

3.0 Methods and Materials

3.1 Design of an on-pond chamber to capture volatilised ammonia

Initially the dimensions of both the facultative and maturation ponds were taken to design an on-pond chamber to collect the volatilized ammonia leaving from the surface of the ponds.

The on-pond apparatus consisted of a perspex box (33cm×34cm×48cm) attached to a steel ladder structure (5.66m long for the maturation and 7m long for the facultative). These were attached using four extension nails and 16 nuts and bolts. The box was connected to tubing leading to the gas collection apparatus (Figure 3.7), a series of different tubing was used including 30m of 8mm bore, 1m of 16mm bore and 1m of 5mm bore. Connectors were used to connect the different diameters of the tubing. The tubing was attached to the perspex box using a jubilee clip. Constructing the apparatus required a drill, a pair of pliers, a wrench, a screwdriver, welding equipment, a saw and silica gel to complete the operation. The resulting structure can be seen in Figure 3.1 and Figure 3.2.

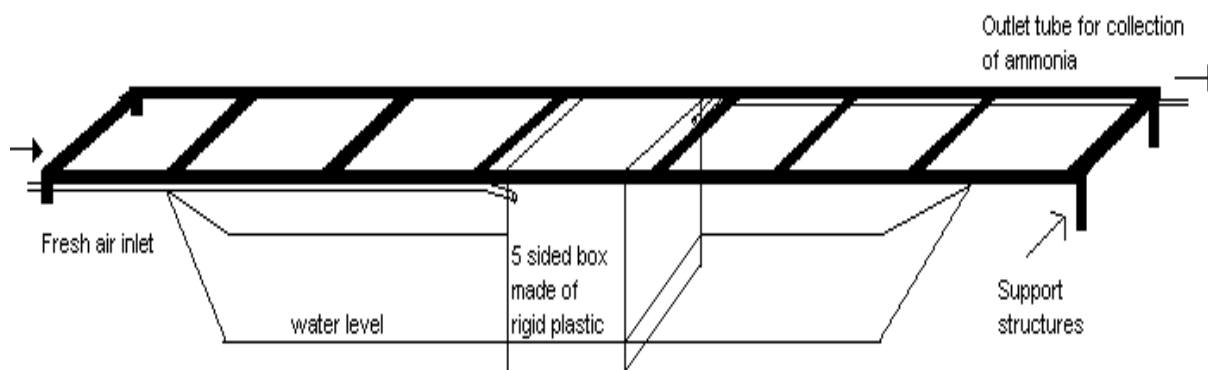


Figure 3.1. Basic diagram of the Mark I apparatus used to collect ammonia emissions from the top of the ponds (not to scale).



Figure 3.2. Photograph of Mark I box design

Higher legs were put on one end of the ladder to account for difference in elevation of the different sides of the ponds. However alterations were needed to be made to the Mark I box structure, as shown Figures 3.3 and Figure 3.4.

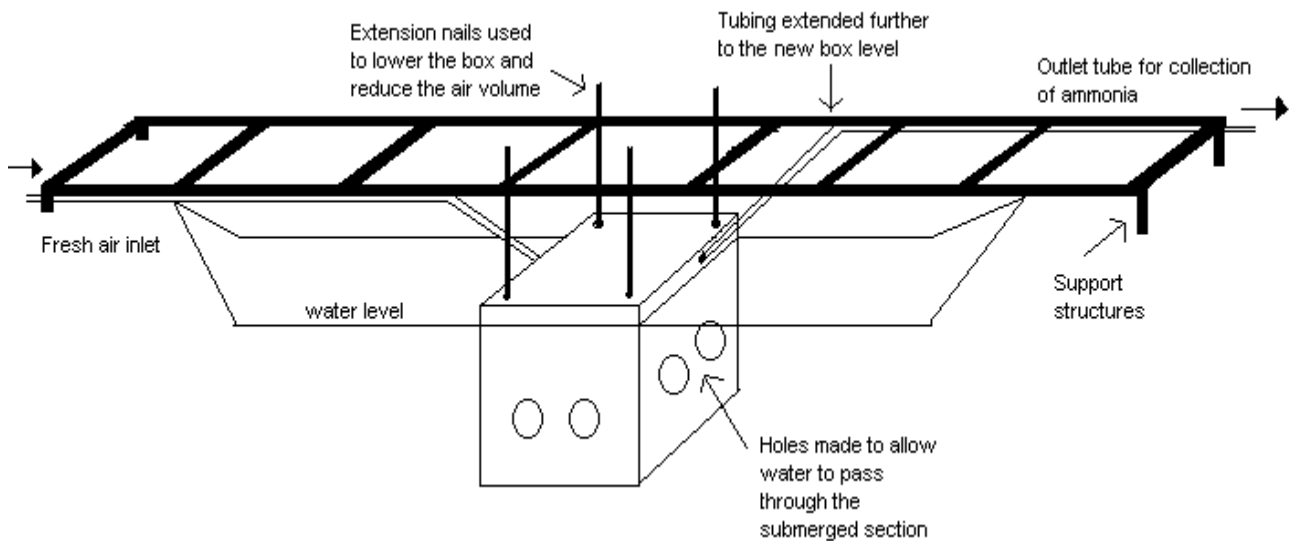


Figure 3.3. Mark II of the box apparatus made for ammonia collection (not to scale).



Figure 3.4. Photograph showing the Mark II amendments made to the Mark I box

Initially the volume of air contained in the box above the water surface was deeper, but due to the build up of condensation inside the box and an unnecessary large volume, this was altered. The inlet hole in the box was connected to tubing of 8mm bore and open to the outside air. The outlet hole was enlarged to allow easier gas transfer and connected to tubing of 16mm bore which led to the collection flasks. Large holes were made in the walls of the box that were now submerged into water, to allow the free flow of the pond water beneath the area being tested, but also allowing versatility in case the box had to be enlarged. The final dimensions of the box above the water were 34cm by 33cm and 15cm deep, giving a surface area of 0.1122m^2 .

After continued surveillance of the box design, it was found that a significant amount of condensation was collecting inside the box. There was no effective way of removing this without further alterations to the box design. These alterations can be seen in Figures 3.5 and 3.6.

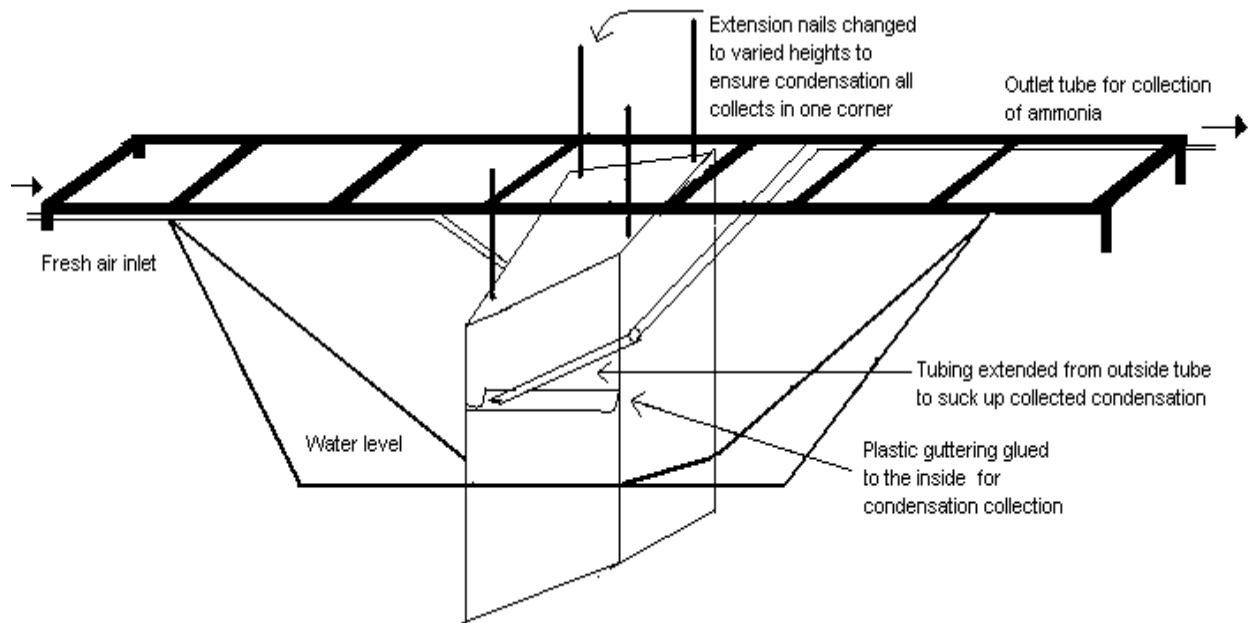


Figure 3.5. Mark III design with a gutter to collect condensation droplets (not to scale).



Figure 3.6. Photograph of the Mark III box design

The extension nails were altered so that one corner was lower than the other three corners. This allowed the condensation to run down the slope now created on the roof of the box and into the lowest corner. Along that edge of the box, a gutter was created by sawing a plastic pipe in half. This was glued onto the interior of the box using araldite rapid action glue. The gutter was taped into place and left to dry and hold for 1 day. Another piece of tubing was inserted into outlet pipe and fastened into place using glue, the other end rested in the lowest point of the guttering. From this

design, the condensation runs into the gutter where it is sucked into the interior tube, along the exterior tubing and into the collection flasks with the boric acid. If there is no condensation being collected the system sucks air through as in the original design.

As can be seen in Figures 3.4 and 3.6, a YSI 6820 sonde probe was attached to the steel ladder to measure the pH, temperature and dissolved oxygen (DO) conditions at the surface of the pond. The sonde probe was attached to a 30m cable line which led into the shed and attached to a YSI 610-dm display logger, which recorded the results every three hours over the sample weeks. The cable was secured to the steel ladder using cable ties. The results were downloaded from the logger to a computer using YSI “EcoWatch” version 3.12.10 software.

3.2 Ammonia collection flask design

The air was sucked through the box by a peristaltic pump into flasks containing boric acid (2%) (20g of boric acid powder per litre of distilled water). There were three conical flasks in series, each containing 100ml of the boric acid solution. In the top of each conical flask was a rubber bung (31mm diameter) with two 5mm bore holes in the middle. Through these holes, glass tubing with a diameter of 5mm was inserted, one leading into the boric acid, the other trapping and transferring any ammonia that did not dissolve in the first flask into the second and third. This was to ensure that any ammonia that escaped from the first flask would be captured in one of the subsequent ones, as seen in Figure 3.7.

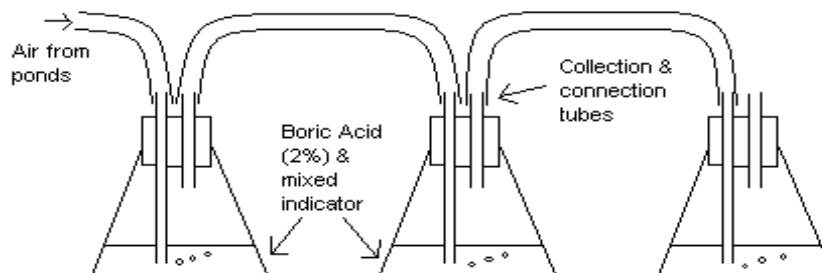


Figure 3.7. Collection flasks trapping ammonia in boric acid.

Mixed indicator solution (consisting of methyl red indicator and methylene blue indicator dissolved in propan-2-ol) was initially added to the 2% boric acid solution to indicate the presence of ammonia; as indicated by a colour change from

purple to green. However, after site and laboratory tests it was established that the indicator did not change colour in the presence of volatilised ammonia, so the mixed indicator was left out of the boric acid solution. This meant that titration could not be used to measure the amount of ammonia present. Instead an ammonia probe was used.

At the end of the one-week sampling period the samples in the three flasks, the influent sample and the effluent sample were transferred into collection bottles and taken to the laboratory for testing.

3.3 Ammonia nitrogen determination using the ion selective electrode

Standards for the expected range of ammonia were prepared. This was done by serial dilution. Ammonium chloride solution was prepared to make three standards: 20mg/l, 10mg/l and 1 mg/l. The 20mg/l standard was made by pipetting 20ml of 100mg/l concentration to a new Nessler tube and adding 80ml of distilled water; for the 10mg/l standard 10ml of the 100mg/l solution and 90 ml of distilled water were used; and for the 1mg/l standard 10ml of the 10mg/l solution and 90 ml of distilled water were mixed together..

The samples of boric acid in the collection flasks were poured into a measuring cylinder to measure the content of boric acid remaining after any evaporation or condensation addition, ensuring the cylinder was effectively washed with distilled water in between samples. Fifty ml of solution was then taken from each collection flask and added to three Nessler tubes; this was also done with 50ml of the pond influent and effluent samples.

Lithium acetate was added to all of these tubes to ensure they had the same salt content. One ml was pipetted into each of the 50ml samples, using a 5ml pipette; 2ml was pipetted into each of the 100ml standards, except for the 10mg/l solution which received 1.8ml as 10 ml was used in the serial dilution. During these procedures the pipette heads were changed between each task to ensure that cross-contamination did not occur.

All of the tubes were placed in a water bath at just above room temperature (usually 25°C) to equilibrate; the temperature measured with a mercury-in-glass thermometer. During this time each Nessler tube was stirred to ensure uniform distribution of its contents, making sure the stirrer was cleaned with distilled water and dried with paper towels before stirring the next tube.

After the solutions were equilibrated the ammonia probe was used to read the ammonia concentration in each tube. The probe was connected to a pH meter and the voltage (mV) reading recorded. The probe was put in each of the standard solutions, stirring gently until the mV reading stabilized. Between each measurement the probe was rinsed with distilled water and dried to ensure no cross-contamination occurred. The standard readings were then used to prepare a calibration graph (Figure A.1 in the Appendix). All four of the samples were then tested in the same fashion.

3.4 Nitrate and nitrite analysis

Samples of the influent and effluent were filtered using Whatman GSC 90mm filter paper, ensuring that the filtration apparatus was thoroughly cleaned between uses. Each water sample was poured into a universal bottle so they were $\frac{3}{4}$ full. These were stored in a freezer to ensure the sample concentrations did not alter prior to analysis. A Dionex DX500 ion analyser was used for fortnightly analyses of the nitrate and nitrite concentrations.

3.5 Total Kjeldahl nitrogen (TKN) analyses

Samples were taken from each of the conical flasks (1, 2 & 3) as well as from the effluent and influent. Fifty ml of each sample was poured into the distillation flasks which were standing in a rack, as well as a blank which was filled with distilled water. Four glass beads were added to each flask as an anti-bumping agent, and one Kjeldahl tablet was also added to each flask.

Gloves and eye protection were used while pipetting 10ml of concentrated sulphuric acid to each of the flasks. The rack of flasks was then fastened to the digestion block for heating. This was attached to the gas scrubbing apparatus and the heater switched on.

The flasks were boiled briskly until the water in the sample had evaporated significantly and the volume was reduced, taking care that no noxious fumes escaped. After completion of digestion the flasks were allowed to cool in the rack.

The samples were then prepared for distillation. Each sample was poured into a 100ml measuring cylinder (excluding the glass beads), and topped up to 100ml with

distilled water, ensuring that no residue was left in the cylinder. The new sample was poured back into the flasks. This was done for all of the samples and the blank.

The reservoirs of distilled water and NaOH attached to the BUCHI still were checked to ensure they were above the minimum level and the connecting hoses checked. An empty duran bottle was placed in the receiving arm of the still and a flask containing distilled water was placed in the distiller. The still was then set to either “cleaning” or “preheating” to ensure there was no other residue in the machine. The samples can be tested when the liquid emptied into the duran bottle (if it did not, then preheating or cleaning was performed again).

The blank flask was inserted into the still using a pair of tongs, and a duran bottle with 50ml of boric acid indicator solution was placed in the receiving arm. The machine was then set to TKN distillation after 100ml of distilled water and 100ml of NaOH had been added to the distillation flask. After the cycle was run, the duran bottle was removed and used for titration. This procedure was done for all of the sample flasks.

The titration was done using 0.01M of sulphuric acid in a burette fastened in a clamp. The duran bottle containing the blank sample was placed underneath the burette tap and one drop was added at a time while being continuously mixed. When the sample had changed from a green to a light purple colour, the tap was closed and the total amount of sulphuric acid used in the titration was recorded. The duran bottle containing the blank was kept as a colour guide until the other samples had been titrated. Titration for the other samples was then performed and the results recorded.

The amounts of sulphuric acid used for the samples were compared with that made for the blank, and the final amounts of nitrogen in the samples were calculated. This was done using the following equation for liquid samples:

$$\text{NH}_3\text{-N (mg/l)} = [(A - B) \times 280] / [\text{volume of sample, ml}]$$

where:

A = volume of acid titrated for sample (ml)

B = volume of acid titrated for blank (ml)