SEN 01 Investigating the complex problem of different environmental conditions that induce the production of Domoic Acid with feedback control system

SEN 01.1 Overview
We have developed an electrochemical sensor for the detection of Domoic Acid (DA) a marine toxin. Also we are investigating the conditions that induce the production of the toxin by the pseudo nitzschia algae. Domoic Acid is a neurotoxin analogous to the excitatory neurotransmitter glutamate. DA has a higher affinity to the glutamate receptor. Binding of DA to the receptor causes prolonged receptor activation and constant influx of ions to the neurons. This leads to the increase in intercellular calcium concentration, resulting in sustained activation of calcium sensitive enzymes, eventually leading to energy depletion, neural swelling and cell death. The affected neurons are mainly located in hippocampus, explaining the most striking effect of DA poisoning which is short-term memory loss.

DA is produced by different species of the diatom pseudo nitzschia. The toxin can enter the marine food chain by shellfish such as mussels when they uptake and filter their food out of water. This water can contain both diatoms themselves and the toxin, which is released to the water column. The toxin accumulates in the digestive gland and certain tissues of shellfish and when the shellfish is consumed by other animals such as sea birds and sea lions which lead to Amnestic shellfish poisoning (ASP). The symptoms of the disease are nausea, vomiting, head bobbing and confusion. In severe cases ASP will lead to death both in animals and in human.

Domoic Acid is a secondary metabolite it has no primary function or any function in the defense mechanism. 11 species of pseudo nitzschia produces DA. Not all the toxic species produce the same amount of toxin and although all of the 11 different species are capable of producing DA many non-toxic blooms of these species have also occurred around the world suggesting that the toxicity is inducible by the environment. The conditions that trigger the production of DA by the algae can be divided in two broad categories of nutritional and non-nutritional conditions. Examples of non-nutritional conditions are geographical location of the bloom, biological factors such as existence of certain species of extracellular bacteria flora and physical factors such as light intensity and temperature of water. Our group has been interested in resolving two issues regarding pseudo nitzschia. First we would like to be able to detect DA at low concentrations and from low sample volumes. And second we are interested in knowing what nutritional conditions trigger the production of Domoic Acid.

We have developed an electrochemical sensor that is capable of detecting DA from 2 μl of sample up to 5 pg/ml. This sensitivity is comparable to ELISA which has a detection limit of 10 pg/ml but ELISA needs a minimum volume of 50 μl of sample for detection. The advantage in using the electrochemical sensor other than better sensitivity is that we can get the DA form lower number of cells which will give us more in dept information about the behavior of the toxin producing algae cells. More information on the results of this sensor will be given out in the accomplishment section. And the second part of work which is still ongoing is covered in the system description and experiments section.

SEN 01.2 Approach
There are multiple nutrients necessary for the growth of pseudo nitzschia. The imbalance of the concentration of each of these nutrients can be a trigger factor in the production of the toxin. In order to study this phenomena one have to look into the combination of various possible concentrations of a set of nutrients which creates a large testing parameter space. For example if we pick to study the effect of only 6 nutrients and each at 10 different levels of concentration we will have a parameter space of 106. As can be seen by the simple example identifying the combinations of multiple nutrients is a major challenge.

When handling complex biological problems, there are two different approaches that can be taken: a top down approach and a bottom up approach. In the bottom up approach the mechanisms through which functional properties arise in the interaction of known components is studied. In our case the goal is to understand everything about the DA production mechanism such as the biosynthetic pathway, the precursors of DA synthesis, the involvement of different enzymes and their kinetics to understand the roles of various environmental stresses which are all very challenging tasks.

In the top down approach the goal is to discover the conditions that induce the toxin production using an iterative cycle. The output of the cell is monitored with the change in the nutrient concentrations as the input to the cell. And the input is changed until the cell is producing the desired output.

In our study we would like to use the top down approach and in order to do that we are going to use a closed loop optimization scheme instead of the trial and error method which requires testing a very large number of all the possible combinations. With the help of the search algorithm we effectively look for combinations that manipulate the algae cell toward triggering the DA production.
SEN 01.3 System(s) Description and/or Experiments

There are multiple studies done on pseudo nitzschia to understand the effect of different nutritional factors on DA. Some of the observations from these studies are as follows:

- The Production of DA is generally lower in the exponential growth phase and it increases as the cells go to the stationary phase.
- Limitation of silicon (Si) cause increased DA production; Si is required in the cell wall of the diatom also in other metabolic processes.
- Limitation of phosphorous (P) also triggers production of DA; P has a key role in the structure of the membrane of the cell, in production of lipids and also in the cell bioenergetics.
- Cells grown under low Iron (Fe) also produce excessive amount of DA in the exponential phase, the high production of DA results in excretion of DA in the culture solution and causes an increase in the uptake of Fe. This phenomenon suggests that DA works as a chelator for metal ions for the algae cell.
- Cell grown under both limitation and excess of copper (Cu) show increased amount of DA production.

In all of these studies all the conditions have been kept constant and only the condition under investigation has been changed. We are interested in studying all of these conditions at the same time.

Search algorithm

The search algorithm is the essential part to solving the large scale optimization problem linking the output to the next inputs to the system. Two different types of stochastic search algorithms the gur game and the differential evolution (DE) have been successfully used in our lab with different cell systems. For this study DE is going to be used. DE requires very few parameters to be set and it is a very simple and reliable search method.

Culture media

Culture media that is often used to grow pseudo nitzschia is F/2 medium. F/2 medium consists of five main components: nitrate, phosphate, silicate, metal solution and vitamin solution. The metal solution and vitamin solution both consist of other multiple components with the total of 13 components. There are various studies that have been done in understanding the effects of these nutritional factors on production of DA but almost all of them have taken only one or at most two of the parameters into account. We would like to utilize the feedback search algorithm to look at all of the ingredients at the same time and study the effects and changes made by the nutrients on production of DA.

In order to keep the experiments simple and the uncertainties low we are going to start this project at macro scale and using 75 ml culture flasks to culture the algae in. And later we can switch to using the micro chambers for growing the cells in. We will start the iterations by culturing cells under different conditions and we would measure the DA content of the cells after 7 days of culture using Elisa kits (later the electrochemical sensor) the amount of DA produced by the condition will be an input to the algorithm and the algorithm will give us new conditions as the output to test. Then we will culture cell under the new conditions and this will continue until we have found different conditions that cause production of DA. After this point we would like to reverse the trend and see if we can start with toxic producing cultures and see if we can reduce the toxicity or eliminate it using the same technique.

SEN 01.4 Accomplishments

The main accomplishment of this year was with the electrochemical sensor part for detection of DA. Detection can be done using only 2 μl of sample and the sensitivity of 5 pg/ml in buffer Figure 2 and Figure 3. The same sensor was tested using cell lysis solution spiked with different concentration of DA. And DA can be detected up to 50 pg/ml (Figure 4). Using only 2 μl of sample is very important especially when growing the cells in the micro chips developed by Dr Yu-Chong Tai group at Caltech. The sample volume is only 0.5 μl. For the detection we used gold electrodes prepared with the mixed self assembled monolayer of biotin thiol and 11-Mercaptoundecanoic acid (OH-thiol).
DA is immobilized on the surface with utilization of neutroavidin-DA conjugates. For the competition step unknown concentration of DA and the HRP conjugated antibody is added to the sensor surface and the DA added to the surface will inhibit the binding of the Antibody to the DA on the surface. After the competition step the substrate is added to the sensor surface and the electric current is read by the electrochemical reader (Figure 5).

Different dynamic ranges and sensitivity limits can be achieved by changing the concentration of immobilized DA on surface and the concentration of antibody used. The general rule for the competitive detection is that for detecting lower antigen concentration we have to sacrifice the width of the dynamic range of the sensor. Our results as shown in Figure 2 and Figure 3 are from two different concentration combinations of DA and antibody used on the surface.

**SEN 01.5 Future Directions**
The future work is implementing the feedback system control on the algae system first at the macro scale and after that we would like to try to use the same concept in the micro scale using the microchip developed by Caltech and the electrochemical sensor developed by us for the same purpose.