4 Material and Methods

4.1 Subsurface flow constructed wetland

This experiment was performed on the wetlands situated at the rooftop of the department for Civil Engineering at the Polytechnic University of Catalunya in Barcelona, Spain. The laboratory, where samples were measured, is in the lower floors of the same building. A prompt analysis could be made. The wetlands are part of “NEWWET” a research project sponsored by the Spanish ministry for education and science. The project was sponsored for three years and subsequently prolonged for another three years until 2008. The principal aim is to study the clogging process and develop ways to avoid it. Another aim is studying different aspects that are important for the wetland modeling. In this study the reaeration rate is of interest. The wetlands are subsurface flow constructed wetlands. For the time of approximately two years they were fed with primary treated wastewater. After not being used for around one year they were run again with tap water.

4.1.1 Dimensioning and construction

The CWs are small high density polyethylene containers filled with gravel and planted. The inside dimension of the containers are \(1,10 \times 0,69 \times 0,45\) m.

![Figure 4.1: left: inlet of the CW; right: outlet of the CW [Mot06]](image)

By reinforcing the boxes with plastic sheets, the container wall was protected from damage by the pressure increase due to gravel weight and growing roots, was prevented. The inlet to the wetland is a polyethylene tube in form of a T. The length of the tube feeds
Batch operating is positive for the redox potential in the wetland.

### 4.2 Quantification of propane

The method used for the measurement works with a tracer gas. As mentioned before a tracer should neither be produced or consumed in the system. In proceeding studies different tracer gases have been used. Beneath others radioactive tracers, sulfur hexafluoride, propane and ethane were employed. [HWG04] In this study propane is used as tracer because it is neither produced or consumed in the wetland. [TG90]

#### 4.2.1 Propane

Pure propane is expensive. So it was decided to use propane camping gas. The propane camping gas “propano comercial” was bought from repsol. According to the client service the composition is:

<table>
<thead>
<tr>
<th>substance</th>
<th>volume%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethane ($C_2H_6$)</td>
<td>0,46</td>
</tr>
<tr>
<td>Propane ($C_3H_8$)</td>
<td>87,48</td>
</tr>
<tr>
<td>Isobutane ($iC_4H_{10}$)</td>
<td>6,3</td>
</tr>
<tr>
<td>Butanenormal ($nC_4H_{10}$)</td>
<td>5,59</td>
</tr>
</tbody>
</table>

The medium relative density of this composition is 1,6199652. The density of each of the gases in this composition in comparison to air is:

Propane gas can be measured with gas chromatography. Propane that is dissolved in water can be measured either with purge and trap or a headspace technique. The purge and trap technique is a method in which the dissolved propane is stripped out by bubbling the water with helium. The out coming helium stream is led through a absorber material in a liquid nitrogen bath. The tracer gas, propane, condenses in the trap and is later revaporized and measured. For the measuring a gas chromatograph is used to separate the gases, than the quantity is found out by the detector response and a calibration line. This method takes a lot of time. Also special material that is not commonly found in most laboratories is necessary. Headspace analysis is cheaper, faster and reliable results are obtained. Thats why headspace technique is applied in this work. [TG90]
Table 4.2: information about the density of “propano comercial” from U. F. Envasado S. A. C., Repsol

<table>
<thead>
<tr>
<th>substance</th>
<th>density relatively to air</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethane((C_2H_6))</td>
<td>1.049</td>
</tr>
<tr>
<td>Propane((C_3H_8))</td>
<td>1.562</td>
</tr>
<tr>
<td>Isobutane((iC_4H_{10}))</td>
<td>2.064</td>
</tr>
<tr>
<td>Butane((nC_4H_{10}))</td>
<td>2.091</td>
</tr>
</tbody>
</table>

4.2.2 Headspace analysis

For the headspace analysis a water sample is taken. In the sample vial a headspace is created. By shaking the vial, an equilibrium is achieved between the gas concentration in the water and in the gas phase. With a syringe a sample is taken from the gas phase and injected in a gas chromatograph. The concentration is determined by the response of the chromatograph and a calibration line.

Handling of the Sample

The sample bottles used in this study have a volume of 250 ml, much bigger than those that are usually used for headspace vials. It was chosen because a bigger sample can be taken. This makes it possible to vary the headspace volume. If the water volume is bigger than the headspace volume it is possible to detect low concentrations of gas dissolved in the water phase.

The closure heads are special gas tight valves. The weight of each bottle with the valve was documented. The bottle volume was determined measuring the weight of the bottle first empty than completely filled with water. After taking the sample the weight of each bottle was measured. By this the exact water volume and the volume of the headspace could be determined for each sample.

All water samples were taken with a flexible tubes to keep the contact between sampling water and air as short and as few as possible in order to avoid the transfer of gases. Another advantage of the tube filling is that turbulence is low. That also keeps the gas transfer down. The end of the tube was held on the bottom of the sample bottle to avoid contact between the sample water and air. The bottle were filled in between Half or 2/3 full and then they were rapidly closed with the gas tight valves. The air volume left in the bottle is the headspace. This handling was chosen instead of the handling applied in the study of John R. Thene. He filled the bottle completely, transported it to the laboratory and then opened the bottle again and created the headspace. [TG90].

In our study the bottle were filled half way and closed immediately. The air left in the bottle formed the headspace.

In the experiment to determine the R the temperature of the sample and the laboratory
the wetland over its whole width with influent water. The tube is perforated on a single line with holes of a diameter of about 2 mm in a distance of about 3 cm far-between.

The outlet is a rigid perforated tube on the bottom of the wetland on the opposite side to the inlet. The outlet tube is connected to a vertical PVC pipe that made it possible to change the altitude of the water in the wetland. The chosen water level was 0.25 cm from the bottom of the container. This locates the water level in about 2 cm below the gravel bed high. The granatical gravel that fills the container has an average diameter of 5 mm and a porosity of 40%

![Figure 4.2: wetland situated on the roof top](image)

The wetland is planted with common reed, phragmites australis. The roots of the plants are completely developed and extended through the whole gravel bed.

### 4.1.2 Operation of the wetland

The wetland is batch operated. The water was distributed every 4 hours. The inlet volume depends on the residential time that was wanted. The residential time HRT is calculated like this:
Figure 4.3: sample bottle

were the same as the experiment located in the laboratory. If samples had a different temperature they were left until their temperature would be adapted to the laboratory temperature. To prepare the samples for measuring, their weight was documented and an equilibrium between the gas phase and the liquid phase was installed.

To establish the equilibrium the samples were shaken wildly by hand. The shaking was performed in a way that bubbles would be formed and the water would flush turbulently from one side to the other. Each sample bottle was shaken by hand. A study has shown that samples that were not shaken would not reach equilibrium also they were let apart for 18 hours. Shaking the sample only 15 second brought some in equilibrium and shaking the samples 30 second guaranteed a equilibrium in each sample bottle. To be sure that equilibrium was reached the bottles were shaken 1 minute. Approximately a 95% of the dissolved propane goes from the liquid to the gaseous phase once the bottle is in equilibrium. [TG90] All samples were analyzed within a time margin of 24 hours. A test was made to verify that a slite variance of volume of the headspace in the bottle would not change the result. The range of the different volumes was from half water half air to 2/3 water and 1/3 air in the sample bottle. Different samples with varying volumes were taken at the same point with the same propane concentration.

4.2.3 Chromatography of gases

The propane was analyzed with a gas chromatograph. The chromatograph used in this study is the model Trace GC 2000 of ThermoQuest. It consists in an injector, a column
and a detector.\cite{Que01}

The injector is a split/splitless injector that was kept at a temperature of 120°C. The sample was injected with a gas-tight syringe of Hamilton with a volume from 0.1 to 1 ml.

A carrier gas circulates through the gas chromatograph. It takes the injected probe from the injector, through the column and to the detector. In this study detection was made with a TCD and due to this, a carrier gas, that has a different conductivity than the sample gases, had to be selected. Commonly used carrier gases are H\textsubscript{2}, N\textsubscript{2} or helium. Helium, with a thermal conductivity of 0.152 W/(m K), was selected as carrier gas. The carrier gas velocity was fixed at 30 ml/min. The TCD detects a broad range of gases, it is sensitive to every gas with a thermal conductivity that is different to the carrier gas.

The column acts to separate the gas mixture into its different gas components. The column used in the study is a Porapack N (80/100) column. It is a stainless steel packed column the separation mechanism is the molecular size of the gas components. The huger the molecular the longer the retention time, see Figure (4.5).

Different oven temperatures, from 32°C to 100°C, were tried. For the analysis a isothermal temperature of 100°C was chosen. This temperature still ensures a good separation of the important components in the gas mixture and the retention time is short. Propane
Table 4.3: Thermal conductivities for different gases, at $0^\circ$C, where $\lambda = \text{cal/(cm*sec*}^\circ\text{C)}$

<table>
<thead>
<tr>
<th>gas</th>
<th>Thermal conductivity ($\lambda \times 10^7$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helium</td>
<td>3363</td>
</tr>
<tr>
<td>Methane</td>
<td>720</td>
</tr>
<tr>
<td>N$_2$</td>
<td>580</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>343</td>
</tr>
</tbody>
</table>

is detected at a peak retention time of approximately 3 minutes. The detector is a TCD, a thermal conductivity detector. As the name implies it detects gases because of their different conductivities in respect to the carrier gas. An advantage of TCD is that it detects a broad number of gases provided that their thermal conductivity is different to the carrier gas. A disadvantage is that it is not as sensitive as a flame ionization detector FID that is commonly used for the detection of propane. In this study the TCD is an appropriate detector because the propane concentrations are high enough to be detected well and other gases, that are produced in the wetland, can be detected. The TCD was run at a temperature of 160$^\circ$C. The chromatograph is connected to a computer with the software ChromLab trace GC. The data of the TCD was processed by the software, which produced a graph that reflected the difference in the conductivity of the detected gases in comparison to helium. The output of the computer was in form of a graph with different peaks. Each peak is correspondend to a gas that passed the column. The peaks have different retention times for different gases and different peak areas for different mass fractions of the gases in the sample.

4.2.4 Interpretation of the peaks

The peaks could be determined comparing their retention time with the retention time of a defined standart and air. A typical graph obtained for a sample of the output of the wetland is seen in figure (4.6). The gases that were found with this method were N$_2$, CH$_3$, CO$_2$, and C$_3$H$_8$. The peak of CH$_3$ was so close to the peak of N$_2$ and its concentration in the wetland were so low that it was not determined properly. For its determination another method with a lower oven temperature would have been necessary. The method (oven temperature: 100$^\circ$C, TCD: 160$^\circ$C) chosen for this study left the peak of propane far from the peaks of the other gases. Propane could be determined without an interference of the other gases. Another reason why this method was selected was that the measurement time could be limited to 5 minutes. In this time all the gases that were in the sample passed the column of the chromatograph. The peak of propane had almost a symmetric form and just a little tailing (4.7).
Table 4.4: retention times for the different gases detected with chromatography [Que01]

<table>
<thead>
<tr>
<th>Detected gas</th>
<th>Peak retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_2$</td>
<td>0.4 – 0.5</td>
</tr>
<tr>
<td>$CO_2$</td>
<td>0.7 – 0.9</td>
</tr>
<tr>
<td>$C_3H_8$</td>
<td>2.98 – 3.02</td>
</tr>
</tbody>
</table>

4.2.5 Reliability of the results

To see if the results obtained in this study are reliable and to see if the method that was applied was appropriate, the variation coefficient was calculated. For this calculation one measuring point was measured more times with the same method. The arithmetic mean was calculated for the obtained values. It was determined with the following formula:

$$\bar{Y}_i = \frac{1}{n} \sum_{k=1}^{n} y_k$$

(4.2)

The standard deviation was calculated like this:
4.2. QUANTIFICATION OF PROPANE

Figure 4.6: Typical output graph of the chromatograph; first peak: $\text{N}_2$, second peak: $\text{CO}_2$, third peak: $\text{C}_3\text{H}_8$

\[
S_{yi} = \sqrt{\frac{1}{n-1} \sum_{k=1}^{n} (y_k - \bar{Y}_i)}
\]  

(4.3)

$\bar{Y}_i$ arithmetic mean

n number of measurements

$y_k$ value of measurement k

$S_{yi}$ standard deviation

$t$ student t factor

With these values the variation coefficient was determined. The value was given in percent because it is easier to interpret:

\[
v_{\text{percent}} = \left( \frac{S_{yi}}{\bar{Y}_i} \right) \times 100
\]  

(4.4)

4.2.6 Calibration line

To determine the propane concentration of an injected volume a calibration line was established. The calibration line relates the output of the chromatograph, the peak
area, to a certain known propane concentration. A certified gas standard with 14.95% ± propane in helium was bought. Several volumes of this defined gas mixture were injected to the chromatograph. As the same syringe was used for the calibration and for the measurement the results were more precise.

The volumes injected were chosen procuring that the detector responses for the sample are in the same range as the calibration line. Volumes from 0.025 to 0.6 ml were injected. Each volume was injected 4 times and the results were interpolated to get reliable results. By this the detector response, a peak area, could be related to a known injected mass of propane. The injected propane mass was calculated with the law for ideal gases:

$$p \times V = \frac{m}{M} R \times T \quad (4.5)$$

$p$ standard pressure is 1 atm

$M$ the molar mass of propane is 44

$R$ gas constant

$T$ Temperature

$V$ standard volume of one mole gas in standard condition is 22.4

With the propane mass and the corresponding peak area a calibration line was obtained.
4.2.7 Calculation of the concentration in the sample

With the calibration line the mole of propane that was injected can be deduced from the peak area shown by TCD. The mass of propane that was injected can be calculated with the injected volume of the syringe. The concentration in the syringe is the concentration of the headspace:

\[
C_{\text{headspace}} (\mu g/L) = \frac{M_{\text{propane}}}{V_{\text{syringe}}} \tag{4.6}
\]

- \(M_{\text{propane}}\): Mass of propane injected to the chromatograph, obtained with the peak area and the calibration line.
- \(V_{\text{syringe}}\): Volume injected to the chromatograph with the gas tight syringe.

With the syringe a volume, 0.6 ml, was taken from the headspace. This causes a dilution of the headspace. It can be corrected with the dilution correction factor \(F\) that is multiplied with the headspace concentration:

\[
F = \prod_{k=1}^{j} \left(1 + \frac{V_{\text{syringe}}}{V_{\text{headspace},i}}\right) \tag{4.7}
\]

- \(j\): Number of injections.
- \(V_{\text{headspace},i}\): Volume of the headspace in sample \(i\).

The concentration in the water phase of the sample is calculated with the law of henry and dalton (3.21) and the law for ideal gases (4.5). [TG90]

Figure 4.8: calibration line obtained for defined volumes of a gas standard
4.3 Measurement of the dissolved oxygen

The dissolved oxygen was detected with a sensor for oxygen YSI 58. The instrument works with a cathode surrounded by a special KCl solution and separated from the water sample by an oxygen permeable membrane. The dissolved oxygen passes through the membrane and then is detected and consumed by the cathode. The oxygen follows the concentration gradient to the cathode. The oxygen that passes the membrane is proportional to the absolute pressure of the oxygen in the water sample, because the oxygen in the KCl solution is consumed immediately and hence that the oxygen concentration is always zero. The sensor was calibrated with the winkler test. [Sup]

4.3.1 Winkler

This analysis is named after Lajos Winkler who developed this method in his doctoral thesis in 1888.

Figure 4.9: Winkler test
4.3. MEASUREMENT OF THE DISSOLVED OXYGEN

The DO is precipitated and fixed with manganese in alkaline solution.

\[ 2Mn^{2+} + 4OH^- + O_2 \rightarrow 2MnO(OH)_2 \downarrow \]  \hspace{1cm} (4.8)

In strong acidic solution Mn\(^{3+}\) is generated proportional to the DO that had been dissolved in the sample.

\[ 2MnO(OH)_2 + 2e^- + 8H^+ \rightarrow 2Mn^{2+} + 6H_2O \]  \hspace{1cm} (4.9)

The Mn\(^{3+}\) ions oxidize a amount of iodide ions that is equivalent to the DO in the water sample.

\[ 2Mn^{3+} + 2I^- \rightarrow 2Mn^{2+} + I_2 \]  \hspace{1cm} (4.10)

The iodine is quantified by titration with a 0.01 molar thiosulfate solution. The endpoint is a color change from yellow to colorless.

\[ I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-} \]  \hspace{1cm} (4.11)

With the titration volume the oxygen can be calculated. Reckoning the stoichiometry relation, 1 ml of 0.01 molar thiosulfate solution is equivalent to 0.08 mg O\(_2\).

\[ C_{O_2} = \frac{V_{\text{titration}} \times r}{V_{\text{sample}} - V_{\text{reactives}}} \]  \hspace{1cm} (4.12)

\( C_{O_2} \) \hspace{0.5cm} concentration of O\(_2\) in the sample in mg/l

\( V_{\text{titration}} \) \hspace{0.5cm} volume of thiosulfate that had to be added until reaching the color change

\( r \) \hspace{0.5cm} stoichiometry factor, mass O\(_2\) : volume of the thiosulfate = 80 mg/l

\( V_{\text{sample}} \) \hspace{0.5cm} volume of the water in the sample bottle

\( V_{\text{reactives}} \) \hspace{0.5cm} volume of the reagents added to the sample

This method can be used for oxygen concentrations \( \geq 0.5 \) mg/l.

Proceeding of the measurement  The reagents were prepared in the following way:

- manganese reactive: 100g MnCl\(_2\)*4H\(_2\)O were dissolved in 150 ml de-ionized water
- iodine reactive: 70g NaOH and 60g KI were dissolved in 150 ml water
- 0.01 molar thiosulfate solution was prepared

A winkler bottle with a defined volume was filled with the sample water in such a way that it had little contact with the atmosphere. The bottle was filled completely so that bubbles removed. To a sample volume of 300 ml, 3 ml of the manganese reactive and than 3 ml of the iodine reactive were added and the bottle was closed again. A brown
precipitate is formed. The bottle is put in the dark and left for at least ten minutes so that the precipitate settles to the bottle. 5 ml of concentrated phosphoric acid are added to the bottle and the sample is titrated immediately, when turning to a light yellow some drops of starch solution are added. After the color changes to blue and the endpoint of titration, the change from color to clear can be seen more easily.

4.4 Method for the determination of the mass transfer coefficient

As explained before, R is the relation between the mass transfer factors K of oxygen and propane (3.30). To determine the R, a water body was saturated with propane. Then the decreasing propane concentration and the increasing oxygen in the water body were measured. By this the K for propane (3.33) and the K for oxygen (3.33) could be calculated.

4.4.1 Set up

Figure 4.10: Experimental set up for the determination of R
The different components of the experimental setup for the determination of \( R \) can be seen in figure (4.10) with:

1. thermometer
2. diffusor for bubbling the water body with propane, connected to the propane bottle by a tube
3. oxygen sensor model YSI 58
4. magnet stirrer, later situated under the water recipient
5. winkler bottles for oxygen analysis
6. headspace bottles with gas tight valve
7. recipient filled with tap water

The water in the recipient was put aside until it reached ambiance temperature. Then the diffusor and the oxygen sensor were collocated in the water body of the tank. The water was bubbled with propane for about two hours until most of the DO was gone. The oxygen outstripping was stopped when the sensor showed an oxygen concentration of 0.8 mg/l and the propane concentration in water was circa 50 mg/l.

The diffusor was removed and a tube for sampling was placed on the bottom of the recipient a magnet stirrer was located that moved the water body gently without causing huge movements on the water surface. The stirrer made it possible to assume that the liquid phase was homogeneous. The gas phase was homogeneous too as the whole experiment was situated in an extractor hood in the laboratory. Doing this ensured that the propane was transported away immediately and would not accumulate on the water surface and change the concentration gradient.

Then samples were taken. Oxygen was measured in two different ways, continuously with the sensor and discontinuously with winkler method. Propane was measured with headspace analysis. Sample were taken with a tube, out of the middle of the water body. The tube was flushed three times with its own volume before taking the samples. With the tube it was possible to take the samples with only very little contact to air as the tube end was brought to the bottom of the sample bottles and was held under the water surface while filling the bottle.

Two experiments with a different mixing level were made. One with the magnet stirrer at a higher velocity, that caused a faster oxygen - propane exchange. In the fast experiment samples were taken every half an hour, for six hours. In the second experiment the stirrer moved very slowly and samples were taken every hour for nine hours.

With the results the specific \( K \) for this experiment for oxygen and propane were calculated (3.33), (3.31) and with them \( R \) was calculated(3.30). Experimental condition,
like the water - gas exchange surface, water body volume, change of the water volume by sampling and temperature affected both, the oxygen and the propane transfer. Thats why they did not have to be considered in the calculation. Also the ratio oxygen - propane was shown to be independent of temperature (in between 20 - 30°C), turbulence and the presence of impurities [MVHJ06].

4.5 Method for the determination of the oxygen transfer rate

The tracer gas method was applied on a constructed wetland to find the oxygen transfer ratio (OTR) for this wetland. Tap water with a high propane concentration was let in the wetland. By measuring the propane concentration at the inlet and at the outlet of the wetland the oxygen entrance could be calculated.

4.5.1 Construction and implementation of the experiment

The set up of the experiment basically consists in a water tank that is connected to a wetland. In the tank tap water was saturated with propane.

![Figure 4.11: Experimental set up for the determination of OTR](image)

Two different tank sizes were used, small 10 liter tanks and a huge 250 liter tank. With these tanks different operating modes for the inlet could be tried. The wetland was batch operated with different residential times. When batch operating was implemented on the small tanks, the tanks were filled, saturated with propane and then emptied entirely to the wetland. The huge tank was saturated once and the water was let into the wetland.
4.5. Method for the determination of the oxygen transfer rate

subsequently batched by a peristaltic pump. The tank, the peristaltic pump and the wetland were connected by flexible plastic tubes.

![Experimental setup](image)

**Figure 4.12:** Foto of the experimental set up for the determination of OTR

1. differently sized tanks for the propane saturated water
2. peristaltic pump
3. flexible plastic tubes
4. propane bottle in a protection cabin
5. wetland

4.5.2 Propane saturation of the influent water

The saturation of the tap water was carried out in the different water tanks. A diffusor, and a oxygen sensor were collocated in the tank. The diffusor was a very simple model as it is commonly used for the oxygen supplement in aquaria. It was connected to the propane bottle with a standardized gas tube conform to Din standard. The propane left the bottle through a security valve. The water was bubbled with propane until 0,0 oxygen were detected with the oxygen sensor. It was assumed that at this point all the oxygen was substituted by propane. In order to avoid rising pressure during the bubbling
process, gas was able to escape through a tube. When the bubbling was stopped the bottle was made gas tight so that no oxygen could enter and no propane could escape. A tube for the outlet was connected to the peristaltic pump.

### 4.5.3 Pressure compensation at the tanks

As the tank was made gas tight, but water was pumped out, a low pressure was created. Low pressure causes the propane to leave and the concentration in the water decreases. For this experiment a constant oxygen concentration is necessary. To compensate the leaving water, propane gas was led in the tank. The propane was pumped in a balloon that was connected to the tank with a plastic tube.

![Figure 4.13: left: tank with balloon for pressure compensation; right: filling the balloon with gas](image)

With this technique a constant propane concentration could be established. The balloons have a slightly higher partial pressure than the atmosphere. This causes a higher transfer of propane to the water by diffusion. Diffusion is a slow process and the contact area between air and water was not so huge. The water was taken from the bottom, the experiment lasted a short time and the balloon pressure decreased as the bottle was emptied emptying the bottle. Because of that the higher balloon pressure was neglected.
4.5. METHOD FOR THE DETERMINATION OF THE OXYGEN TRANSFER RATE

4.5.4 Sampling

Samples were taken for later headspace analysis, the handling is described above. Four different sampling points were chosen. Two at the inlet, one in the middle and one at the outlet. The two inlet and the middle sample point were co-located in the gravel bed. The outlet sample was taken from the outlet tube. The inlet sample was not taken in the inlet tube because the oxygen transfer at the perforated tube, that is co-located 5 cm above the gravel bed, was not of interest for this study, but the oxygen transfer in the wetland was to be measured. The inlet samples were taken in the gravel bed at two parallel points. The concentration should be the same in both points as the perforated inlet tube is assumed to distribute the water homogeneously over the whole length of the gravel bed. For the sampling flexible plastic tubes were fixed at the points that are indicated in figure (4.14). The tubes were fixed with a pole, approximately 4 cm beneath the water level. The water level was at circa 4 cm beneath the gravel level. The draw of the water was effected by a difference of height between sampling point and sample bottle.

A small clamp regulated the water flow. Before taking the sample the tube was flushed with at least 3 times its volume. This ensured that the old sample water was gone. The sample bottle was filled with sample water, shaken, emptied and subsequently filled with the sample water. Care was taken so that water loss would not have an effect on the experiment. After taking the samples the bottles were closed immediately and measured within 24 hours.

4.5.5 Retention time

The velocities of the inlet water were varied depending on the retention time the water was meant to have in the wetland. The theoretical hydraulic retention time was calculated with the pore volume of the gravel bed and the sizes of the wetland. For the determination of the pore volume a glass measuring cup was filled with the gravel. A
Figure 4.15: photo of a sampling out of the middle of the wetland

defined volume of water, 1 liter, was measured. The water was added slowly to the gravel until water and gravel had the same level. Now the rest of the water was measured. By the difference the volume of the added water volume could be calculated. The porosity of the gravel bed is the volume of added water per volume of the measuring cup. It was found to be 40%.

This value was obtained for the pure gravel without plants roots and sludge. As the wetlands where run for several years the real hydraulic retention time may be different because of clogging. With the measured concentrations at the outlet of the wetland a graph can be obtained. It is possible to deduce the real HRT from this value.

4.5.6 Calculation of oxygen transfer

First the mass transfer coefficient $K$ of propane (5.4.1) was calculated with the measured propane concentration at the inlet and at the outlet. These values were put in the formula (3.33) and $K_{la,propane}$ was calculated.

$K_{la,propane}$ is related to $K_{la,oxygen}$ with $R$. The value for $R$ was not taken from the literature but it was measured again 5.2.2.

The oxygen transfer rate was calculated with the formula (3.39). For $C_s$ the maximum
4.5. METHOD FOR THE DETERMINATION OF THE OXYGEN TRANSFER RATE

Oxygen concentration in water at 20°C (9.2 mg/l) was taken and C was no oxygen concentration at all (0 mg/l). The surface area of the wetland is 0.759 m² and the pore water volume is 84 l. There was no constant temperature during the experiments due to the long run, the variations between day and night did affect. Because of that, $K_{la,oxygen}$ could not be adapted to a temperature of 20°C with the formula for the temperature correction (3.34)