalyzed by catalase.

(1) $O_2 \rightarrow O_2^{\bullet-} \rightarrow H_2O_2 \rightarrow HO^{\bullet} + H_2O \rightarrow H_2O$

(2) $O_2^{\bullet-} + 2\ H_2 \rightarrow H_2O_2 + O_2$

(3) $2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$

The types of damage caused by oxidative stress include DNA damage and mutagenesis, as well as membrane and protein damage.

The presence of these electrophiles changes the intracellular oxidation state. Two genes, *oxyR* and *soxR*, exist to detect such shifts in redox potential. Redox regulation is defined as the control of protein activity by oxidation and reduction and is a major means of

cellular control (as reviewed in Demple, 1996 and Pomposiello and Demple, 2001). The first step in protecting cells against damage is sensing the oxidants, achieved by the proteins OxyR and SoxR. These proteins are bound to the promoter region of the genes under their control such that the conformational change occurring upon oxidation allows transcription of these response genes (as reviewed in Pomposiello and Demple, 2001).

As reviewed in, for example, Storz and Imlay (1999) and Pomposiello and Demple (2001), OxyR is activated and deactivated by the formation and reduction of disulfide bonds. Peroxide exercises direct control over the activation of OxyR by reducing pairs of sulfur-hydrogen bonds to disulfide bonds. When oxidized by an electrophilic compound, the OxyR protein self-regulates the *oxyR* gene, as well as *dps* (protein to bind iron and DNA), *gorA* (GSH reductase), *grxA* (glutaredoxin), *katG* (peroxidase), *ahpCF* (NADPH reductase), and *fur* (iron binding protein to prevent iron transport). Additionally, through its control of *oxyS* and production of OxyS protein, OxyR controls other regulatory genes such as *rpoS* and *flhA*. The majority of the information on the OxyR system has been obtained from *E. coli*, but homologs have been found in many other species. After oxidation, OxyR is returned to the reduced state by the GSH-glutaredoxin-1 (*grxA*) system (as reviewed in Storz and Imlay, 1999 and Pomposiello and Demple, 2001).

The SoxR protein is constitutively expressed at a minimal level within the cell and functions through the activity of its [2Fe-2S] centers, which can be oxidized and reduced by a one-electron shift (Ding and Demple, 1997) as reviewed in, for example, Storz and Imlay (1999) and Pomposiello and Demple (2001). Superoxide exercises direct control over the activation of SoxR by shifting the oxidation state of Fe^{+} to Fe^{2+} in the iron-sulfur centers. The controlling compound for oxidation may be superoxide or depletion of NADPH. The

SoxR responds in the presence of heavy metals, antibiotics, and organic compounds, all superoxide-generating compounds, and also responds to nitric oxide (Zheng and Storz, 2000). When activated by an oxidant, SoxR initiates expression of the *soxS* gene. The genes under control of the *soxRS* regulon are *sodA* (superoxide dismutase to deactivate oxidants), *zwf* (glucose-6-phosphate dehydrogenase to recreate the NADPH pool), *fldA* and *fldB* (flavodoxins to reduce metals in constituent groups on intracellular molecules), *fpr* (NADPH reductase), *fur* (iron binding protein to prevent iron transport), *nfo* (DNA repair gene), *arcAB* (efflux pump to remove toxic chemicals), and *micF* (reduces porin expression and thus cell permeability). All of these genes that are controlled by SoxR work to prevent or mitigate damage caused by oxidants. These genes were elucidated in *E. coli*, but many other bacterial strains contain SoxR homologs (as reviewed in Storz and Imlay, 1999 and Pomposiello and Demple, 2001).

Another type of intracellular oxidative stress arises from methylglyoxal, an electrophilic byproduct of metabolism, also called pyruvaldehyde, pyruvic aldehyde, 2-oxopropanal, 2-ketoproprion-aldehyde, or acetyl-formaldehyde. Methylglyoxal generation occurs during upswings in the electron donor concentration, accumulating in starving cells that are suddenly exposed to high concentrations of carbon source. The pathways for glucose oxidation become overwhelmed, and methylglyoxal is formed to release some of the excess carbonaceous energy (Kalapos, 1999). It has been shown (Ness *et al*., 1997) that the glutathione-gated potassium efflux system evolved as a mechanism of protecting the cell from electrophilic damage from methylglyoxal.

Glutathione-gated potassium efflux (GGKE)

Reduced glutathione (GSH) (N-(N-L-γ-glutamy-L-cysteinyl)glycine) is constitutively expressed in the cell (reviewed in Storz and Imlay, 1999). Due to the activity of its sulfhydryl group, GSH acts in a sacrificial role to bind electrophiles and oxidizes into GSSG, as well as oxidized glutathione-S-conjugates (GSX) (Apontoweil and Berends, 1975 a and b). GSX triggers the cell stress response of potassium efflux through the glutathione-gated potassium efflux (GGKE) mechanism controlled by the KefB and KefC membrane transport proteins (Apontoweil and Berends, 1975 a and b; Booth *et al*., 1993; Elmore *et al.*, 1990, Ferguson *et al.*, 1995; Munro *et al.*, 1991). GGKE shuttles potassium ions out of the cell in an ion exchange for hydrogen into the cell. The transport of these hydrogen ions dissipates the proton gradient, but acidifies the cytoplasm, causing the DNA to supercoil protecting it from oxidative damage (Ferguson *et al*., 2000).

The glutathione control system is involved in the OxyR stress response, in that OxyR is reduced by enzymatic reaction with glutaredoxin 1 (Grx1), produced through OxyR control to reduce oxidized glutathione back to the reduced form, GSH (Zheng *et al*., 1998). Because the production of Grx1 is regulated by OxyR, the reduction of OxyR is autoregulated. Zheng and Storz (1998) presented a conceptual equation for this reaction obtained through both molecular and biochemical studies:

$$\text{OxyR (ox)} + 8\ \text{GSH} \leftrightarrow \text{OxyR (red)} + 4\ \text{GSSG}$$

Interestingly, OxyR is reduced by sacrificially oxidizing GSH into GSSG (Zheng and Storz 1998). Further, the redox potential of OxyR was found to be –185 mV, much higher than the typical unstressed cell redox potential, indicating that OxyR is primarily in the reduced form normally (Zheng and Storz, 1998).

Overall, bacteria respond to electrophilic stress, detected by OxyR, SoxR, and glutathione, by a number of protective mechanisms. The GGKE system is linked to the OxyR system through the reduction of the oxidized proteins back to their reduced forms. Although SoxR and OxyR control many intracellular responses, such as the activation of protective genes, the GGKE system differs in its interaction with the Kef potassium efflux channels because the system triggers an observable extracellular effect. The efflux of potassium appears to cause deflocculation in wastewater sludge flocs, a typical symptom of process upset caused by toxic shock.

Real time information on wastewater toxicity

The key to successful control of toxic shock events is awareness of their impending arrival at the plant as well as the causative toxin and its potential effect. Recognizing that a threat is approaching gives the operators time to respond in order to mitigate the threat. Control of wastewater plants is typically performed by operators with the help of computerized systems such as the Supervisory Control and Data Acquisition (SCADA) system. However, these systems are limited by the lack of real-time monitoring devices (for example, Dieu, 2001). Parameters that can be evaluated quickly include flowrate, pH, temperature, and dissolved oxygen, which are inadequate to accurately predict plant functionality (Bungay and Andrews, 1970, Olsson and Andrews, 1981). The five-day BOD test clearly takes too much time to be helpful in identifying problems in treatment. Even the chemical oxygen demand (COD) test, which can be completed in a few hours, is too long to wait to mediate an upset because the upset has already begun by the time the effluent COD spike is seen. Therefore, these tests fail as process control techniques. One simple way to

use existing technology to improve process control is the use of the dissolved oxygen (DO) profile. The variations in DO along the length of the biological reactor comprise the DO profile. Monitoring this profile permits operators to reduce the energy used to aerate the tank and to estimate the oxygen uptake rate. Shifts in the uptake rate as an alteration in the respirometry response can signal process upset (Olsson and Andrews, 1981, Bergeron and Paice, 2001).

Most control systems use "feed-back" setups, in that data from the effluent determines if treatment is effective (Bungay and Andrews, 1970). In order to effectively control plant function, "feed-forward" control is necessary to alert operators to potential problems, which would link real-time on-line sensors to global control systems within the plant (Bungay and Andrews, 1970). The limitation on these control systems is the lack of real-time sensors for causative factors in process upset. Lab analyses of the components in the influent are time consuming and costly, particularly when the target compound is unknown. The use of biosensors to provide real-time feedback on toxicity or process conditions shows promise for improving process control in wastewater treatment.

Immobilized cells as biological elements in biosensors

A biosensor is generally defined as an analytical tool that pairs a biological component with a detection system to produce a measurable response, which can be quantified into the amount of analyte present in a sample (Belkin, 2003). The response is often measured electrochemicalyl or optically. The types of biological components that can be used are enzymes, antibodies, proteins, nucleic acids, or whole cells. Whole cells are less expensive to use because their components require no isolation and purification, as in the cell

component assay systems. Also, whole cells provide information on the bioavailability of the toxins within the sample instead of simply reporting the concentration present. However, whole cells have a certain limited lifespan, require longer response times than isolated components, and may show significant variability between cell batches (Ramanathan, 2000).

Reporter genes

Much work to date with whole cell biosensors has focused on genetically engineering bacterial strains to elicit specific responses to particular compounds. Three types of reporter genes are typically used: *lux, luc,* and *gfp*. First, bacterial luciferase (*lux*) produces the oxyluciferin protein by combining the substrate luciferin with oxygen in the presence of the luciferase enzyme. The oxyluciferin product is in the excited state and produces a photon, which can be detected and used to quantify the response (Hastings, 1983). The yield of this reaction is low, only about 0.1 yield units, and it consumes reducing power, the precursor to ATP formation. Further, the reaction requires a substantial amount of oxygen to proceed, limiting its use to aerobic conditions only (Lewis *et al.,* 1998). The *lux* gene was originally isolated from three bacterial species: *Vibrio* and *Photobacterium*, both marine, and *Photoorhabdu*s, a terrestrial species (Keane *et al.,* 2002). Second, firefly luciferase (*luc*) was isolated from the beetle *Photinus pyralis* and also produces oxyluciferin, but requires a two-step enzyme reaction and consumes ATP. This reaction has a yield of 0.88 (Lewis *et al.,* 1998) and a wide linear range. However, the reaction only proceeds below 30^{o}C (Keane *et al.,* 2002), so it is less frequently used in bacterial work, as the cells typically require higher temperatures for optimal growth. Third, green fluorescent protein (*gfp*) from the jellyfish *Aequorea victoria* requires a much smaller amount of oxygen than *luc* or *lux* and the light

chromophore is directly incorporated within the crystal structure. Production of the response protein is much slower than the luciferase-based systems (Keane *et al.,* 2002).

The selection of the reporter gene for a particular application is typically based on the desired time response of the reporter. Luciferase systems, in which the fluorescent proteins are broken down in a short time, are used for time-based assays in which repeated measurements are needed. Green fluorescent protein systems are best for single measurement studies where one yes or no answer is desired because hours are required to produce and properly fold the protein. Additionally, once generated, the fluorescent signal remains robust for much longer than the luciferase signals, resulting in a longer measuring window with a constant signal output (Keane *et al.,* 2002).

Two methods exist for adding response genes to cells, which can be classified as "lights on" and "lights off", or induced and constitutive. Inserting the reporter gene into a general cell maintenance section of the genome will result in constant production of green fluorescence, while reduced intensity indicates some type of insult has occurred (Belkin, 2003). Alternately, the reporter can be linked with a promoter that regulates gene expression of a desired reporting characteristic, such as DNA repair. In this setup, an increase in light level functions as the indicator (Belkin, 2003).

Genetically engineered strains in biosensors

Several groups have used genetically modified strains to create biosensors specific to certain chemicals. A biosensor using *Pseudomonas aeruginosa* containing the *lux* operon to respond to polycyclic aromatic hydrocarbons (PAHs) was created by immobilizing the cells in poly(vinyl alcohol) (Philp *et al.,* 2003), which showed good repeatability on standards but

variable results on real samples. Also, *E. coli, P. fluorescens,* and *P. putida* with a *lux* reporter gene were used to construct a biosensor to respond to PAHs via bioluminescence (Reid *et al.,* 1998) and, although real samples were not tested, the sensor showed a stable, linear, and dose-dependent response to standards. A heavy metal responsive plasmid was constructed using *luc* to respond with production of luciferase and inserted into *Stapholococcus aureus* and *Bacillis subtilis* (Taurianinen *et al.,* 1998). This sensor responded within three hours to lab samples and responsitivity was not reduced by freeze-drying the cells before use. Kohler *et al.* (2000) used *E. coli* modified with the *lux* operon to make a biosensor for detecting 4-chlorobenzoic acid by immobilizing cells in alginate in a microtiter plate, but more work was needed to optimize the system before real samples can be tested. Strains of *Pseudomonas* and *Archromobacter* were used to perform preliminary work towards creating a biosensor for surfactants using amperometric detection (Taranova *et al.* 2002). Gu and Choi (2002) used freeze-dried *E. coli* with a reporter bound to the phenol degradation pathway to create a biosensor for phenol detection in a complete portable field-ready kit. A range of phenols were tested with rapid, repeatable results, but no results from field samples were reported.

Hansen *et al.* (2000) used *E. coli* to create a biosensor for detecting mercury by fusing the P_{mer}, a promoter induced by Hg, and the *mer* reporter gene for Hg to *luxCDABE, lacZYA*, and *gfp*. These constructs produce luminescence, beta-galactosidase, and green fluorescent protein, respectively. Each construct was cloned into a delivery vector to permit its insertion into any bacterial strain desired. *P. aeruginosa* was used as an example. Other groups (Ramanathan *et al.,* 1997; Lyngberg *et al.*, 1999; Rasmussen *et al.,* 2000; Selifonova *et al.,* 1993) had performed similar research with the *mer* reporter gene encoded on a plasmid, but

mixed results were obtained because the plasmids were unstable. However, Petanen and Romantschuk (2002) used plasmids with a *luc* response to mercury and arsenite in *P. fluorescens* and obtained results comparable to traditional analytical methods, but much faster.

Howbrook *et al.* (2001) created a whole cell biosensor for glucose using *E. coli* with a luminescence reporter bound to the *katG* gene, which is tightly controlled by the OxyR system such that it responds to oxidative stress. As proof of concept, the glucose oxidase enzyme was added to the sensor to produce H_2O_2 when glucose is present, thus creating oxidative stress within the cell to trigger the OxyR response system and initiating the *lux* reporter. Any such oxidase enzyme could be included to target sensor specificity for a wide variety of compounds. The assays were conducted in liquid culture with samples generated in the laboratory.

Creating a general response biosensor is typically approached by developing a panel of bacteria, each responsive to a different threat. Panels of *E. coli* bacteria were engineered to create sensors for heat shock, oxidative stress, and protein damage (Belkin *et al.,* 1997), DNA damage (Belkin, 2003), oxidative damage, membrane damage, DNA damage, and protein damage (Kim and Gu, 2003), and heat shock, oxidative stress, fatty acids, peroxides, and genotoxicity (Premkumar *et al.,* 2003). A *lux*-containing *E. coli* strain immobilized in poly (vinyl alcohol) (PVA) was used to create a slow release biosensor for general stress where the PVA matrix was dissolved using KCl over time so that new cells were being used for each toxicity test (Horsburgh *et al.,* 2002). Biosensors for genotoxicants were created by inserting a *lux* reporter into the *recA* promoter region for DNA damage repair in *E. coli*.

(Polyak *et al.,* 1997; Belkin, 2003; Polyak, 2001). Elasri *et al.* (2000) created a similar sensor using *P. aeruginosa*.

Two well-known assays for genotoxicants and cytotoxicants are the SOS-*Lux* test and the *Lac*-Fluoro test. The SOS-*Lux* test was created by adding the *lux* reporter under the control of the SOS DNA damage repair system to identify genotoxicants. The *Lac*-Fluoro test uses constitutive expression of *gfp*, which is inserted to report cell maintenance function and to indicate cytotoxins. Decreases in fluorescence shows cytotoxicity, or impacts to cell metabolism and function (Baumstark-Khan *et al.,* 2001).

The toxicity of heavy metals (chromium, zinc, copper, nikel, and arsenic) was evaluated using a microtiter plate setup and two strains of *Salmonella typhimurion*, one modified for the SOS-*Lux* and one for the *Lac*-Fluoro test (Rabbow *et al.,* 2002). Improvements to the sensitivity of the SOS-Lux test were obtained by inserting the reporter plasmid into *E. coli* containing a *tolC* mutation, which made the membrane more permeable to hydrophobic substances. The sensitivity was further improved by using a *S. typhimurion* strain with a defect in the cell membrane, which again increases the membrane permeability (Rettberg *et al.,* 2001). The SOS-*Lux* and the *Lac*-Fluoro tests developed by Rabbow *et al.* (2002) have been applied in practice on the international space station to determine toxicity of recycled water supplies on the station. A different sensor formulation with the similar genetic modifications has been used to determine the threat from radiation on the space station (Rabbow *et al.*, 2003).

On the whole, genetically engineered strains used in biosensors have not been commercialized to a large degree. The difficulty in overcoming the genetically modified organism (GMO) regulations makes such sensors primarily useful only in laboratory settings,

where the threat of accidental release is not applicable. Despite their advantages in terms of robust, repeatable responses, engineered strains have yet to be widely commercialized.

Non-engineered strains

One drawback of the majority of work to date is that most sensors employ genetically modified bacteria as the reporter. A notable exception is the commercial Microtox® assay, which uses naturally luminescent *Vibrio fischerii* bacteria. Non-engineered cells do not mandate such strict controls on release as those that have genetic modifications. While engineered cells may yield a more robust response, wild-type cells facilitate eventual commercialization of a sensor. Additionally, the use of indigenous wild type cells more accurately reflects the conditions under observation than the use of an engineered lab strain. Using wild-type cells means that the intrinsic cell processes must be harnessed to detect the compounds of interest or to determine if the cells are inhibited.

BOD sensors typically do not require genetic engineering to create a functional sensor since the concentration of oxygen in a sample can be easily monitored. These sensors are typically constructed using an immobilized bacterial culture contained between a dialysis membrane and a gas permeable membrane. The sample fluid diffuses through the dialysis membrane and passes through the culture where some of the oxygen in the sample is consumed. The amount of oxygen remaining in the sample is measured using a dissolved oxygen electrode placed above the gas permeable membrane. The respiration rate of the culture is calculated from the difference in oxygen concentrations between the amount in the sample initially and that detected by the electrode after exposure to the cells (Liu and Mattiasson 2002). Current work (Heim *et al.* 1999) has focused on combining two pure

cultures to expand the limited range of compounds detected when one pure culture is used, or an ensemble of bacteria, which shows unstable response over time as different species dominate (Tan and Wu 1999). In general, these sensors are complicated to apply because the respiration rate shifts depending on the amount of substrate present in the sample. Adjusting for this variation, this type of BOD sensor has been shown to be as effective as the traditional five-day BOD test but with a response within minutes. However, the variability between sensors of the same construction is significant (Liu and Mattiasson 2002).

In addition to BOD sensors, amperometric biosensors have been developed that function by binding cells to a screen printed electrode to measure the transfer of electrons during substrate consumption. One example of this type of sensor was developed by Skladal *et al* (2002) to create a phenol sensitive biosensor. *Pseudomonas* strains were immobilized on a screen-printed electrode such that the uptake of phenol as a carbon and energy source by the cells can be detected by the electrode as electrons are transferred. The sensor showed a rapid, reproducible response. However, the sensing surface had to be prepared and used on the same day and the response varied depending on the ionic strength of the sample.

Natural products of cells can also be monitored to determine if they are inhibited. For example, Guven *et al*. (2003) created a bioassay for inhibition of enzyme biosynthesis to monitor the concentrations of two enzymes, one produced by *E. coli* and the other produced by *Bacillus subtilis.* Colorimetric tests were used to monitor beta-galactosidase synthesis in *E coli*, which is created during consumption of glucose and is metabolism related, and alpha-amylase synthesis in *B. subtilis*, which is created during late log stage and is growth phase related. Changes in the concentrations of these enzymes indicated the presence of toxic compounds. The system worked very well for pesticides, the model organics tested, but was

less repeatable with heavy metals. This type of assay takes much longer than an engineered "lights on" or "lights off" strain, because up to eight hours are required to determine if a decrease has occurred in the enzyme synthesis.

Cell components in biosensors

The use of cell components (purified enzymes) in biosensors is an attractive alternative to whole, living cells, which require a constant supply of nutrients and oxygen. These systems typically consist of the sensing element (enzyme) bound to an electrode such that the change in concentration of a product is converted into an electric signal monitored by a detector (reviewed in Karube and Nomura, 2000). An example of such a system was created by Moser *et al*. (2002) in which oxidase enzymes specific to glucose, lactate, glutamate, and glutamine were immobilized into a flow through microfluidic device. The response was rapid and repeatable and a single sensor could perform continuous monitoring of all four compounds of interest. However, isolation of the enzymes used in this type of sensor is costly and time consuming and enzymes alone are less stable than whole cells (Karube *et al.*, 1995).

Using whole cells that have been killed provides a useful method of preserving the enzymes and eliminates the purification step. Tan and Zhenrong (1998) used thermally killed *Bacillus subtilis* cells immobilized in a membrane bound to the end of a DO probe to create a sensor for BOD. The BOD reported by the sensor correlated well with BOD_5 assays performed on identical samples, with results available within half an hour. This type of biosensor functions by incorporating the active components necessary to catalyze the

oxidation of carbon sources, specifically, enzymes and cofactors, but without including the difficult and time consuming step of isolating these enzymes from living cells.

Microfluidic sensors

These macroscale sensors using whole cells or cell components can be improved by miniaturization. Reducing the size of a sensor to the microscale to create microelectromechanical systems (MEMS) presents several advantages in sensing ability. Microsensors require much lower sample and analyte volumes, present a more rapid response than full-scale sensors, and can be made disposable because of the small size and volumes involved, thus creating a highly cost-effective analysis system. However, the robustness of these systems to harsh environments has not been proven and they have also been slow to commercialize.

Several groups have used microfluidic technology to create biosensors. One prominent example relavent to environmental engineering is the class of BOD sensors created by Yang *et al.* (1996, 1997), where whole cells were immobilized directly onto the surface of a microscale DO probe. The cells used were *Trichosporon cutaneum*, useful for BOD sensors because they consume a wide range of carbon sources, not a limited few compounds. In this way, they report a BOD closer to the BOD_5 than what would be reported by a sensor using a strain such as *Pseudomonas*, which prefers to grow on acetate. With the sensor, this group was able to achieve sensor BOD to BOD_5 ratios of 0.65 to 1.70 with real wastewater samples and a repeatability of plus or minus eight percent was reported. The response time was twenty minutes and the sensor was linear for a BOD range of 0.2 to 18 mg/L BOD. Additionally, because it is on the microscale, the device is smaller and easy to

handle. Such a sensor would be useful to indicate spikes in influent and effluent BOD; for such an application, the limited precision of the device is less of a factor because the purpose of the sensor is to provide nearly real-time monitoring, not reportable BOD values.

In addition to whole cells, enzymes are commonly used within the microscale sensors as reporters for the presence of certain compounds. Zhan *et al.* (2002) immobilized a pH reporting dye and enzymes to catalyze specific reactions in a microreactor within a microfluidic channel array to create a biosensor for potentially any compound of interest. Adding the correct enzymes created a pH shift, reported by the dye, and the magnitude of the shift correlated to the concentration of the analyte that reacted with the enzymes. Moser *et al.* (2002) created a bio-MEMS using enzymes bound within a flow channel with electrodes to report changes in current as the enzymes reacted with the species of interest, which were glucose, lactate, glutamate, and glutamine. Petrou *et al.* (2002, 2003) created a bio-MEMS sensor for glucose and lactate by immobilizing enzymes in a microchannel and measuring the response with a dialysis probe made of polyacrilonitrile fiber. Zimmerman *et al.* (2003) created a flow-through glucose sensor by immobilizing glucose oxidase enzyme in polyvinyl alcohol (PVA) polymerized by UV light within a microfluidic system. Due to high fabrication temperatures associated with the anodic bonding between wafer sheets, it was important that the enzymes were immobilized in situ after device fabrication.

These types of sensors were designed for medical applications, such as monitoring the concentrations of sugars in blood samples. Although the concept could be modified for application in environmental engineering processes by changing the enzymes used in the sensors, this design is less useful for general monitoring. Such sensors are primarily useful for monitoring the concentration of a few known compounds, and so could be applied to

sensing compounds known to be present in environmental samples. However, such sensors do not address the need for monitoring a wide range of toxic compounds.

Immobilization Matrices

The immobilization of cells within a microfluidic sensor is critical to the functionality of the system. Using a biofilm of cells adhered to the bottom of the flow channel is likely to be unstable, as cells and chunks of film can slough off with time and foul the detector or clog the channel. Immobilizing cells within hydrogels prevents sloughing, provides some degree of protection for the cells, and exposes the cells to an environment similar to that within a biofilm (Junter *et al.*, 2002). There are two primary types of hydrogels, those that are chemically polymerizable and those that are photopolymerizable.

Chemically polymerized hydrogels, such as sodium alginate, are useful for macroscale work by forming beads from the gel precursor solution (Drury *et al.*, 2004). However, downsizing the bead to microscale may prove difficult to form. Therefore, photopolymerized polymers are typically used for microfluidic applications because they can easily be formed into a variety of shapes in situ by using various photomasks to control what areas of the polymer precursor are polymerized. First, Beebe *et al.* (2000) created various shapes of hydrogels by polymerizing acrylic acid and methacrylate in situ in a microchannel, showing the ease of handling with the photopolymer. Further, this group used pH-sensitive polymers to control flow within a microfluidic channel matrix by placing polymers of varying compositions at critical points in the flow matrix such that shifts in pH would cause expansion or contraction of the polymer patch and, thus, either allow or block flow through particular regions of the channels. This passive method of flow control simplifies

microfluidic componentry, but for biological systems, the pH extremes required to change the flow regime would prove detrimental to the cells in the system. Moving from flow control to the development of a reporting system, Zhan *et al.* (2002) immobilized the glucose oxidase enzyme within several micropatches in a microfluidic device. The device reported the concentration of glucose by shifts in the intensity of the reporter dye co-immobilized with the enzyme. This sensor would be used for medical applications, but still shows the usefulness of the photopolymer in microfluidic work.

Enzymes as well as viable cells have been immobilized within photopolymers. Koh *et al.* (2002) encapsulated mammalian cells within polyethylene glycol (PEG) diacrylate (PEG-DA) with Darocure 1173 as the photoinitiator polymerized with UV light. Cells were viable up to a week after polymerization based on observations using the LIVE/DEAD viability/cytotoxicity fluorescence staining. This application was for tissue engineering and not biosensor development. However, Heo *et al.* (2003) immobilized *E. coli* cells in PEG-DA within a microfluidic channel constructed of polydimethylsiloxane (PDMS) using photopolymerized micropatches of gel spanning the height of the channel, but not the width, to permit flow around the edges of the hydrogels. The authors attempted to prove cell viability using LIVE/DEAD staining and 2', 7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF-AM) staining. The LIVE/DEAD stain was ineffective because the hydrogel fluoresced strongly green, so live cells could not be observed above the background fluorescence. Additionally, the BCECF-AM stain indicated enzyme activity, not true viability, because enzymes remain active after a cell is no longer considered viable. This work did not develop a field-deployable sensor with the photopolymer, but simply tried to prove viability within the matrix.

One difficulty associated with the photopolymerizable systems is mixing the cells with the gel precursor solution because the components are highly viscous and the shear stresses on the cells during mixing may increase cell mortality. Modifications to the gel structure have been undertaken in an attempt to create a low molecular weight hydrogel based on alginate. Kong *et al.* (2003) created different molecular weight alginates by irradiation and the viscosity of each was measured by shear stress and shear rate. Results showed that higher radiation doses reduced the molecular weight most significantly, but doses in the mid-range of those tested made the gels stiffer because only the flexible connectors of the gel matrix broke during radiation, leaving the strong bonds intact. At low doses, no change in stiffness was observed. Osteoblasts were mixed with the gels before polymerization. The lower molecular weight gels resulted in greatly improved viability as measured visually by microscopy using the Trypan blue stain, which cannot enter cells with intact membranes, but stains the nuclei of cells with compromised membranes.

Overall, the photopolymerizable system is preferable for microfluidic work because it can be formed into a variety of shapes by changing the photomask prior to polymerization. Photopolymerizable polymers have been used to successfully immobilize both eukaryotic and prokaryotic cells, as well as to immobilize enzymes for the creation of a sensor. However, the major challenges that users of photopolymerizable polymers face are the toxicity of the polymer components and the difficulty visualizing cells within the polymer microscopically, because the polymer tends to absorb stains such as the green stain of the LIVE/DEAD package.

Scope of Research

Based on this review of biosensors developed previously, it is clear that the need for a non-engineered bacterial sensor for a range of toxic compounds has not yet been addressed. Many groups have created compound-specific sensors using both whole cells as well as purified enzymes, but no general stress response sensor has been developed yet. Microtox® uses a non-engineered strain, but quantifies toxicity, not stress. In wastewater treatment, electrophilic stress results in process upset and reduced treatment capacity. To reduce instances of process upset, a sensor that responds to electrophilic insult quantified by monitoring the intrinsic stress response of the immobilized cells, not by engineering a known response into the cells, can be developed.

The objective of this work is to evaluate the performance of an immobilized bacterial culture that will comprise the biological sensing element within a biosensor for detecting electrophilic compounds based on activation of the GGKE mechanism. The biosensor will consist of bacterial cells immobilized in a hydrogel matrix in a microfluidic flow through setup. In its ultimate application, the biosensor will direct water to be monitored over the immobilized cells. Any electrophilic toxins in the flow will trigger potassium efflux from the cells. The potassium concentration will be measured using an ion-selective film (Kopelman *et al.*, 1997) over which the flow will pass both before and after contacting the cells. The change in potassium will be monitored and, if efflux is indicated, the device will implicate the presence of electrophilic toxins. For wastewater treatment applications, electrophilic toxins have been linked to deflocculation and, thus, to process upset. A positive response by the sensor would give plant operators early warning of process upset potential in the influent so that the toxic slug could be rerouted to storage and slowly fed in for treatment to avoid

upset. For surface, ground, or drinking water applications, many chemical agents that could be used to threaten homeland security are classified as electrophiles, such as nerve agents, and could be effectively sensed. On the whole, the sensor under development would improve process stability at wastewater plants and improve confidence in water supply at drinking water plants.

To determine the feasibility of a biosensor based on the intrinsic potassium efflux stress response, the potassium efflux potential of polymer-immobilized bacterial culture was evaluated experimentally by comparing to non-immobilized planktonic cultures. Furthermore, a model was developed to determine of the number of cells needed to efflux detectable levels of potassium, while estimating the oxygen requirement for these cells to determine if oxygen would be limiting within the system. Three matrices, alginate, a photopolymerizable polymer, and a thermally reversible gel, were evaluated as possible hydrogels for immobilization. Both the viability and the potassium efflux from immobilized cells were elucidated. This work will become the basis for the incorporation of the immobilized cells into the microfluidic device.

References

Apontoweil, P. and W. Berends. (1975a) Glutathione biosythesis in *Escherichi coli* K-12: properties of the enzymes and regulation. *Biochimica et Biophysica Acta* **399** (1), 1-9

Apontoweil, P. and W. Berends. (1975b) Isolation and initial characterization of Glutathione-deficient mutants of *Escherichia coli* K-12. *Biochimica et Biophysica Acta*. **399** (1), 10-22

Baumstark-Khan, Christa, Angela Rode, Petra Rettberg, and Gerda Horneck. (2001) Application of the *Lux-Fluoro* test as bioassay for combined genotoxicity and cytotoxicity measurements by means of recombinant *Salmonella typhimurium* TA1535 cells. *Analytica Chimica Acta* **437**, 23–30

Baumstark-Khan, Christa, Riaz A. Khan, Petra Rettberg, and Gerda Horneck. (2003) Bacterial *Lux-Fluoro* test for biological assessment of pollutants in water samples from urban and rural origin. *Analytica Chimica Acta* **487**, 51–60

Booth, Ian R., R.M. Douglas, G.P. Ferguson, A.J. Lamb, A.W. Munro, and G.Y. Ritchie. (1993) Chapter 11E: K^+ Efflux systems. Bakker, Evert P., Editor. *Alkali Cation Transport Systems in Prokaryotes*. Boca Raton, Florida: CRC Press, Inc. (291-308)

Bott, Charles B., and Nancy G. Love. (In Press) Implicating the Glutathione-Gated Potassium Efflux System as a Cause of Electrophile-Induced Activated Sludge Deflocculation. *Applied and Environmental Microbiology*

Bott, Charles B., and Nancy G. Love. (2002) Investigating a mechanistic cause for activated-sludge deflocculation in response to shock loads of toxic electrophilic chemicals. *Water Environment Research* **(74)** 3, 306-315

Beebe, David J., Jeffrey S. Moore, Joseph M. Bauer, Qing Yu, Robin H. Liu, Chelladural Devadoss, and Byung-Ho Jo. (2000) Functional hydrogel structures for autonomous flow control inside microfluidic channels. *Letters to Nature* **404** 588-590

Belkin, Shimshon. (2003) Microbial whole-cell sensing systems of environmental pollutants. *Current Opinion in Microbiology* **6** 206–212.

Belkin, Shimshon, Dana R. Smulski, Sara Dadon, Amy C. Vollmer, Tina K. Van Dyk, and Robert A. Larossa. A panel of stress-responsive luminous bacteria for the detection of selected classes of toxicants. (1997) *Water Research* **(31)** 12, 3009-3016

Bergeron, J., M. Paice. (2001) Monitoring of Activated Sludge Systems Using a Combination of Specific Oxygen Uptake Rates and Specific Adenosine Triphosphate Measurements. *Water Qual. Res. J. Can*. **(36)** 4, 659-685

Bott, Charles B. and Nancy G. Love. (2002) Investigating a mechanistic cause for activated-sludge deflocculation in response to shock loads of toxic electrophilic chemicals. *Water Environment Research* **(74)** 3, 306-315

Bungay, HR and JF Andrews. (1970) Dynamics of microbial processes. *Water and Sewage Works* **(117)** 3, p 77-80

Demple, Bruce. (1996) Redox signaling and gene control in the *Escherichia coli soxRS* oxidative stress regulon-a review. *Gene* **179**, 53-57.

Dieu, Ba. (2001) Application of the SCADA system in wastewater treatment plants. *ISA Transactions* **40**, 267-281

Ding, H.G. and Demple, B. (1997) In vivo kinetics of a redox-regulated transcriptional switch. *Proc. Natl. Acad. Sci. U.S.A.* **94,** 8445-8449

Drury, Jeanie L., Robert G. Dennis, David J. Mooney. (2004) The tensile properties of alginate hydrogels. *Biomaterials* **24**, 3187-3199

Elasri, Mohamed O., Tricia Reid, Steven Hutchens, Robert V. Miller. (2000) Response of a *Pseudomonas aeruginosa* biofilm community to DNA-damaging chemical agents. *FEMS Microbiology Ecology* **33**, 21-25

Elmore, J.J., A.J. Lamb, G.Y. Ritchie, R.M. Douglas, A.W. Munro, A. Gajewska, and I.R. Booth. (1990) Activation of potassium efflux from *Escherichia coli* by glutathione metabolites. *Molecular Microbiology*. **4**(3) 405-412

Ferguson, Gail P., John R. Battista, Annettee T. Lee, and Ian R. Booth. (2000) Protection of the DNA during the exposure of *Escherichia coli* cells to a toxic metabolite: the role of the KefB and KefC potassium channels. *Molecular Microbiology* **35**(1), 113-122

Ferguson, Gail, P. D. McLaggan, and I.R. Booth. (1995) Potassium channel activation by glutathione-S-conjugates in Escherichia coli. *Moecular Microbiology* **17**(6), 1025-1033

Ferguson, Gail P., A.W. Munro, R.M. Douglas, D. McLaggan, and I.R. Booth. (1993) Activation of potassium channels during metabolic detoxifiction in *Escherichia coli*. *Molecular Microbiology*. **9**(6) 1297-1303

Gu, Man Bock, and Sue Hyung Choi. (2002) A portable toxicity biosensor using freeze-dried recombinant bioluminescent bacteria. *Biosensor and Bioelectronics* **17** 433-440

Guven, Kemal, Songul Togrul, Fikret Uyar, Sifa Ozant, David I. De Pomerai. (2003) A comparative study of bioassays based on enzyme biosynthesis in *Escherichia coli* and *Bacillus subtilis* exposed to heavy metals and organic pesticides. *Enzyme and Microbial Technology* **32**, 658–664

Hansen, Lars Hestbjerg and Soren Johannes Sorensen. (2000) Versatile biosensor vectors for detection and quantification of mercury. *FEMS Microbiology Letters* **193**, 123-127.

Hastins, J.W. (1983) Biological diversity, chemical mechanisms, and the evolutionary origins of bioluminescent system. *J. Mol. Evol.* **19**, 309-321

Heim S, Schnieder I, Binz D, Vogel A, Bilitewski U. (1999) Development of an automated microbial sensor system. *Biosens Bioelectron* **14**, 187–93.

Heo, Jinseok, K. Joseph Thomas, Gi Hun Seong, and Richard M. Crooks. (2003) A microfluidic bioreactor based on hydrogel-entrapped E. coli: cell viability, lysis, and intracellular reactions. *Anal. Chem.*

Higgins, M.J. and Novak, J.T. (1997a) Dewatering and settling of activated sludges: The case for using cation analysis. *Water Environment Research* **69** (2), 225-232

Higgins, M.J. and Novak, J.T. (1997b) The effect of cations on the settling and dewatering of activated sludges: Laboratory results. *Water Environment Research* **69** (2), 215-224

Horsburgh, Alison M., D.P. Mardlin, N.L. Turner, R. Henkler, N. Strachan, L.A. Glover, G.I. Paton, K. Kilham. (2002) On-line microbial biosensing and fingerprinting of water pollutants. *Biosensors and bioelectronics* **17**, 495-501

Howbrook, D. N., J.M. Lynch, N.J. Bainton. (2001) An oxidative stress-responsive biosensor: responses to hydrogen peroxide generated by an extracellular enzyme. *Enzyme and Microbial Technology* **29**, 521–526

Junter, GA, I Coquet, S Vilain, T Jouenne. (2002) Immobilized-cell physiology: current data and the potentialities of proteomics. *Enzyme Microb Technol* **31**, 201-212

Kalapos, M.P. (1999) Methylglyoxal in living organisms chemistry, biochemistry, toxicology and biological implications. *Toxicology Letters* **110** 145-175.

Karube, Isao, and Yoko Nomura. (2000) Enzyme sensors for environmental samples. *Journal of Molecular Catalysis B: Enzymatic* **10**, 177-181

Karube, Isao, Yoko Nomura, and Yoshiko Arikawa. (1995) Biosensor for environmental control. *Trends in Analytical Chemistry.* **14** (7), 295-299

Keane, Angela, Pauline Phoenix, Subhasis Ghoshal, Peter C.K. Lau. (2002) Exposing culprit organic pollutants: A review. *Journal of Microbiological Methods* **49**, 103-119

Kim, Byoung Chan, and Man Bock Gu. (2003) A bioluminescent sensor for high throughput toxicity classification. *Biosensors and Bioelectyronics* **18**, 1015-1021

Koh, Won-Gun, Alexander Revzin, and Michael V. Pishko. (2002) Poly(ethylene glycol) hydrogel microstructures encapsulating living cells. *Langmuir* **18**, 2459-2462.

Kohler, Sabine, Till T. Bachmann, Jutta Schmitt, Shimshon Belkin, Rolf D. Schmid. (2000) Detection of 4-chlorobenzoate using immobilized recombinant *Escherichia coli* reporter strains. *Sensors and Actuators B* **70**, 139–144.

Kong, Hun Joon, Molly K. Smith, and David J. Mooney. (2003) Designing alginate hydrogels to maintain viability of immobilized cells. *Biomaterials* **24**, 4023-4029

Kopelman, Raoul, Michael Shortreed, and Sunil Dourado. (1997) Development of a fluorescent optical potassium-selective ion sensor with ratiometric response for intracellular applications. *Sensors and Actuators B* **38-39** 8-12

Lewis, J.C., A. Feltus, C.M. Ensor, S. Ramanathan, S. Daunert. (1998) Applications of reporter genes. *Anal. Chem.* **70**, 579A-585A

Liu, Jing and Bo Mattiasson. (2002) Microbial BOD sensors for wastewater analysis. *Water Research* **36**, 3786–3802

Love, Nancy G. and C.B. Bott. WERF Project 99-WWF-2 Report: A review of and needs survey of upset early warning devices. Water Environment Research Foundation (2000)

Lyngberg, O.K., Stemke, D.J., Schottel, J.L. and Flickinger, M.C. (1999) A single-use luciferase-based mercury biosensor using Escherichia coli HB101 immobilized in a latex copolymer film. *J. Ind. Microbiol. Biotechnol.* **23** 668-676.

Munro, Andrew W., G.Y. Ritchie, A.J. Lamb, R.M. Douglas, and Ir. R. Booth. (1991) The cloning and DNA sequence of the gene for the glutathione-regulated potassium efflux system KefC of *Escherichia coli*. *Molecular Microbiology*. 5(3) 607-616

Murthy, SN, JT Novak, RD De Haas. (1998) Monitoring cations to predict and improve activated sludge settling and dewatering properties of industrial wastewaters. *Water Science and Technology.* **38** (3), 119-126

Moser, Isabella, Gerhard Jobst, and Gerald A. Urban. (2002) Biosensor arrays for simultaneous measurement of glucose, lactate, glutamate, and glutamine. *Biosensors & Bioelectronics* **17**, 297–302.

Ness, L.S., Ferguson, G.P., Nikolaev, Y., and Booth, I.R. (1997) Survival of *Escherichia coli* cells exposed to iodoacetate and chlorodinitrobenzene is independent of the glutathione-gated K^+ efflux systems KefB and KefC. *Applied and Environmental Microbiology* **63** (10), 4083-4086.

Olsson G. and J.F. Andrews. (1981) Dissolved Oxygen Control in the Activated Sludge Process. *Water Science and Technology* **13** (10), 341-347

Petanen, Tiina and Martin Romantschuk. (2002) Use of bioluminescent bacterial sensors as an alternative method for measuring heavy metals in soil extracts. *Analytica Chimica Acta* **456**, 55–61

Petrou, P.S., I. Moser, G. Jobst. (2002) BioMEMS device with integrated microdialysis probe and biosensor array. *Biosensors and Bioelectronics* **17**, 859- 865

Petrou, P.S., I. Moser, G. Jobst. (2003) Microdevice with integrated dialysis probe and biosensor array for continuous multi-analyte monitoring. *Biosensors and Bioelectronics* **18**, 613- 619

Philp, Jim C., Severine Balmand, Eva Hajto, Mark J. Bailey, Siouxsi Wiles, Andrew S. Whiteley, Andrew K. Lilley, Janos Hajto, and Sandra A. Dunbar. (2003) Whole cell immobilized biosensor for toxicity assessment of a wastewater treatment plant treating phenolics-containing waste. *Analytica Chimica Acta*

Polyak, B., E. Bassis, A. Novodvorets, S. Belkin, and R.S. Marks. (1997) Optical fiber bioluminescent whole-cell microbial biosensors to genotoxicants. *Water Science and Technology* **42** (1-2), 305-311

Polyak, Boris S., Efim Bassis, Alex Novodvorets, Shimshon Belkin, Robert S. Marks. (2001) Bioluminescent whole cell optical fiber sensor to genotoxicants: system optimization. *Sensors and Actuators B* **74**, 18-26

Pomposiello, Pablo J and Bruce Demple. (2001) Redox operated genetic switches: the SoxR and OxyR transcription factors. *Trends in Biotechnology* **19** (3), 109-114

Premkumar, J. Rajan, Rachel Rosen, Shimshon Belkin, and Ovadia Lev. (2002) Sol–gel luminescence biosensors: Encapsulation of recombinant *E. coli* reporters in thick silicate films. *Analytica Chimica Acta* **462**, 11–23

Rabbow, Elke, Petra Rettberg, Christa Baumstark-Khan, and Gerda Horneck. (2002) SOS-*LUX*- and LAC-*FLUORO*-TEST for the quantification of genotoxic and/or cytotoxic effects of heavy metal salts. *Analytica Chimica Acta* **456**, 31–39

Rabbow, E., P. Rettberg, C. Baumstark-Khan, G. Homeck. (2003) The SOS-LUX-LAC-FLUORO toxicity test on the international space station (ISS). *Adv. Space Res.* 31(6) 1513-1524

Ramanathan, S., M. Ensor, and S. Daunert. (1997) Bacterial biosensors for monitoring toxic metals. *Trends Biotechnol.* **15**, 500-506

Rasmussen, L.D., S.J. Strensen, R.R. Turner, and T. Barkay. (2000) Application of a mer-lux biosensor for estimating bioavailable mercury in soil. *Soil Biol. Biochem.* **32**, 639-646

Reid, Brian J., Kirk T. Semple, Christopher J. Macleod, Jedda J. Weitz, Graeme I. Paton. (1998) Feasibility of using prokaryote biosensors to assess acute toxicity of polycyclic aromatic hydrocarbons. *FEMS Microbiology Letters* **169**, 227-233

Rettberg, P., K. Bandel, C. Baumstark-Khan, and G. Horneck. (2001) Increased sensitivity of the SOS-LUX-Test for the detection of hydrophobic genotoxic substances with *Salmonella typhimurium* TA1535 as host strain. *Analytica Chimica Acta* **426**, 167–173

Selifonova, O., R. Burlage, and T. Barkay. (1993) Bioluminescent sensors for detection of bioavailable mercury(II) in the environment. *Appl. Environ. Microbiol.* **59**, 3083-3090

Skladal, Petr, Natalya O. Morozova, and Anatoly N. Reshetilov. (2002) Amperometric biosensors for detection of phenol using chemically modified electrodes containing immobilized bacteria. *Biosensors and Bioelectronics* **17**, 867-873

Storz, Gisela and James A Imlay. (1999) Oxidative stress. *Current Opinion in Microbiology* **2**, 188-194

Strachan, Gillian, Sara Preston, Helena Maciel, Andrew J. R. Porter, and Graeme I. Paton. (2001) Use of bacterial biosensors to interpret the toxicity and mixture toxicity of herbicides in freshwater. *Wat. Res*. **35** (14), 3490–3495

Tan, T.C., F. Li, K.G. Neoh, Y.K. Lee. (1992) Microbial membrane modified dissolved oxygen probe for rapid biochemical oxygen demand measurement. *Sens Actuators B* **8**, 167–172.

Tan, T. C. and Quan Zhenrong. (1998) Response characteristics of a dead-cell BOD sensor. *Water Research* **32** (3), 801-807

Tan, T.C., and C. Wu. (1999) BOD sensors using multi-species living or thermally killed cells of a BODSEED microbial culture. *Sens Acutators B* **54**, 252–260.

Taranova, L., I. Semenchuk, T. Manolov, P. Iliasov, and A. Reshetilov. (2002) Bacteria-degraders as the base of an amperometric biosensor for detection of anionic surfactants. *Biosensors and Bioelectronics* **17**, 635- 640

Taurianinen, Sisko, Matti Karp, Wei Chang, and Marko Virta. (1998) Luminescent bacterial sensor for cadmium and lead. *Biosensor and Bioelectronics* **13**, 931-938

Yang, Z., H. Suzuki, S. Sasaki, I. Karube. (1996) Disposable sensor for biochemical oxygen demand. *Appl Microbiol Biotechnol* **46** (1), 10–14.

Yang, Z., H. Suzuki, S. Sasaki, S. McNiven, I. Karube. (1997) Comparison of the dynamic transient- and steady-state measuringmethods in a batch type BOD sensing system. *Sens Acutators B* **45**, 217–222.

Zhan, Wei, Gi Hun Seong, and Richard M. Crooks. (2002) Hydrogel-based microreactors as a functional component of microfluidic systems. *Anal Chem* **74**, 4647-4652

Zheng, M., Aslund F, Storz G. (1998) Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* **279**, 1718-1721

Zheng, Ming and Gisela Storz. (2000) Redox sensing by prokaryotic transcription factors. *Biochemical Pharmacology* **59**, 1-6

Zimmerman, Stefan, Doerte Feinbork, Albert W. Flounders, and Dorian Liepmann. (2003) In-device enzyme immobilization: wafer-level fabrication of an integrated glucose sensor. *Sensors and Actuators B* **99**(1), 163-173

Evaluating strategies for integrating bacterial cells into a biosensor designed to detect process upset
Part A: viability and activity

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Abstract: The biological element of a biosensor to predict process upset in wastewater treatment plants is characterized. The bacterial culture was immobilized in a range of hydrogel polymers: a calcium alginate, a series of photopolymerizable polymers, and a thermally-reversible gel. Oxygen uptake rates and LIVE/DEAD staining were used to determine the activity and viability of the immobilized cultures. The alginate showed good viability, but poor mechanical stability in that the matrix dissolved after five days. The photopolymerizable polymers resulted in high mortality, although the matrix remained stable. The thermal polymer showed extended viability up to 12 days after immobilization, and the material did not deteriorate with time.

1. Introduction

Biosensors show promise for real time monitoring of toxicity in environmental samples. A biosensor is generally defined as an analytical tool that pairs a biological element with a detection system to produce a measurable response, which can be quantified into the amount of analyte present in a sample (Belkin, 2003). The response is typically measured by electrochemical or optical means. Biological elements are typically enzymes, antibodies, proteins, nucleic acids, or whole cells (Belkin, 2003). Whole cells have a certain limited lifespan, have longer response times than isolated components, and may show significant variability between cell batches (Ramanathan *et al.*, 2000). On the other hand, whole cells are less expensive to use because their components require no isolation and purification as in the cell component assay systems. Also, whole cells provide information on the bioavailability of toxins being monitored instead of simply reporting the concentration present. Overall, whole cell biological elements provide a means to monitor more elaborate biological responses than biosensors based on *in vitro* biochemical responses.

For general environmental monitoring, many compounds may be responsible for the toxicity of a sample, and so development of sensors that indicate many different toxic compounds are required. Creating a general response biosensor is typically approached by developing a panel of bacteria, each responsive to a different threat. Panels of *E. coli* bacteria were engineered to create sensors for heat shock, oxidative stress, and protein damage (Belkin *et al.,* 1997), DNA damage (Belkin, 2003), and oxidative damage, membrane damage, DNA damage, and protein damage (Kim and Gu, 2003). A field-ready biological element was developed by Premkumar *et al.* (2003), who immobilized bacteria that were engineered to report on heat shock, oxidative stress, fatty acids, peroxides, and genotoxicity in thick silicate films, which maintained viability and activity for over a month (Premkumar *et al.*, 2003).

On the whole, genetically engineered strains used in biosensors have not been commercialized to a large degree. The difficulty in overcoming the genetically modified organism (GMO) regulations makes such sensors primarily useful only in laboratory settings, where the threat of accidental release is minimized. Despite their advantages in terms of robust, repeatable responses, engineered strains have yet to be widely commercialized.

Non-engineered cells do not mandate such strict controls on release as those that have genetic modifications. While engineered cells may yield a more robust response, wild-type cells facilitate eventual commercialization of a sensor, exemplified by the Microtox® assay, which uses naturally luminescent *Vibrio fischerii* bacteria. Additionally, the use of indigenous, wild-type cells may more accurately reflect the conditions under observation than the use of an engineered lab strain. Using wild-type cells means the intrinsic cell

processes must be harnessed to detect the compounds of interest or determine if the cells are inhibited.

The purpose of this work was to explore strategies for immobilizing non-engineered bacterial cells for inclusion into a biosensor designed to predict process upset in wastewater treatment plants. Process upset is commonly characterized by such symptoms as ineffective biological oxygen demand (BOD) removal, ineffective nitrification, deflocculation, and sludge bulking (Love and Bott, 2000). These process effects observed during an upset event result in poor treatment and possibly permit violations. Developing a biosensor based on the natural cell physiological response to chemical insult would give an early warning that toxic chemicals were present. This warning would significantly benefit wastewater treatment operations by allowing operators time to respond to the approaching threat, and could also clarify the type of operational adjustment needed to prevent or remediate treatment process damage.

For this study, bacterial cells were immobilized in a range of polymer matrices and cell viability and activity was assessed. Immobilization of the bacterial cells was important because it keeps the cell concentration relatively constant by maintaining cell viability while reducing cell division. Additionally, immobilized cells cannot slough off as a biofilm layer and clog downstream channels in the device. Such clogging is particularly problematic if a liquid microfluidic conveyance system is used. Chemically polymerized hydrogels, such as sodium alginate, are useful for macroscale work and form beads from the gel precursor solution (Drury *et al.*, 2004). Photopolymerized polymers have also been used for microfluidic applications because they can easily be formed into a variety of shapes in situ by using various photomasks to control the areas of the precursor that are polymerized (Beebe *et*

al., 2000; Heo *et al.*, 2003). Further, viable cells have been immobilized within photopolymers. One type of photopolymer, polyethylene glycol (PEG) diacrylate (PEG-DA) with Darocure 1173 as the photoinitiator polymerized with UV light, has been used to immobilize *E. coli* cells (Heo *et al.*, 2003) within a microfluidic channel constructed of polydimethylsiloxane (PDMS). The authors attempted to prove cell viability using LIVE/DEAD staining, but found the stain ineffective because the hydrogel fluoresced strongly green, so live cells could not be observed above the background fluorescence. Overall, photopolymers have been used for cell immobilization because it is easy to form them into a variety of shapes *in situ*, while the alginate is suited only for macroscale applications because the ability to control the shape of the polymerized product is limited.

In the current work, a set of three polymers was investigated for use as the immobilization matrix in the biological element of the biosensor: a calcium alginate, a series of photopolymerizable polymers, and a thermally-reversible gel. The specific goals of the work were to evaluate viability and activity of immobilized cells to determine a preferred matrix for further study of detectable physiological responses to chemical perturbations.

2. Materials and Methods

2.1 Bacterial culturing

2.1.1 Growth conditions

Pseudomonas aeruginosa was isolated previously from a local wastewater treatment plant and was grown in a mineral salt medium denoted PA M9. This medium consisted of NaH_2PO_4, 3.0 g; $Na_2HPO_4*7H_2O$, 6.0 g; NH_4Cl, 1.0 g; NaCl, 0.50 g; $MgSO_4^{2-}*7H_2O$, 0.246 g; $CaCl_2$, 0.0147g; $FeSO_4*7H_2O$, 2.5 mg; $ZnCl_2$, 0.25 mg; $MnSO_4*H2O$, 0.185 mg; $CuSO_4$, 0.030 mg; $NaMoO_4*2H_2O$, 0.006 mg; $CoCl_2*6H_2O$, 0.001mg; H_3BO_3, 0.03 mg; glacial

acetic acid, 0.89 mL per liter solution. A second medium denoted PA BT was used to improve alginate stability when it was the immobilizing hydrogel. The PA BT media consisted of: NaH_2PO_4, 0.3 g; $Na_2HPO_4*7H_2O$, 0.6 g; NH_4Cl, 0.1 g; NaCl, 0.05 g; Bis Tris, 1.0 g; $MgSO_4^{2-}*7H_2O$, 0.246 g; $CaCl_2$, 0.0147g; $FeSO_4*7H_2O$, 2.5 mg; $ZnCl_2$, 0.25 mg; $MnSO_4*H2O$, 0.185 mg; $CuSO_4$, 0.030 mg; $NaMoO_4*2H_2O$, 0.006 mg; $CoCl_2*6H_2O$, 0.001mg; H_3BO_3, 0.03 mg; and glacial acetic acid, 0.89 mL per liter solution. After preparing media but before autoclaving, the pH was adjusted to 7.0 using 50% w/v NaOH (approximately 3 mL per liter PA M9 media, and about 4.5 mL per liter PA BT media).

Escherichia coli strain K-12, ATCC 29947, was grown on mineral salt medium M9. This media consisted of NaH_2PO_4, 3.0 g; $Na_2HPO_4*7H_2O$, 6.0 g; NH_4Cl, 1.0 g; NaCl, 0.50 g; $MgSO_4^{2-}*7H_2O$, 0.246 g; $CaCl_2$, 0.0147g; Thiamine HCl, 1.0 mg; and D-glucose, 2.0 g per liter solution. No pH adjustment was required for this media. All media components were obtained from Fisher Scientific (Pittsburgh, PA).

Potassium stock was eliminated from the culture media to reduce the dilution required for the potassium efflux experiments. Potassium contamination from the sodium phosphate buffers, sodium chloride, and sodium hydroxide appeared to be adequate for growth requirements, and resulted in media concentration of about 2 mg/L.

Cultures were maintained in 250 mL Erlenmeyer flasks using a gyratory water bath shaker (Model G76, New Brunswick Scientific, Edison, NJ) set at 37^{o}C and were transferred to fresh media every two days using a 1:100 dilution factor. To expand the volume of culture, cells were transferred at the appropriate dilution to a five liter jug that was aerated using an aquarium pump and diffuser with magnetic stirring placed in an incubator at 37^{o}C. Monthly, cultures were restarted from frozen stock by streaking frozen culture on Luria-

Bertani (LB) agar plates consisting of: NaCl, 10g; tryptone/peptone, 10 g; yeast extract, 5 g; and Bacto agar, 10 g per liter solution. The streaked plate was incubated overnight at 37°C. Then, a single colony was placed aseptically into fresh sterile media.

2.1.2 Growth Curve Preparation

A growth curve was determined for each strain using sidearm flasks containing 100 mL growth media incubated in a rotary water bath shaker maintained at 37°C. Transmittance through the media was measured at 590 nm on a spectrophotometer (Spectronic 20, Bausch and Lomb, Philadelphia, PA). Separate growth curves were determined spectrophotometrically for cultures grown in five liter batches.

2.2 Immobilization polymer preparations

2.2.1 Alginate immobilization

The calcium alginate hydrogel matrix, a copolymer of β-D-mannuronate (M-residue) and α-L-guluronate (G-residue) (reviewed in Rehm 1998), was used as the preliminary immobilization matrix for the cells because this matrix has been used extensively by others (for example, Elasri *et al.*, 2000; Kohler, 2000; Polyak *et al.*, 1997, 2001; Webb *et al*., 1997). To form alginate beads, five liters *P. aeruginosa* were grown overnight to mid-log growth state. The cells were concentrated by centrifugation in multiple bottles for 20 minutes at 4420 x g and the supernatant was discarded. The cells were resuspended in about 20 mL of fresh media and divided into aliquots of equal cell number for the immobilization. The resuspended bacterial concentrate was added to a 2% solution (w/v) of sodium alginate (Protanal LF10/60, FMC BioPolymer, Philadelphia, PA, USA) in water using approximately a 10:1 (v/v) ratio of alginate to cell solution. The solution was well mixed and slowly

dropped by a 21-gauge syringe into a 10% solution (w/v) of calcium chloride in water to form spheres. The beads were rinsed with nanopure water and placed in PA BT media.

PA BT media was used for alginate bead experiments because the beads were found to be unstable in the PA M9 media. The central modification for the BT media was the substantial reduction in sodium concentration. Because the alginate polymerizes by substitution of calcium for sodium within the polymer matrix, it was thought that ion exchange could compromise alginate stability and return the beads to the liquid state, which occurred within an hour in the PA M9 media.

The number of cells per batch was determined by dividing the cell concentration by the number of beads per batch. Cell concentration was determined by plating triplicate serial dilutions in sterile nanopure water of the resuspended culture on Luria-Bertani (LB) agar. The plates were incubated overnight at 37^{o}C, and the cell count was determined by counting the number of colonies per plate and correcting for the dilution factor.

2.2.2 Photo-polymer immobilization

The photopolymerization procedure made it very difficult to create immobilizing beads; therefore, flat disks approximately 0.5 cm in diameter and 0.1 cm in thickness were formed instead. The resuspended bacterial concentrate was added to one of two versions of the photopolymer:

- Photo 1 contained a pre-mixed solution of 5% (w/v) bis(2,4,6-trimethylbenzoyl)-phenylphophineoxide (Irgacure 819, Ciba, Suffolk, VA, USA) with poly(ethylene glycol) (PEG) (MW 400) containing acrylate ends (SR 344, Sartomer, Exton, PA, USA) mixed with the bacterial concentrate to yield a 25% (w/v) polymer solution.

- Photo 2 contained a pre-mixed solution of 1% (w/v) Irgacure 819 with PEG mixed with the bacterial concentrate to yield a 22 % (w/v) polymer solution.

For both compositions, the cells were concentrated into a volume of about 8 mL of media as described in Section 2.2.1, and the concentrated cell solution was mixed with the polymer precursor. The mixture was dropped by pipette onto a plastic sheet and cured for 10 minutes using an aquarium lamp with peak emission at 420 nm. The cured disks were removed from the sheet and rinsed with nanopure water, and then placed in PA M9 media.

A third photopolymer composition, Photo 3, was created using a different initiator. This solution contained the initiator 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone (sold as Irgacure 2959, Ciba, Suffolk, VA, USA) in the Irgacure 819 PEG monomer base. The solutions were premixed as 1% (w/v) of Irgacure 2959 in PEG. The bacterial concentrate was then added and mixed as described above to yield a 22% (w/v) polymer solution. Curing was performed using the same lamp with a cure time of 15 minutes. The disks were rinsed and placed in PA M9 media immediately afterwards. The cell concentration per batch was calculated as described in Section 2.2.1.

2.2.3 Thermal polymer immobilization

A thermal polymer was also tested. As with the photopolymer, small beads were difficult to generate. To form the thermal polymer sphere, the cells were concentrated into about 20 mL of media as described in Section 2.2.1. The concentrated cell solution was mixed at room temperature in a scintillation vial with a 15% (w/v) solution of liquid thermal polymer, N-isopropylacrylamide-co-acrylic acid (NIPA-co-Aac), a copolymer with 98 mole percent NIPA and 2 mole percent AAc, to result in a 10% (w/v) polymer solution. The vial was equilibrated in a water bath at 40^{o}C for about five minutes until the mixture solidified

into a white gel-like sphere about 1.5 cm in diameter. The somewhat spherical gel was removed from the vial and wrapped in single mesh fabric (21 holes per inch) to facilitate handling. The gel was then placed in the gyratory water bath shaker in PA M9 media at 40^{o}C to maintain the polymer in the gelled state. The cell concentration in the matrix was determined by cell counts, as described in Section 2.2.1.

2.3 Cell activity and viability experiments

Bacterial activity and viability within the planktonic and immobilized cultures were evaluated respirometrically using oxygen uptake rates and microscopically using a LIVE/DEAD® stain, respectively. The LIVE/DEAD® stain has been widely used to determine the viability of cells in a population by comparing the number that stain red, representing dead cells, to the number that stain green, representing live cells.

2.3.1 Oxygen uptake rates

Oxygen uptake rates (OURs) were used to determine the activity of planktonic and immobilized bacteria over time, with the procedure shown schematically in Figure 1. Measurements were performed with planktonic cultures in parallel with immobilized cultures at time zero, and continued with immobilized cultures over time. For alginate-immobilized cultures, beads were prepared and wrapped in mesh fabric (21 holes per inch) to facilitate handling. Beads were maintained aerobically under sterile conditions in 250 mL Erlenmeyer flasks containing 100 mL of PA BT media, and were transferred to fresh media daily. To determine the OUR, the mesh-bundled beads were transferred from the growth flask to a 300 mL BOD bottle, which was brought to a volume of 300 mL with media and sealed with the dissolved oxygen (DO) probe (Orion 97-08-99, Beverly, MA). The decrease in dissolved oxygen concentration was monitored over time using a computerized data acquisition system

(LabView version 6.0, National Instruments, Austin, TX) with data collected by an attached meter (Accument Research AR25 Dual Channel ph/Ion Meter, Fisher Scientific, Pittsburgh, PA). After the oxygen uptake rate analysis, the mesh-bundled beads were transferred to a sterile flask with fresh media so that measurements could be performed at a later time using the same immobilized cells. All SOUR tests were performed in duplicate.

To determine OURs for the photopolymer immobilized cells, the disks were placed in 300 mL BOD bottles and PA M9 media was added. The bottles were sealed with the DO probes, and the experiment proceeded as described above.

To determine OURs for the thermal polymer immobilized cells, the fabric-bundled gel was removed from its maintenance flask and transferred to a 300 mL BOD bottle. PA M9 media at 40^{o}C was added to the bottles, which were sealed with the DO probes. The test proceeded as above, except that the bottle was immersed in a 40^{o}C water bath for the duration of the SOUR test. To compensate for the temperature variation, a planktonic control tested at day zero was evaluated at the same temperature.

2.3.2 LIVE/DEAD® staining

The LIVE/DEAD® BacLight™ Bacterial Viability Kit (Molecular Probes, Eugene, Oregon, USA) was used with a fluorescent microscope (Zeiss Axioscope 2 Plus, Thornwood, NY, USA) and camera system (Zeiss AxioCam MRm, Thornwood, NY, USA) to visually compare the numbers of viable and nonviable cells at 20 and 40x magnification. The kit contents were mixed 1:1 and three microliters of the mixture were added to each milliliter of cells to be stained. To stain all gels, an aliquot of the gel was placed in a microcentrifuge tube with 1 mL of water and stained with the same amount of dye.

3. Results and Discussion

3.1 Oxygen uptake rates (OURs) show extended activity in thermal gels, low activity in photopolymer, and activity until material failure in alginate gels

OURs performed on cells immobilized in the first formulation of the photopolymer (Photo 1) showed near-zero activity after polymerization, as shown in Figure 2. Therefore, the concentration of initiator was reduced as much as possible without compromising curing quality or time. This new formulation, which contained 1% rather than 5% initiator, was polymer Photo 2. Unfortunately, this reduction still resulted in very low activity (Figure 2). Therefore, the initiator was changed to Irgacure 2959 at 1% (w/v) (polymer Photo 3), but the results continued to show low activity (Figure 2). Changing the cell type may improve the activity, but the current combination of cells and photo-matrices resulted in an immediate and severe reduction in cell respirometric activity when immobilized in the matrix.

In comparison, cell activity was good in the alginate and thermal matrices, although the OUR results indicated more limited activity initially as compared to planktonic controls containing the same number of cells. Figure 2 summarizes the OUR results from these matrices relative to planktonic controls measured at t=0. Because several groups show elevated OURs relative to control, oxygen diffusion into the polymers appeared uninhibited. In the alginate, the increased OURs probably indicate cell division and colonization within the structure, as observed by Mater *et al.* by microscopy of alginate beads containing immobilized *Pseudomonas* strains. This colonization occurs within the pores of the alginate matrix, which are large enough to permit cell division. The pore size of the thermal polymer is unknown. Because the OURs in the thermal gel also increase over the control, cell division may also be occurring within the polymer structure. In the alginate matrix, the rates

increase consistently, which supports the hypothesis that the cells are using the alginate as a carbon source, creating space for cell division as they consume the structure within which they are contained. Perhaps because of this consumption, the matrix deteriorated within a week of bead manufacture, preventing quantification of the long-term viability of the alginate-immobilized cells. However, an experiment performed to determine whether the cells could use alginate as a sole carbon source showed no difference in initial oxygen uptake rates between immobilized cells (0.050 mg DO per cell per minute) and planktonic cells (0.045 mg DO per liter per minute), both in media without the acetate carbon source. By day two, the OUR for immobilized cells had dropped to 0.019 mg DO per cell per minute, much lower than control, indicating that the cells were losing activity because the carbon source was unacceptable. Perhaps the alginate is metabolized along with HAC, but alone the cells do not consume the polymer.

The thermal polymer showed excellent material stability, with no deterioration observed for over two weeks. The oxygen uptake rates fluctuate relative to the control, as shown in Figure 2, but on the whole the cells remained viable. Clearly, the thermal polymer is a good choice for the immobilization matrix because it is nontoxic to the cells and supports extended viability. However, integrating the thermal polymer into the biosensor is a challenge because of the temperature requirements of this matrix. To overcome this constraint, the sensor requires a small heater to maintain the temperature at the appropriate level (for example, by incorporating titanium/platinum films to permit temperature measurement and regulation, as reviewed in Erickson and Li, 2004). Alternately, the polymer could be modified to shift the critical temperature to a range below ambient (about 25^{o}C). In either case, the thermal polymer showed the most promise for cell activity and,

thus, for inclusion in the biosensor.

3.2 LIVE/DEAD® staining is effective in determining immobilized viability in thermal polymer

The stain was applied to cells immobilized in all matrices tested, but was only effective for the thermal polymer. The spherical bead structure of the alginate prevented the microscope camera from focusing on a single plane of bacteria. Attempts to flatten to beads to permit adequate observation showed a strong background fluorescence with the green stain, making observations of live cells difficult (Figure 3). The photopolymer matrix also fluoresces very strongly green, making observations of live cells difficult, as shown in Figure 3. The thermal polymer showed the ideal response to the dye, in that zero background fluorescence was observed after allowing the polymer to liquefy at room temperature.

The staining of the thermal and photopolymers reinforces the results obtained from the OUR experiments. As shown in Figure 3, all the bacteria immobilized in the photopolymer stain red, whether or not the cells were dropped on the surface of the polymer or mixed with it prior to polymerization. The images shown are typical of those from the other photopolymer formulations. Because the background fluorescence is so strongly green, one could argue that live cells are present that cannot be observed with this method. While that is possible, the extremely low OURs combined with the number of red cells observed in the photopolymers support the conclusion that the survivability in the matrix was very low. The thermal polymer shows good viability, even after 10 days within the matrix, because green cells dominate over red. On the whole, these results replicate the SOUR data, showing that the photopolymer has low viability, while the thermal polymer cells remain mostly viable.

4. Conclusions

A series of experiments were conducted in this work to identify the most appropriate polymer matrix for bacterial cell immobilization for a biosensor. The ideal matrix is physically and chemically stable over several weeks, non-toxic to the cells, and will not limit the diffusion of oxygen, compounds necessary for cell growth, or compounds, which would trigger a stress response. Three matrices were examined here: a calcium alginate, several photopolymerizable hydrogels, and a thermal polymer.

The sodium alginate matrix was unstable, although bacterial consumption of the matrix as a substrate was not validated as the cause. The material degraded within a week, rendering it incapable of containing the bacteria. The photopolymer was toxic to the cells, resulting in significant cell death immediately after polymerization. Changes to the concentration and the composition of the initiator did not reduce the matrix toxicity. Finally, the thermal polymer matrix was non-toxic, stable, and did not hinder oxygen diffusion. The only limitation on the incorporation of the thermal polymer within the microfluidic sensor is the need to maintain a higher-than-ambient temperature in the cell chamber (40^{o}C). However, adding a micro-scale heater such as that reviewed in Erickson and Li (2004) would meet this need. Alternatively, the polymer could be modified to change its immobilization temperature. By modifying the polymer structure, the critical temperature could be reduced below ambient, and thus reduce the need for a heating element. On the whole, even without such chemical modifications the thermal polymer shows great potential for incorporation in the biosensor.

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References

Beebe, David J., Jeffrey S. Moore, Joseph M. Bauer, Qing Yu, Robin H. Liu, Chelladural Devadoss, and Byung-Ho Jo. (2000) Functional hydrogel structures for autonomous flow control inside microfluidic channels. *Letters to Nature* **404** 588-590

Belkin, Shimshon. (2003) Microbial whole-cell sensing systems of environmental pollutants. *Current Opinion in Microbiology* **6** 206–212

Belkin, Shimshon, Dana R. Smulski, Sara Dadon, Amy C. Vollmer, Tina K. Van Dyk, and Robert A. Larossa. A panel of stress-responsive luminous bacteria for the detection of selected classes of toxicants. (1997) *Water Research* **(31)** 12, 3009-3016

Drury, Jeanie L., Robert G. Dennis, David J. Mooney. (2004) The tensile properties of alginate hydrogels. *Biomaterials* **24**, 3187-3199

Elasri, Mohamed O., Tricia Reid, Steven Hutchens, Robert V. Miller. (2000) Response of a *Pseudomonas aeruginosa* biofilm community to DNA-damaging chemical agents. *FEMS Microbiology Ecology* **33**, 21-25

Erickson, David, and Dongqing Li. (2004) Integrated microfluidic devices. Analytica Chimica Acta **507,** 11–26

Gu, Man Bock, and Sue Hyung Choi. (2002) A portable toxicity biosensor using freeze-dried recombinant bioluminescent bacteria. *Biosensor and Bioelectronics.* **17,** 433-440

Heim S, Schnieder I, Binz D, Vogel A, Bilitewski U. (1999) Development of an automated microbial sensor system. *Biosens Bioelectron* **14**, 187–93

Heo, Jinseok, K. Joseph Thomas, Gi Hun Seong, and Richard M. Crooks. (2003) A microfluidic bioreactor based on hydrogel-entrapped E. coli: cell viability, lysis, and intracellular reactions. *Anal. Chem.* **75**(1) 22-26

Horsburgh, Alison M., D.P. Mardlin, N.L. Turner, R. Henkler, N. Strachan, L.A. Glover, G.I. Paton, K. Kilham. (2002) On-line microbial biosensing and fingerprinting of water pollutants. *Biosensors and bioelectronics* **17**, 495-501

Kim, Byoung Chan, and Man Bock Gu. (2003) A bioluminescent sensor for high throughput toxicity classification. *Biosensors and Bioelectyronics* **18**, 1015-1021

Kohler, Sabine, Till T. Bachmann, Jutta Schmitt, Shimshon Belkin, Rolf D. Schmid. (2000) Detection of 4-chlorobenzoate using immobilized recombinant *Escherichia coli* reporter strains. *Sensors and Actuators B* **70,** 139–144

Liu, Jing and Bo Mattiasson. (2002) Microbial BOD sensors for wastewater analysis. *Water Research* **36**, 3786–3802

Love, N. G., Bott, C.B. (2000). WERF Project 99-WWF-2 Report: A review of and needs survey of upset early warning devices. *Water Environment Research Foundation*.

Mater, Denis D.G., Jose E. Nava Saucedo, Nicole Truffaut, Jean-Noel Barbotin, Daniel Thomas. (1999) Conjugative plasmid transfer between *Pseudomonas* strains wtihin alginate bead microcosms: effect of the internal gel structure. *Biotechnology and Bioengineering* **65**(1), 34-42

Philp, Jim C., Severine Balmand, Eva Hajto, Mark J. Bailey, Siouxsi Wiles, Andrew S. Whiteley, Andrew K. Lilley, Janos Hajto, and Sandra A. Dunbar. (2003). Whole cell immobilized biosensor for toxicity assessment of a wastewater treatment plant treating phenolics-containing waste. *Analytica Chimica Acta* **487**(1) 61-74

Polyak, B., E. Bassis, A. Novodvorets, S. Belkin, and R.S. Marks. (1997) Optical fiber bioluminescent whole-cell microbial biosensors to genotoxicants. *Water Science and Technology*. **42** (12), 305-311

Polyak, Boris S., Efim Bassis, Alex Novodvorets, Shimshon Belkin, Robert S. Marks. Bioluminescent whole cell optical fiber sensor to genotoxicants: system optimization. (2001) *Sensors and Actuators B* **74,** 18-26

Premkumar, J. Rajan, Rachel Rosen, Shimshon Belkin, and Ovadia Lev. (2002) Sol–gel luminescence biosensors: Encapsulation of recombinant *E. coli* reporters in thick silicate films. *Analytica Chimica Acta* **462**, 11–23

Ramanathan, S., M. Ensor, and S. Daunert. (1997) Bacterial biosensors for monitoring toxic metals. *Trends Biotechnol.* **15**, 500-506

Rehm, Bernd H.A. (1998) Alginate lyase from Pseudomonas aeruginosa CF1/M1 prefers the hexameric oligomannuronate as substrate. *FEMS Microbiology Letters* **165**, 175-180

Reid, Brian J., Kirk T. Semple, Christopher J. Macleod, Jedda J. Weitz, Graeme I. Paton. (1998) Feasibility of using prokaryote biosensors to assess acute toxicity of polycyclic aromatic hydrocarbons. *FEMS Microbiology Letters* **169**, 227-233

Tan, T.C., and C. Wu. (1999) BOD sensors using multi-species living or thermally killed cells of a BODSEED microbial culture. *Sens Acutators B* **54**, 252–260

Taurianinen, Sisko, Matti Karp, Wei Chang, and Marko Virta. (1998) Luminescent bacterial sensor for cadmium and lead. *Biosensor and Bioelectronics* **13**, 931-938

Webb O. F., P.R. Beinkowski, U. Matrubutham, F.A. Evans, A. Heitzer, G.S. Sayler. (1997) Kineticis and response of a *Pseudomonas fluorescens* HK44 biosensor. *Biotechnology and Bioengineering*. **54** (5), 491-502

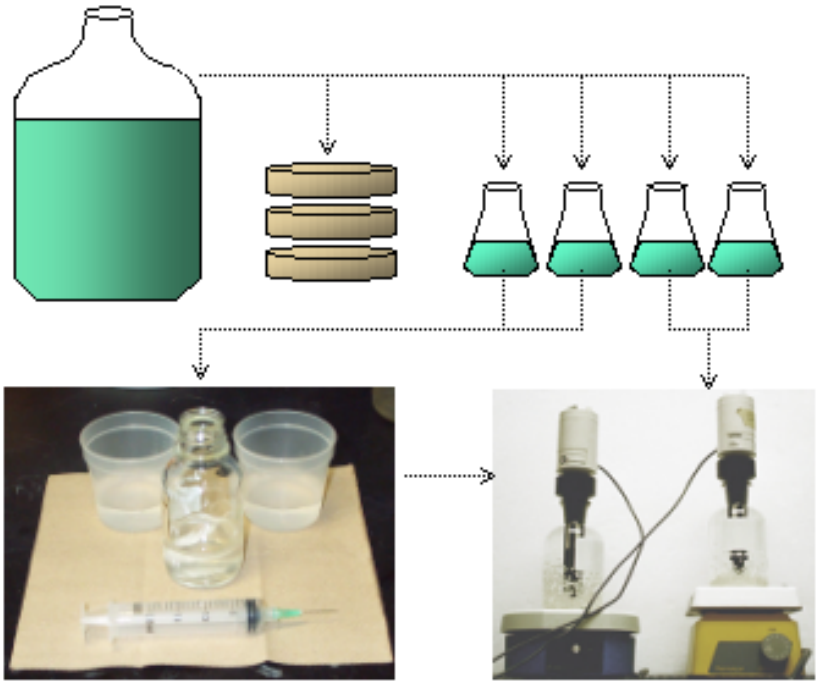

Figure 1: Diagram of the OUR experiment protocol. Cells were grown up in 5 L jugs, concentrated by centrifugation, resuspended, and an aliquot was taken for plate counts. Next, the concentrated cells were divided into four equal batches, with two sets being immobilized in the polymer, and two remaining in the planktonic state. Here, alginate immobilization is shown as an example. The oxygen uptake rates were determined using oxygen probes and a computerized data acquisition system in duplicate for the immobilized cells as well as the planktonic cells.

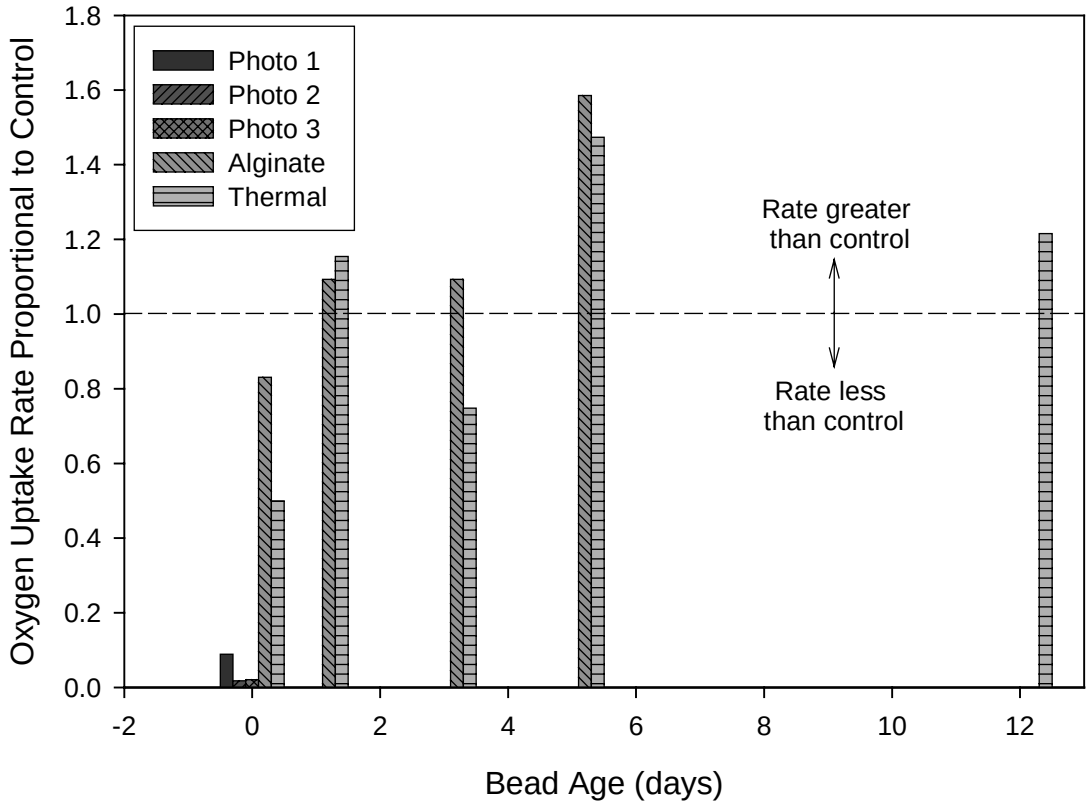

Figure 2: OUR tests for cells immobilized in each polymer matrix. Data is normalized to a planktonic control with equal cell density by dividing the observed rate within the gels by the average rate observed in the controls at t=0. Photopolymers show near zero oxygen uptake, while thermal and alginate matrices show extended viability. The alginate deteriorated within a week, so further testing with that matrix was impossible.

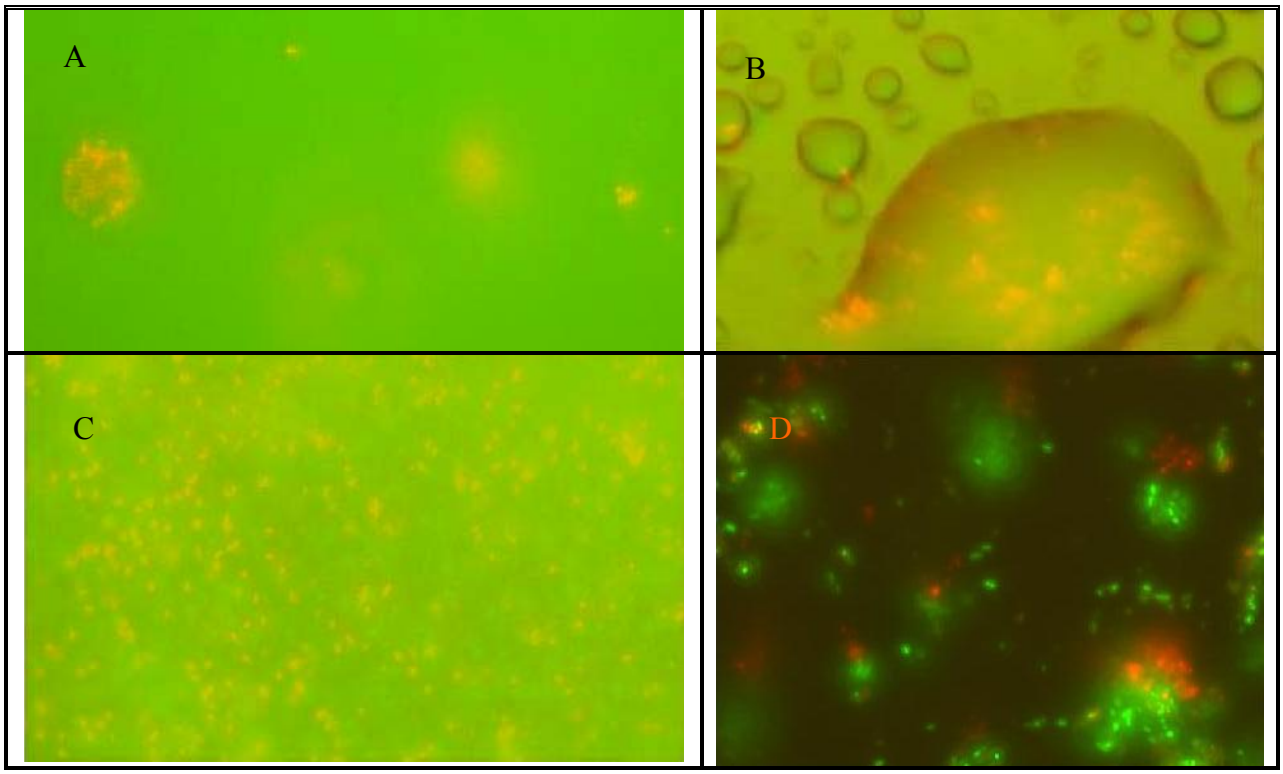

Figure 3: Representative LIVE/DEAD® stains of immobilized cells in photopolymer Photo 1 (A and B), alginate (C), and thermal polymer (D). Cells were dropped on the surface of the polymer before polymerization in Image B, while in A cells were completely mixed with the polymer before polymerization. Photopolymer and alginate images were taken immediately after polymerization, while Image D shows the thermal polymer 10 days after immobilization.

Evaluating strategies for integrating bacterial cells into a biosensor designed to detect electrophilic toxins
Part B: potassium efflux

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Abstract: The biological element of a biosensor to detect electrophilic compounds by harnessing the glutathione-gated potassium efflux (GGKE) stress response system through the detection of effluxed potassium is characterized. The potassium efflux caused by a model electrophile is elucidated in planktonic cultures of *Psuedomonas aeruginosa* and *Escherichia coli*. *P. aeruginosa* was selected for further study because it is an environmentally relevant strain and effluxed comparable potassium per cell to *E. coli*. The bacterial culture was immobilized in two hydrogel polymers: a calcium alginate and a thermally-reversible gel. The potassium efflux from immobilized cells caused by N-ethyl maleimide, a model electrophile, was elucidated. The efflux from the alginate and thermal polymers was strong initially, but deteriorated with time, probably due to potassium limitation in the media.

1. Introduction

Real-time monitoring of toxicity in environmental samples is primarily performed through biosensors or bioassays, because laboratory analytical techniques take more time and require knowledge of the compound of interest in the sample. A number of whole-cell biosensors have been developed using genetically modified bacterial strains that respond to particular compounds, such as phenol (Philp *et al.,* 2003; Gu and Choi, 2002), polycyclic aromatic hydrocarbons (PAHs) (Reid *et al.,* 1998), and heavy metals (Taurianinen *et al.,* 1998). One drawback with most whole cell biosensors is that they employ genetically modified bacteria as the reporter, the use of which is prohibited in many locations. One notable exception is the commercial Microtox® assay, which uses naturally luminescent *Vibrio fischerii* bacteria. Non-engineered cells do not mandate strict controls on release whereas those that have genetic modifications are heavily regulated. Additionally, the use of indigenous wild type cells more accurately reflects the conditions under observation than do

engineered lab strains. With wild-type cells the intrinsic cellular physiology must be harnessed to detect the presence of compounds of interest or to determine if the cells are inhibited or stressed.

Electrophiles are a class of compounds that have been shown to trigger measurable stress responses in bacterial cells (Bott and Love, 2002). Industrially relevant electrophilic (oxidative) toxins include a range of chemicals, such as heavy metals and organic compounds containing chloro- and imide- constituents. This class of toxins, commonly found in industrial wastewater, is believed to impair biomass function in biological treatment plants when present at shock loads, and can result in process upset (Bott and Love, 2002). The most common modes of process upset are ineffective biological oxygen demand (BOD) removal, ineffective nitrification, deflocculation, and sludge bulking (Love and Bott, 2000). Although there is substantial speculation about the origin of these upsets, operators generally have insufficient information to identify the source of the problem. However, when asked, operators suggested that heavy metals and toxic organic compounds (which include electrophilic chemicals) are among the most common sources of these upsets (Love and Bott, 2000).

It is speculated that electrophilic chemicals cause process upset by triggering stress response mechanisms in the biomass. Activated sludge deflocculation, one common component of process upset, has been linked to bacterial activation of a stress response called the glutathione-gated potassium efflux (GGKE) response (Bott and Love, 2002). This mechanism is initiated when an electrophile enters the cell and the reaction between the electrophile and glutathione (N-(N-L-γ-glutamy-L-cysteinyl)glycine) activates potassium efflux pumps located in the cell membrane (Apontoweil and Berends, 1975 a and b) (shown

in Figure 1). GGKE transports potassium ions out of the cell in exchange for hydrogen ions transported into the cell, which acidify the cytoplasm and cause activation of secondary stress responses that protect cells from oxidative damage (Ferguson *et al*., 2000).

In biomass, the GGKE response results in an increase in extracellular potassium, adding additional monovalent cations in the floc matrix, and increasing the intrafloc monovalent to divalent cation ratio (Bott and Love, 2002). This ratio is an important predictor of floc strength (Higgins and Novak, 1997 a and b). Disrupting that ratio makes flocs unstable and results in loss of biomass from the system (Murthy *et al*., 1998). This type of upset encompasses the modes of upsets observed with electrophilic shock, namely, ineffective BOD removal, deflocculation, and sludge bulking. The development of sensors around this GGKE response to detect electrophilic compounds entering sewers or wastewater treatment plants would improve process operation and effluent quality by predicting the process effect linked to such upset events, and thus allow the adjustment of process operations to accommodate and minimize the process impact.

The goal of this project was to develop a biosensor based on the GGKE stress response system. By monitoring bulk potassium as an indicator of the stress experienced by the cells, the biosensor will monitor for electrophilic compounds that have been shown to cause deflocculation in the activated sludge process. The microscale biosensor under development includes a whole cell biological element, potassium detection membranes, and microfluidic componentry that compose a lab-on-a-chip device.

Within the biological element, bacterial cells will be immobilized in a polymer matrix. Immobilization of the bacterial cells is important because it as compared to a biofilm, it keeps the cell concentration more constant by maintaining the viability of the

immobilized cells while reducing cell division. Additionally, immobilized cells cannot slough off as a biofilm layer could and clog the microfluidic channels in the device.

The focus of this work was on the design and characterization of the biological element of the biosensor, with the specific goal of quantifying the impact of immobilization on the rate and degree of potassium efflux in response to a model electrophile. In the current work, two polymers were investigated for use as the immobilization matrix: a calcium alginate and a thermally-reversible gel. In the completed sensor, the concentration of potassium will be monitored as an indicator of bacterial stress. By utilizing the intrinsic response of wild type cells and monitoring the change in potassium ion efflux from them, the device is devoid of genetically modified cells and its eventual use and commercialization are simplified greatly.

2. Materials and Methods

2.1 Bacterial culturing

2.1.1 Growth conditions

Pseudomonas aeruginosa was isolated previously from a local wastewater treatment plant and was grown in a mineral salt medium denoted PA M9. This medium consisted of NaH_2PO_4, 3.0 g; $Na_2HPO_4*7H_2O$, 6.0 g; NH_4Cl, 1.0 g; NaCl, 0.50 g; $MgSO_4^{2-}*7H_2O$, 0.246 g; $CaCl_2$, 0.0147g; $FeSO_4*7H_2O$, 2.5 mg; $ZnCl_2$, 0.25 mg; $MnSO_4*H2O$, 0.185 mg; $CuSO_4$, 0.030 mg; $NaMoO_4*2H_2O$, 0.006 mg; $CoCl_2*6H_2O$, 0.001mg; H_3BO_3, 0.03 mg; glacial acetic acid, 0.89 mL per liter solution. A second medium denoted PA BT was used to improve alginate stability when it was the immobilizing hydrogel. The PA BT media consisted of: NaH_2PO_4, 0.3 g; $Na_2HPO_4*7H_2O$, 0.6 g; NH_4Cl, 0.1 g; NaCl, 0.05 g; Bis Tris, 1.0 g; $MgSO_4^{2-}*7H_2O$, 0.246 g; $CaCl_2$, 0.0147g; $FeSO_4*7H_2O$, 2.5 mg; $ZnCl_2$, 0.25 mg;

$MnSO_4$*H2O, 0.185 mg; $CuSO_4$, 0.030 mg; $NaMoO_4$*$2H_2O$, 0.006 mg; $CoCl_2$*$6H_2O$, 0.001mg; H_3BO_3, 0.03 mg; and glacial acetic acid, 0.89 mL per liter solution. After preparing media but before autoclaving, the pH was adjusted to 7.0 using 50% w/v NaOH (approximately 3 mL per liter PA M9 media, and about 4.5 mL per liter PA BT media).

Escherichia coli strain K-12, ATCC 29947, was grown on mineral salt medium M9. This media consisted of NaH_2PO_4, 3.0 g; Na_2HPO_4*$7H_2O$, 6.0 g; NH_4Cl, 1.0 g; NaCl, 0.50 g; $MgSO_4^{2-}$*$7H_2O$, 0.246 g; $CaCl_2$, 0.0147g; Thiamine HCl, 1.0 mg; and D-glucose, 2.0 g per liter solution. No pH adjustment was required for this media. All media components were obtained from Fisher Scientific (Pittsburgh, PA).

Potassium stock was eliminated from the culture media to reduce the dilution required for the potassium efflux experiments. Potassium contamination from the sodium phosphate buffers, sodium chloride, and sodium hydroxide appeared to be adequate for growth requirements, and resulted in media concentration of about 2 mg/L.

Cultures were maintained in 250 mL Erlenmeyer flasks using a gyratory water bath shaker (Model G76, New Brunswick Scientific, Edison, NJ) set at 37^{o}C and were transferred to fresh media every two days using a 1:100 dilution factor. To expand the volume of culture, cells were transferred at the appropriate dilution to a five liter jug that was aerated using an aquarium pump and diffuser with magnetic stirring placed in an incubator at 37^{o}C. Monthly, cultures were restarted from frozen stock by streaking frozen culture on Luria-Bertani (LB) agar plates consisting of: NaCl, 10g; tryptone/peptone, 10 g; yeast extract, 5 g; and Bacto agar, 10 g per liter solution. The streaked plate was incubated overnight at 37^{o}C. Then, a single colony was placed aseptically into fresh sterile media.

2.1.2 Growth Curve Preparation

A growth curve was determined for each strain using sidearm flasks containing 100 mL growth media incubated in a rotary water bath shaker maintained at 37^{o}C. Transmittance through the media was measured at 590 nm on a spectrophotometer (Spectronic 20, Bausch and Lomb, Philadelphia, PA). Separate growth curves were determined spectrophotometrically for cultures grown in five liter batches.

2.2 Immobilization polymer preparations

2.2.1 Alginate immobilization

The calcium alginate hydrogel matrix, a copolymer of β-D-mannuronate (M-residue) and α-L-guluronate (G-residue) (reviewed in Rehm, 1998), was used as the preliminary immobilization matrix for the cells because this matrix has been used extensively by others (for example, Elasri *et al.,* 2000; Kohler, 2000; Polyak *et al.,* 1997, 2001; Webb *et al.,* 1997). To form alginate beads, five liters *P. aeruginosa* were grown overnight to mid-log growth state. The cells were concentrated by centrifugation in multiple bottles for 20 minutes at 4420 x g and the supernatant was discarded. The cells were resuspended in about 20 mL of fresh media and divided into aliquots of equal cell number for the immobilization. The resuspended bacterial concentrate was added to a 2% solution (w/v) of sodium alginate (Protanal LF10/60, FMC BioPolymer, Philadelphia, PA, USA) in water using approximately a 10:1 (v/v) ratio of alginate to cell solution. The solution was well mixed and slowly dropped by a 21-gauge syringe into a 10% solution (w/v) of calcium chloride in water to form spheres. The beads were rinsed with nanopure water and placed in PA BT media.

PA BT media was used for alginate bead experiments because the beads were found to be unstable in the PA M9 media. The central modification for the BT media was the

substantial reduction in sodium concentration. Because the alginate polymerizes by substitution of calcium for sodium within the polymer matrix, it was thought that ion exchange could compromise alginate stability and return the beads to a liquid state, which occurred within an hour in the PA M9 media.

The number of cells per batch was determined by dividing the cell concentration by the number of beads per batch. Cell concentration was determined by plating triplicate serial dilutions in sterile nanopure water of the resuspended culture on Luria-Bertani (LB) agar. The plates were incubated overnight at 37^{o}C, and the cell count was determined by counting the number of colonies per plate and correcting for the dilution factor.

2.2.2 Thermal polymer immobilization

A thermal polymer was also tested. As with the photopolymer, small beads were difficult to generate. To form the thermal polymer immobilization gel, the cells were concentrated into about 20 mL of media as described in Section 2.2.1. The concentrated cell solution was mixed at room temperature in a scintillation vial with a 15% (w/v) solution of liquid thermal polymer, N-isopropylacrylamide-co-acrylic acid (NIPA-co-Aac), a copolymer with 98 mole percent NIPA and 2 mole percent AAc, to result in a 10% (w/v) polymer solution. The vial was equilibrated in a water bath at 40^{o}C for about five minutes until the mixture solidified into a white gel-like sphere about 1.5 cm in diameter. The somewhat spherical gel was removed from the vial and wrapped in single mesh fabric (21 holes per inch) to facilitate handling. The gel was then placed in the gyratory water bath shaker in PA M9 media at 40^{o}C to maintain the polymer in the gelled state. The cell concentration in the matrix was determined by cell counts, as described in Section 2.2.1.

2.3 Potassium Efflux Experiments

Potassium efflux experiments at various time points on the growth curve with planktonic cultures were carried out by growing five liters *P. aeruginosa* or *E. coli* overnight to the desired growth state in the appropriate growth medium. The cells were concentrated by centrifugation in multiple bottles for 20 minutes at 4420 x g and the supernatant was discarded. The cells were resuspended in fresh media, combined, and the volume was adjusted to 700 mL with fresh media. Samples were taken from the well mixed flask for soluble potassium and plate counts. The resuspended culture was divided evenly among six flasks with 100 mL per flask. The flasks were placed on a multiple position stir plate and aerated using an aquarium pump to ensure oxygenation during the experiment. One milliliter automatic pipetter tips were placed on the end of the air tubing in each flask to reduce bubble size and to prevent contamination of the tubing. Each of three flasks was shocked with 1 mL of a 5 mg/mL N-ethyl malemide (NEM) stock, resulting in a dose of 50 mg/L of NEM. Three control flasks remained undosed with NEM. Samples for potassium measurement were taken over a one-hour time period by filtering the media through 0.2 μm nitrocellulose MCE filters (25 mm diameter, Fisher Scientific, Pittsburgh, PA). Samples were acidified with concentrated nitric acid for preservation, and then diluted 1:10 with nanopure water. One milliliter of diluted sample was removed and replaced with 1 mL of 12.7 g/L cesium chloride stock solution (Alfa Aesar, Ward Hill, MA) to minimize interference from sodium. Potassium standards were prepared using a potassium reference solution (Fisher Scientific, Fairlawn, NJ) and the same concentration of cesium chloride was added to standards. The samples were analyzed on an Atomic Absorption Spectrometer (AA) (5100 PC Atomic Absorption Spectrometer, Perkin Elmer, Norwalk, CT). All glassware used in the potassium

measurements was prepared by acid washing in 10% nitric acid, followed by triple rinsing with nanopure water. Error for potassium efflux potential was calculated from the standard deviation of averages for analysis from three flasks and included standard deviation from the cell counts. Cell counts were determined by plating serial dilutions in sterile nanopure water of the resuspended culture on Luria-Bertani (LB) agar. The plates were incubated overnight at 37^{o}C, and the cell count was determined by counting the number of colonies per plate and correcting for the dilution factor.

Potassium efflux experiments with immobilized cultures (procedure shown in Figure 2) were carried out by growing 5 L culture overnight to mid-log growth state (absorbance of the media was between 0.10 and 0.20 at 590 nm) in PA BT media. The polymer immobilization was carried out as described in Section 2.2. Once complete, the immobilized cells were equally divided among six flasks containing 100 mL media each. The experiment on the potassium concentration effluxed per cell after a shock with NEM proceeded in the same manner as described for the planktonic samples, but the experiment time was extended to two hours.

2.4 Alginate bead material stability testing

The material stability of the alginate beads after exposure to equivalent concentrations of potassium to that observed during efflux was determined using a texture analyzer (TA-XT2i Texture Analyzer, Texture Technologies Corporation, Scarsdale, NY). The instrument was operated to measure force in compression, with a test speed of 0.1mm/s using the 5 kg load cell. Rupture was deemed as the peak of the force curve before reaching the stage.

To determine the effect of potassium on the bead stability, alginate beads without immobilized cells were incubated for two weeks in deionized water containing potassium at a range bracketing the values that would be observed per bead from cell efflux. The maximum efflux per cell was scaled down based on the cell loading per bead to determine the potassium dose per bead. Ten beads were used for each concentration, and the concentrations selected were 0.3 and 0.6 mg potassium per 1.1 mL of liquid to bracket the possible range of efflux potential. The control consisted of 10 beads in 1.1 mL deionized water with no added potassium.

3. Results and Discussion

3.1 Planktonic efflux experiments show no difference between bacterial strains

A series of potassium efflux experiments were conducted on planktonic cultures of *P. aeruginosa* and *E. coli* across their growth curves to determine which strain effluxed the most potassium per cell, and to determine which growth state elicits the strongest efflux response. A typical result from a batch efflux experiment is shown in Figure 3. Figure 4 shows the series of batch experiments that were performed to compare the potassium efflux potential of the two strains at various growth states on media containing comparable potassium concentration. The average K^+ efflux in planktonic cultures grown to mid log state was $(4.50 \pm 0.4) \times 10^{-10}$ mg K^+ per cell for *P. aeruginosa* and $(5.54 \pm 0.8) \times 10^{-10}$ mg K^+ per cell for *E. coli,* while cells in late log phase averaged a potassium efflux of $(3.07 \pm 0.4) \times 10^{-10}$ mg K^+ per cell for *P. aeruginosa* and $(4.15 \pm 0.3) \times 10^{-10}$ mg K^+ per cell for *E. coli*. Although it appears that late log phase (0.20-0.25 and 0.25-0.30 absorbance units measured at 590 nm for *E. coli* and *P. aeruginosa*, respectively) experiments produced somewhat lower efflux potential than mid log phase cells (0.025-0.20 and 0.025-0.25 absorbance units

measured at 590 nm for *E. coli* and *P. aeruginosa*, respectively) no difference exists in the average efflux potentials based on a statistical t-test between mid and late log phase experiments with either culture. Further, no difference exists between the average efflux potentials between the cultures at either growth state. Since the activated sludge *P. aeruginosa* isolate used in these experiments effluxes comparable K^+ on average per cell, we continued to use *P. aeruginosa* in further experiments because it may more accurately represent conditions at wastewater plants than the laboratory *E. coli* strain.

3.2 Material failure in alginate beads cannot be attributed to K^+ efflux

Because the alginate matrix degraded after only five days of polymerization, experiments were conducted to determine if the potassium efflux from the cells caused the breakdown of the polymer structure. The alginate polymerizes by substitution of calcium for sodium within the matrix, and matrix stability was hypothesized to be affected by re-equilibration in sodium. Because of the similarity in the properties of sodium and potassium, it was thought that the potassium effluxed from the cells interacts with the matrix and aids in its degradation. However, the concentration of potassium that would be effluxed into the bulk liquid had no effect on the bead stability as measured by a texture analyzer to measure force in compression, as shown in Figure 5.

3.2 Potassium efflux from alginate-immobilized cells is reduced as compared to planktonic cultures, but nearly equal for thermal polymer-immobilized cells

Experiments to determine the amount of potassium effluxed per cell while immobilized were carried out with *P. aeruginosa* at mid-log growth state in calcium alginate beads. Sample results from initial efflux experiments conducted with alginate-immobilized cells are shown in Figure 6. A t-test showed that the potassium concentration at point four

was statistically different from the initial value, indicating that efflux occurred. The efflux potential with the immobilized culture was $(1.3 \pm 0.1) \times 10^{-10}$ mg K^+ per cell, as shown in Table 1, which is much lower than the efflux from planktonic cultures. However, efflux was significantly reduced in experiments conducted on the same immobilized cells after five days (shown in Figure 7), probably because the maintenance media had a very low potassium concentration, and the cells were unable to replenish their internal potassium stocks. The large variability and the reduced initial efflux potential observed in the data from the alginate immobilized cells is likely due to electrophilic diffusion limitations into the beads and K^+ out of the beads, although these limitations were not explored or quantified.

On the other hand, the efflux potential from the thermal polymer-immobilized cells was higher than that from the alginate and comparable to the results for planktonic culture, as shown in Table 1. The efflux observed one day after immobilization was $(4.6 \pm 0.8) \times 10^{-10}$ mg K^+/cell, four times greater than that of the alginate-immobilized cells (Figure 8). As in the alginate, the efflux potential decreased with time. Figure 9 shows the efflux results from cells immobilized for five days. The figure shows no clear efflux trend, although the control seems to be uptaking potassium. Again, it is thought that the reduced efflux potential with time is attributed to the low-potassium maintenance media.

Table 1: The efflux potential from immobilized P. aeruginosa in two polymer matrices on day one as compared to the average of four planktonic experiments

Matrix	**Efflux Potential (mg K^+ / cell)**	**n**
None (Planktonic)	$(4.50 \pm 0.4) \times 10^{-10}$	4
Alginate	$(1.3 \pm 0.1) \times 10^{-10}$	1
Thermal	$(4.6 \pm 0.8) \times 10^{-10}$	1

4. Conclusions

A series of experiments were conducted in this work to select an appropriate polymer matrix for bacterial cell immobilization within the biosensor under development. The ideal matrix will be physically and chemically stable over several weeks, will be non-toxic to the cells, and will not limit the diffusion of oxygen, compounds necessary for cell growth, or the electrophiles which would trigger a stress response. Two matrices were examined here: a calcium alginate and a thermal polymer.

Both polymers showed stable initial efflux results, but later efflux experiments on the polymers resulted in unstable and reduced efflux, perhaps because the potassium concentration in the matrix was limited, thus preventing the cells from replenishing their potassium stocks. The sodium alginate matrix was materially unstable and unable to contain the bacterial cells after five days, but the cause could not be attributed to the potassium effluxed from the cells. Efflux from the alginate beads was four times lower than that from the thermal or the planktonic cells, and the variability was much higher. The thermal polymer was non-toxic, stable, and the initial efflux potential from thermal polymer-immobilized cells was nearly equal to that from planktonic cells. The only limitation on the incorporation of the thermal polymer within the microfluidic sensor is the need to maintain a higher-than-ambient temperature in the cell chamber. On the whole, the thermal polymer shows great potential for incorporation in the biosensor.

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References

Apontoweil, P., Berends, W. (1975a). Glutathione biosythesis in *Escherichia coli* K-12: properties of the enzymes and regulation. *Biochimica et Biophysica Acta,* **399** (1), 1-9.

Apontoweil, P., Berends, W. (1975b). Isolation and initial characterization of glutathione-deficient mutants of *Escherichia coli* K-12. *Biochimica et Biophysica Acta*. **399**(1), 10-22.

Bott, C. B. and Love, N. G. (2002) Investigating a mechanistic cause for activated sludge deflocculation in response to shock loads of toxic electrophilic chemicals. *Water Environment Research*. **74,** 306-315.

Bott, Charles B. (2001) Elucidating the role of toxin-induced microbial stress responses in biological wastewater treament process upset. Diss. Virginia Polytechnic Insitute and State University.

Elasri, Mohamed O., Tricia Reid, Steven Hutchens, Robert V. Miller. (2000) Response of a *Pseudomonas aeruginosa* biofilm community to DNA-damaging chemical agents. *FEMS Microbiology Ecology* **33**, 21-25

Erickson, David, and Dongqing Li. (2004) Integrated microfluidic devices. Analytica Chimica Acta **507,** 11–26

Ferguson, G., McLaggan P. D., Booth, I.R. (1995). Potassium channel activation by glutathione-S-conjugates in Escherichia coli. *Molecular Microbiology.* **17**(6), 1025-1033.

Gu, Man Bock, and Sue Hyung Choi. (2002) A portable toxicity biosensor using freeze-dried recombinant bioluminescent bacteria. *Biosensor and Bioelectronics.* **17,** 433-440

Higgins, M.J. and Novak, J.T. (1997a) Dewatering and settling of activated sludges: The case for using cation analysis. *Water Environment Research* **69** (2), 225-232

Higgins, M.J. and Novak, J.T. (1997b) The effect of cations on the settling and dewatering of activated sludges: Laboratory results. *Water Environment Research* **69** (2), 215-224

Love, N. G., Bott, C.B. (2000). WERF Project 99-WWF-2 Report: A review of and needs survey of upset early warning devices. *Water Environment Research Foundation*.

Kohler, Sabine, Till T. Bachmann, Jutta Schmitt, Shimshon Belkin, Rolf D. Schmid. (2000) Detection of 4-chlorobenzoate using immobilized recombinant *Escherichia coli* reporter strains. *Sensors and Actuators B* **70,** 139–144

Murthy, SN, JT Novak, RD De Haas. (1998) Monitoring cations to predict and improve activated sludge settling and dewatering properties of industrial wastewaters. *Water Science and Technology.* **38** (3), 119-126

Philp, Jim C., Severine Balmand, Eva Hajto, Mark J. Bailey, Siouxsi Wiles, Andrew S. Whiteley, Andrew K. Lilley, Janos Hajto, and Sandra A. Dunbar. (2003). Whole cell immobilized biosensor for toxicity assessment of a wastewater treatment plant treating phenolics-containing waste. *Analytica Chimica Acta* **487**(1) 61-74

Polyak, B., E. Bassis, A. Novodvorets, S. Belkin, and R.S. Marks. (1997) Optical fiber bioluminescent whole-cell microbial biosensors to genotoxicants. *Water Science and Technology*. **42** (12), 305-311

Polyak, Boris S., Efim Bassis, Alex Novodvorets, Shimshon Belkin, Robert S. Marks. Bioluminescent whole cell optical fiber sensor to genotoxicants: system optimization. (2001) *Sensors and Actuators B* **74,** 18-26

Rehm, Bernd H.A. (1998) Alginate lyase from Pseudomonas aeruginosa CF1/M1 prefers the hexameric oligomannuronate as substrate. *FEMS Microbiology Letters* **165**, 175-180

Reid, Brian J., Kirk T. Semple, Christopher J. Macleod, Jedda J. Weitz, Graeme I. Paton. (1998) Feasibility of using prokaryote biosensors to assess acute toxicity of polycyclic aromatic hydrocarbons. *FEMS Microbiology Letters* **169**, 227-233

Taurianinen, Sisko, Matti Karp, Wei Chang, and Marko Virta. (1998) Luminescent bacterial sensor for cadmium and lead. *Biosensor and Bioelectronics* **13**, 931-938

Webb O. F., P.R. Beinkowski, U. Matrubutham, F.A. Evans, A. Heitzer, G.S. Sayler. (1997) Kineticis and response of a *Pseudomonas fluorescens* HK44 biosensor. *Biotechnology and Bioengineering*. **54** (5), 491-502

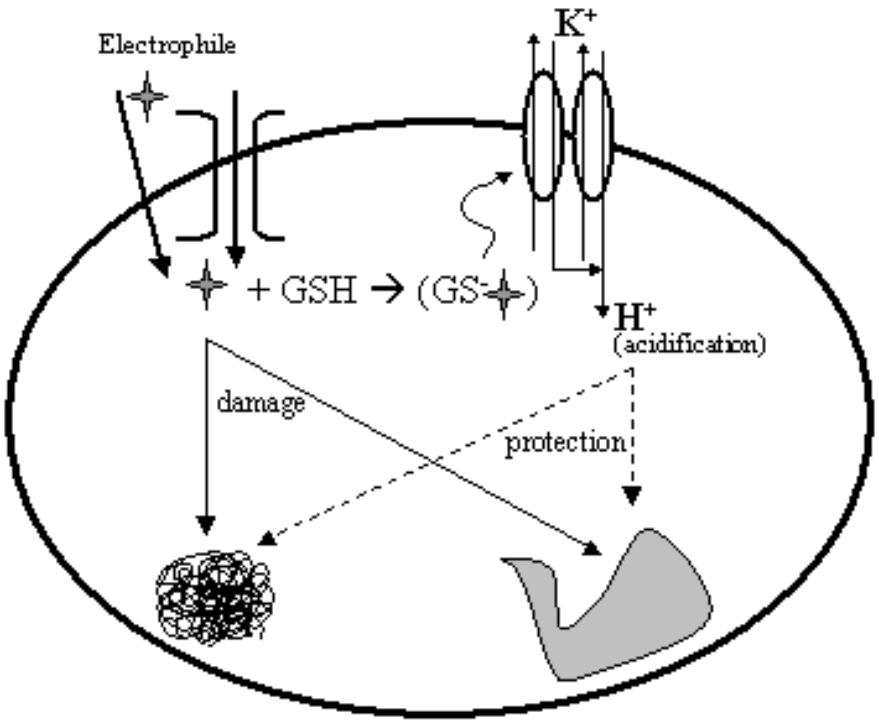


Figure 1: The glutathione-gated potassium efflux (GGKE) system. When an electrophile enters a cell, it can damage DNA and proteins by oxidation. Cells have developed the GGKE stress response to reduce this type of damage. Reduced glutathione, GSH, reacts with the electrophile to create a glutathione-electrophile conjugate. The presence of this conjugate within the cells triggers efflux channels that efflux potassium outside the cell while concurrently importing hydrogen ions. These hydrogen ions acidify the cytoplasm, and the pH drop affords protection from the electrophile for the DNA and proteins within the cell. Overall, the GGKE stress response system is characterized by potassium efflux in response to an electrophilic challenge. (After Dr. Charles Bott, 2001)

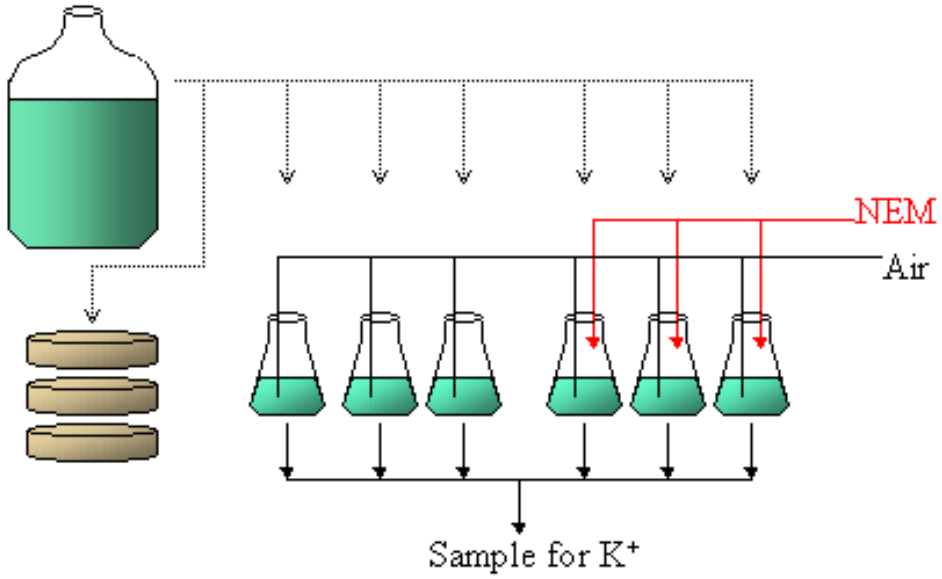


Figure 2: The experimental setup for potassium efflux experiments. Culture was grown to the appropriate growth state in a 5 L jug, then concentrated by centrifugation and resuspended. An aliquot for plate counts was removed. The concentrated culture was divided among six flasks, which were aerated and, at time=0, dosed with NEM. Samples for potassium were taken over time to determine the increase in potassium concentration due to cell efflux.

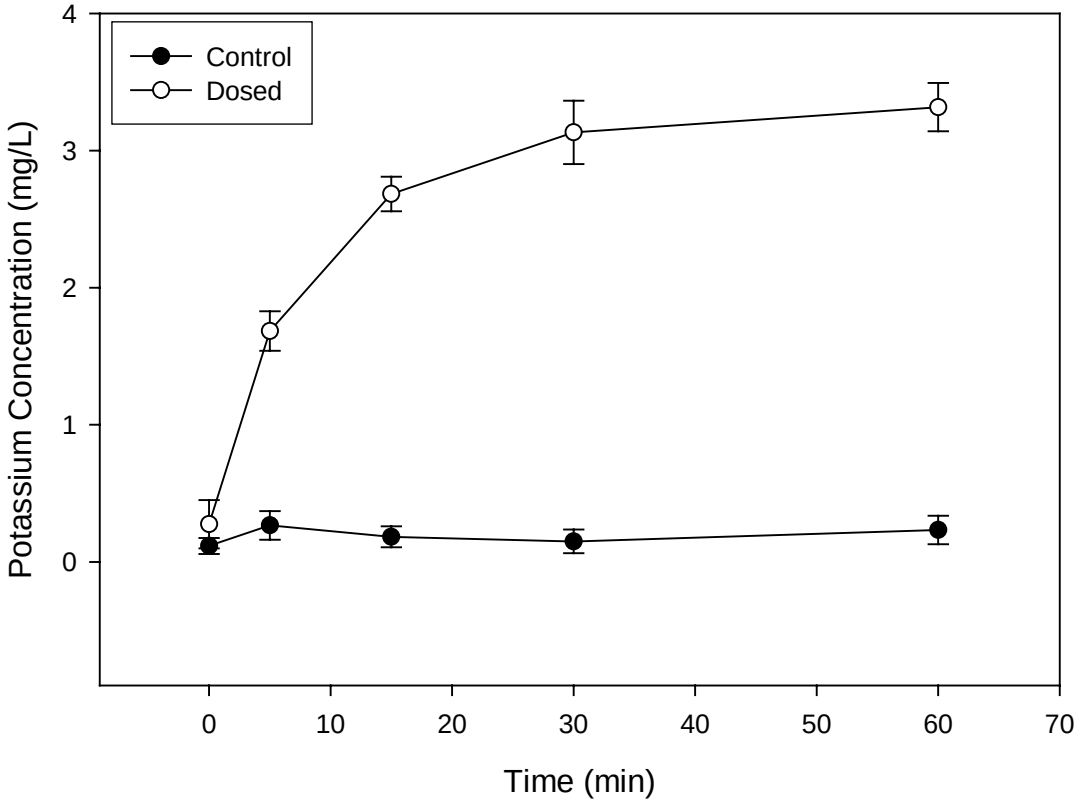


Figure 3. Batch K^+ efflux experiment with planktonic *P. aeruginosa* at an absorbance (measured at 590 nm) of 0.215 after exposure to 50 mg/L N-ethylmaleimide (NEM) versus an unshocked control. Error bars represent the standard deviation of the averages for three flasks.

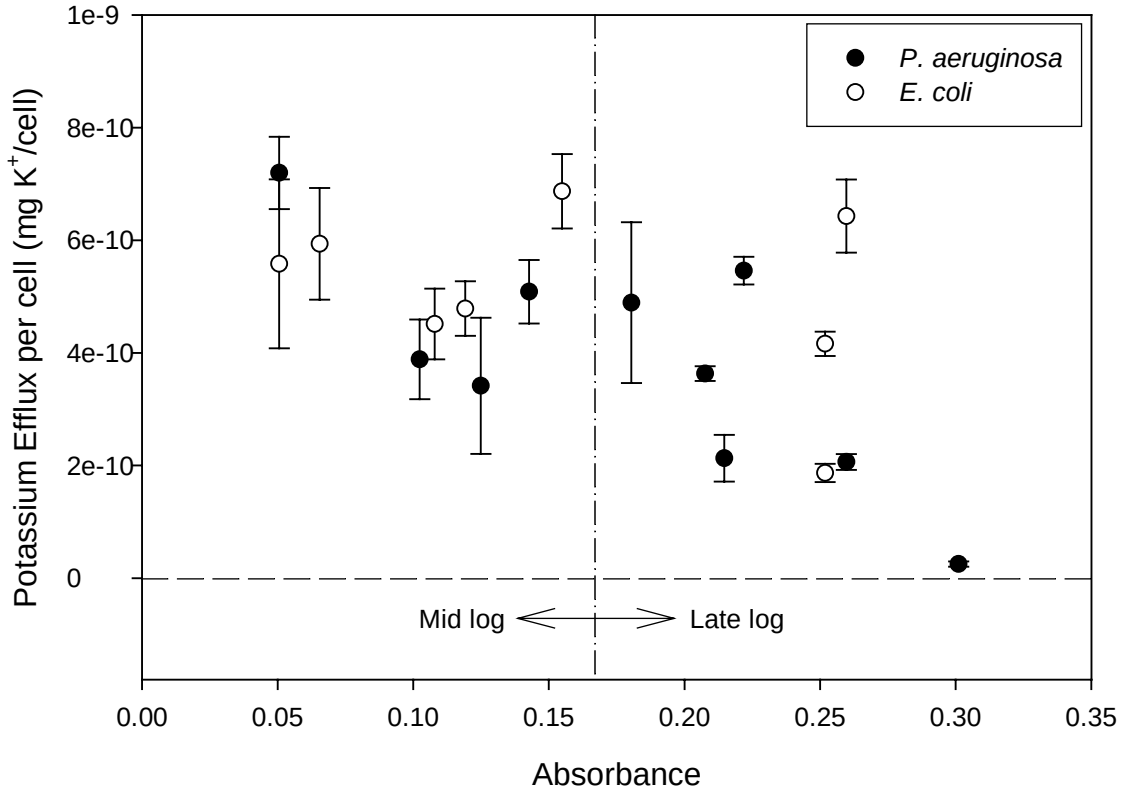


Figure 4. K^+ efflux potentials (mg K^+ effluxed per cell) versus planktonic culture density, measured as absorbance (at 590 nm), for *P. aeruginosa* and *E. coli*.

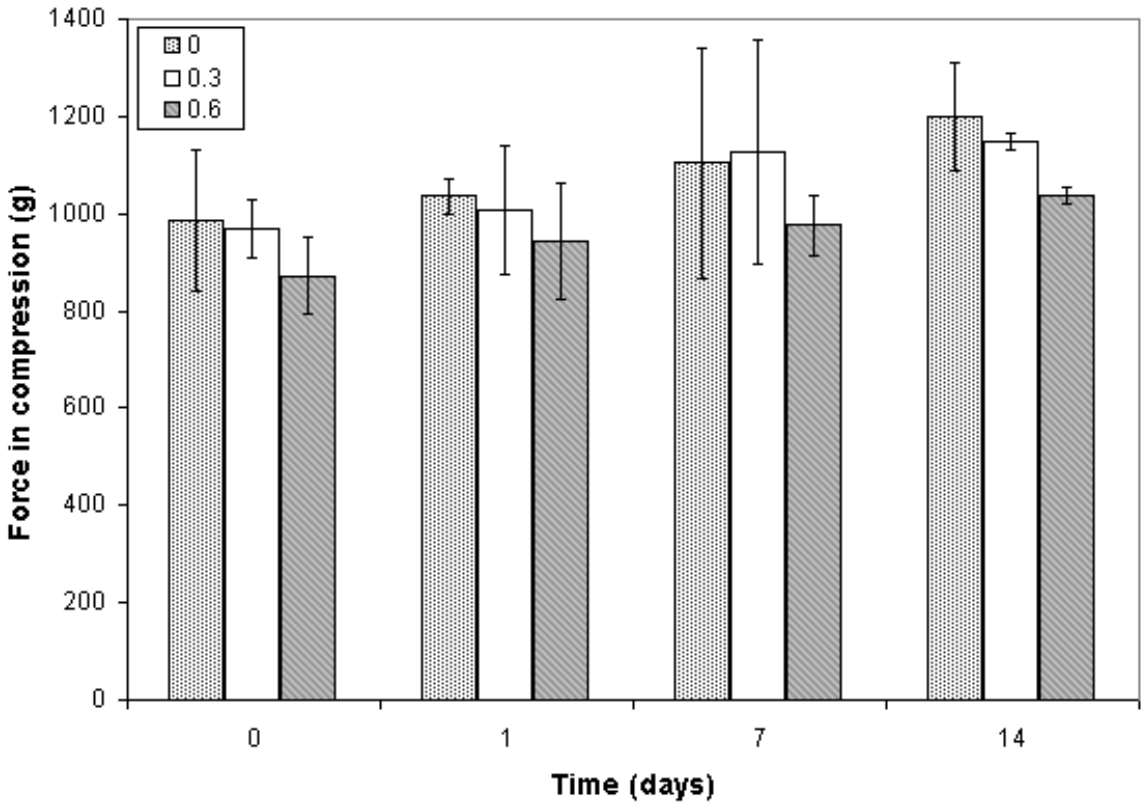


Figure 5: No significant difference is observed in the force required to rupture for alginate beads incubated over time with potassium concentrations comparable to that effluxed from immobilized cells.

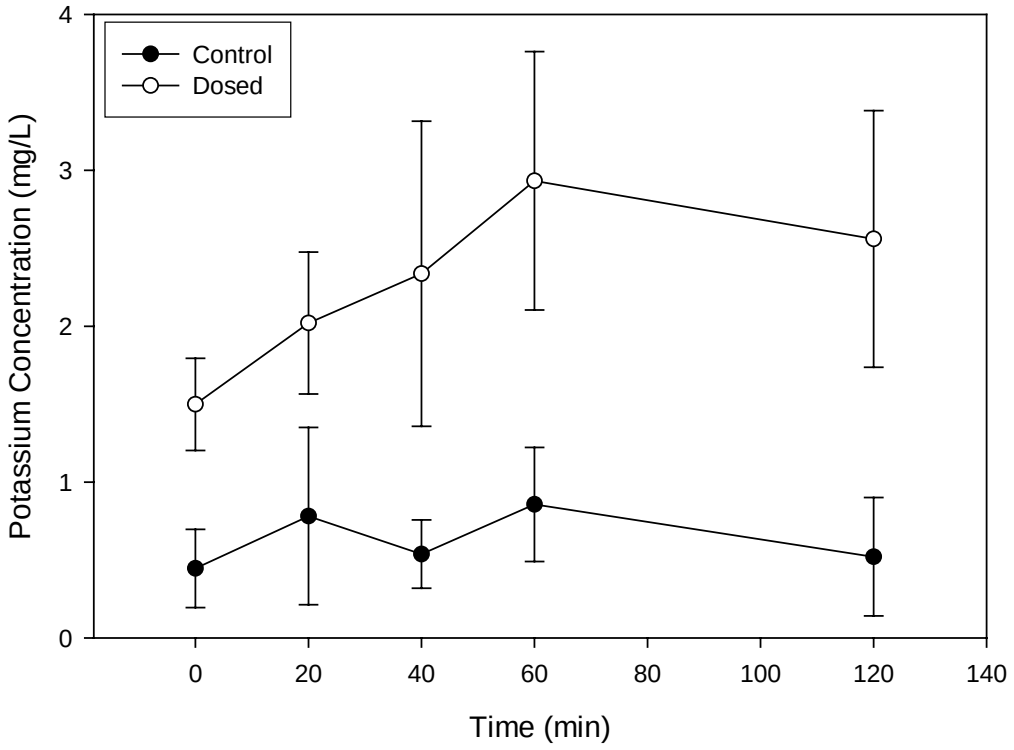


Figure 6. K^+ efflux ($(1.3 \pm 0.1) \times 10^{-10}$ mg K^+ per cell) at t=1 day from *P. aeruginosa* immobilized in calcium alginate beads after exposure to 50 mg/L NEM versus an unstressed, immobilized control.

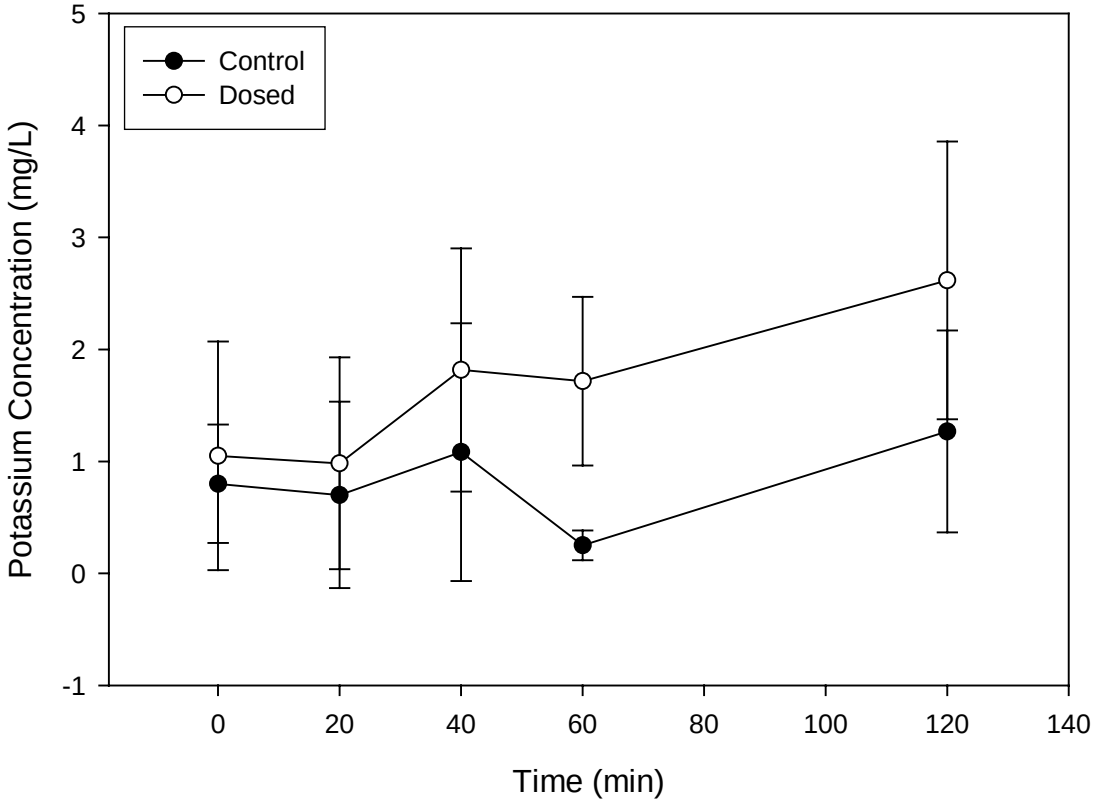


Figure 7: K^+ efflux $((1.3 \pm 0.1) \times 10^{-10}$ mg K^+ per cell) at t=5 days from *P. aeruginosa* immobilized in calcium alginate beads after exposure to 50 mg/L NEM versus an unstressed, immobilized control.

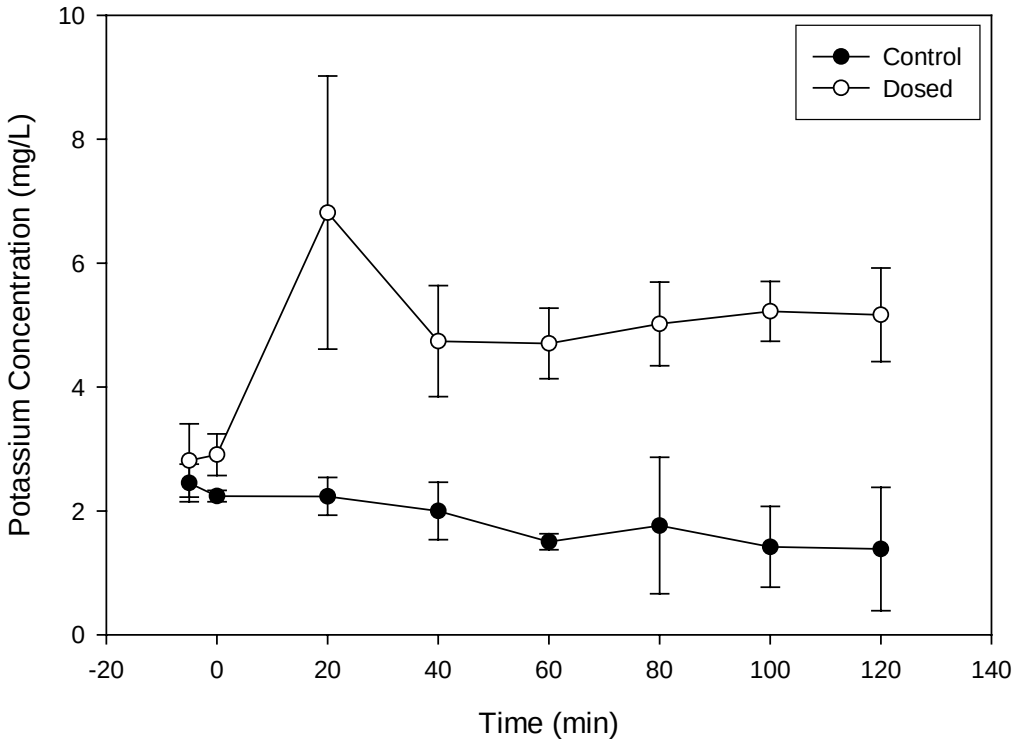


Figure 8. K^+ efflux ((4.6 $\pm$ 0.8) x 10^{-10} mg K^+/cell) at t=1day from *P. aeruginosa* immobilized in thermal polymer gels after exposure to 50 mg/L NEM versus an unstressed, immobilized control.

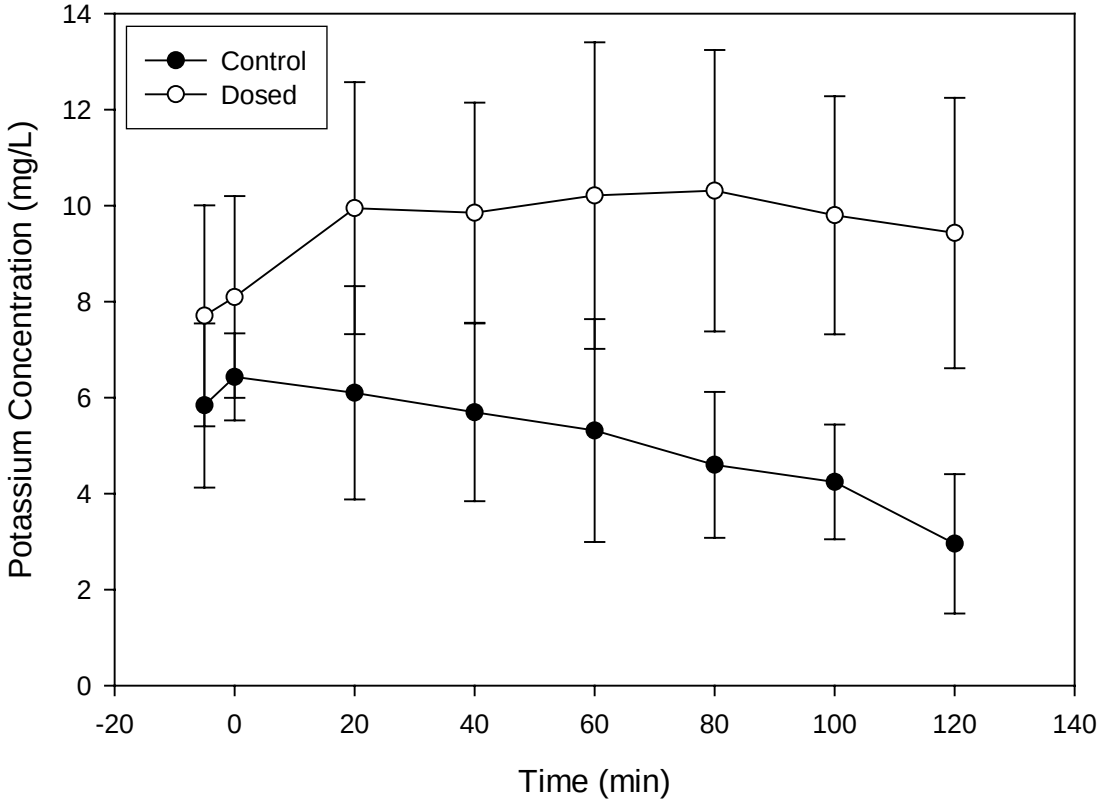


Figure 9: K^+ efflux ((4.6 $\pm$ 0.8) x 10^{-10} mg K^+/cell) at t=5 days from *P. aeruginosa* immobilized in thermal polymer gels after exposure to 50 mg/L NEM versus an unstressed, immobilized control.

Chapter 4: Engineering Significance

Engineering Significance

Properly functioning wastewater treatment plants are vital to human health and water quality. However, even the most efficient plant may have instances of process upset, characterized by deflocculation and poor nutrient and BOD removal, that result in the failure of the treatment train. During such an event, operators typically are unaware that upset has begun until the event is well underway and the damage to the biological treatment step has been done. Providing operators with advanced warning when an upset is about to occur would allow them to initiate protective measures to ensure the continued functioning of the treatment process. Such advance warning would be possible with the biosensor under development here.

This biosensor will be the first to harness the GGKE stress response mechanism to link the efflux of potassium from immobilized cells with the presence of an electrophile in the sample. The change in potassium concentration across the sensor caused by potassium efflux from immobilized cells will be monitored; and, at a certain threshold increase, activation of the GGKE system will be inferred. Because the system is activated by electrophiles, the sensor will not be useful for upset events caused by other classes of compounds, such as oil and grease. Since many upsets can be linked to electrophilic shock and potassium efflux and, thus, to deflocculation, the sensor will serve a vital role in improving the functionality of wastewater treatment plants under the influence of toxic chemical shocks.

Incorporating this sensor in the real-time analyses of plant influent will inform operators of potential upset due to electrophilic shock. However, it will not tell the whole

story of the potential for upset events because it is only useful for predicting electrophilic shock upset. Combining this sensor with other real-time sensors, such as sensors measuring the influent's effect on oxygen uptake rates within the bioreactor, would provide a more complete picture of the upset potential of the influent. Adding sensors for other effects on the biomass to create a sensor network that reponds to plant influent in real time would significantly improve the operator's control over process operations by providing information on the types of effects the influent may cause in the biomass. Although such a sensor would provide limited information on the cause of the effect, the early warning allows operators time to reroute the suspect influent or initiate treatment steps to mitigate the toxic effects of the influent.

In addition to using an array of biosensors to monitor the plant influent, such sensors might be applied to test the effluent from industries that discharge to the sewer system. This testing could be conducted as a prerequisite for discharge to the sewer, to ensure the safety of the large quantities of wastewater generated from industrial activities. Alternately, daily samples of industrial wastewater could be collected and the testing could be performed on the stored samples after an upset event, or after the upset warning sensors at the plant were triggered, in an attempt to find the cause of the upset. Such a system would work well in an area containing a few high volume industrial contributions where the testing of stored samples would take minimal time. As the number of industries increases, the time to determine the source of the cause increases, as does the age of the sample tested, reducing the reliability of the results. Clearly, for such attempts to link the causative industry to an upset event, the results of the biosensor must be proven and reliable. Extensive tests to determine the response of the sensor against background shifts in pH and temperature must be

performed to rule out the possibility of false positive results. On the whole, an array of biosensors to monitor for process upset by electrophilic shock and by other modes of upset would improve the state of wastewater treatment by allowing operators time to respond to the threat, and by potentially enabling them to link the each upset with its cause.

Appendix A: Data for Chapter 2

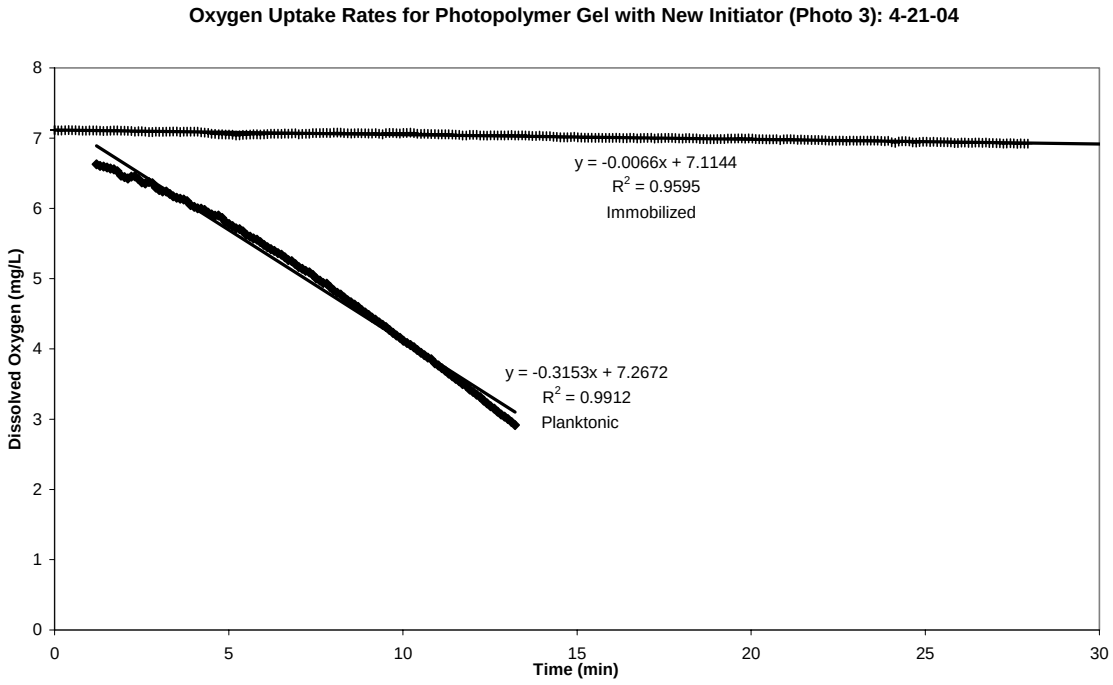
A.1 Data for oxygen uptake rate experiments

Data averaged from Probe A and B
Presented in Chapter 2, Figure 2

A.1.1 Photopolymerizable polymer: Photo 3, 4-21-04, Book 2, Page 87

Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)
1.2	6.628	0	7.114
1.3	6.604	0	7.115
1.4	6.5955	0.1	7.108
1.5	6.582	0.2	7.1095
1.6	6.568	0.3	7.1115
1.7	6.5565	0.4	7.1155
1.8	6.5285	0.5	7.113
1.9	6.4665	0.6	7.1105
2	6.446	0.7	7.109
2.1	6.4255	0.8	7.1065
2.2	6.4525	0.9	7.107
2.3	6.458	1	7.108
2.4	6.4165	1.1	7.1095
2.5	6.37	1.2	7.108
2.6	6.354	1.3	7.1055
2.7	6.3725	1.4	7.1
2.8	6.358	1.5	7.103
2.91	6.2965	1.6	7.1055
3	6.2605	1.7	7.109
3.1	6.2405	1.8	7.108
3.21	6.241	1.9	7.1055
3.31	6.2055	2	7.106
3.41	6.1725	2.1	7.1045
3.51	6.151	2.2	7.1005
3.61	6.1375	2.3	7.092
3.71	6.126	2.4	7.097
3.81	6.103	2.5	7.097
3.91	6.048	2.6	7.092
4.01	6.021	2.7	7.087
4.11	6.0005	2.81	7.0855
4.21	5.9955	2.91	7.093
4.31	5.9795	3.01	7.0895
4.41	5.939	3.11	7.0895
4.51	5.9155	3.21	7.092
4.61	5.894	3.31	7.0925
4.71	5.899	3.41	7.095
4.81	5.8635	3.51	7.0945
4.91	5.805	3.61	7.0865
5.01	5.7755	3.71	7.0915
5.11	5.748	3.81	7.093
5.21	5.716	3.91	7.092
5.31	5.704	4.01	7.093
5.41	5.6715	4.11	7.0905
5.51	5.6235	4.21	7.0825
5.61	5.5965	4.31	7.0785
5.71	5.5705	4.41	7.074
5.81	5.5535	4.51	7.0615
5.91	5.519	4.61	7.0605
6.01	5.4805	4.71	7.054
6.11	5.4485	4.81	7.053
6.21	5.42	4.91	7.0515
6.31	5.396	5.01	7.052
6.41	5.363	5.11	7.0475
6.51	5.3405	5.21	7.037
6.61	5.3035	5.31	7.041
6.71	5.264	5.41	7.0455
6.81	5.248	5.51	7.048
6.91	5.206	5.61	7.052
7.01	5.1645	5.71	7.054
7.11	5.1385	5.81	7.0555
7.21	5.109	5.91	7.05
7.31	5.0905	6.01	7.0535
7.411	5.0525	6.109	7.058
7.511	5.003	6.21	7.0625
7.612	4.971	6.31	7.063
7.712	4.9385	6.41	7.0615
7.812	4.93	6.51	7.059
7.912	4.8805	6.61	7.0645
8.012	4.834	6.711	7.0635
8.113	4.8	6.811	7.0645
8.212	4.774	6.911	7.0675
8.313	4.7315	7.011	7.0535
8.413	4.692	7.111	7.059
8.512	4.663	7.212	7.063
8.613	4.6295	7.312	7.0635
8.713	4.596	7.412	7.07
8.814	4.554	7.512	7.07
8.913	4.518	7.612	7.07
9.014	4.485	7.712	7.073
9.114	4.4435	7.812	7.0745
9.214	4.416	7.913	7.0725
9.314	4.377	8.013	7.0785
9.414	4.343	8.113	7.0775
9.515	4.311	8.213	7.0765
9.615	4.269	8.313	7.071
9.715	4.2265	8.414	7.069
9.815	4.189	8.513	7.067
9.915	4.158	8.613	7.069
10.02	4.114	8.714	7.0705
10.12	4.0825	8.814	7.069
10.22	4.0535	8.914	7.069
10.32	4.022	9.014	7.065
10.42	3.982	9.114	7.0655
10.52	3.9465	9.22	7.0725
10.62	3.9095	9.31	7.064
10.72	3.875	9.42	7.054
10.82	3.852	9.52	7.0715
10.92	3.795	9.62	7.075
11.02	3.764	9.72	7.074
11.12	3.7255	9.82	7.069
11.22	3.688	9.92	7.0665
11.32	3.6485	10	7.0735
11.42	3.6095	10.1	7.0745
11.52	3.574	10.2	7.076
11.62	3.538	10.3	7.0705
11.72	3.498	10.4	7.064
11.82	3.4715	10.5	7.0605
11.92	3.421	10.6	7.0625
12.02	3.3855	10.7	7.061
12.12	3.3495	10.8	7.057
12.22	3.314	10.9	7.056
12.32	3.266	11	7.0555
12.42	3.225	11.1	7.0555
12.52	3.185	11.2	7.0555
12.62	3.148	11.3	7.0535
12.72	3.105	11.4	7.0505
12.82	3.0665	11.5	7.047
12.92	3.0355	11.6	7.0455
13.02	3.002	11.7	7.036
13.12	2.9595	11.8	7.037
13.22	2.9145	11.9	7.0445
		12	7.0455
		12.1	7.044
		12.2	7.037

Time (min)	Av DO for Immobilized (mg/L)
12.32	7.037
12.42	7.0325
12.52	7.0385
12.62	7.0385
12.72	7.037
12.82	7.041
12.92	7.036
13.02	7.0405
13.12	7.042
13.22	7.038
13.32	7.038
13.42	7.0385
13.52	7.0355
13.62	7.033
13.72	7.031
13.82	7.032
13.92	7.033
14.02	7.029
14.12	7.029
14.22	7.0295
14.32	7.02
14.42	7.016
14.52	7.011
14.62	7.0115
14.72	7.011
14.82	7.0125
14.92	7.0205
15.02	7.0095
15.12	7.0085
15.22	7.0055
15.32	7.006
15.42	7.0055
15.52	7.003
15.62	7.004
15.72	7.0035
15.83	7.0035
15.93	7.0025
16.02	7.003
16.13	7.004
16.23	7.0015
16.33	6.9995
16.43	6.998
16.53	7.005
16.63	7.0035
16.73	6.996
16.83	7
16.93	7.0025
17.03	7.0025
17.13	7.005
17.23	6.9935
17.33	7.0005
17.43	7.0005
17.53	7.0015
17.63	7.003
17.73	7.0035
17.83	7.0005
17.93	6.9975
18.03	6.993
18.13	6.998
18.23	6.998
18.33	6.9975
18.43	6.995
18.53	6.988
18.63	6.988
18.73	6.9835
18.83	6.9845
18.93	6.9835
19.03	6.988
19.13	6.988
19.23	6.9865
19.33	6.994
19.43	6.994
19.53	6.994
19.63	6.9965
19.73	6.996
19.83	6.9895
19.93	6.9955
20.03	6.9925
20.13	6.987
20.23	6.9855
20.33	6.9845
20.43	6.982
20.53	6.983
20.63	6.983
20.73	6.985
20.83	6.9715
20.93	6.9815
21.03	6.9855
21.13	6.9845
21.23	6.981
21.33	6.975
21.43	6.979
21.53	6.9765
21.63	6.977
21.73	6.972
21.83	6.9725
21.93	6.973
22.03	6.971
22.13	6.968
22.23	6.9635
22.33	6.9615
22.43	6.966
22.54	6.9655
22.64	6.9655
22.74	6.96
22.84	6.9635
22.94	6.966
23.04	6.962
23.14	6.9635
23.24	6.9645
23.34	6.966
23.44	6.965
23.54	6.966
23.64	6.964
23.74	6.9575
23.84	6.956
23.94	6.961
24.04	6.9425
24.14	6.934
24.24	6.9505
24.34	6.955
24.44	6.955
24.54	6.952
24.64	6.94
24.7	6.9365
24.8	6.944
24.9	6.9465
25	6.946
25.1	6.9455
25.2	6.9475
25.3	6.944
25.4	6.944
25.5	6.9415
25.6	6.942
25.7	6.9385
25.8	6.9295
25.9	6.9305
26	6.9365
26.1	6.9365
26.2	6.934
26.3	6.932
26.4	6.9355
26.5	6.9345
26.6	6.9335
26.7	6.935
26.8	6.9315
26.9	6.9305
27	6.9265
27.1	6.927
27.2	6.921
27.3	6.9255
27.4	6.9265
27.5	6.9235
27.6	6.918
27.7	6.923



A.1.2 Photopolymerizable polymer: Photo 2, 3-18-04, Book 2, Page 77

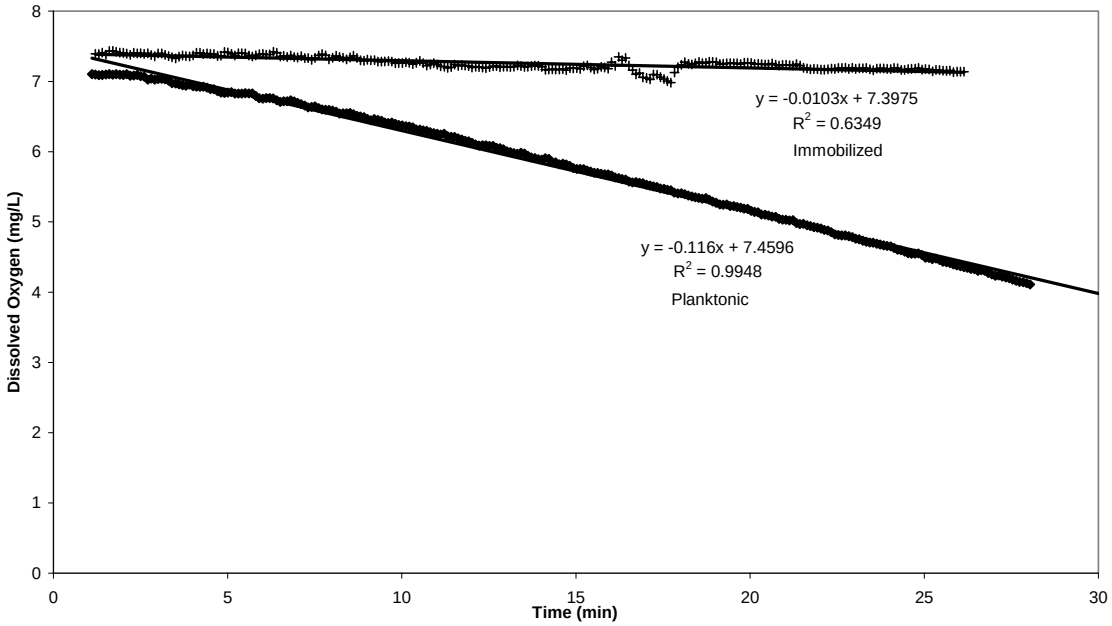
Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)
1.102	7.1025	1.2	7.3925
1.202	7.097	1.3	7.3895
1.302	7.0895	1.4	7.409
1.402	7.091	1.5	7.3795
1.502	7.096	1.6	7.431
1.602	7.1025	1.7	7.4345
1.703	7.0955	1.8	7.4195
1.803	7.1035	1.9	7.4075
1.903	7.0935	2	7.405
2.003	7.0925	2.1	7.394
2.103	7.102	2.2	7.3765
2.203	7.0795	2.3	7.4045
2.303	7.083	2.4	7.3965
2.404	7.0915	2.5	7.4
2.504	7.073	2.6	7.3915
2.603	7.0675	2.7	7.399
2.704	7.025	2.8	7.374
2.804	7.0385	2.91	7.356
2.905	7.026	3	7.3985
3.004	7.034	3.11	7.397
3.104	7.0355	3.21	7.376
3.205	7.0285	3.31	7.3585
3.305	7.0095	3.41	7.343
3.405	6.974	3.51	7.3275
3.505	6.9695	3.61	7.35
3.605	6.9575	3.71	7.3685
3.706	6.9435	3.81	7.3595
3.805	6.9375	3.91	7.355
3.905	6.954	4.01	7.3705
4.006	6.93	4.11	7.406
4.106	6.9255	4.21	7.407
4.21	6.9205	4.31	7.39
4.31	6.9255	4.41	7.398
4.41	6.9085	4.51	7.3915
4.51	6.8885	4.61	7.3885
4.61	6.888	4.71	7.3635
4.71	6.8605	5.71	7.354
4.81	6.841	5.81	7.3725
4.91	6.835	5.91	7.3845
5.01	6.84	6.01	7.3915
5.11	6.846	6.11	7.38
5.21	6.825	6.21	7.384
5.31	6.8275	6.31	7.4195
5.41	6.827	6.41	7.4065
5.51	6.8315	6.51	7.355
5.61	6.8305	6.61	7.3635
5.71	6.83	6.71	7.3485
5.81	6.792	6.81	7.3695
5.91	6.759	6.91	7.346
6.01	6.7555	7.01	7.339
6.11	6.7625	7.11	7.3595
6.21	6.7635	7.21	7.3305
6.31	6.756	7.31	7.332
6.41	6.7325	7.41	7.3065
6.51	6.7075	7.51	7.333
6.61	6.718	7.61	7.367
6.71	6.718	7.71	7.389
6.81	6.732	7.81	7.371
6.91	6.7145	7.91	7.313
7.01	6.693	8.01	7.3365
7.11	6.6775	8.11	7.362
7.21	6.6565	8.21	7.344
7.31	6.6295	8.312	7.336
7.41	6.637	8.413	7.3145
7.51	6.6445	8.512	7.3385
7.61	6.62	8.613	7.363
7.71	6.6005	8.713	7.333
7.81	6.5965	8.813	7.2985
7.91	6.592	8.913	7.2935
8.01	6.583	9.013	7.3085
8.11	6.5695	9.114	7.2985
8.21	6.5405	9.214	7.301
8.31	6.5465	9.314	7.2825
8.41	6.5455	9.414	7.283
8.51	6.548	9.514	7.284
8.61	6.531	9.615	7.288
8.71	6.51	9.714	7.283
8.81	6.499	9.814	7.26
8.91	6.4915	9.915	7.2605
9.01	6.4655	10.02	7.262
9.11	6.445	10.12	7.273
9.21	6.462	10.22	7.264
9.31	6.452	10.32	7.243
9.41	6.441	10.42	7.2635
9.51	6.413	10.52	7.291
9.61	6.407	10.62	7.2595
9.71	6.4135	10.72	7.224
9.81	6.396	10.82	7.241
9.91	6.38	10.92	7.259
10	6.3735	11.02	7.2375
10.1	6.361	11.12	7.2155
10.2	6.356	11.22	7.209
10.3	6.3435	11.32	7.1935
10.42	6.3145	11.42	7.2035
10.51	6.3125	11.52	7.24
10.61	6.3005	11.62	7.2215
10.72	6.2945	11.72	7.2315
10.82	6.2785	11.82	7.224
10.92	6.265	11.92	7.218
11.02	6.2565	12.02	7.1975
11.12	6.2435	12.12	7.2095
11.22	6.254	12.22	7.2045
11.32	6.2175	12.32	7.1995
11.42	6.211	12.42	7.189
11.52	6.21	12.52	7.2045
11.62	6.1875	12.62	7.215
11.72	6.1715	12.72	7.2195
11.82	6.1555	12.82	7.2025
11.92	6.148	12.92	7.2085
12.02	6.1245	13.02	7.211
12.12	6.102	13.12	7.205
12.22	6.0865	13.22	7.2095
12.32	6.0905	13.32	7.226
12.42	6.09	13.42	7.2175
12.52	6.083	13.52	7.207
12.62	6.0825	13.62	7.209
12.72	6.052	13.72	7.218
12.82	6.0495	13.82	7.2245
12.92	6.0335	13.92	7.2205
13.02	6.0055	14.02	7.2075
13.12	5.994	14.12	7.167
13.22	5.9795	14.22	7.169
13.32	5.971	14.32	7.172
13.42	5.985	14.42	7.168

Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)
13.52	5.973	14.52	7.168
13.62	5.942	14.62	7.174
13.72	5.9185	14.72	7.162
13.82	5.909	14.82	7.191
13.92	5.8925	14.92	7.186
14.02	5.8865	15.02	7.191
14.12	5.9075	15.12	7.179
14.22	5.898	15.22	7.215
14.32	5.8475	15.32	7.228
14.42	5.845	15.42	7.1965
14.52	5.821	15.52	7.168
14.62	5.829	15.62	7.1815
14.72	5.817	18.03	7.236
14.82	5.7975	18.13	7.274
14.92	5.7685	18.23	7.249
15.02	5.7545	18.33	7.241
15.12	5.752	18.43	7.257
15.22	5.7505	18.53	7.275
15.32	5.731	18.63	7.2595
15.42	5.715	18.73	7.2605
15.52	5.698	18.83	7.278
15.62	5.6965	18.93	7.2765
15.72	5.683	19.03	7.273
15.82	5.6785	19.13	7.248
15.92	5.676	19.23	7.251
16.02	5.6615	19.33	7.2555
16.12	5.6335	19.43	7.258
16.22	5.624	19.53	7.257
16.32	5.61	19.63	7.255
16.42	5.604	19.73	7.248
16.52	5.574	19.83	7.2645

Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)
16.6	5.5645	19.93	7.2635
16.7	5.565	20.03	7.262
16.8	5.553	20.13	7.245
16.9	5.5525	20.23	7.249
17	5.5295	20.33	7.2445
17.1	5.516	20.43	7.2395
17.2	5.506	20.53	7.242
17.3	5.4955	20.63	7.2525
17.4	5.4765	20.73	7.236
17.5	5.4695	20.83	7.224
17.6	5.4565	20.93	7.232
17.7	5.452	21.03	7.236
17.8	5.4165	21.13	7.23
17.9	5.4055	21.23	7.225
18	5.406	21.33	7.243
18.1	5.393	21.43	7.2255
18.2	5.3745	21.53	7.1925
18.3	5.3615	21.63	7.183
18.4	5.35	21.73	7.1735
18.5	5.341	21.83	7.1785
18.6	5.33	21.93	7.174
18.7	5.337	22.03	7.167
18.8	5.316	22.13	7.1655
18.9	5.2905	22.23	7.1725
19	5.273	22.33	7.181
19.1	5.2475	22.43	7.1775
19.2	5.244	22.53	7.1895
19.3	5.249	22.63	7.1895
19.4	5.226	22.73	7.1935
19.5	5.2245	22.83	7.177
19.6	5.21	22.93	7.1925

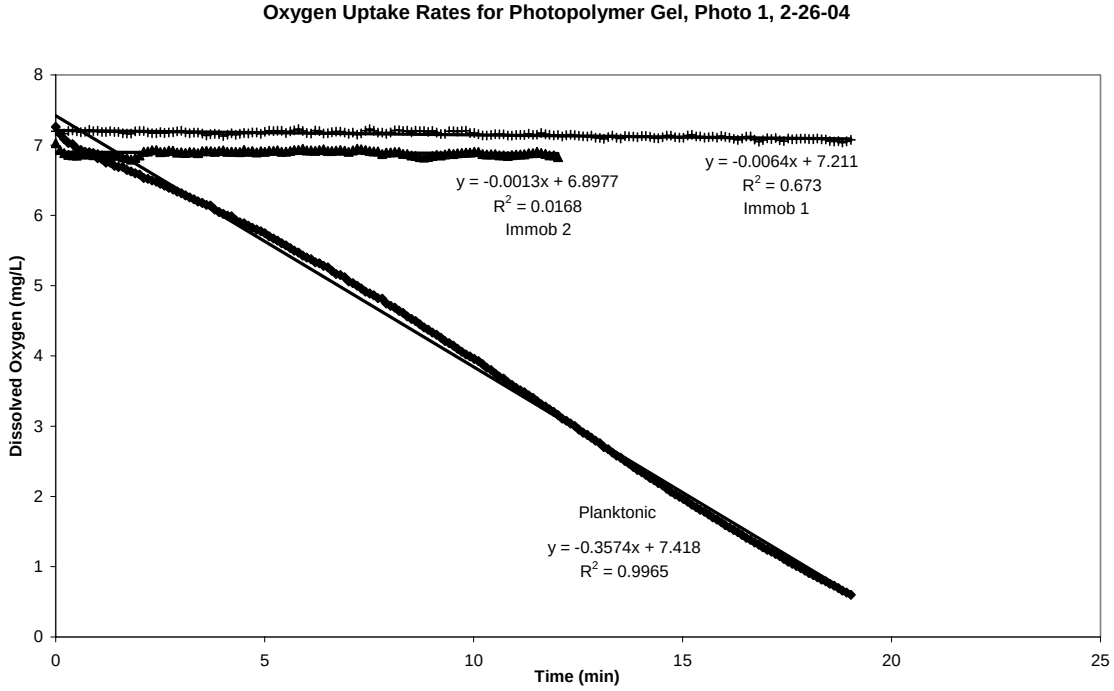
Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)
19.73	5.2015	23	7.1795
19.83	5.1875	23.1	7.187
19.93	5.184	23.2	7.187
20.03	5.157	23.3	7.1895
20.13	5.1425	23.4	7.1735
20.23	5.135	23.5	7.159
20.33	5.1045	23.6	7.181
20.43	5.103	23.7	7.1845
20.53	5.0895	23.8	7.1835
20.63	5.075	23.9	7.1775
20.73	5.073	24	7.176
20.83	5.0445	24.1	7.1895
20.93	5.0325	24.2	7.1865
21.03	5.031	24.3	7.164
21.13	5.0205	24.4	7.1625
21.23	5.0225	24.5	7.174
21.33	4.982	24.6	7.179
21.43	4.9685	24.7	7.181
21.53	4.971	24.8	7.191
21.63	4.9525	24.9	7.167
21.73	4.939	25	7.174
21.83	4.9265	25.1	7.17
21.93	4.9165	25.2	7.1425
22.03	4.906	25.3	7.165
22.13	4.8865	25.4	7.16
22.23	4.878	25.5	7.151
22.33	4.855	25.6	7.15
22.43	4.8215	25.7	7.1545
22.53	4.812	25.8	7.1325
22.63	4.8085	25.9	7.1375
22.73	4.807	26	7.1375

Oxygen Uptake Rates for Photopolymer Gel, Photo 2, 3-18-04



A.1.3 Photopolymerizable polymer: Photo 1, 2-26-04, Book 2, Page 72

Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)
2.1	6.531	2.104	6.9035
2.2	6.52	2.203	6.911
2.3	6.498	2.304	6.927
2.4	6.48	2.404	6.9295
2.5	6.449	2.504	6.902
2.6	6.422	2.604	6.9045
2.7	6.395	2.704	6.922
2.81	6.367	2.804	6.9225
2.91	6.338	2.905	6.8935
3	6.316	3.004	6.898
3.11	6.285	3.105	6.89
3.21	6.255	3.205	6.9
3.31	6.23	3.305	6.898
3.41	6.198	3.405	6.886
3.51	6.177	3.505	6.913
3.61	6.149	3.606	6.9195
3.71	6.14	3.706	6.912
3.81	6.081	3.805	6.9055
3.91	6.049	3.906	6.917
4.01	6.023	4.006	6.923
4.11	5.995	4.106	6.9035
4.21	5.988	4.206	6.907
4.31	5.932	4.306	6.9045
4.41	5.9	4.407	6.902
4.51	5.885	4.506	6.933
4.61	5.851	4.606	6.9055
4.71	5.824	4.707	6.9155
4.81	5.797	4.807	6.8965
4.91	5.775	4.907	6.9115
5.01	5.746	5.007	6.9055
5.11	5.702	5.107	6.9185
5.208	5.67	5.208	6.918
5.307	5.64	5.307	6.9265
5.408	5.603	5.407	6.9045
5.508	5.572	5.508	6.9075
5.609	5.533	5.608	6.916
5.708	5.496	5.708	6.929
5.808	5.472	5.808	6.934
5.909	5.428	5.908	6.946
6.009	5.398	6.009	6.923
6.109	5.376	6.109	6.924
6.209	5.332	6.209	6.9175
6.309	5.317	6.309	6.9305
6.41	5.28	6.409	6.9425
6.51	5.259	6.51	6.9155
6.609	5.212	6.609	6.9155
6.71	5.165	6.71	6.921
6.81	5.15	6.81	6.9295
6.911	5.119	6.91	6.921
7.01	5.06	7.01	6.904
7.11	5.035	7.11	6.9245
7.211	4.999	7.211	6.9465
7.311	4.96	7.311	6.9415
7.41	4.915	7.41	6.9295
7.511	4.888	7.511	6.9145
7.611	4.861	7.611	6.91
7.712	4.823	7.712	6.909
7.811	4.812	7.811	6.873
7.911	4.747	7.911	6.8935
8.012	4.713	8.012	6.895
8.111	4.686	8.111	6.9
8.212	4.641	8.212	6.9025
8.312	4.612	8.312	6.875
8.412	4.561	8.412	6.873
8.512	4.524	8.512	6.8525
8.612	4.497	8.612	6.845
8.713	4.45	8.713	6.829
8.813	4.404	8.813	6.822
8.913	4.372	8.913	6.8315
9.013	4.331	9.013	6.8465
9.113	4.299	9.113	6.861
9.214	4.259	9.213	6.851
9.314	4.21	9.314	6.8655
9.413	4.183	9.413	6.88
9.514	4.144	9.514	6.873
9.614	4.102	9.614	6.8835
9.715	4.058	9.714	6.89
9.814	4.02	9.814	6.894
9.914	3.99	9.914	6.8915
10.02	3.956	10.02	6.9055
10.12	3.926	10.12	6.901
10.22	3.882	10.21	6.873
10.32	3.831	10.32	6.868
10.42	3.799	10.42	6.869
10.52	3.748	10.52	6.8685
10.62	3.704	10.62	6.855
10.72	3.676	10.72	6.8565
10.82	3.622	10.82	6.846
10.92	3.589	10.92	6.858
11.02	3.545	11.02	6.8635
11.12	3.51	11.12	6.868
11.22	3.48	11.22	6.869
11.32	3.439	11.32	6.884
11.42	3.402	11.42	6.8875
11.52	3.356	11.52	6.9045
11.62	3.312	11.62	6.89
11.72	3.28	11.72	6.8745
11.82	3.24	11.82	6.8555
11.92	3.201	11.92	6.8545
12.02	3.158	12.02	6.829



A.1.4 Alginate oxygen uptake rates

Alginate polymer: day zero, 4-18-04, Book 2, Page 84

Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)	Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)	Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)
1.203	7.8395	1.202	7.417	4.307	7.4315	4.307	7.252	7.412	7.284	7.411	7.02
1.303	7.8295	1.303	7.4145	4.408	7.398	4.407	7.2225	7.512	7.297	7.512	7.0165
1.403	7.7675	1.402	7.416	4.508	7.418	4.508	7.2235	7.613	7.2915	7.612	7.017
1.503	7.756	1.502	7.388	4.607	7.445	4.607	7.226	7.713	7.2785	7.712	6.9965
1.603	7.714	1.603	7.417	4.708	7.487	4.707	7.2285	7.812	7.2815	7.812	6.993
1.704	7.693	1.703	7.407	4.808	7.4765	4.808	7.222	7.913	7.2695	7.912	6.9765
1.803	7.684	1.803	7.397	4.909	7.477	4.907	7.226	8.013	7.262	8.013	6.9745
1.903	7.7265	1.903	7.4195	5.008	7.4725	5.008	7.208	8.113	7.246	8.113	6.982
2.004	7.688	2.003	7.373	5.109	7.461	5.108	7.1965	8.213	7.2385	8.213	6.977
2.104	7.623	2.104	7.3795	5.209	7.4595	5.208	7.1935	8.314	7.239	8.313	6.957
2.204	7.7085	2.203	7.3805	5.308	7.4565	5.308	7.1835	8.414	7.2165	8.413	6.942
2.304	7.7565	2.304	7.382	5.409	7.4555	5.408	7.188	8.514	7.193	8.513	6.944
2.405	7.6905	2.404	7.3855	5.509	7.4355	5.509	7.169	8.614	7.1855	8.613	6.9415
2.505	7.679	2.504	7.3695	5.61	7.457	5.609	7.156	8.714	7.173	8.714	6.9195
2.604	7.687	2.604	7.3475	5.709	7.404	5.708	7.154	8.815	7.1955	8.814	6.863
2.705	7.66	2.704	7.342	5.809	7.437	5.809	7.1515	8.915	7.157	8.913	6.7805
2.805	7.6205	2.805	7.334	5.91	7.418	5.909	7.144	9.014	7.1455	9.014	6.7545
2.905	7.601	2.905	7.3425	6.01	7.378	6.01	7.149	9.115	7.141	9.114	6.796
3.005	7.574	3.004	7.3335	6.11	7.3805	6.109	7.1295	9.215	7.091	9.215	6.784
3.105	7.5825	3.105	7.3255	6.21	7.4035	6.209	7.1125	9.315	7.1235	9.314	6.7915
3.206	7.5815	3.205	7.337	6.31	7.3765	6.31	7.1065	9.415	7.127	9.414	6.773
3.306	7.59	3.306	7.3205	6.411	7.3655	6.41	7.104	9.515	7.095	9.515	6.7595
3.406	7.554	3.405	7.313	6.51	7.357	6.51	7.1025	9.616	7.0815	9.615	6.762
3.506	7.562	3.505	7.285	6.611	7.3345	6.61	7.0885	9.715	7.0825	9.715	6.7485
3.606	7.5555	3.606	7.2595	6.711	7.3415	6.71	7.0785	9.816	7.0805	9.815	6.745
3.707	7.5545	3.705	7.2825	6.811	7.378	6.811	7.0635	9.916	7.084	9.915	6.7535
3.806	7.5545	3.806	7.3015	6.911	7.3565	6.911	7.055	10.02	7.0795	10.02	6.729
3.907	7.5265	3.906	7.2715	7.011	7.3195	7.011	7.061	10.12	7.0665	10.12	6.7265
4.007	7.48	4.007	7.2475	7.112	7.3125	7.111	7.0485	10.22	7.063	10.22	6.744
4.107	7.4575	4.107	7.2595	7.212	7.2875	7.212	7.0335	10.32	7.0345	10.32	6.7305
4.207	7.439	4.206	7.2605	7.312	7.297	7.312	7.022	10.42	7.0215	10.42	6.712

Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)
10.5	6.995	10.52	6.6945
10.6	6.995	10.62	6.689
10.7	7.001	10.72	6.69
10.8	6.976	10.82	6.6675
10.9	6.9835	10.92	6.664
11	6.9595	11.02	6.6835
11.1	6.944	11.12	6.719
11.2	6.948	11.22	6.6835
11.3	6.924	11.32	6.6435
11.4	6.945	11.42	6.653
11.5	6.909	11.52	6.664
11.6	6.891	11.62	6.6445
11.7	6.906	11.72	6.643
11.8	6.918	11.82	6.648
11.9	6.895	11.92	6.658
12	6.874	12.02	6.646
12.1	6.8755	12.12	6.63
12.2	6.8525	12.22	6.6505
12.3	6.8235	12.32	6.655
12.4	6.799	12.42	6.6415
12.5	6.807	12.52	6.602
12.6	6.808	12.62	6.592
12.7	6.804	12.72	6.6
12.8	6.78	12.82	6.5995
12.9	6.762	12.92	6.614
13	6.784	13.02	6.6395
13.1	6.7685	13.12	6.6665
13.2	6.7485	13.22	6.669
13.3	6.7475	13.32	6.668
13.4	6.7325	13.42	6.641
13.5	6.7205	13.52	6.629

Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)
13.62	6.6995	13.62	6.5985
13.72	6.6815	13.72	6.593
13.82	6.6775	13.82	6.5775
13.92	6.6945	13.92	6.5655
14.02	6.67	14.02	6.557
14.12	6.647	14.12	6.554
14.22	6.6605	14.22	6.543
14.32	6.6595	14.32	6.5355
14.42	6.64	14.42	6.514
14.52	6.603	14.52	6.5025
14.62	6.6185	14.62	6.5005
14.72	6.6125	14.72	6.491
14.82	6.5955	14.82	6.4775
14.92	6.6115	14.92	6.463
15.02	6.6025	15.02	6.465
15.12	6.586	15.12	6.455
15.22	6.565	15.22	6.445
15.32	6.5395	15.32	6.426
15.42	6.527	15.42	6.4155
15.53	6.511	15.52	6.415
15.63	6.502	15.62	6.4065
15.73	6.4855	15.72	6.3775
15.83	6.4955	15.82	6.3905
15.93	6.4955	15.93	6.3775
16.03	6.494	16.02	6.379
16.13	6.4805	16.13	6.364
16.23	6.452	16.23	6.3435
16.33	6.4315	16.33	6.3485
16.43	6.434	16.43	6.3465
16.53	6.4205	16.53	6.3205
16.63	6.4085	16.63	6.324

Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)
16.7	6.4025	16.73	6.326
16.8	6.3665	16.83	6.3075
16.9	6.363	16.93	6.294
17	6.362	17.03	6.288
17.1	6.352	17.13	6.2725
17.2	6.367	17.23	6.2665
17.3	6.3435	17.33	6.259
17.4	6.3445	17.43	6.2475
17.5	6.323	17.53	6.2495
17.6	6.3225	17.63	6.23
17.7	6.309	17.73	6.2295
17.8	6.274	17.83	6.22
17.9	6.2745	17.93	6.2215
18	6.26	18.03	6.206
18.1	6.264	18.13	6.198
18.2	6.2365	18.23	6.188
18.3	6.2355	18.33	6.1765
18.4	6.217	18.43	6.1765
18.5	6.196	18.53	6.172
18.6	6.1825	18.63	6.1535
18.7	6.165	18.73	6.151
18.8	6.174	18.83	6.141
18.9	6.166	18.93	6.141
19	6.1585	19.03	6.1365
19.1	6.13	19.13	6.1155
19.2	6.112	19.23	6.1075
19.3	6.085	19.33	6.1085
19.4	6.1145	19.43	6.0955
19.5	6.1015	19.53	6.082
19.6	6.114	19.63	6.062
19.7	6.0785	19.73	6.056

Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Immobilized (mg/L)
19.8	6.057	19.83	6.057
19.9	6.0715	19.93	6.064
20	6.065	20.03	6.051
20.1	6.067	20.13	6.042
20.2	6.035	20.23	6.037
20.3	6.0015	20.33	6.0105
20.4	5.988	20.43	6.015
20.5	5.98	20.53	6.0125
20.6	5.9715	20.63	5.993
20.7	5.9735	20.73	5.979
20.8	5.9675	20.83	5.976
20.9	5.9625	20.93	5.9795
21	5.9575	21.03	5.953
21.1	5.9265	21.13	5.921
21.2	5.91	21.23	5.944
21.3	5.9135	21.33	5.941
21.4	5.891	21.43	5.895
21.5	5.89	21.53	5.91
21.6	5.8795	21.63	5.905
21.7	5.851	21.73	5.916
21.8	5.8665	21.83	5.906
21.9	5.856	21.93	5.8975
22	5.8495	22.03	5.881
22.1	5.8265	22.13	5.884
22.2	5.818	22.23	5.872
22.3	5.7965	22.33	5.846
22.4	5.802	22.43	5.831
22.5	5.791	22.54	5.8435
22.6	5.781	22.64	5.8395
22.7	5.759	22.74	5.8365
22.8	5.744	22.84	5.829

Time (min)	Av DO for Immobilized (mg/L)
23	5.812
23.1	5.805
23.2	5.785
23.3	5.79
23.4	5.779
23.5	5.7765
23.6	5.7715
23.7	5.757
23.8	5.741
23.9	5.7385
24	5.718
24.1	5.7065
24.2	5.713
24.3	5.675
24.4	5.613
24.5	5.6215
24.6	5.668
24.7	5.675
24.8	5.671
24.9	5.6655
25	5.6525
25.1	5.6395
25.2	5.612
25.3	5.6045
25.4	5.605
25.5	5.5965
25.6	5.587
25.7	5.5255
25.8	5.4855
25.9	5.5295
26	5.55

Time (min)	Av DO for Immobilized (mg/L)
26.14	5.554
26.24	5.5345
26.34	5.5355
26.44	5.5265
26.54	5.5195
26.64	5.5065
26.74	5.4975
26.84	5.483
26.94	5.4695
27.04	5.4355
27.14	5.3965
27.24	5.3825
27.34	5.3655
27.44	5.349
27.54	5.3235
27.64	5.297
27.74	5.338
27.84	5.349
27.94	5.3195
28.04	5.2995

Alginate polymer: days 1, 3, and 5

Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 3 (mg/L)	Time (min)	Av DO for Day 5 (mg/L)
1.2	8.1605	1.303	8.395	1.202	7.9995
1.3	8.2055	1.403	8.37	1.302	7.973
1.4	8.2475	1.502	8.362	1.401	7.952
1.5	8.2805	1.603	8.353	1.502	7.91
1.6	8.287	1.703	8.343	1.602	7.8935
1.7	8.267	1.803	8.3305	1.703	7.885
1.8	8.251	1.903	8.3205	1.803	7.869
1.9	8.236	2.003	8.305	1.903	7.8665
2	8.2365	2.104	8.2805	2.003	7.8615
2.1	8.223	2.204	8.2635	2.103	7.8405
2.2	8.204	2.303	8.25	2.203	7.827
2.3	8.175	2.404	8.2325	2.303	7.811
2.4	8.1565	2.504	8.2185	2.404	7.8
2.51	8.1405	2.604	8.213	2.504	7.7785
2.61	8.118	2.704	8.191	2.603	7.7685
2.71	8.089	2.805	8.1785	2.704	7.746
2.81	8.0815	2.905	8.164	2.804	7.733
2.91	8.058	3.004	8.1435	2.905	7.722
3.01	8.0485	3.105	8.1305	3.004	7.712
3.11	8.0365	3.205	8.1325	3.104	7.6975
3.21	8.014	3.306	8.1175	3.205	7.6795
3.31	8.0085	3.405	8.0905	3.305	7.6655
3.41	7.9875	3.505	8.0635	3.405	7.653
3.51	7.9765	3.606	8.0575	3.505	7.646
3.61	7.9675	3.706	8.0535	3.606	7.629
3.71	7.957	3.806	8.0195	3.706	7.6135
3.81	7.9465	3.906	8.0215	3.805	7.5975
3.91	7.9325	4.006	7.988	3.906	7.587
4.01	7.924	4.107	7.9565	4.006	7.5705
4.11	7.9025	4.206	7.9525	4.107	7.563
4.21	7.888	4.307	7.949	4.206	7.553

Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 3 (mg/L)	Time (min)	Av DO for Day 5 (mg/L)
4.307	7.8775	4.407	7.925	4.306	7.532
4.408	7.872	4.508	7.907	4.407	7.5005
4.508	7.8585	4.607	7.8845	4.507	7.492
4.607	7.847	4.707	7.869	4.607	7.492
4.708	7.8295	4.808	7.868	4.707	7.493
4.808	7.8175	4.908	7.8545	4.807	7.4845
4.909	7.81	5.008	7.847	4.907	7.4675
5.008	7.7985	5.108	7.8405	5.007	7.459
5.108	7.797	5.208	7.819	5.108	7.447
5.209	7.785	5.308	7.806	5.208	7.4335
5.309	7.778	5.408	7.7925	5.308	7.4125
5.409	7.767	5.509	7.786	5.408	7.4065
5.509	7.76	5.609	7.7435	5.508	7.3925
5.609	7.7545	5.709	7.7465	5.609	7.39
5.709	7.746	5.809	7.7355	5.708	7.377
5.809	7.7365	5.909	7.716	5.808	7.3575
5.91	7.7315	6.01	7.7025	5.909	7.356
6.01	7.7235	6.109	7.678	6.009	7.343
6.11	7.704	6.209	7.6645	6.109	7.333
6.21	7.6955	6.31	7.662	6.209	7.319
6.31	7.6905	6.41	7.638	6.309	7.3095
6.411	7.6815	6.51	7.615	6.41	7.296
6.51	7.663	6.61	7.5995	6.51	7.281
6.611	7.653	6.711	7.5555	6.609	7.2645
6.711	7.65	6.811	7.5125	6.71	7.2505
6.811	7.6425	6.911	7.4775	6.81	7.241
6.911	7.63	7.011	7.462	6.911	7.225
7.011	7.622	7.111	7.448	7.01	7.2185
7.112	7.613	7.211	7.4205	7.111	7.2095
7.212	7.6025	7.312	7.4085	7.211	7.198
7.312	7.592	7.411	7.3905	7.311	7.173

Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 3 (mg/L)	Time (min)	Av DO for Day 5 (mg/L)
7.412	7.577	7.512	7.3755	7.41	7.163
7.512	7.5675	7.612	7.3595	7.51	7.1515
7.613	7.568	7.712	7.339	7.61	7.14
7.713	7.5635	7.812	7.344	7.71	7.1275
7.812	7.5535	7.912	7.3365	7.81	7.103
7.913	7.5405	8.013	7.337	7.91	7.0935
8.013	7.54	8.113	7.3495	8.01	7.086
8.113	7.5325	8.212	7.3435	8.11	7.079
8.213	7.52	8.313	7.3315	8.21	7.049
8.313	7.5115	8.413	7.3265	8.31	7.037
8.414	7.498	8.514	7.3195	8.41	7.0185
8.514	7.492	8.613	7.3155	8.51	7.0125
8.614	7.48	8.713	7.2915	8.61	6.994
8.714	7.4715	8.814	7.2615	8.71	6.9825
8.814	7.4605	8.913	7.252	8.81	6.96
8.915	7.447	9.014	7.232	8.91	6.95
9.014	7.4365	9.114	7.205	9.01	6.938
9.115	7.4245	9.214	7.193	9.11	6.917
9.215	7.4195	9.314	7.1825	9.21	6.902
9.315	7.412	9.414	7.1805	9.31	6.8925
9.415	7.4025	9.515	7.159	9.41	6.8865
9.515	7.3955	9.615	7.136	9.51	6.8815
9.616	7.3845	9.715	7.1335	9.61	6.863
9.715	7.376	9.815	7.1055	9.72	6.831
9.816	7.3645	9.915	7.085	9.81	6.8245
9.916	7.3585	10.02	7.0855	9.91	6.81
10.02	7.354	10.12	7.0605	10	6.799
10.12	7.344	10.22	7.0555	10.1	6.7925
10.22	7.3345	10.32	7.0245	10.2	6.784
10.32	7.3265	10.42	7.0085	10.3	6.762
10.42	7.313	10.52	7.003	10.4	6.751

Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 3 (mg/L)	Time (min)	Av DO for Day 5 (mg/L)
10.5	7.3075	10.62	6.999	10.52	6.73
10.6	7.3005	10.72	6.9805	10.62	6.717
10.7	7.291	10.82	6.965	10.72	6.6995
10.8	7.2805	10.92	6.957	10.82	6.679
10.9	7.2705	11.02	6.95	10.92	6.653
11	7.2655	11.12	6.922	11.02	6.644
11.1	7.2445	11.22	6.893	11.12	6.6235
11.2	7.2385	11.32	6.883	11.22	6.6055
11.3	7.2335	11.42	6.8565	11.32	6.591
11.4	7.219	11.52	6.8535	11.42	6.572
11.5	7.2085	11.62	6.844	11.52	6.5665
11.6	7.2055	11.72	6.8225	11.62	6.5695
11.7	7.1995	11.82	6.8	11.72	6.562
11.8	7.1905	11.92	6.7915	11.82	6.5515
11.9	7.1805	12.02	6.78	11.92	6.5295
12	7.1715	12.12	6.756	12.02	6.5115
12.1	7.164	12.22	6.751	12.12	6.4895
12.2	7.1555	12.32	6.727	12.22	6.4835
12.3	7.1465	12.42	6.7105	12.32	6.4695
12.4	7.131	12.52	6.68	12.42	6.455
12.5	7.1105	12.62	6.675	12.52	6.443
12.6	7.099	12.72	6.669	12.62	6.4235
12.7	7.0975	12.82	6.6605	12.72	6.413
12.8	7.0935	12.92	6.6335	12.82	6.3955
12.9	7.089	13.02	6.6215	12.92	6.3775
13	7.073	13.12	6.6175	13.02	6.3655
13.1	7.0635	13.22	6.607	13.12	6.338
13.2	7.056	13.32	6.575	13.22	6.3175
13.3	7.0365	13.42	6.5685	13.32	6.3115
13.4	7.028	13.52	6.55	13.42	6.292
13.5	7.021	13.62	6.5345	13.52	6.283

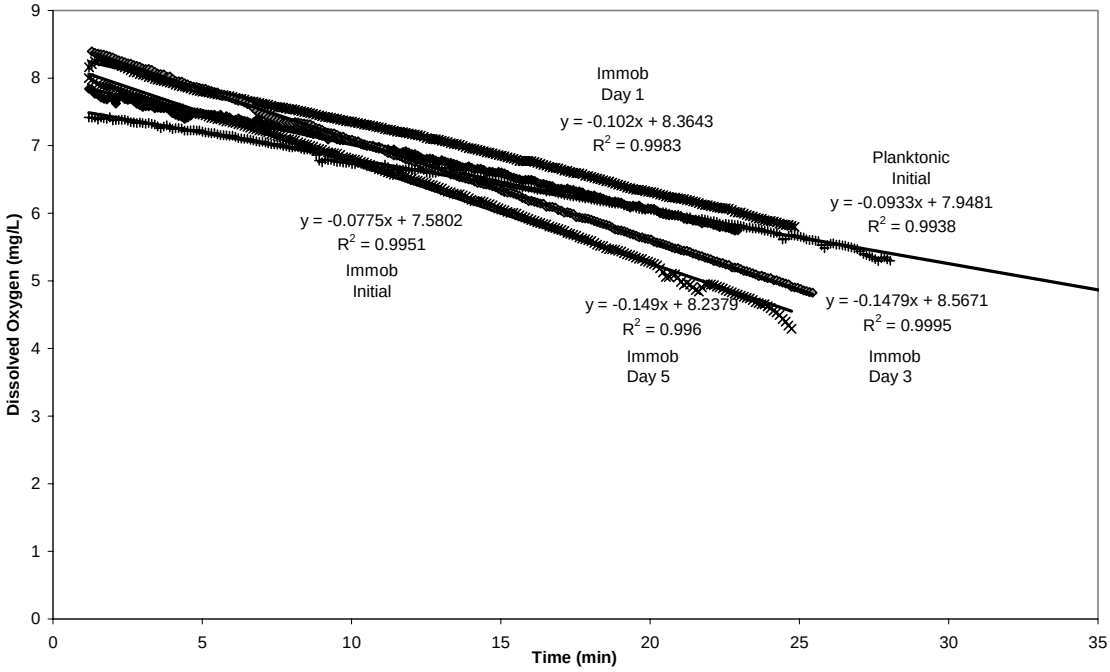
Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 3 (mg/L)	Time (min)	Av DO for Day 5 (mg/L)
13.62	7.0125	13.72	6.527	13.6	6.2805
13.72	6.9955	13.82	6.5125	13.7	6.265
13.82	6.976	13.92	6.5015	13.8	6.248
13.92	6.9625	14.02	6.495	13.9	6.223
14.02	6.9595	14.12	6.4715	14	6.199
14.12	6.9515	14.22	6.451	14.1	6.183
14.22	6.938	14.32	6.4475	14.2	6.178
14.32	6.927	14.42	6.4325	14.3	6.177
14.42	6.916	14.52	6.4255	14.4	6.1685
14.52	6.901	14.62	6.416	14.5	6.147
14.62	6.895	14.72	6.4	14.6	6.135
14.72	6.8875	14.82	6.3855	14.7	6.116
14.82	6.872	14.92	6.363	14.8	6.0905
14.92	6.8605	15.02	6.336	14.9	6.0725
15.02	6.851	15.12	6.3155	15	6.0575
15.12	6.842	15.22	6.3065	15.1	6.0435
15.22	6.826	15.32	6.3055	15.2	6.021
15.32	6.8145	15.42	6.292	15.3	6.0065
15.42	6.807	15.52	6.275	15.4	6.007
15.53	6.7915	15.62	6.2565	15.5	5.9945
15.63	6.77	15.72	6.228	15.6	5.971
15.72	6.769	15.82	6.2115	15.7	5.953
15.83	6.7735	15.93	6.1835	15.8	5.938
15.93	6.7745	16.02	6.1855	15.9	5.9205
16.03	6.77	16.12	6.171	16	5.9
16.13	6.7575	16.23	6.159	16.1	5.885
16.23	6.745	16.33	6.144	16.2	5.868
16.33	6.7325	16.43	6.142	16.3	5.8575
16.43	6.713	16.53	6.097	16.4	5.841
16.53	6.7045	16.63	6.0955	16.5	5.835
16.63	6.6865	16.73	6.0935	16.6	5.804

Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 3 (mg/L)	Time (min)	Av DO for Day 5 (mg/L)
16.73	6.676	16.83	6.085	16.73	5.7935
16.83	6.6725	16.93	6.047	16.82	5.7815
16.93	6.6655	17.03	6.0395	16.93	5.767
17.03	6.646	17.13	6.008	17.03	5.7535
17.13	6.6285	17.23	6.016	17.13	5.739
17.23	6.628	17.33	6.0025	17.23	5.7105
17.33	6.6115	17.43	5.9905	17.33	5.6975
17.43	6.6	17.53	5.9425	17.43	5.6825
17.53	6.5925	17.63	5.9415	17.53	5.671
17.63	6.5895	17.73	5.9285	17.63	5.643
17.73	6.578	17.83	5.9255	17.73	5.6245
17.83	6.5535	17.93	5.92	17.83	5.6155
17.93	6.55	18.03	5.9035	17.93	5.6015
18.03	6.538	18.13	5.887	18.03	5.5905
18.13	6.5255	18.23	5.872	18.13	5.5635
18.23	6.5125	18.33	5.856	18.23	5.5355
18.33	6.5045	18.43	5.8415	18.33	5.519
18.43	6.49	18.53	5.8305	18.43	5.513
18.53	6.4745	18.63	5.8135	18.53	5.4835
18.63	6.4705	18.73	5.7905	18.63	5.469
18.73	6.454	18.83	5.7605	18.73	5.468
18.83	6.4375	18.93	5.7505	18.83	5.461
18.93	6.424	19.03	5.746	18.93	5.447
19.03	6.4105	19.13	5.7345	19.03	5.433
19.13	6.398	19.23	5.718	19.13	5.4105
19.23	6.3785	19.33	5.696	19.23	5.393
19.33	6.3715	19.43	5.684	19.33	5.381
19.43	6.3605	19.53	5.668	19.43	5.364
19.53	6.3465	19.63	5.6465	19.53	5.3445
19.63	6.3465	19.73	5.643	19.63	5.3295
19.73	6.3405	19.83	5.625	19.73	5.325

Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 3 (mg/L)	Time (min)	Av DO for Day 5 (mg/L)
19.83	6.33	19.93	5.6195	19.8	5.295
19.93	6.319	20.03	5.61	19.9	5.2845
20.03	6.3145	20.13	5.581	20	5.266
20.13	6.3065	20.23	5.5655	20.1	5.2515
20.23	6.29	20.33	5.5555	20.2	5.231
20.33	6.277	20.43	5.544	20.3	5.1835
20.43	6.2675	20.53	5.53	20.4	5.1245
20.53	6.253	20.63	5.515	20.5	5.058
20.63	6.2405	20.73	5.4945	20.6	5.06
20.73	6.233	20.83	5.48	20.7	5.1035
20.83	6.2315	20.93	5.476	20.8	5.095
20.93	6.2205	21.03	5.4535	20.9	5.0505
21.03	6.212	21.13	5.445	21	4.9845
21.13	6.2085	21.23	5.4415	21.1	4.9415
21.23	6.1955	21.33	5.4275	21.2	4.9695
21.33	6.188	21.43	5.403	21.3	4.9455
21.43	6.1735	21.53	5.3945	21.4	4.905
21.53	6.1635	21.63	5.375	21.5	4.8645
21.63	6.149	21.73	5.3655	21.6	4.8445
21.73	6.133	21.83	5.345	21.7	4.902
21.83	6.1235	21.93	5.3355	21.8	4.953
21.93	6.1205	22.03	5.3185	21.9	4.9485
22.03	6.105	22.13	5.297	22	4.9455
22.13	6.0975	22.23	5.287	22.1	4.9355
22.24	6.088	22.33	5.27	22.2	4.9165
22.33	6.0805	22.43	5.2635	22.3	4.903
22.44	6.071	22.53	5.2415	22.4	4.8885
22.54	6.0705	22.63	5.235	22.5	4.8635
22.64	6.069	22.74	5.1995	22.6	4.8475
22.74	6.0465	22.83	5.1955	22.7	4.8305
22.84	6.02	22.94	5.1925	22.8	4.81

Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 3 (mg/L)	Time (min)	Av DO for Day 5 (mg/L)
22.94	6.011	23.04	5.1735	22.93	4.799
23.04	6.0115	23.14	5.1495	23.04	4.78
23.14	5.9945	23.24	5.1435	23.14	4.7645
23.24	5.975	23.34	5.1285	23.23	4.751
23.34	5.96	23.44	5.112	23.34	4.7405
23.44	5.9525	23.54	5.094	23.44	4.7175
23.54	5.9335	23.64	5.072	23.54	4.6845
23.64	5.922	23.74	5.0775	23.64	4.6725
23.74	5.9195	23.84	5.0585	23.74	4.655
23.84	5.8975	23.94	5.0395	23.84	4.653
23.94	5.89	24.04	5.042	23.94	4.6255
24.04	5.887	24.14	5.012	24.04	4.597
24.14	5.877	24.24	5.01	24.14	4.5585
24.24	5.865	24.34	4.9915	24.24	4.535
24.34	5.8475	24.44	4.975	24.34	4.496
24.44	5.8355	24.54	4.96	24.44	4.436
24.54	5.8275	24.64	4.9475	24.54	4.3965
24.64	5.83	24.74	4.913	24.64	4.3405
24.74	5.8145	24.84	4.9035	24.74	4.293
24.84	5.799	24.94	4.898		
		25.04	4.878		
		25.14	4.859		
		25.24	4.8495		
		25.34	4.835		
		25.44	4.8265		

Oxygen Uptake Rates for Alginate Gels: Experiment Begun 4-18-04



Sample	Date	Gel Age (days)	DO Uptake Rate (mg/L/min)
Plank Control	4/18/04	0	0.0933
Gel	4/18/04	0	0.0775
Gel	4/19/04	1	0.1020
Gel	4/21/04	3	0.1479
Gel	4/23/04	5	0.149

A.1.5 Thermal polymer oxygen uptake rates

Thermal polymer: planktonic control, and polymer on days 0, 1, 5, 8, 10, and 14

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cells counts 4.00E+09 2.90E+09 3.80E+09

ave 3.57E+09 cells per mL

tot vol 12 mL

cells per batch 7.13E+09

Time (min)	Av DO for Planktonic (mg/L)	Time (min)	Av DO for Day 0 (mg/L)	Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 3 (mg/L)	Time (min)	Av DO for Day 8 (mg/L)	Time (min)	Av DO for Day 10 (mg/L)	Time (min)	Av DO for Day 14 (mg/L)
2.303	6.3045	2.303	5.823	3.505	4.8945	2.504	2.6735	3.104	6.559	2.505	5.0645	2.504	4.8605
2.404	6.2575	2.404	5.7615	3.605	4.874	2.603	2.633	3.204	6.5205	2.604	5.0725	2.604	4.799
2.504	6.2195	2.504	5.8605	3.706	4.894	2.704	2.6765	3.305	6.642	2.704	5.085	2.704	4.833
2.604	6.187	2.603	5.7265	3.805	4.886	2.804	2.651	3.405	6.645	2.805	5.0785	2.804	4.8
2.704	6.1245	2.704	5.781	3.906	4.902	2.904	2.663	3.504	6.671	2.905	5.0395	2.905	4.8115
2.804	6.0875	2.804	5.8545	4.006	4.917	3.004	2.641	3.605	6.705	3.005	5.0405	3.004	4.7955
2.905	6.034	2.905	5.959	4.106	4.835	3.104	2.586	3.705	6.628	3.105	5.027	3.105	4.778
3.004	5.9995	3.004	5.839	4.206	4.843	3.205	2.544	3.806	6.6005	3.205	5.085	3.205	4.8155
3.104	5.9535	3.104	5.761	4.306	4.84	3.305	2.544	3.905	6.646	3.306	5.0605	3.306	4.791
3.205	5.895	3.205	5.8145	4.407	4.846	3.405	2.5545	4.005	6.685	3.405	5.097	3.405	4.816
3.305	5.835	3.305	5.8915	4.506	4.8055	3.505	2.509	4.106	6.689	3.506	5.038	3.505	4.7885
3.405	5.7815	3.405	5.823	4.607	4.8	3.605	2.4655	4.206	6.651	3.606	5.035	3.606	4.768
3.505	5.7395	3.505	5.7565	4.707	4.8345	3.706	2.477	4.306	6.67	3.706	5.0735	3.706	4.775
3.605	5.6625	3.605	5.6575	4.807	4.751	3.805	2.433	4.406	6.5825	3.806	5.038	3.806	4.7685
3.706	5.606	3.706	5.545	4.907	4.772	3.905	2.4245	4.506	6.666	3.906	5.0665	3.906	4.795
3.805	5.554	3.805	5.61	5.007	4.81	4.006	2.4045	4.607	6.589	4.007	5.0345	4.006	4.7725
3.906	5.523	3.905	5.68	5.107	4.764	4.106	2.373	4.706	6.6225	4.107	5.064	4.107	4.7575
4.006	5.477	4.006	5.641	5.208	4.7445	4.206	2.3475	4.806	6.607	4.207	5.021	4.206	4.753
4.106	5.414	4.106	5.5465	5.307	4.715	4.306	2.3245	4.907	6.6995	4.307	5.067	4.307	4.8105
4.206	5.3455	4.206	5.5795	5.408	4.774	4.406	2.2895	5.006	6.695	4.407	5.033	4.407	4.753
4.306	5.298	4.306	5.6695	5.508	4.7495	4.507	2.308	5.107	6.6495	4.508	5.066	4.508	4.7765
4.407	5.2435	4.406	5.491	5.608	4.692	4.606	2.253	5.207	6.6605	4.607	4.957	4.607	4.7615
4.507	5.1755	4.507	5.492	5.708	4.633	4.707	2.245	5.308	6.683	4.708	4.9805	4.707	4.7515
4.606	5.1	4.606	5.5205	5.808	4.6255	4.807	2.2485	5.407	6.707	4.808	4.988	4.808	4.709
4.707	5.047	4.707	5.413	5.909	4.6905	4.907	2.1955	5.507	6.6765	4.908	4.968	4.907	4.6745
4.807	5.0125	4.807	5.383	6.009	4.7555	5.007	2.1685	5.608	6.6095	5.008	4.9015	5.008	4.6965
4.907	4.9525	4.907	5.443	6.109	4.624	5.107	2.1675	5.708	6.6295	5.108	4.82	5.108	4.674
5.007	4.911	5.007	5.3545	6.209	4.655	5.208	2.15	5.807	6.712	5.209	4.81	5.208	4.6985
5.107	4.8145	5.107	5.2985	6.309	4.6635	5.307	2.1215	5.908	6.6445	5.308	4.793	5.308	4.643
5.208	4.756	5.208	5.315	6.41	4.5915	5.407	2.0635	6.008	6.6095	5.408	4.8205	5.408	4.6665
5.308	4.7075	5.308	5.3785	6.51	4.651	5.508	2.073	6.109	6.6335	5.509	4.802	5.509	4.679

Time (min)	Av DO for Plankto	Time (min)	Av DO for Day 0 (mg/L)	Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 3 (mg/L)	Time (min)	Av DO for Day 8 (mg/L)	Time (min)	Av DO for Day 10 (mg/L)	Time (min)	Av DO for Day 14 (mg/L)
5.408	4.629	5.407	5.2425	6.609	4.6575	5.608	2.049	6.208	6.567	5.609	4.822	5.609	4.662
5.508	4.551	5.508	5.3435	6.71	4.617	5.708	1.9835	6.308	6.5895	5.709	4.8225	5.708	4.607
5.608	4.4845	5.608	5.2595	6.81	4.5615	5.808	1.962	6.409	6.6405	5.809	4.777	5.809	4.5935
5.708	4.4535	5.708	5.226	6.911	4.4995	5.908	1.92	6.509	6.6115	5.909	4.8145	5.909	4.571
5.808	4.3745	5.808	5.2505	7.01	4.5055	6.009	1.937	6.609	6.4795	6.01	4.7745	6.01	4.568
5.909	4.303	5.908	5.2455	7.11	4.525	6.109	1.9325	6.709	6.5255	6.109	4.7325	6.109	4.627
6.009	4.24	6.009	5.1945	7.211	4.4885	6.209	1.882	6.809	6.5405	6.21	4.742	6.209	4.5925
6.109	4.1705	6.109	5.237	7.311	4.55	6.309	1.8665	6.91	6.5235	6.31	4.711	6.31	4.602
6.209	4.0935	6.208	5.1765	7.411	4.4615	6.41	1.834	7.01	6.5745	6.411	4.7465	6.41	4.612
6.309	4.0115	6.309	5.081	7.511	4.4375	6.51	1.8085	7.11	6.5995	6.51	4.744	6.51	4.5395
6.41	3.935	6.409	4.983	7.612	4.525	6.609	1.8145	7.21	6.651	6.61	4.735	6.61	4.5965
6.51	3.8655	6.51	5.0075	7.712	4.409	6.71	1.7475	7.31	6.5315	6.711	4.7115	6.711	4.5535
6.609	3.8105	6.609	5.0185	7.811	4.464	6.81	1.7315	7.41	6.6905	6.811	4.71	6.811	4.5815
6.71	3.731	6.709	4.9545	7.912	4.3785	6.911	1.7205	7.51	6.6135	6.911	4.698	6.911	4.5775
6.81	3.6645	6.81	5.0705	8.012	4.3735	7.01	1.6715	7.611	6.5645	7.011	4.6655	7.011	4.5395
6.911	3.577	6.91	5.0445	8.113	4.399	7.11	1.666	7.711	6.6005	7.111	4.7015	7.111	4.531
7.01	3.5165	7.01	4.9875	8.212	4.3195	7.211	1.652	7.811	6.5885	7.212	4.7215	7.212	4.538
7.11	3.448	7.11	4.889	8.312	4.3905	7.311	1.6285	7.911	6.6295	7.312	4.675	7.312	4.5395
7.211	3.367	7.21	4.9545	8.413	4.3	7.411	1.592	8.011	6.603	7.412	4.627	7.411	4.4765
7.311	3.297	7.311	4.9005	8.512	4.2915	7.511	1.559	8.111	6.548	7.512	4.662	7.512	4.519
7.411	3.221	7.41	4.8595	8.613	4.2225	7.611	1.534	8.211	6.5075	7.612	4.6605	7.612	4.5065
7.511	3.1675	7.511	4.749	8.713	4.2695	7.712	1.5395	8.311	6.583	7.713	4.6945	7.713	4.5215
7.611	3.072	7.611	4.7695	8.813	4.307	7.811	1.486	8.412	6.559	7.812	4.6695	7.812	4.5065
7.712	2.991	7.711	4.8325	8.913	4.249	7.912	1.4565	8.512	6.461	7.913	4.672	7.912	4.5175
7.811	2.903	7.811	4.759	9.013	4.234	8.012	1.4405	8.612	6.541	8.013	4.6745	8.013	4.486
7.912	2.854	7.911	4.666	9.114	4.211	8.111	1.411	8.712	6.5265	8.113	4.659	8.113	4.492
8.012	2.7635	8.012	4.7235	9.214	4.1285	8.212	1.4005	8.812	6.491	8.213	4.6345	8.213	4.4615
8.111	2.6635	8.111	4.637	9.314	4.2435	8.312	1.391	8.913	6.521	8.313	4.62	8.313	4.4145
8.212	2.598	8.211	4.6145	9.414	4.159	8.412	1.319	9.012	6.5175	8.414	4.561	8.414	4.459
8.312	2.5305	8.312	4.628	9.514	4.1555	8.512	1.329	9.113	6.572	8.514	4.5565	8.514	4.467
8.413	2.4615	8.412	4.6205	9.615	4.216	8.612	1.305	9.213	6.565	8.614	4.571	8.613	4.4

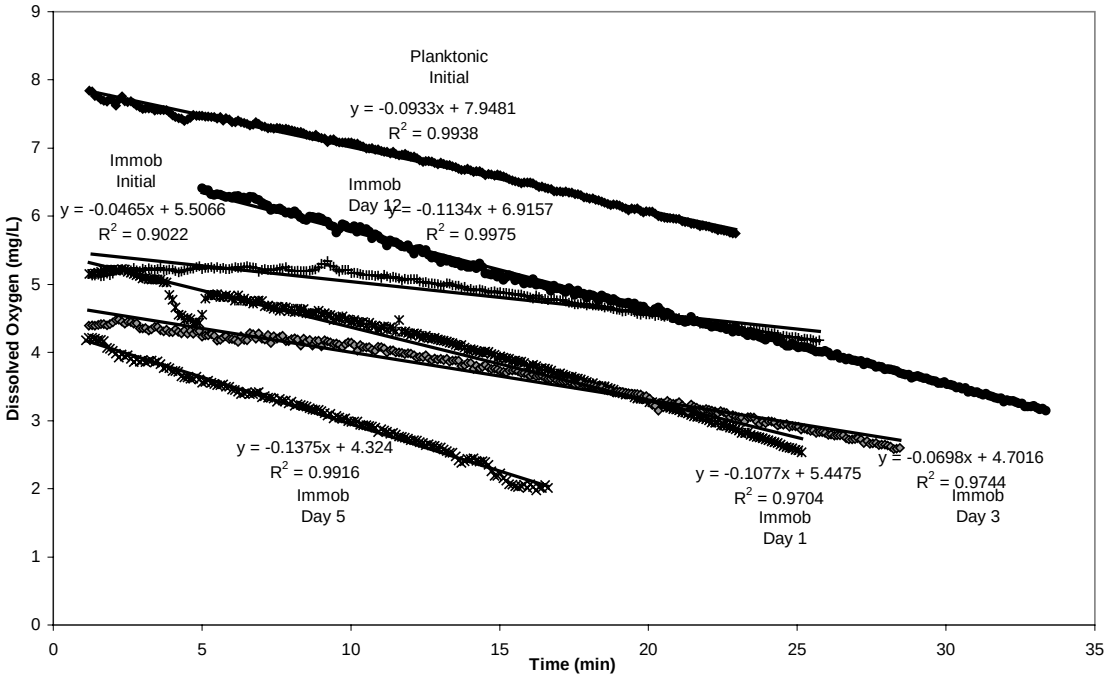
Time (min)	Av DO for Plankto	Time (min)	Av DO for Day 0 (mg/L)	Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 3 (mg/L)	Time (min)	Av DO for Day 8 (mg/L)	Time (min)	Av DO for Day 10 (mg/L)	Time (min)	Av DO for Day 14 (mg/L)
8.512	2.41	8.512	4.5735	9.715	4.1485	8.713	1.2725	9.313	6.5955	8.714	4.578	8.714	4.425
8.612	2.306	8.612	4.559	9.815	4.0635	8.813	1.2375	9.413	6.5455	8.814	4.5865	8.814	4.415
8.713	2.237	8.712	4.4665	9.915	4.1085	8.913	1.2255	9.513	6.507	8.915	4.544	8.914	4.431
8.813	2.1585	8.813	4.5165	10.015	4.058	9.013	1.202	9.613	6.5845	9.014	4.513	9.014	4.3955
8.913	2.096	8.913	4.4285	10.115	4.0845	9.113	1.1745	9.714	6.5625	9.115	4.487	9.114	4.4125
9.013	1.9935	9.012	4.398	10.215	4.027	9.214	1.135	9.813	6.6375	9.215	4.462	9.215	4.37
9.113	1.9365	9.113	4.4385	10.315	4.042	9.314	1.0875	9.914	6.495	9.314	4.512	9.314	4.362
9.214	1.8505	9.213	4.432	10.416	3.994	9.413	1.0885	10.014	6.51	9.415	4.505	9.415	4.4085
9.314	1.7755	9.314	4.401	10.516	3.992			10.114	6.435	9.515	4.4745	9.515	4.383
9.414	1.6715	9.413	4.4085	10.616	4.0125			10.214	6.49	9.616	4.4795	9.615	4.351
9.514	1.636	9.513	4.295	10.716	4.0035			10.314	6.55	9.715	4.496	9.715	4.32
9.614	1.553	9.614	4.2415	10.816	3.972			10.415	6.5185	9.816	4.502	9.815	4.348
9.715	1.4855	9.714	4.2525	10.916	3.9425			10.515	6.533	9.916	4.448	9.916	4.345
9.814	1.385	9.814	4.1935	11.016	3.8685			10.614	6.549	10.016	4.4695	10.016	4.3095
9.915	1.301	9.914	4.191	11.117	3.9115			10.715	6.523	10.116	4.468	10.116	4.342
10.015	1.2475	10.014	4.223	11.217	3.891			10.815	6.502	10.216	4.4685	10.216	4.33
10.115	1.156	10.115	4.102	11.316	3.881			10.915	6.5035	10.317	4.4545	10.316	4.2945
10.215	1.073	10.214	4.06	11.417	3.8825			11.015	6.521	10.417	4.3805	10.417	4.3225
		10.315	4.0035	11.517	3.8275			11.115	6.5365	10.517	4.408	10.516	4.2775
		10.415	3.973	11.618	3.8575			11.216	6.5895	10.617	4.3665	10.617	4.252
		10.514	4.0855	11.717	3.8385			11.316	6.46	10.717	4.3615	10.717	4.284
		10.615	3.994	11.818	3.8185			11.416	6.453	10.818	4.3135	10.817	4.3105
		10.715	3.8445	11.918	3.8165			11.516	6.5395	10.917	4.295	10.917	4.2345
		10.816	3.953	12.018	3.7885			11.616	6.4355	11.017	4.289	11.017	4.258
		10.915	3.9	12.118	3.7705			11.717	6.542	11.118	4.2595	11.118	4.234
		11.015	3.9155	12.218	3.8105			11.816	6.5655	11.218	4.251	11.218	4.2465
		11.116	3.871	12.319	3.775			11.917	6.5665	11.318	4.24	11.317	4.1785
		11.216	3.879	12.419	3.737			12.017	6.487	11.418	4.23	11.418	4.1945
		11.316	3.7305	12.518	3.7145			12.117	6.471	11.518	4.2205	11.518	4.206
		11.416	3.7695	12.619	3.689			12.217	6.568	11.619	4.226	11.618	4.2285
		11.516	3.7295	12.719	3.6725			12.317	6.4975	11.718	4.1605	11.718	4.2255

Time (min)	Av DO for Day 0 (mg/L)	Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 8 (mg/L)	Time (min)	Av DO for Day 10 (mg/L)	Time (min)	Av DO for Day 14 (mg/L)
11.617	3.7955	12.819	3.6855	12.418	6.4925	11.819	4.214	11.818	4.2395
11.717	3.748	12.919	3.6945	12.517	6.462	11.919	4.213	11.919	4.2175
11.817	3.635	13.019	3.6595	12.617	6.485	12.019	4.1885	12.019	4.1605
11.917	3.523	13.12	3.624	12.718	6.4675	12.119	4.168	12.119	4.151
12.017	3.6535	13.22	3.554	12.818	6.5395	12.219	4.175	12.219	4.17
12.118	3.5295	13.32	3.573	12.918	6.567	12.32	4.191	12.319	4.1965
12.217	3.52	13.42	3.547	13.018	6.4785	12.42	4.1485	12.42	4.1295
12.318	3.5215	13.521	3.5775	13.118	6.4195	12.52	4.191	12.519	4.1645
12.418	3.4855	13.62	3.573	13.219	6.519	12.62	4.202	12.62	4.148
12.517	3.5455	13.72	3.529	13.318	6.4855	12.72	4.1625	12.72	4.0995
12.618	3.4955	13.821	3.5055	13.418	6.5255	12.821	4.1735	12.821	4.1335
12.718	3.419	13.921	3.512	13.519	6.489	12.92	4.1595	12.92	4.125
12.818	3.4905	14.021	3.4625	13.619	6.4035	13.021	4.167	13.02	4.0895
12.918	3.4025	14.121	3.4235	13.719	6.4645	13.121	4.1905	13.121	4.0735
13.018	3.329	14.221	3.366	13.819	6.4695	13.221	4.17	13.22	4.0955
13.119	3.279	14.322	3.35	13.919	6.4255	13.321	4.1675	13.321	4.1015
13.219	3.2875	14.422	3.3205	14.02	6.4825	13.421	4.1695	13.421	4.0825
13.318	3.316	14.521	3.349	14.12	6.4125	13.522	4.134	13.521	4.1075
13.419	3.223	14.622	3.372	14.22	6.4555	13.622	4.1445	13.622	4.033
13.519	3.1245	14.722	3.338	14.32	6.3935	13.722	4.1595	13.721	4.0445
13.62	3.1625	14.823	3.3205	14.42	6.4825	13.822	4.104	13.822	4.037
13.719	3.165	14.922	3.278	14.521	6.4645	13.922	4.122	13.922	4.0645
13.819	3.1495	15.022	3.2855	14.62	6.5405	14.023	4.106	14.021	4.077
13.92	3.056	15.123	3.309	14.72	6.4595	14.122	4.0985	14.122	4.053
14.02	3.126	15.222	3.264	14.821	6.4005	14.223	4.12	14.222	4.0175
14.12	3.055	15.323	3.23	14.92	6.397	14.323	4.098	14.323	4.074
14.22	2.9595	15.423	3.243	15.021	6.45	14.422	4.0925	14.422	4.0705
14.32	2.9745	15.524	3.225	15.121	6.401	14.523	4.0625	14.522	4.0505
14.421	2.8985	15.623	3.2105	15.222	6.483	14.623	4.061	14.623	4.001
14.521	2.9455	15.723	3.149	15.321	6.42	14.724	4.0565	14.723	4.041
14.621	2.923	15.824	3.076	15.421	6.4385	14.823	4.064	14.823	3.9905

Time (min)	Av DO for Day 0 (mg/L)	Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 8 (mg/L)	Time (min)	Av DO for Day 10 (mg/L)	Time (min)	Av DO for Day 14 (mg/L)
14.721	2.875	15.924	3.103	15.522	6.4355	14.923	4.066	14.923	3.9845
14.821	2.7995	16.024	3.026	15.622	6.374	15.024	4.0555	15.024	4.0195
14.921	2.8815	16.124	3.057	15.722	6.4445	15.124	4.017	15.124	4.0025
15.021	2.722	16.224	3.012	15.822	6.446	15.224	4.044	15.224	3.98
15.122	2.7285	16.325	3.0075	15.922	6.449	15.324	4.015	15.324	3.972
15.222	2.709	16.424	2.9985	16.023	6.4315	15.424	4.0345	15.424	3.977
15.321	2.708	16.525	3.008	16.122	6.3525	15.525	4.03	15.525	3.9875
15.422	2.68	16.625	2.966	16.223	6.4155	15.625	4.0025	15.625	3.9425
15.522	2.641	16.725	2.9475	16.323	6.5035			15.724	3.976
15.622	2.666	16.826	2.9625	16.423	6.4585			15.825	3.961
15.722	2.558	16.925	2.9795	16.524	6.49			15.925	3.9535
		17.026	2.964	16.623	6.4355			16.025	3.9325
		17.126	2.9455	16.723	6.4635			16.125	3.907
		17.226	2.918	16.824	6.4905			16.225	3.896
		17.326	2.949	16.924	6.4035			16.326	3.9085
		17.426	2.9505	17.024	6.397			16.426	3.924
		17.527	2.936	17.124	6.316			16.526	3.8975
		17.626	2.904	17.225	6.499			16.626	3.877
		17.727	2.9015	17.324	6.401			16.726	3.8985
		17.827	2.898	17.424	6.447			16.826	3.8555
		17.927	2.8735	17.525	6.44			16.926	3.8765
		18.027	2.8435	17.625	6.4805			17.027	3.9105
		18.127	2.8725	17.726	6.38			17.127	3.8655
		18.228	2.8685	17.825	6.39			17.227	3.846
		18.328	2.8775	17.925	6.433			17.327	3.856
		18.427	2.862	18.026	6.386			17.427	3.847
		18.528	2.8205	18.125	6.38			17.528	3.8075
		18.628	2.7765	18.226	6.48			17.627	3.836
		18.729	2.7785	18.326	6.414			17.728	3.821
		18.828	2.82	18.426	6.4765			17.828	3.841
		18.928	2.778	18.527	6.3605			17.928	3.8005

Time (min)	Av DO for Day 1 (mg/L)	Time (min)	Av DO for Day 8 (mg/L)	Time (min)	Av DO for Day 14 (mg/L)
19.029	2.799	18.626	6.444	18.028	3.7955
19.129	2.7695	18.727	6.3315	18.128	3.82
19.229	2.768	18.827	6.341	18.229	3.754
19.329	2.771	18.927	6.4305	18.329	3.76
19.429	2.7705	19.027	6.314	18.429	3.7575
19.53	2.7515	19.127	6.3955	18.529	3.7355
19.629	2.706	19.228	6.3575	18.629	3.7435
19.73	2.738	19.327	6.2725	18.729	3.7675
19.83	2.727	19.427	6.403	18.829	3.743
19.931	2.734	19.528	6.3755	18.93	3.7305
20.03	2.691	19.628	6.409	19.03	3.73
20.13	2.663	19.728	6.3595	19.13	3.7015
20.231	2.6505	19.828	6.281	19.23	3.7005
20.331	2.67	19.928	6.3115	19.33	3.7195
20.431	2.641	20.029	6.342	19.431	3.7095
20.531	2.6365	20.128	6.3705	19.531	3.711
20.631	2.616	20.228	6.315	19.63	3.6765
20.732	2.6425	20.329	6.303	19.731	3.655
20.831	2.5925	20.429	6.3405	19.831	3.661
20.932	2.592	20.529	6.3225	19.931	3.687

Oxygen Uptake Rates for Thermal Polymer Gels: Experiment Begun 4-18-04



Sample	Date	Gel Age (days)	DO Uptake Rate (mg/L/min)
Plank Control	4/18/04	0	0.0933
Gel	4/18/04	0	0.0465
Gel	4/19/04	1	0.1077
Gel	4/21/04	3	0.0698
Gel	4/23/04	5	0.1375
Gel	4/30/04	12	0.1134

A.2 **Alginate as a carbon source experiment**, 5-22-04, Book 2, Page 91

Referenced in Chapter 2, Section 3.1

Planktonic control with HAC

Time (min)	Average
0.6	4.0265
0.701	3.8775
0.801	3.7255
0.902	3.569
1.002	3.403
1.101	3.267
1.202	3.1185
1.302	2.979
1.403	2.8415
1.502	2.6805
1.602	2.542
1.703	2.394
1.803	2.252
1.903	2.096
2.003	1.953
2.103	1.814
2.204	1.6725
2.303	1.531
2.404	1.369
2.504	1.228
2.604	1.091
2.704	0.948
2.804	0.797
2.905	0.6625

Planktonic control without HAC

Time	Average	Time	Average	Time	Average	Time	Average	Time	Average	Time	Average	Time	Average	Time	Average	Time	Average	Time	Average
1.102	7.395	4.207	7.115	7.312	6.9505	10.416	6.7915	13.521	6.645	16.625	6.504	19.73	6.3655	22.834	6.225	25.94	6.0935	29.045	5.9575
1.202	7.3725	4.307	7.11	7.411	6.9395	10.517	6.7905	13.621	6.641	16.726	6.494	19.83	6.355	22.935	6.221	26.039	6.09	29.145	5.954
1.302	7.3525	4.407	7.1075	7.512	6.939	10.616	6.789	13.72	6.635	16.826	6.501	19.931	6.3485	23.035	6.223	26.14	6.0825	29.245	5.955
1.403	7.3325	4.507	7.1035	7.612	6.9335	10.717	6.779	13.821	6.6325	16.925	6.489	20.031	6.3475	23.136	6.2115	26.24	6.0825	29.345	5.945
1.502	7.317	4.607	7.0945	7.712	6.932	10.817	6.776	13.921	6.6275	17.026	6.484	20.13	6.345	23.235	6.2065	26.34	6.073	29.445	5.943
1.603	7.3055	4.707	7.0855	7.812	6.9215	10.917	6.7715	14.022	6.6175	17.126	6.4805	20.231	6.342	23.335	6.203	26.44	6.068	29.545	5.9355
1.703	7.295	4.808	7.084	7.912	6.913	11.017	6.7565	14.121	6.619	17.226	6.483	20.331	6.3325	23.436	6.2	26.541	6.066	29.645	5.9295
1.803	7.2815	4.908	7.077	8.013	6.908	11.117	6.7595	14.221	6.614	17.326	6.4695	20.431	6.33	23.536	6.2015	26.641	6.0625	29.746	5.931
1.903	7.2665	5.008	7.0665	8.113	6.9055	11.218	6.7545	14.322	6.6115	17.426	6.465	20.531	6.3285	23.635	6.192	26.74	6.0555	29.846	5.924
2.003	7.2635	5.108	7.0635	8.212	6.902	11.318	6.7545	14.422	6.6085	17.527	6.464	20.631	6.3225	23.736	6.1845	26.841	6.05	29.946	5.919
2.104	7.2655	5.208	7.0585	8.313	6.8965	11.417	6.7415	14.522	6.603	17.626	6.463	20.732	6.315	23.836	6.181	26.941	6.0495	30.046	5.9125
2.204	7.2455	5.309	7.047	8.413	6.8945	11.518	6.736	14.622	6.6005	17.727	6.4555	20.831	6.3135	23.937	6.177	27.042	6.045	30.146	5.9095
2.303	7.234	5.408	7.048	8.514	6.883	11.618	6.73	14.722	6.593	17.827	6.45	20.932	6.3025	24.036	6.174	27.142	6.0385	30.247	5.909
2.404	7.223	5.508	7.044	8.613	6.8815	11.717	6.73	14.823	6.5795	17.927	6.4455	21.032	6.3055	24.137	6.167	27.242	6.0325	30.346	5.905
2.504	7.225	5.609	7.038	8.713	6.8755	11.818	6.7275	14.922	6.5825	18.028	6.443	21.132	6.296	24.237	6.164	27.342	6.032	30.447	5.8985
2.605	7.2115	5.709	7.0295	8.814	6.8765	11.918	6.718	15.023	6.5795	18.127	6.4405	21.233	6.2945	24.337	6.167	27.442	6.019	30.547	5.895
2.704	7.208	5.809	7.027	8.913	6.864	12.019	6.713	15.123	6.575	18.228	6.429	21.332	6.2885	24.437	6.16	27.542	6.016	30.647	5.89
2.805	7.2035	5.909	7.024	9.014	6.8625	12.118	6.714	15.224	6.556	18.328	6.4215	21.433	6.2905	24.537	6.153	27.642	6.0165	30.747	5.8835
2.905	7.1885	6.009	7.0195	9.114	6.8555	12.218	6.703	15.323	6.552	18.428	6.4245	21.533	6.28	24.638	6.1475	27.743	6.0145	30.847	5.8815
3.004	7.1935	6.11	6.9965	9.214	6.852	12.319	6.7045	15.423	6.5525	18.528	6.418	21.632	6.2775	24.738	6.146	27.843	6.0115	30.948	5.876
3.105	7.1775	6.209	7.001	9.314	6.845	12.419	6.6975	15.524	6.554	18.628	6.413	21.733	6.272	24.838	6.139	27.942	6.0075	31.048	5.872
3.205	7.1735	6.31	7.0065	9.414	6.8365	12.519	6.6945	15.624	6.5535	18.729	6.4035	21.833	6.2745	24.938	6.1325	28.043	5.9995	31.147	5.865
3.306	7.17	6.41	6.996	9.515	6.8335	12.619	6.6935	15.724	6.544	18.829	6.3995	21.934	6.2685	25.038	6.131	28.143	5.995	31.248	5.865
3.405	7.1545	6.51	6.988	9.615	6.835	12.719	6.682	15.824	6.539	18.929	6.401	22.034	6.2565	25.139	6.125	28.244	5.9945	31.348	5.859
3.505	7.153	6.61	6.9855	9.715	6.827	12.82	6.67	15.924	6.5355	19.029	6.395	22.133	6.2545	25.238	6.126	28.344	5.9845	31.448	5.8575
3.606	7.147	6.71	6.9845	9.815	6.8245	12.92	6.6775	16.024	6.533	19.129	6.3875	22.234	6.255	25.339	6.1195	28.443	5.9795	31.548	5.853
3.706	7.1525	6.811	6.97	9.915	6.8175	13.02	6.6765	16.124	6.527	19.229	6.3875	22.334	6.2465	25.439	6.1115	28.544	5.983	31.649	5.847
3.806	7.1455	6.911	6.9635	10.016	6.8185	13.12	6.668	16.225	6.5175	19.329	6.3835	22.434	6.245	25.539	6.108	28.644	5.9755	31.749	5.8485
3.906	7.133	7.011	6.965	10.116	6.812	13.22	6.654	16.325	6.517	19.43	6.379	22.534	6.2365	25.639	6.102	28.744	5.9685	31.849	5.8435
4.007	7.1295	7.111	6.9625	10.216	6.806	13.32	6.6535	16.425	6.503	19.53	6.376	22.635	6.2355	25.739	6.0985	28.844	5.965	31.949	5.8385
4.107	7.1215	7.211	6.946	10.316	6.794	13.42	6.652	16.525	6.499	19.629	6.3665	22.735	6.225	25.84	6.095	28.944	5.963	32.049	5.833

Time	Average
32.15	5.827
32.25	5.8255
32.349	5.819
32.45	5.8145
32.55	5.8155
32.651	5.8085
32.75	5.806
32.85	5.8
32.951	5.7935
33.05	5.7925
33.151	5.784
33.251	5.787
33.352	5.784
33.451	5.774
33.551	5.7715
33.652	5.767
33.752	5.762
33.853	5.7585
33.952	5.7565
34.052	5.7535
34.153	5.7445
34.252	5.741
34.353	5.7395
34.453	5.7375
34.554	5.729
34.653	5.725
34.753	5.7155
34.854	5.718
34.954	5.7135
35.055	5.7045
35.154	5.704

Time	Average
35.254	5.699
35.355	5.6945
35.454	5.6865
35.555	5.681
35.655	5.6795
35.755	5.681
35.855	5.6715
35.955	5.666
36.056	5.664
36.156	5.665
36.256	5.663
36.356	5.654
36.456	5.6515
36.557	5.6465
36.656	5.647
36.757	5.6415
36.857	5.632
36.957	5.6305
37.057	5.632
37.157	5.6225
37.258	5.6175
37.358	5.6175
37.457	5.614
37.558	5.604
37.658	5.5975
37.759	5.5965
37.858	5.593
37.959	5.585
38.059	5.5845
38.159	5.5825
38.259	5.579

Time	Average
38.359	5.5755
38.46	5.575
38.56	5.567
38.659	5.561
38.76	5.5585
38.86	5.5535
38.96	5.55
39.06	5.5415
39.161	5.5435
39.261	5.5365
39.361	5.533
39.461	5.531
39.561	5.5255
39.661	5.528
39.762	5.518
39.861	5.51
39.962	5.501
40.062	5.5045
40.161	5.5
40.262	5.4965
40.362	5.4935
40.463	5.489
40.562	5.4875
40.662	5.484
40.763	5.477
40.863	5.478
40.964	5.4695
41.063	5.4645
41.163	5.461
41.264	5.4605
41.363	5.452

Time	Average
41.464	5.451
41.564	5.446
41.665	5.445
41.764	5.441
41.864	5.436
41.965	5.4325
42.065	5.423
42.166	5.42
42.265	5.42
42.365	5.4145
42.466	5.4095
42.565	5.407
42.666	5.406
42.766	5.396
42.866	5.392
42.966	5.389
43.066	5.3865
43.167	5.3825
43.267	5.3775

Alginate immobilized cells, no HAC, day zero

Time	Average
1.102	7.3835
1.202	7.3835
1.302	7.382
1.403	7.3545
1.502	7.3415
1.603	7.352
1.703	7.352
1.803	7.3275
1.903	7.3055
2.003	7.321
2.104	7.319
2.204	7.345
2.303	7.3375
2.404	7.3205
2.504	7.316
2.605	7.3195
2.704	7.328
2.805	7.3165
2.905	7.3005
3.004	7.306
3.105	7.2995
3.205	7.268
3.306	7.2375
3.405	7.2415
3.505	7.2475
3.606	7.269
3.706	7.279
3.806	7.2505
3.906	7.2175
4.007	7.2495
4.107	7.223
4.206	7.236
4.307	7.198

Time	Average
4.407	7.1725
4.508	7.191
4.607	7.203
4.707	7.199
4.808	7.186
4.908	7.1785
5.008	7.177
5.108	7.1635
5.209	7.1535
5.309	7.145
5.408	7.1325
5.509	7.1355
5.609	7.1335
5.709	7.1355
5.809	7.127
5.909	7.1395
6.01	7.1245
6.11	7.103
6.21	7.103
6.31	7.1015
6.41	7.0965
6.51	7.0805
6.61	7.056
6.711	7.0695
6.811	7.0565
6.911	7.052
7.011	7.05
7.111	7.0395
7.212	7.0215
7.312	7.015
7.412	7.02
7.512	7.0135
7.612	7.0145

Time	Average
7.713	7.0025
7.812	6.971
7.913	6.976
8.013	6.9795
8.113	6.971
8.213	6.9585
8.313	6.9605
8.414	6.948
8.514	6.9285
8.613	6.9295
8.714	6.9215
8.814	6.926
8.915	6.8995
9.014	6.859
9.114	6.881
9.215	6.879
9.314	6.887
9.415	6.866
9.515	6.8895
9.615	6.88
9.715	6.9055
9.815	6.898
9.916	6.8905
10.016	6.873
10.116	6.871
10.216	6.877
10.316	6.8675
10.417	6.8575
10.517	6.8495
10.617	6.822
10.717	6.8145
10.817	6.8135
10.917	6.82

Time	Average
11.017	6.8225
11.118	6.816
11.218	6.8085
11.318	6.8015
11.418	6.7815
11.518	6.7725
11.619	6.7825
11.718	6.788
11.818	6.773
11.919	6.768
12.019	6.751
12.119	6.7435
12.219	6.7435
12.319	6.7385
12.42	6.734
12.519	6.7255
12.62	6.731
12.72	6.7115
12.82	6.701
12.92	6.705
13.02	6.6935
13.121	6.6925
13.221	6.6785
13.32	6.6745
13.421	6.6715
13.521	6.671
13.622	6.6725
13.721	6.649
13.822	6.625
13.922	6.582
14.022	6.521
14.122	6.4595
14.222	6.4265

Time	Average
14.323	6.417
14.422	6.4255
14.522	6.407
14.623	6.3835
14.723	6.38
14.823	6.376
14.923	6.3835
15.023	6.403
15.124	6.3955
15.224	6.394
15.324	6.382
15.424	6.373
15.524	6.3535
15.625	6.342
15.724	6.3345
15.825	6.337
15.925	6.3335
16.025	6.332
16.125	6.4225
16.225	6.5525
16.326	6.5785
16.426	6.559
16.526	6.5545
16.626	6.551
16.726	6.5305
16.826	6.526
16.926	6.5225
17.027	6.5165
17.127	6.5205
17.226	6.4985
17.327	6.4975
17.427	6.488
17.528	6.4825

Time	Average
17.628	6.4795
17.727	6.4695
17.828	6.4685
17.928	6.467
18.028	6.4535
18.128	6.4555
18.229	6.445
18.329	6.4415
18.428	6.429
18.529	6.4275
18.629	6.4225
18.73	6.4115
18.829	6.409
18.929	6.4
19.03	6.404
19.13	6.39
19.23	6.3915
19.33	6.3815
19.431	6.3695
19.531	6.3715
19.63	6.3605
19.731	6.363
19.831	6.3555
19.932	6.3435
20.031	6.3405
20.131	6.326
20.232	6.328
20.332	6.32
20.432	6.318
20.532	6.3235
20.632	6.3065
20.733	6.3005
20.832	6.3055

Time	Average
20.933	6.298
21.033	6.2805
21.133	6.284
21.233	6.2845
21.333	6.283
21.434	6.264
21.534	6.252
21.633	6.26
21.734	6.2495
21.834	6.2525
21.934	6.2395
22.034	6.2315
22.134	6.2375
22.235	6.226
22.335	6.2215
22.435	6.2185
22.535	6.219
22.635	6.208
22.736	6.203
22.835	6.2005
22.936	6.189
23.036	6.184
23.136	6.1805
23.236	6.1665
23.336	6.17
23.437	6.167
23.537	6.168
23.637	6.156
23.737	6.157
23.837	6.1415
23.937	6.1525
24.037	6.1315
24.138	6.1325

Time	Average
24.238	6.1275
24.338	6.1285
24.438	6.125
24.538	6.118
24.639	6.118
24.739	6.1145
24.838	6.104
24.939	6.1025
25.039	6.0895
25.139	6.081
25.239	6.0715
25.34	6.0745
25.44	6.0665
25.539	6.0705
25.64	6.0625
25.74	6.0575
25.841	6.059
25.941	6.045
26.041	6.048
26.141	6.0385
26.241	6.0275
26.341	6.017
26.441	6.024
26.542	6.0155
26.642	6.009
26.742	5.998
26.842	6.009
26.942	6.0005
27.043	5.9965
27.142	5.9915
27.242	5.979
27.343	5.981
27.443	5.976

Time	Average
27.543	5.9605
27.643	5.9615
27.744	5.955
27.844	5.948
27.943	5.9515
28.044	5.9525
28.144	5.9365
28.244	5.9365
28.344	5.927
28.444	5.924
28.545	5.921
28.645	5.9155
28.745	5.91
28.845	5.9025
28.945	5.906
29.045	5.8925
29.145	5.89
29.246	5.8885
29.346	5.884
29.447	5.877
29.546	5.871
29.646	5.867
29.747	5.8655
29.847	5.8595
29.947	5.8585
30.047	5.8585
30.147	5.851
30.247	5.846
30.347	5.841
30.448	5.827
30.548	5.834
30.647	5.8285
30.748	5.828

Time	Average
30.848	5.816
30.949	5.807
31.049	5.813
31.148	5.809
31.249	5.8035
31.349	5.785
31.449	5.78
31.549	5.785
31.65	5.775
31.75	5.7755
31.85	5.7775
31.95	5.77
32.05	5.769
32.151	5.764
32.25	5.7505
32.35	5.7475
32.451	5.749
32.551	5.7385
32.651	5.7335
32.751	5.7235
32.851	5.736
32.952	5.715
33.051	5.712
33.152	5.715
33.252	5.7095
33.352	5.697
33.452	5.693
33.552	5.695
33.653	5.688
33.753	5.6885
33.853	5.685
33.953	5.676
34.053	5.6735

Time	Average
34.154	5.675
34.253	5.6645
34.354	5.655
34.454	5.6525
34.554	5.653
34.654	5.6505
34.754	5.6415
34.855	5.632
34.955	5.6195
35.054	5.6235
35.155	5.616
35.255	5.6135
35.355	5.6125
35.455	5.617
35.555	5.6065
35.656	5.594
35.756	5.5945
35.856	5.596
35.956	5.58
36.057	5.5745
36.157	5.5685
36.256	5.5615
36.357	5.5605
36.457	5.5575
36.557	5.5585
36.657	5.548
36.757	5.5575
36.858	5.5435
36.958	5.5365
37.058	5.5385
37.158	5.5355
37.259	5.533
37.359	5.534

Time	Average
37.458	5.5265
37.559	5.519
37.659	5.5155
37.759	5.506
37.859	5.5095
37.959	5.507
38.06	5.506
38.16	5.5
38.26	5.4875
38.36	5.4755
38.461	5.4815
38.561	5.473
38.66	5.4655
38.761	5.465
38.861	5.468
38.961	5.4675
39.061	5.457
39.161	5.448
39.262	5.446
39.361	5.445
39.462	5.4455
39.562	5.433
39.662	5.425
39.763	5.424
39.862	5.4245
39.963	5.4145
40.063	5.4105
40.163	5.4065
40.263	5.4045
40.363	5.3995
40.464	5.394
40.563	5.391
40.664	5.384

Alginate immobilized cells, no HAC, day 2

Time	Average
1.301	9.7275
1.401	9.7395
1.5	9.7345
1.601	9.7325
1.701	9.7475
1.802	9.76
1.901	9.7265
2.002	9.758
2.102	9.753
2.202	9.7475
2.302	9.75
2.402	9.766
2.503	9.7535
2.603	9.753
2.702	9.7515
2.803	9.747
2.903	9.7445
3.003	9.746
3.103	9.748
3.203	9.74
3.304	9.7275
3.404	9.738
3.504	9.7375
3.604	9.74
3.705	9.746
3.805	9.7385
3.904	9.7495
4.005	9.7355
4.105	9.715
4.206	9.7245
4.305	9.7205
4.405	9.726

Time	Average
4.506	9.721
4.606	9.7215
4.705	9.72
4.806	9.73
4.906	9.7125
5.006	9.7115
5.106	9.709
5.206	9.716
5.307	9.715
5.406	9.7005
5.507	9.6995
5.607	9.6955
5.707	9.693
5.807	9.6965
5.907	9.6895
6.008	9.6845
6.108	9.6775
6.207	9.681
6.308	9.6605
6.408	9.6785
6.509	9.6625
6.609	9.661
6.708	9.6515
6.809	9.6505
6.909	9.642
7.01	9.6405
7.109	9.649
7.209	9.65
7.31	9.652
7.41	9.6605
7.51	9.685
7.61	9.6675

Time	Average
7.71	9.6645
7.811	9.656
7.91	9.6535
8.011	9.6335
8.111	9.6355
8.21	9.6375
8.311	9.639
8.411	9.632
8.512	9.643
8.611	9.627
8.712	9.63
8.812	9.6285
8.912	9.6285
9.012	9.6245
9.112	9.632
9.213	9.627
9.313	9.6275
9.413	9.622
9.513	9.627
9.613	9.6295
9.714	9.617
9.814	9.6115
9.913	9.6
10.014	9.5995
10.114	9.61
10.214	9.606
10.314	9.597
10.414	9.5975
10.515	9.5925
10.615	9.607
10.715	9.5895
10.816	9.5955

Time	Average
10.916	9.6025
11.015	9.5965
11.116	9.593
11.216	9.6
11.316	9.599
11.416	9.6015
11.516	9.5955
11.617	9.598
11.717	9.6
11.817	9.6
11.917	9.5915
12.017	9.589
12.118	9.5795
12.217	9.5815
12.318	9.5795
12.418	9.5745
12.518	9.57
12.618	9.562
12.718	9.571
12.819	9.577
12.918	9.594
13.018	9.579
13.119	9.571
13.219	9.5745
13.319	9.5865
13.419	9.5775
13.519	9.569
13.62	9.558
13.719	9.568
13.82	9.5645
13.92	9.567
14.02	9.561

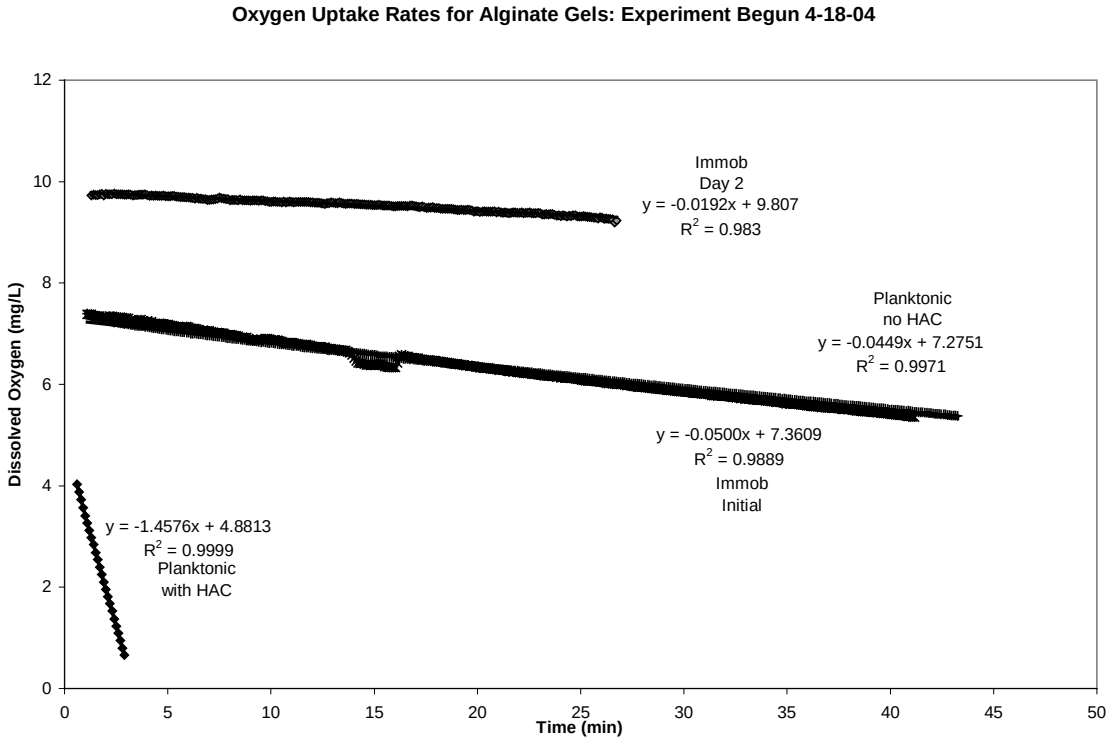
Time	Average
14.12	9.555
14.22	9.5585
14.321	9.5535
14.421	9.551
14.521	9.5475
14.621	9.54
14.721	9.5465
14.822	9.545
14.922	9.5355
15.021	9.5355
15.122	9.5315
15.222	9.5425
15.323	9.5385
15.422	9.524
15.522	9.5305
15.623	9.5245
15.722	9.526
15.823	9.5205
15.923	9.513
16.023	9.511
16.124	9.5155
16.223	9.519
16.324	9.5165
16.424	9.5155
16.524	9.519
16.624	9.5115
16.724	9.525
16.825	9.523
16.924	9.5195
17.025	9.5145
17.125	9.501
17.225	9.4955

Time	Average
17.325	9.509
17.425	9.497
17.526	9.4815
17.626	9.4895
17.726	9.486
17.826	9.491
17.926	9.4765
18.027	9.481
18.126	9.4765
18.227	9.4605
18.327	9.4665
18.427	9.4645
18.527	9.462
18.627	9.4605
18.728	9.462
18.828	9.4545
18.927	9.4435
19.028	9.44
19.128	9.441
19.229	9.443
19.328	9.44
19.428	9.4375
19.529	9.445
19.629	9.4395
19.729	9.43
19.829	9.4105
19.929	9.4155
20.029	9.418
20.129	9.4025
20.23	9.4085
20.33	9.416
20.43	9.4105

Time	Average
20.53	9.4065
20.63	9.4075
20.731	9.4105
20.83	9.4125
20.93	9.3905
21.031	9.404
21.131	9.392
21.232	9.3835
21.331	9.387
21.431	9.38
21.532	9.3775
21.632	9.381
21.732	9.3895
21.832	9.387
21.932	9.3795
22.032	9.3835
22.132	9.3895
22.233	9.377
22.333	9.3845
22.433	9.379
22.533	9.3875
22.633	9.377
22.733	9.375
22.833	9.3785
22.933	9.3835
23.034	9.3785
23.134	9.354
23.233	9.3545
23.334	9.358
23.434	9.354
23.535	9.3565
23.635	9.3565

Time	Average
23.734	9.3405
23.835	9.3265
23.935	9.3345
24.035	9.32
24.135	9.3355
24.235	9.3285
24.336	9.309
24.435	9.3295
24.536	9.322
24.636	9.3315
24.736	9.3315
24.837	9.31
24.936	9.312
25.037	9.313
25.137	9.3185
25.236	9.3135
25.337	9.3015
25.437	9.3025
25.538	9.294
25.638	9.2905
25.737	9.2845
25.838	9.275
25.938	9.2965
26.038	9.288
26.138	9.264
26.238	9.271
26.339	9.264
26.438	9.2645
26.539	9.252
26.639	9.195
26.74	9.2305

Summary plot from alginate degradation experiment



Sample	Date	DO Uptake Rate (mg/L/min)
Plank with HAC	5/22/04	1.4576
Plank no HAC	5/22/04	0.0449
Gel Initial no HAC	5/22/04	0.0500
Gel Day 2 no HAC	5/24/04	0.0192

A.3 t-tests to determine statistical difference

t tests for planktonic potassium efflux experiments: (units = mg K^+/cell):
Referenced in Chapter 3
Data:

late log PA	mid log PA
2.13E-10	5.09E-10
5.46E-10	5.61E-10
2.52E-11	3.89E-10
2.06E-10	3.42E-10
4.89E-10	
3.63E-10	

late log EC	mid log EC
4.16E-10	4.51327E-10
1.87E-10	6.87023E-10
6.43E-10	4.78676E-10
	5.93559E-10
	5.58081E-10

Compare late log PA to mid log PA
t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	3.07151E-10	4.49969E-10
Variance	3.83649E-20	1.04178E-20
Observations	6	4
Hypothesized Mean Di	0	
df	8	
t Stat	-1.505553529	
P(T<=t) one-tail	0.085300189	
t Critical one-tail	1.85954832	
P(T<=t) two-tail	0.170600377	
t Critical two-tail	2.306005626	

tcalc < t crit, so accept null hypothesis. No difference exists.

Compare late log EC to mid log EC
t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	4.15329E-10	5.53733E-10
Variance	5.19538E-20	8.87291E-21
Observations	3	5
Hypothesized Mean D	0	
df	2	
t Stat	-1.001652612	
P(T<=t) one-tail	0.211007083	
t Critical one-tail	2.91998731	
P(T<=t) two-tail	0.422014166	
t Critical two-tail	4.302655725	

tcalc < t crit, so accept null hypothesis. No difference exists.

Compare late log PA to late log EC
t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	3.07151E-10	4.15329E-10
Variance	3.83649E-20	5.19538E-20
Observations	6	3
Hypothesized Mean Di	0	
df	4	
t Stat	-0.702517739	
P(T<=t) one-tail	0.260543522	
t Critical one-tail	2.131846486	
P(T<=t) two-tail	0.521087044	
t Critical two-tail	2.776450856	

tcalc < t crit, so accept null hypothesis. No difference exists.

Compare mid log PA to mid log EC
t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	4.49969E-10	5.53733E-10
Variance	1.04178E-20	8.87291E-21
Observations	4	5
Hypothesized Mean D	0	
df	6	
t Stat	-1.568050427	
P(T<=t) one-tail	0.083958121	
t Critical one-tail	1.943180905	
P(T<=t) two-tail	0.167916242	
t Critical two-tail	2.446913641	

tcalc < t crit, so accept null hypothesis. No difference exists.

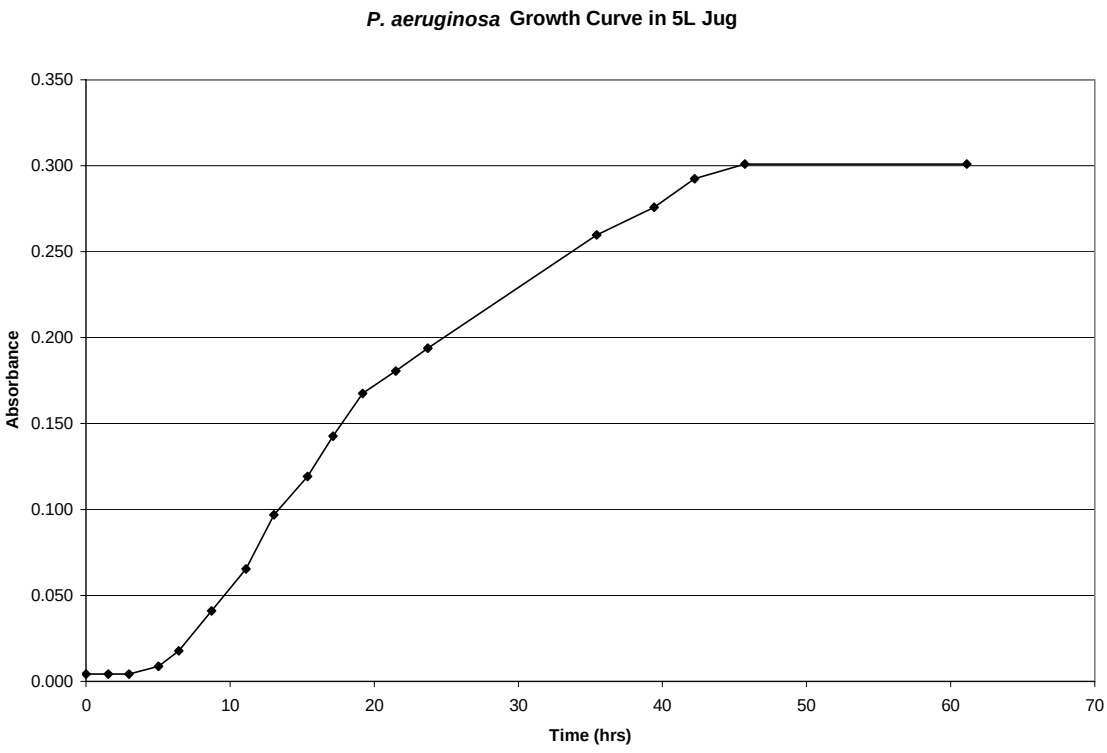
Appendix B: Data for Chapter 3

B.1 Growth Curve Data

B.1.1 *Psuedomonas aeruginosa*

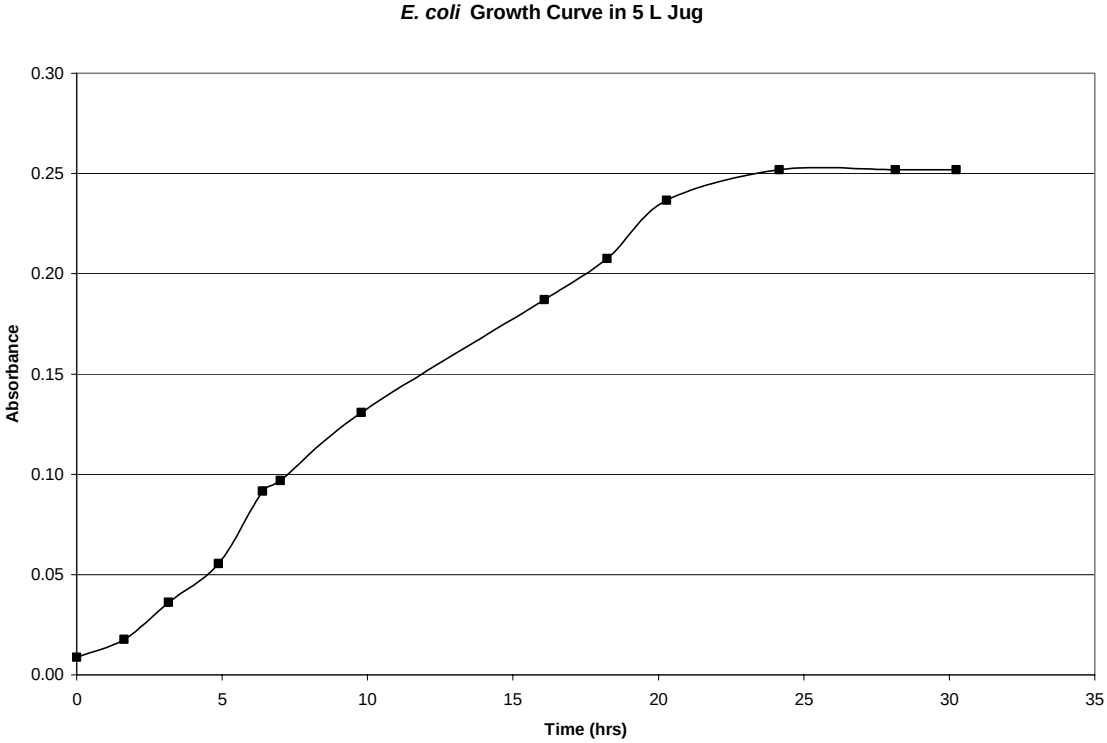
7-22-03, Book 2, Page 12

Time	Trans 1	Abs 1
0.0	99	0.004
1.5	99	0.004
3.0	99	0.004
5.0	98	0.009
6.4	96	0.018
8.7	91	0.041
11.1	86	0.066
13.0	80	0.097
15.4	76	0.119
17.1	72	0.143
19.2	68	0.167
21.5	66	0.180
23.7	64	0.194
35.4	55	0.260
39.4	53	0.276
42.2	51	0.292
45.7	50	0.301
61.1	50	0.301



B.1.2 *Escherchia coli*
8-14-03, Book 2, Page 16

Time	Transmittance	Absorbance
0.0	98	0.0088
1.6	96	0.0177
3.2	92	0.0362
4.9	88	0.0555
6.4	81	0.0915
7.0	80	0.0969
9.8	74	0.1308
16.1	65	0.1871
18.2	62	0.2076
20.3	58	0.2366
24.2	56	0.2518
28.2	56	0.2518
30.2	56	0.2518



B.2 Data for potassium efflux per cell for planktonic cultures

B.2.1 Planktonic efflux from ***P. aeruginosa***

Summary table of efflux data for planktonic *P. aeruginosa*

Trans	Abs	Cell Counts			Av cell count	StdDev cell count	delta K			av delta K	Std Dev delta K	delta K / cell	Error on Efflux
61	0.2147	1.7E+09	1.2E+09	1.4E+09	1.4E+09	2.5E+08	3.2	2.8	3.3	3.07	0.284	2.13E-10	4.1E-11
72	0.1427	1.3E+09	1.1E+09	1.1E+09	1.2E+09	8.1E+07	5.5	6.5	5.7	5.88	0.501	5.09E-10	5.6E-11
60	0.2218	1.2E+09	1.3E+09	1.2E+09	1.2E+09	4.2E+07	7.0	6.6	6.7	6.73	0.202	5.46E-10	2.5E-11
50	0.3010	9.0E+09	7.2E+09	8.4E+09	8.2E+09	9.2E+08	2.2	2.3	1.7	2.07	0.321	2.52E-11	4.8E-12
55	0.2596	1.3E+09	1.1E+09	1.2E+09	1.2E+09	7.6E+07	2.5	2.6	2.5	2.48	0.058	2.06E-10	1.4E-11
91	0.0410	2.7E+08	3.3E+08	3.2E+08	3.1E+08	3.2E+07	1.7	1.7	1.9	1.73	0.104	5.61E-10	6.7E-11
79	0.1024	7.30E+08	6.20E+08	6.70E+08	6.7E+08	5.5E+07	2.6	3.1	2.2	2.62	0.425	3.89E-10	7.1E-11
75	0.1249	7.70E+08	7.70E+08	8.60E+08	8.0E+08	5.2E+07	1.8	2.7	3.7	2.73	0.950	3.42E-10	1.2E-10
66	0.1805	1.84E+09	1.72E+09	1.23E+09	1.6E+09	3.2E+08	6.8	7.0	9.7	7.81	1.644	4.89E-10	1.4E-10
62	0.2076	1.78E+09	1.82E+09	1.86E+09	1.8E+09	4.0E+07	6.4	6.8	6.7	6.61	0.190	3.63E-10	1.3E-11

The following sets of data were obtained by acidifying the samples with nitric acid, diluting, and analyzing on the AA. Data shown has been corrected for dilution and for machine drift during analysis. Control flasks are indicated by "C", and dosed flasks by "F." Potassium concentration is reported in mg/L.

Potassium efflux from transmittance = 61, 6-20-03, Book 1, Page 99

Time	C1	C2	C3	F1	F2	F3	av control	av dose
0	0.15	0.05	0.15	0.15	0.40	--	0.12	0.28
5	0.15	0.30	0.35	1.85	1.60	1.60	0.27	1.68
15	0.10	0.25	0.20	2.80	2.55	2.70	0.18	2.68
30	0.25	0.10	0.10	3.40	3.00	3.00	0.15	3.13
60	0.35	0.20	0.15	3.30	3.15	3.50	0.23	3.32

Potassium efflux from transmittance = 72, 7-18-03, Book 2, Page 9

Time	C1	C2	C3	F1	F2	F3	av control	av dose	std dev cont	std dev dose
0	2.30	2.35	2.65	2.90	3.10	2.70	2.43	2.90	0.189	0.200
5	1.75	1.85	1.60	6.05	5.70	7.25	1.73	6.33	0.126	0.813
15	2.15	1.85	1.65	7.70	8.00	8.80	1.88	8.17	0.252	0.569
30	1.70	1.35	1.70	8.75	8.60	7.95	1.58	8.43	0.202	0.425
60	1.55	1.60	1.60	8.40	9.55	8.40	1.58	8.78	0.029	0.664

Potassium efflux from transmittance = 60, 7-19-03, Book 2, Page 10

Time	C1	C2	C3	F1	F2	F3	av control	av dose	std dev cont	std dev dose
0	1.40	2.05	1.15	1.60	1.70	1.80	1.53	1.70	0.465	0.100
15	3.00	1.80	2.85	7.80	7.75	8.65	2.55	8.07	0.654	0.506
30	2.30	2.25	2.05	8.10	7.70	7.85	2.20	7.88	0.132	0.202

Initial 1.15

Potassium efflux from transmittance = 50, 7-24-04, Book 2, Page 11

Time	C1	C2	C3	F1	F2	F3	av control	av dose	std dev cont	std dev dose
0	0.05	0.05	0.20	0.55	0.15	0.55	0.10	0.42	0.087	0.231
20	0.10	0.15	0.10	1.50	1.30	1.50	0.12	1.43	0.029	0.115
40	0.05	0.05	0.10	2.15	2.40	2.20	0.07	2.25	0.029	0.132
60	0.05	0.05	0.05	2.75	2.45	2.25	0.05	2.48	0.000	0.252

Initial 3.15

Potassium efflux from transmittance = 55, 7-29-03, Book 2, Page 14

Time	C1	C2	C3	F1	F2	F3	av control	av dose	std dev cont	std dev dose
0	0.20	0.30	0.15	0.25	0.20	0.20	0.22	0.22	0.076	0.029
20	0.25	0.30	0.15	1.60	1.40	1.55	0.23	1.52	0.076	0.104
40	0.20	0.30	0.20	2.25	2.45	2.25	0.23	2.32	0.058	0.115
60	0.20	0.15	0.15	2.70	2.75	2.65	0.17	2.70	0.029	0.050

Initial 3.15

Potassium efflux from transmittance = 91, 7-31-03, Book 2, Page 15

Time	C1	C2	C3	F1	F2	F3	av control	av dose	std dev cont	std dev dose
0	2.10	1.85	2.05	2.10	2.00	1.90	2.00	2.00	0.132	0.100
20	1.75	2.00	1.65	2.10	1.95	2.15	1.80	2.07	0.180	0.104
40	1.35	1.50	1.40	2.20	2.75	2.50	1.42	2.48	0.076	0.275
60	1.65	0.80	0.65	2.30	2.35	2.50	1.03	2.38	0.539	0.104

Initial 3.15

Potassium efflux from transmittance = 79, 11-23-03, Book 2, Page 53

Time	C1	C2	C3	F1	F2	F3	av control	av dose	std dev cont	std dev dose
0	2.30	0.60	3.25	1.55	0.95	1.25	2.05	1.25	1.343	0.300
5	0.90	0.95	1.40	1.55	1.95	2.35	1.08	1.95	0.275	0.400
15	0.65	0.80	0.75	3.45	3.05	3.20	0.73	3.23	0.076	0.202
30	0.75	0.65	0.70	3.25	4.00	3.85	0.70	3.70	0.050	0.397

Potassium efflux from transmittance = 75, 11-23-03, Book 2, Page 54

Time	C1	C2	C3	F1	F2	F3	av control	av dose	std dev cont	std dev dose
0	0.95	0.70	1.35	1.80	1.20	0.85	1.00	1.28	0.328	0.480
5	3.80	0.95	2.00	2.95	2.95	1.95	2.25	2.62	1.441	0.577
15	1.80	0.85	0.45	4.30	3.55	3.65	1.03	3.83	0.693	0.407
30	2.20	1.10	1.05	3.60	3.90	4.55	1.45	4.02	0.650	0.486

Potassium efflux from transmittance = 66, 1-2-04, Book 2, Page 59

Time	C1	C2	C3	F1	F2	F3	av control	av dose	st dev cont	st dev dose
0	1.90	1.95	1.29	1.80	2.54	1.51	1.71	1.95	0.369	0.530
20	1.34	1.90	1.59	5.08	5.62	5.18	1.61	5.30	0.281	0.287
40	1.80	2.77	2.72	8.17	8.71	9.01	2.43	8.63	0.546	0.423
60	7.00	2.10	3.13	8.56	9.50	11.21	4.08	9.76	2.584	1.343

Initital 2.36

Potassium efflux from transmittance = 62, 1-17-04, Book 2, Page 63

Time	C1	C2	C3	F1	F2	F3	av control	av dose	st dev cont	st dev dose
0	0.51	0.85	1.43	0.89	0.95	0.55	0.93	0.80	0.466	0.214
20	0.58	1.89	1.58	1.79	3.72	3.92	1.35	3.14	0.684	1.178
40	3.47	0.55	0.62	5.35	4.82	6.42	1.55	5.53	1.666	0.815
60	2.01	0.93	1.70	7.29	7.72	8.82	1.55	7.94	0.555	0.790

Initital 2.15

B.2.2 Planktonic efflux from ***E. coli***

Summary table of efflux data for planktonic *E. coli*

Trans	Abs	Cell Counts			Av cell count	StdDev cell count	delta K			av delta K	Std Dev delta K	delta K / cell	Error on Efflux
56	0.252	1.0E+09	1.1E+09	1.1E+09	1.1E+09	4.0E+07	4.6	4.3	4.4	4.38	0.153	4.161E-10	2.16E-11
56	0.252	1.9E+09	2.1E+09	2.1E+09	2.1E+09	9.6E+07	3.6	4.1	3.9	3.83	0.275	1.870E-10	1.61E-11
78	0.108	1.1E+09	1.3E+09	1.0E+09	1.1E+09	1.4E+08	4.9	5.0	5.5	5.10	0.304	4.513E-10	6.27E-11
70	0.155	9.1E+08	8.2E+08	8.9E+08	8.7E+08	4.7E+07	5.7	5.8	6.6	6.00	0.477	6.870E-10	6.61E-11
55	0.260	1.68E+09	1.44E+09	1.43E+09	1.5E+09	1.4E+08	9.4	9.8	10.1	9.75	0.377	6.429E-10	6.50E-11
76	0.119	4.20E+08	4.40E+08	5.00E+08	4.5E+08	4.2E+07	2.1	2.2	2.3	2.17	0.092	4.787E-10	4.84E-11
86	0.066	1.10E+09	8.00E+08	1.05E+09	9.8E+08	1.6E+08	5.8	6.1	5.7	5.84	0.201	5.936E-10	9.91E-11
89	0.051	1.28E+09	1.32E+09	1.42E+09	1.3E+09	7.2E+07	6.2	6.5	9.7	7.48	1.970	5.581E-10	1.50E-10

The following sets of data were obtained by acidifying the samples with nitric acid, diluting, and analyzing on the AA. Data shown has been corrected for dilution and for machine drift during analysis. Control flasks are indicated by "C", and dosed flasks by "F." Potassium concentration is reported in mg/L.

Potassium efflux from transmittance = 56, 8-15-03, Book 2, Page 17

Time	C1	C2	C3	F1	F2	F3	av control	av dose	st dev cont	st dev dose
0	0.40	0.30	0.25	0.25	0.35	0.30	0.32	0.30	0.466	0.214
20	0.35	0.10	0.40	3.40	3.20	2.80	0.28	3.13	0.684	1.178
40	0.20	0.35	0.25	4.25	4.20	4.55	0.27	4.33	1.666	0.815
60	0.25	0.30	0.20	4.70	4.60	4.90	0.25	4.73	0.555	0.79

Initial 0.35

Potassium efflux from transmittance = 56, 8-20-03, Book 2, Page 18

Time	C1	C2	C3	F1	F2	F3	av control	av dose	st dev cont	st dev dose
0	0.35	0.30	0.25	0.45	0.35	0.75	0.30	0.52	0.466	0.214
20	0.50	0.25	0.30	1.35	1.45	1.80	0.35	1.53	0.684	1.178
40	0.40	0.45	0.45	3.70	3.50	3.80	0.43	3.67	1.666	0.815
60	0.40	0.40	0.25	4.25	4.50	3.95	0.35	4.23	0.555	0.79

Initial 0.40

Potassium efflux from transmittance = 78, 8-22-03, Book 2, Page 20

Time	C1	C2	C3	F1	F2	F3	av control	av dose	st dev cont	st dev dose
0	0.20	0.15	0.15	0.40	2.95	1.70	0.17	1.68	0.466	0.214
20	0.30	0.25	0.15	4.75	4.25	4.55	0.23	4.52	0.684	1.178
40	0.30	0.40	0.30	5.05	5.10	4.65	0.33	4.93	1.666	0.815
60	0.20	0.30	0.40	4.55	4.70	5.60	0.30	4.95	0.555	0.79

Initial 0.15

Potassium efflux from transmittance = 70, 8-23-03, Book 2, Page 21

Time	C1	C2	C3	F1	F2	F3	av control	av dose	st dev cont	st dev dose
0	0.55	0.55	0.50	0.45	1.25	0.70	0.53	0.80	0.466	0.214
20	0.60	0.80	0.45	4.75	5.55	5.10	0.62	5.13	0.684	1.178
40	0.50	0.60	0.30	6.05	6.65	7.45	0.47	6.72	1.666	0.815
60	0.65	0.45	0.35	6.60	6.40	9.25	0.48	7.42	0.555	0.79

Initial 0.90

Potassium efflux from transmittance = 55, 9-25-03, Book 2, Page 33

Time	C1	C2	C3	F1	F2	F3	av control	av dose	st dev cont	st dev dose
0	1.10	1.90	0.70	0.85	1.60	1.85	1.23	1.43	0.466	0.214
20	0.35	0.90	0.75	4.50	4.55	4.75	0.67	4.60	0.684	1.178
40	0.85	1.15	1.25	10.15	8.60	8.40	1.08	9.05	1.666	0.815
60	0.65	0.55	0.75	10.10	10.55	10.85	0.65	10.50	0.555	0.79

Potassium efflux from transmittance = 76, 12-10-03, Book 2, Page 56

Time	C1	C2	C3	F1	F2	F3	av control	av dose	st dev cont	st dev dose
0	0.17	0.47	1.16	0.27	0.74	0.79	0.60	0.60	0.466	0.214
20	0.52	0.93	1.22	1.43	1.55	1.55	0.89	1.51	0.684	1.178
40	0.76	0.64	0.35	2.42	2.07	2.36	0.58	2.29	1.666	0.815
60	1.28	0.93	1.10	3.18	2.48	2.60	1.10	2.75	0.555	0.79

Init 0.33

Potassium efflux from transmittance = 86, 12-12-03, Book 2, Page 57

Time	C1	C2	C3	F1	F2	F3	av control	av dose	st dev cont	st dev dose
0	3.24	0.50	0.68	0.76	0.37	0.65	1.47	0.59	0.466	0.214
20	3.58	1.38	1.96	4.48	4.15	4.42	2.31	4.35	0.684	1.178
40	1.61	1.08	1.61	5.04	5.92	5.31	1.43	5.42	1.666	0.815
60	1.03	0.85	0.91	6.09	6.37	5.98	0.93	6.15	0.555	0.79

Initial 0.70

Potassium efflux from transmittance = 89, 1-6-04, Book 2, Page 60

Time	C1	C2	C3	F1	F2	F3	av control	av dose	st dev cont	st dev dose
0	0.77	0.77	0.82	0.97	0.61	0.72	0.79	0.77	0.466	0.214
20	0.68	1.07	1.75	5.36	5.36	4.95	1.17	5.22	0.684	1.178
40	0.73	1.02	0.77	6.23	6.58	6.33	0.84	6.38	1.666	0.815
60	0.73	0.87	0.68	7.14	7.55	10.46	0.76	8.39	0.555	0.79

Initial 1.04

B.3 Data for efflux from alginate-immobilized *P. aeruginosa*

The following data were obtained by acidifying the samples with nitric acid, diluting, and analyzing on the AA. Data shown has been corrected for dilution and for machine drift during analysis. Control flasks are indicated by "C", and dosed flasks by "F." Potassium concentration is reported in mg/L. Days after immobilization are given (e.g., day 0 = immediately after immobilization and day 1 = 24 hours after immobilization). Cell counts were performed by serial dilutions and plating. Volumes shown with cell counts indicate total volume of culture after resuspension. Total cells = average cell count x resuspension volume. This total was divided equally into six flasks to give cells per flask.

Efflux 2, Day 1
2-4-04, Book 2, Page 69
Presented in Chapter 3, Table 1 and Figure 6

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
0	0.56	0.62	0.16	1.16	1.72	1.61	0.45	1.50	0.251	0.296
20	0.17	1.29	0.88	1.50	2.22	2.34	0.78	2.02	0.569	0.455
40	0.51	0.34	0.77	1.33	2.39	3.29	0.54	2.34	0.219	0.979
60	0.51	1.24	0.83	2.73	2.22	3.84	0.86	2.93	0.366	0.829
120	0.84	0.62	0.10	2.39	1.83	3.45	0.52	2.56	0.380	0.823

Cell Counts	2.46E+10	2.37E+10	2.87E+10 cells per mL
Average	2.57E+10	cells per mL	38 ml total
Total cells	9.75E+11	total cells	

Cells per flask 1.63E+11

Efflux 3, Day 5
1-27-04, Book 2, Page 67
Presented in Chapter 3, Figure 7

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
0	0.63	0.43	1.34	0.66	0.24	2.19	0.80	1.03	0.4764	1.0258
20	0.12	1.70	0.33	0.62	0.24	2.04	0.72	0.97	0.8602	0.9497
40	0.84	0.12	2.25	0.62	1.99	2.79	1.07	1.80	1.0828	1.1001
60	0.22	0.17	0.37	2.01	0.84	2.19	0.26	1.68	0.1048	0.7328
120	2.26	0.43	1.14	2.30	1.49	3.94	1.28	2.58	0.9258	1.2485

Data not included in thesis:

1-26-04, Book 2, Page 66

Efflux 1, Day 0

Time	C1	C2	C3	F1	F2	F3	PD	Av Cont	Av Dose	st dev cont	st dev dose
0	4.55	1.85	5.69	2.11	1.09	1.83	1.16	4.03	1.68	1.9730	0.5289
20	2.39	3.41	8.80	3.60	3.52	1.63	2.85	4.87	2.92	3.4451	1.1154
40	2.19	5.10	2.52	2.86	2.44	2.03	4.06	3.27	2.44	1.5913	0.4104
60	3.41	4.42	4.14	3.87	1.90	2.17	3.72	3.99	2.65	0.5234	1.0676
120	2.32	1.85	2.25	3.80	4.80	3.32	3.79	2.14	3.97	0.2537	0.7583

Cell Counts	2.12E+10	2.41E+10	2.01E+10 cells per mL
Average	2.18E+10	cells per mL	18.8 ml total
Total cells	4.10E+11	total cells	

Cells per flask 6.83E+10

Efflux 2, Day 4

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
0	0.91	0.96	0.55	0.08	0.79	0.48	0.81	0.45	0.2220	0.3526
20	0.50	0.65	0.08	0.92	0.89	1.04	0.41	0.95	0.2951	0.0815
40	1.57	0.75	0.19	2.01	0.73	0.89	0.84	1.21	0.6959	0.6961
60	0.40	0.55	0.66	0.86	0.99	0.79	0.53	0.88	0.1297	0.1030
120	0.50	0.70	0.97	1.91	0.99	4.76	0.72	2.55	0.2348	1.9694

1-27-04, Book 2, Page 67

Efflux 1, Day 0

Time	C1	C2	C3	F1	F2	F3	PD	Av Cont	Av Dose	st dev cont	st dev dose
0	5.80	5.44	5.45	3.85	4.85	5.32	1.85	5.56	4.68	0.2027	0.7499
20	7.30	4.37	4.19	7.65	5.45	7.59	2.59	5.28	6.90	1.7460	1.2511
40	8.51	3.87	3.65	7.92	5.39	6.52	4.45	5.34	6.61	2.7451	1.2685
60	6.87	5.30	3.72	5.65	6.05	7.65	1.39	5.29	6.45	1.5748	1.0587
120	10.01	5.73	5.79	7.85	9.99	8.59	1.59	7.17	8.81	2.4572	1.0843

Cell Counts 9.40E+10 1.07E+11 8.90E+10 cells per mL
Average 9.67E+10 cells per mL 38 ml total
Total cells 3.67E+12 total cells

Cells per flask 6.12E+11

Efflux 2, Day 3

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
0	0.84	0.43	1.01	0.11	0.22	1.91	0.76	0.75	0.2987	1.0066
20	1.19	0.07	0.16	1.11	1.35	2.57	0.47	1.68	0.6239	0.7842
40	0.28	0.33	0.46	1.56	1.45	3.08	0.35	2.03	0.0955	0.9121
60	0.38	0.07	0.31	1.96	1.30	1.86	0.25	1.70	0.1609	0.3576
120	0.38	0.22	1.01	1.81	1.30	2.52	0.54	1.88	0.4167	0.6148

2-4-04, Book 2, Page 69

Efflux 1, Day 0

Time	C1	C2	C3	F1	F2	F3	PD	Av Cont	Av Dose	st dev cont	st dev dose
0	0.13	0.44	1.45	0.57	1.12	2.81	1.25	0.67	1.50	0.694	1.168
20	0.18	1.49	0.79	1.18	3.71	2.92	13.25	0.82	2.60	0.658	1.295
40	1.34	0.07	2.34	2.18	6.57	3.93	25.5	1.25	4.23	1.138	2.213
60	2.60	0.39	1.62	3.79	5.39	3.15	12.75	1.54	4.11	1.108	1.158
120	0.65	0.86	0.23	2.84	3.43	3.71	14.25	0.58	3.33	0.321	0.441

Cell Counts 2.46E+10 2.37E+10 2.87E+10 cells per mL
Average 2.57E+10 cells per mL 15 ml total
Total cells 3.85E+11 total cells

Cells per flask 6.42E+10

Efflux 2, Day 2

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
0	0.56	0.62	0.16	1.16	1.72	1.61	0.45	1.50	0.251	0.296
20	0.17	1.29	0.88	1.50	2.22	2.34	0.78	2.02	0.569	0.455
40	0.51	0.34	0.77	1.33	2.39	3.29	0.54	2.34	0.219	0.979
60	0.51	1.24	0.83	2.73	2.22	3.84	0.86	2.93	0.366	0.829
120	0.84	0.62	0.10	2.39	1.83	3.45	0.52	2.56	0.380	0.823

Efflux 3, Day 5

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
0	0.49	0.38	1.33	1.16	1.05	0.33	0.73	0.85	0.519	0.455
20	1.27	0.72	0.88	1.39	1.67	1.89	0.96	1.65	0.287	0.252
40	1.27	0.21	0.66	0.83	2.84	1.22	0.72	1.63	0.533	1.066
60	0.72	0.16	0.55	1.44	5.19	2.06	0.47	2.90	0.287	2.007
120	2.39	0.94	--	1.22	4.24	1.67	1.67	2.37	--	1.628

B.4 Data for thermallly reversible gel-immobilized *P. aeruginosa*

The following data were obtained by acidifying the samples with nitric acid, diluting, and analyzing on the AA. Data shown has been corrected for dilution and for machine drift during analysis. Control flasks are indicated by "C", and dosed flasks by "F." Potassium concentration is reported in mg/L. Days after immobilization are given (e.g., day 0 = immediately after immobilization and day 1 = 24 hours after immobilization). Cell counts were performed by serial dilutions and plating. Volumes shown with cell counts indicate total volume of culture after resuspension. Total cells = average cell count x resuspension volume. This total was divided equally into six flasks to give cells per flask.

Efflux 1, Day One, 4-19-04, Book 2, Page 86
Presented in Chapter 3, Table 1 and Figure 8

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
-5	2.27	2.80	2.28	2.45	3.50	2.50	2.45	2.81	0.3026	0.5909
0	2.21	2.16	2.34	2.95	2.55	3.22	2.24	2.91	0.0914	0.3355
20	2.05	2.59	2.06	9.34	5.27	5.83	2.23	6.81	0.3052	2.2044
40	1.74	2.53	1.73	5.67	3.88	4.66	2.00	4.74	0.4628	0.8964
60	1.36	1.52	1.62	5.12	4.05	4.94	1.50	4.70	0.1282	0.5711
80	0.94	3.01	1.34	5.73	4.38	4.94	1.76	5.02	1.1003	0.6750
100	0.99	1.10	2.17	5.01	5.77	4.88	1.42	5.22	0.6536	0.4821
120	0.78	2.53	0.84	5.95	4.44	5.11	1.38	5.17	0.9963	0.7567

Cell Counts	3.19E+09	3.54E+09	3.42E+09	cells per mL
Average	3.38E+09	cells per mL		6.0 ml total
Total cells	2.03E+10	total cells		
Cells per flask	3.38E+09			

5-24-04, Book 2, Page 92
Presented in Chapter 3, Figure 9
Efflux 1, Day 3

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
-5	6.83	7.31	6.66	10.51	6.04	5.16	6.93	7.23	0.3331	2.8708
0	7.67	7.15	7.10	11.55	9.95	7.36	7.31	9.62	0.3177	2.1163
20	5.31	5.99	6.06	11.94	9.62	8.49	5.78	10.02	0.4163	1.7581
40	4.15	6.31	6.11	9.19	9.57	8.33	5.52	9.03	1.1943	0.6368
60	4.46	6.06	5.29	8.21	9.46	8.43	5.27	8.70	0.7978	0.6639
80	4.46	6.33	5.18	7.88	9.29	8.86	5.33	8.68	0.9435	0.7227
100	2.88	5.40	4.85	8.43	9.13	8.06	4.38	8.54	1.3222	0.5463
120	2.25	5.12	4.30	8.43	9.46	8.76	3.89	8.88	1.4786	0.5249

Cell Counts	4.60E+10	4.00E+10	4.30E+10	cells per mL
Average	4.30E+10	cells per mL		
16 ml total divided into 8 batches				
Cells per flask	8.60E+10			

Data not included in thesis:

3-31-04, Book 2, Page 82

Efflux 1, Day 1

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
0	0.62	1.19	1.59	2.37	2.20	1.98	1.13	2.18	0.4905	0.1945
20	0.88	0.46	4.87	3.42	2.63	3.77	2.07	3.28	2.4348	0.5847
40	0.30	0.46	1.87	3.31	2.85	2.90	0.88	3.02	0.8628	0.2530
60	0.20	0.30	1.37	3.31	3.07	2.96	0.62	3.11	0.6478	0.1815
80	0.20	0.20	1.48	3.37	3.18	3.67	0.63	3.40	0.7400	0.2464
100	0.41	0.25	0.76	3.81	3.34	3.56	0.47	3.57	0.2598	0.2372
120	0.51	0.25	0.48	4.59	3.29	3.56	0.41	3.81	0.1423	0.6890

Cell Counts 6.90E+10 5.30E+10 6.10E+10 cells per mL
Average 6.10E+10 cells per mL
Total cells 2.32E+12 total cells
11.5 ml total, 8 batches
Cells per flask 3.33E+12

Efflux 2, Day 3

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
-5	0.08	0.55	0.12	0.35	0.17	0.23	0.25	0.25	0.2613	0.0907
0	0.08	0.97	0.07	1.13	0.85	1.02	0.37	1.00	0.5186	0.1439
20	0.40	0.97	0.57	1.30	1.02	1.64	0.65	1.32	0.2952	0.3111
40	0.34	1.71	0.40	1.02	1.86	1.02	0.82	1.30	0.7694	0.4880
60	0.08	0.55	0.07	1.58	1.19	1.13	0.23	1.30	0.2768	0.2469
80	0.13	0.97	0.12	1.13	1.58	1.24	0.41	1.32	0.4872	0.2338
100	0.19	0.61	0.63	1.30	1.30	1.58	0.47	1.39	0.2484	0.1623
120	0.19	0.08	0.29	1.36	1.47	1.41	0.19	1.41	0.1043	0.0553

Efflux 3, Day 7

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
-5	0.34	0.51	0.94	0.33	0.36	0.13	0.60	0.28	0.3095	0.1239
0	1.53	0.45	1.66	0.22	1.39	1.16	1.21	0.93	0.6612	0.6204
20	2.03	1.19	0.55	0.55	0.82	0.53	1.26	0.64	0.7433	0.1600
40	0.40	0.28	0.28	2.60	1.96	2.42	0.32	2.33	0.0671	0.3271
60	0.34	0.45	1.27	0.61	0.42	0.42	0.69	0.48	0.5085	0.1084
80	0.56	0.40	0.77	0.61	0.76	0.65	0.58	0.67	0.1893	0.0804
100	1.36	0.40	0.17	0.50	0.36	0.53	0.64	0.46	0.6314	0.0902
120	0.23	0.11	0.06	0.50	0.65	0.65	0.13	0.60	0.0868	0.0874

4-18-04, Book 2, Page 84 and 86

Efflux 2, Day 3

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
-5	5.43	5.64	3.36	3.30	8.43	8.93	4.81	6.88	1.2600	3.1182
0	8.14	5.47	3.26	3.39	8.14	8.71	5.62	6.75	2.4401	2.9237
20	6.64	5.51	2.80	3.92	8.64	10.64	4.98	7.74	1.9750	3.4521
40	6.05	5.26	2.92	4.11	8.71	8.57	4.75	7.13	1.6293	2.6195
60	5.22	5.22	2.83	4.08	8.57	12.00	4.42	8.22	1.3820	3.9738
80	4.60	7.39	2.58	5.23	9.43	9.93	4.85	8.20	2.4155	2.5791
100	4.18	4.76	2.64	4.45	8.71	15.14	3.86	9.44	1.0968	5.3822
120	4.60	4.30	2.42	4.20	8.79	9.14	3.77	7.38	1.1806	2.7557

Efflux 3, Day 5

Time	C1	C2	C3	F1	F2	F3	Av Cont	Av Dose	st dev cont	st dev dose
-5	5.93	7.50	4.08	5.15	8.33	9.63	5.84	7.70	1.7111	2.3032
0	6.64	7.21	5.44	5.72	8.83	9.73	6.43	8.09	0.9065	2.1016
20	6.93	7.79	3.58	7.08	10.53	12.23	6.10	9.95	2.2216	2.6233
40	5.29	7.71	4.08	7.30	10.53	11.73	5.69	9.85	1.8505	2.2936
60	5.14	7.71	3.08	6.87	10.53	13.23	5.31	10.21	2.3211	3.1935
80	5.21	5.71	2.87	7.37	10.33	13.23	4.60	10.31	1.5202	2.9315
100	4.50	5.29	2.94	6.94	11.13	11.33	4.24	9.80	1.1947	2.4797
120	3.29	4.21	1.37	6.22	10.53	11.53	2.96	9.43	1.4520	2.8193