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Concept of using nanosensors for water quality monitoring in aquaculture

Introduction

The significance of aquaculture in the context of global food security and the socioeconomic development of a country is now fully appreciated worldwide. The demand for nutritive food production continues to increase and will be fulfilled in a major part by aquaculture. Aquaculture farmers resort to intensive farming and use excessive feed than required to cater to the growing demand and attain larger shrimp sizes to earn higher rates in the export market. It has been reported by many researchers, that about 70-80% of nitrogen added as shrimp feed is not retained as shrimp biomass.

Nitrogen components play a key role in aquaculture, owing to their dual role as nutrients and metabolites (ammonia and nitrite). These metabolite levels increase in *L.vannamei* culture ponds due to higher stocking density, particularly in zero water exchange which is preferred by farmers mainly as a preventive measure to combat bacterial/viral diseases. These metabolites increase exponentially over time in grow-out ponds with the increase in biomass resulting in reduced animal growth and in extreme cases causing mortality. Early detection of metabolites and its mitigation reduces the stress of the animals and helps reduce the incidence of diseases.

The current methodology to quantify metabolites is to bring the samples to a lab for analysis and there is no process or technology to reliably analyse the metabolites at the farm itself. Lab facilities are not accessible to all the farmers at the required time and in a number of occasions, crop was lost due to failure to detect and mitigate metabolites at the right time. Hence, monitoring of metabolites on a regular basis using a simple process will empower the farmers to swiftly take necessary mitigation actions. Sensors will overcome the current short comings and effectively meet the requirements of reliability and ease of use. It also has the potential to provide real time data capture and logging facility and send alarms on reaching specified limits to enable immediate action.

Why nanosensors?

Nanotechnology is a fast emerging field with unlimited potential as nanoparticles have high surface volume ratio and large reaction surface. This unique property is due to the small particle size of less than 100 nm. Nanoparticles have an edge over conventional materials due to its enhanced structural, magnetic, electrical and optical properties. Nanotechnology based sensing platforms offer high sensitivity compared to existing platforms with a shorter response time. It enables simultaneous multivariable detections and will miniaturize the sensors to unprecedented levels. They can detect single cells or even atoms, making them far more sensitive than counterparts with larger components. Nanosensors work by changes in spectral absorbance, photoluminescence, chemiluminescence and oxidation-reduction reaction phenomena induced by the interaction between nanomaterials and various analytes.

Applications of Nanosensors

Nanosensors have been successfully used in various applications. Tungsten and Tin oxide (SnO_2) based gas sensors help to detect H_2S, NO_2, CO, CO_2, NO and O_3 even at very low concentrations. Gold nanoparticle based fluorescent and chemical sensor help to detect Hg (II) and organophosphate pesticides respectively in aqueous solution. Single wall carbon nano tube (SWCNT) sensor has been used as optical fiber sensor and paper sensor for detection of toluene and toxin in water respectively. There is also an increased interest in using nanosensors made up of magnetic nanoparticles for detecting location of oil reserves. Semiconducting silicon nanowires configured as field-effect transistors are used for the electrical detection of viruses in solutions.

It is apparent from the literature that nanosensors have been successfully used in the detection of gases, pollutants, contaminants and pathogens in various fields. However, in aquaculture, the potential of nanosensors have not been exploited so far. There are a number of opportunities to deploy nanosensors in aquaculture for the early detection of the metabolites (ammonia, nitrite, sulphide *etc*.) and pathogens (bacteria and viruses) in order to maintain the healthy pond environment. A novel initiative has been made to use nanosensors for the early detection of metabolites to support successful and sustainable aquaculture.

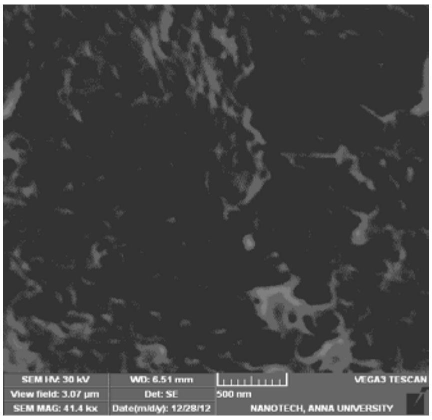
Fabrication of prototype nanosensors

Conventional solid state sensors for NO_2 and NH_3 typically operate at temperatures over 400^oC, and a use of polymers provide only limited sensitivity. Whereas, sensors made from single-wall nanotubes have high sensitivity and a fast response time at room temperature. Moreover, experimental and theoretical studies have found that these nano-meter sized CNTs have novel electronic properties, which can be either metallic or semiconducting depending on their radius and chirality.

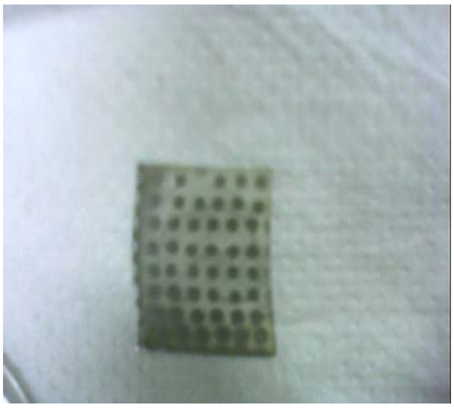
To fabricate nanosensors, SWCNT having 4 nm diameter was dispersed in different solvents (Acetone, chloroform and N,N-Dimethylformamide) and DMF was chosen as the best solvent as it exhibited better dispersion as compared to others. Before coating SWCNT, glass slides were pre-treated with 3-aminopropyltriethoxysilane (APTES) to overcome the problem of SWCNT detachment from the glass slide. During pre treatment, the glass slides were washed in water and acetone and then immersed in 2% APTES solution for 2 hours. APTES treated slides were then dried in air at room temperature.

To coat SWCNT on pre-treated glass slide, 250 µl of the SWCNT suspension was drop casted and spin coated on pre-treated glass slide and dried in hot air oven at 100^0C for 10 min. Nearly 80% wastage of sample was observed in spin coating, hence drop casted slides were chosen and the coating cycle was repeated thrice.

Coated glass slides were characterized using Atomic force microscopy and scanning electron microscopy and it confirmed the presence of SWCNT network. Platinum electrode was fixed on CNT slide and was tested for its response against different concentrations of ammonia and nitrite using multi meter.



SEM image of SWCNT network



Platinum electrode fixed on CNT slide

Estimation of nitrogen metabolites using nanosensors

Current conduction in a semiconductor occurs via free electrons and holes, collectively known as charge carriers. Adding impurity atoms to a semiconducting material, known as "doping", greatly increases the number of charge carriers within it and modify the conductance / resistance, which helps to detect and quantify the metabolites.

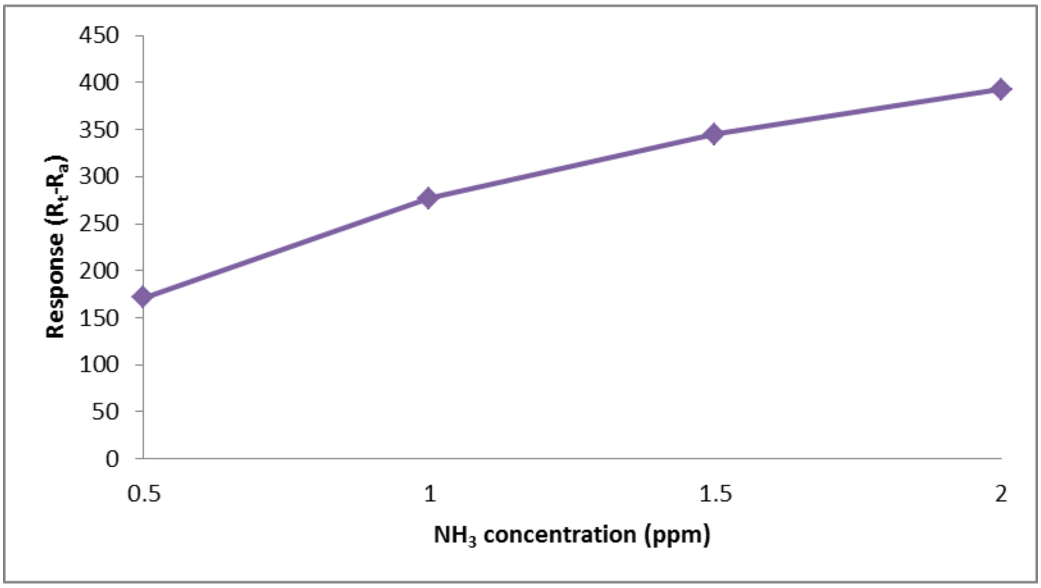
The SWCNT based sensor was tested for its performance at room temperature by spiking NH_3 and NO_2 at different concentrations. The resistance of the sensor increased with addition of NH_3 and decreased with the addition of NO_2. The response was determined as difference (R_t - R_a) between resistance before addition of analyte (R_a) and after addition of analyte (R_t).

Change in resistance of SWCNT with metabolites

Metabolite concentration	R_a	R_t
NH_3 concentration (1.0 ppm)	3.12	280
NO_2 concentration (1.0 ppm)	3.4	0.5

Ra: Resistance of CNT in air Rt: Resistance of CNT in the presence of metabolite

As ammonia is a reducing agent, it transfers electrons to SWCNT and hence, increases resistance and decreases conductivity. Whereas NO_2 is an oxidizing agent and charge transfer (transfer of electrons) takes place from SWCNT to NO_2 resulting in increase in holes, leading to decrease in resistance and increase in conductivity. The sensor response was also determined for increasing concentrations of NH_3 (0.5 ppm to 2.0 ppm) at room temperature. A linear relationship was observed between resistance and metabolite concentration.



Sensor response as a function of NH_3 concentration

Conclusion

The fabricated prototype carbon nanotube sensor works on the principle of oxidation-reduction and measured the ammonia and nitrite concentration through the changes in the electrical resistance. This prototype nanosensor will be developed further and evaluated under different conditions, so that it can be used at field level by the farmers themselves to detect the metabolite concentration in pond water and undertake timely management measures to maintain a healthy pond environment.

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