

Evaluating Bacterial Immobilization in Alginate for Use in a Biosensor

Kaoru Ikuma

Department of Civil and Environmental Engineering

Biosensors are devices used to monitor samples by utilizing biological elements such as bacterial cells. One application of a biosensor is to monitor the stability of wastewater treatment plants. The desirable biosensor in our ongoing research will be in the form of whole bacterial cells that respond to toxins in the water samples, and the response is transmitted as a fluorescent signal that can be detected. In order to utilize bacteria in the biosensor, the bacteria must be immobilized in a matrix that can sustain the cell so that the biosensor can be reused over long time periods and so that the bacteria are not flushed from the sensor. Several polymers have been evaluated as the immobilization matrix for the bacterium of choice in our biosensor - *Pseudomonas aeruginosa*. My research is focused on one of the polymers, alginate, which forms solid beads when put into CaCl_2 . The objectives of my research are to determine how best to immobilize the bacterial cells in the alginate beads, and to evaluate the viability and functionality of the immobilized cells over time (up to one month).

Experiments have been completed to show that the cells can be immobilized in the alginate using our protocol. However, we have determined that the media used to grow the bacterial cells dissolved the alginate beads within an hour. It was necessary to find an appropriate media that can sustain the bacterial cells and at the same time, keep the alginate in the solid form. Different salt concentrations were used to see what caused the beads to dissolve (Figure 1). The alginate beads were stable at both the lower and higher salt concentrations but very unstable in between. When the alginate beads were put into different media with varying salt concentrations (including other nutritional factors such as Ca^{2+} , Mg^{2+} , trace metals, and Fe^{+3}), the results indicated that it was not only the overall salt concentration that determined alginate bead stability, but also the salt composition. Results to date show that the alginate beads are most stable in the media containing very low levels of salt. To see if the bacterial cells can be sustained in this dilute media, a microscopic staining procedure that differentiates between live and dead cells was conducted for the cells that were immobilized in alginate beads. As shown in Figure 2, the cells were well sustained. In addition to the chemical limitations of the long-term immobilization, possible physical constraints were also evaluated. Alginate beads were put in media with aeration through a diffuser. After a week, the alginate beads had deteriorated. Although aerating the immobilized cells is essential to sustaining the cells long term, the air bubbles could cause physical damage to the alginate beads. My ongoing work for this research project is to evaluate the biological aspects of the immobilization. Long-term experiments will be conducted, and the viability of the bacterial cells will be evaluated by using live/dead staining and measuring oxygen uptake rates of the cells. We also hope to test the functionality of the cells to toxins using a fluorescence detection system being developed by others in the college.



Figure 1. Alginate beads in solutions with various salt concentrations.

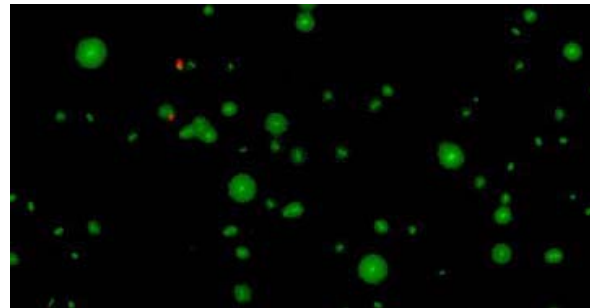


Figure 2. Live/Dead stain shows that dilute media does not adversely impact cell viability. Green cells are alive, whereas red cells are dead. Cells were dissolved from the alginate bead prior to staining to enhance picture resolution.