METHODOLOGY

The study was designed to have three parts: base line study in preparation phase; wetland's characteristic study in phase 1 including wetland's profile study and identification of aquatic plants; wetland's water quality assessment in phase 2 including physical parameter measuring, water sampling and chemical analysis. Time schedule for the study is as follows :

Base line study	: October - November 1997
Phase 1	: December 1997 - January 1998
Phase 2	: February - April 1998

Base Line Study

Base line information of the studied wetland and Mae Hia biogas unit were obtained via different methods and sources: reviewing existing secondary data; interviewing with local workers in Mae Hia Research Station and Training Center; interviewing local people living nearby the studied site; and field observations. The information includes history of the studied wetland and the biogas unit, activities which have been carried out in the area around the wetland, some physical characteristics of the studied site, such as, inlet and outlet channels and fluctuation, flow direction. Other important interested base line data to the study are chemical, physical as well as economical characteristics and properties of the biogas unit which is the main inlet source to the studied wetland.

Phase 1 : Wetland's Characteristic

Profile Study of Wetland

In order to understand characteristics and flow-pattern of the wetland, the profile-pattern showing contour lines of the pond was made. The procedure of making this profile is as follows.

- Strings were laid out in the wetland as shown in Figure 8.
- Using rubber raft, depth of the wetland were measured and recorded at every 5 meters along the strings
- Data form depth-measuring were then used for making the contour line map profile of the wetland.
- Natural vegetation zoning in the wetland was also recorded and the map of vegetation zoning and distribution was then prepared.

Identification of Aquatic Plants

All aquatic plants found in the wetland were photographed at the site and samples were brought back to the herbarium at Biology Deparment, Faculty of Science, Chiang Mai University, for their identification and confirmation. Some botany taxonomy text books and manuals were used for the identification and the samples were compared with specimens in the same herbarium. Finally, all identified samples were confirmed again by experienced botanist.

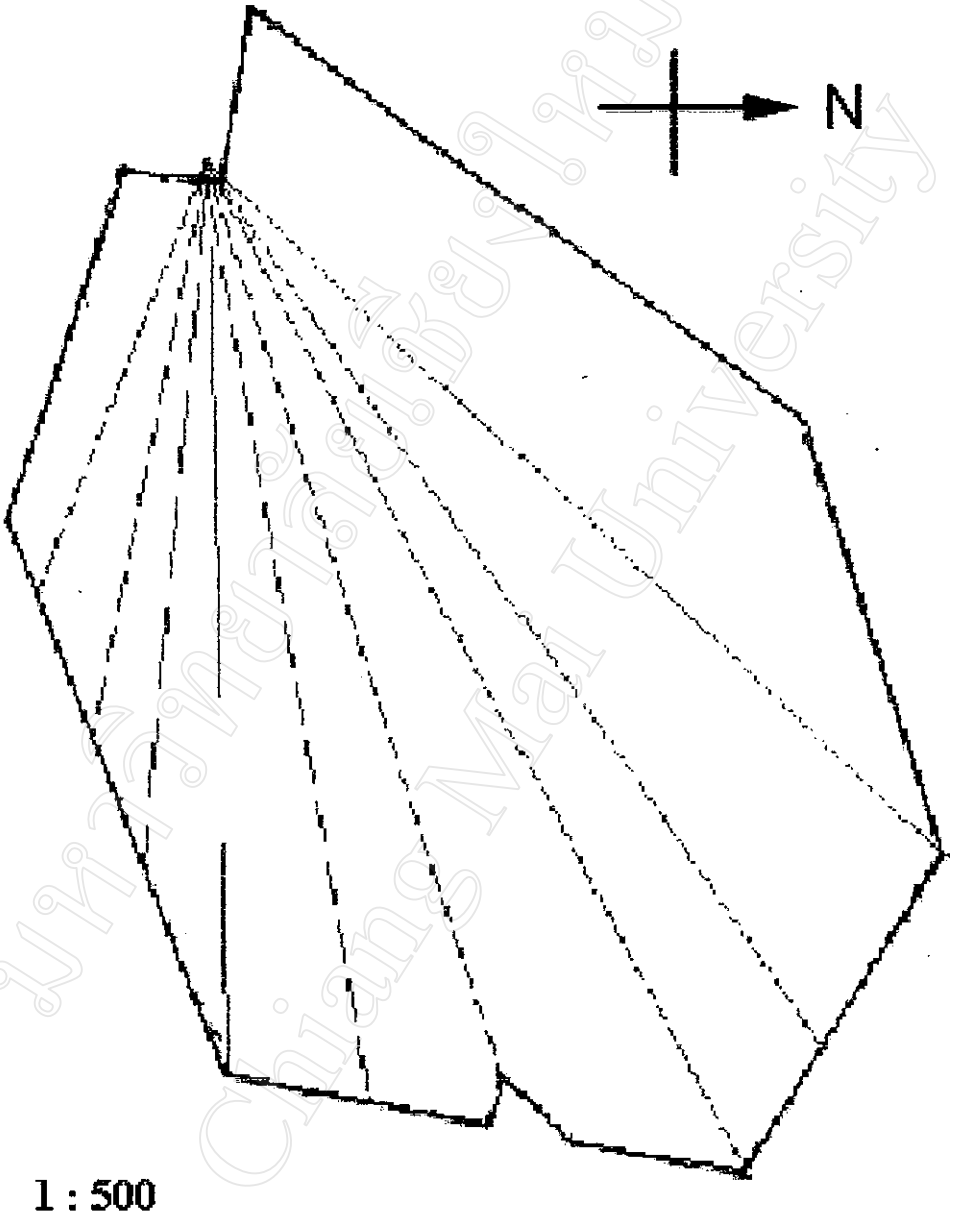

Figure 8. Laying out strings over the wetland for measuring the depth.

Phase 2 : Wetland's Water Quality Assessment

Physical Parameters Measuring

pH and water temperature were measured directly at the sampling sites using pH-meter and thermometer. Depth and flow status of the water at each site were also recorded along with sampling time.

Water Sampling for Chemical Analyses

Five Sampling sites were selected according to the possible flow pattern derived from profile study of the wetland. Figure 9 shows the 5 selected sampling sites: S1- the inlet from pig manure biogas digester, S2 - the inlet from cattle farm, S3 and S4 - mid-wetland in deeper areas, and S5 - the only flooded over outlet of the wetland.

Water samplings were carried out weekly on Monday started from February until almost to the end of April 1998 with a two-week break in the beginning of April. The break was due to the activities occured to disturb the hydrology of the wetland and changed all some major surrounding conditions, especially inlet flow. Some surrounding activities and phenomenon were also recorded, such as the amount of inlet flows, interfering activities around the wetland.

Water samples were collected directly from about 30 cm below water surface at sampling points using 1 L - plastic bottles. All the plastic bottles used were covered with black paint in order to reduce biochemical activities in the water samples while being delivered to the laboratory.

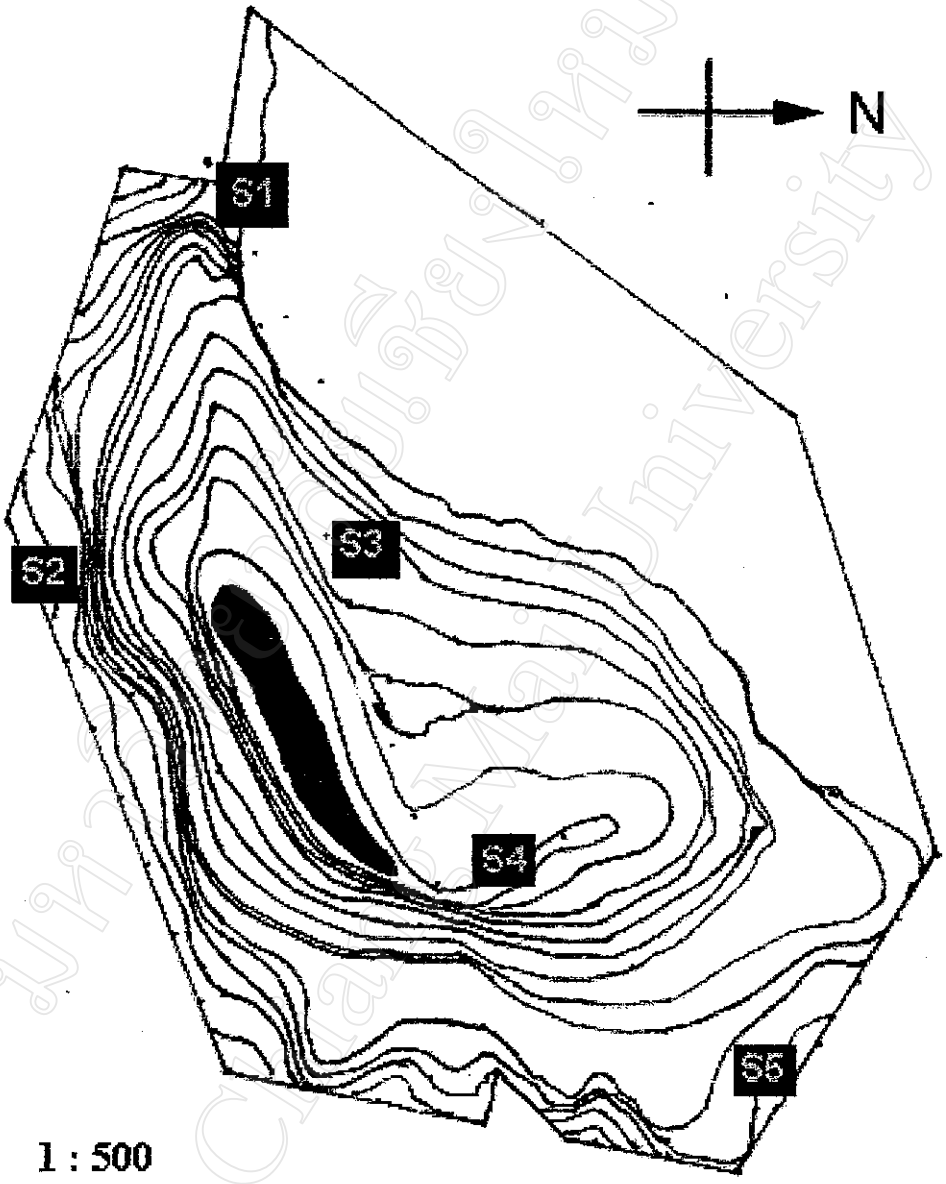

Figure 9. Five selected sampling sites in the studied wetland

(S1- inlet from pig manure biogas unit, S2 - inlet from cattle farm,

S3 and S4 - mid-wetland, S5 - outlet)

Chemical Analyses

After all water samples arrived at the laboratory, the water samples were firstly filtrated using Wattman filtered paper No. 1. Then the analyses for physico-chemical parameters took place. Chemical parameters and analyses methods adopted in this study is shown in table 6.

Table 6. Chemical parameters and analysis methods

Parameter	Method	Replicates
BOD_5	Winkler method / Azide modification	3
COD	Titrimetric method / closed reflux	2
NH_3-N	Nesslerization method / Titration	3
NO_3-N	Phenoldisulphonic acid method	3
PO_4-P	Stannous chloride method	3

Water samples in this study were generally assumed as wastewater drained out from biogas digester and animal farm. The analysis for DO was then missed out since the pretest study done in January showed zero value for this parameter. Furthermore, for analyses of all other possible chemical parameters, all water samples were diluted to suit the method limitation.

BOD_5 analysis method (ศิริเพ็ญ, 2530)

chemicals preparation

1. concentrated sulfuric acid
2. manganous sulfate solution

   - Disolve 480 g of $MnSO_4.4H_2O$ (or 400 g of $MnSO_4.2H_2O$ or 364 g of $MnSO_4.H_2O$) in distilled water, filterd and add distilled water to 1,000 ml. It should be noted that this solution should not produce any color when added to acidified potassium iodide having stacrh solution as indicator.

3. alkali-iodide azide reagent

   - For water sample which has estimated DO-value lower than saturated point, dissolve 500 g of NaOH (or 700 g of KOH) and 135 g of NaI (or 150 g of KI) in 250 ml of distilled water. Add 10 g of $NaNO_3$ dissolved in 40 ml of distilled water, then make up to 1,000 ml. (This solution should not present any color with starch solution when being diluted and acidified.)

   - For water sample which has estimated DO-value higher than saturated point, dissolve 10 g of $NaNO_3$ in 500 ml of distilled water. Add 480 g of NaOH and 75 g of NaI. Shake well until completely dissolved. (This solution appear with cloudy whitish of Na_2CO_3, adding acid to the solution produces toxic hydrazoic acid gas.)

4. starch solution

   - Weight 2 g of starch powder and 0.2 g of salicylic acid, then dissolve them in hot distilled water and make up to 100 ml.

5. standard solution: sodium thiosulfate 0.021 M

   - Dissolve 6.205 g of $Na_2S_2O_3.5H_2O$ in distilled water. Add 1.5 ml of NaOH 6 N (or solid NaOH 0.4 g) and diluted with distilled water to 1,000 ml. Titrate this solution for comparison with potassium bi-iodate to make standardization.

6. standard solution: potassium bi-iodate (0.021 M)

   - Dissolve 812.4 g of $KH(IO_3)_2$ in distilled water and make up to 1,000 ml.

determination

1. Distilled water is aerated overnight or at least 2 hours before the analysis.
2. After arriving at laboratory, water sample is suddenly diluted to 2 % by adding 2 ml of filterd sample into 300 ml BOD bottle and filled with aerated distilled water. Two set of samples are prepared; one set for DO_1 (measured on day 0) analysis carried out on the sampling day, another set for DO_2 analysis (measured on day 5) which is put in the incubator at 20 ± 0.1 °C for five days before the analysis.
3. One ml of $MnSO_4$ is added followed by 1 ml of alkali-iodide-azide reagent.
4. The bottle is closed and shook well until 2/3 of precipatate occurs.
5. The bottle is then repeted shaking and left for 2/3 of precipatate to occur.
6. One ml of conc. H_2SO_4 is added and then the bottle is shook well to completely dissolve the precipitate.

7. Solution obtained from 4. is titrated with $Na_2S_2O_3$ 0.021 M until pale-yeelow color appears.

8. Few drops of starch solution is then added followed by shaking the bottle till blue color appears.

9. The titration is continued until the end point indicated by the disappearing of the blue color.

10. DO as ppm (mg/l) is then calculated using the formula:

DO (mg/l) = ml of $Na_2S_2O_3$ 0.021M x 2 x 100/2* (* dilution)

And BOD_5 as ppm (mg/l) is then calculated from :

$BOD_5 = DO_1 - DO_2$

COD analysis method (Anaerobic Wastewater Treatment, 1993)

chemicals

1. Potassium dichromate 0.0167 M (digestion solution)

- Dissolve 4.913 g of solid $K_2Cr_2O_7$ (dried at 103 °C for 2 hours) in distilled water and make up to 500 ml. Then 167 ml of concentrated sulfuric acid was slowly added followed by 33.3 g of $HgSO_4$. The solution is then stirred up and left to cool down before being diluted by making up with distilled water to 1,000 ml.

2. sulfuric acid reagent

- Add 22 g of Ag_3SO_4 into conc. H_2SO_4 which is normally in 9 lb or 2.65 L - bottle. Allow to be completely dissolved which could take 1 - 2 days. (ratio is 5.5 g of Ag_3SO_4 to 1 kg of conc. H_2SO_4)

3. feroin indicator

4. ferrous ammonium sulfate solution (FAS) 0.10 M

- Dissolve 39.2 g of $Fe(NH_4)2(SO_4)_2.6H_2O$ in a small amonut of distilled water followed with adding 20 ml of conc. H_2SO_4 and let it cool down before making up with distilled water to 1,000 ml.

determination

1. Digestion tubes are rinsed with 20 % H_2SO_4.
2. Five ml of water sample (not filtered and diluted to 40 %) is added to each digestion tube.
3. Three ml of $K_2Cr_2O_7$ 0.0167 M, digestion solution, is added.
4. Seven ml of conc. H_2SO_4 is then slowly added followed by closing the tube-cap tightly and shaking the tube well.
5. All digestion tubes are put in the incubator set at 150 °C for 2 hours.
6. The tubes are taken out and let to cool down to room temperature.
7. Add 2 drops of feroin indicator and titrate with FAS until the end point where the solution's color changes from green-blue to brownish-red.
8. At least two tubes of blank (distilled water) are prepared and passed through all the same procedure with water samples in order to get factor " b " in the caculation formula.

9. COD as ppm (mg/l) is then calculated using formula:

COD (mg/l) = [(a - b) x N x 8 x 1,000] / [ml of water sample]

where a = ml of FAS used in titration with blank

b = ml of FAS used in titration with water sample

N = Normality of FAS

NH_3-N analysis method (ศิริเพ็ญ, 2530)

chemical preparation

1. zinc sulfate solution

   - Dissolve 100 g of $ZnSO_4.7H_2O$ in free-ammonia distilled water and make up to 1,000 ml.

2. sodium hydroxid 6 N

   - Dissolve 240 g of NaOH in 500 ml of distilled water and make up to 1,000 ml.

3. stabilizer reagent (EDTA reagent)

   - Dissolve 50 g of $Na_2EDTA.2H_2O$ in 60 ml of water dissolved in with 10 g of NaOH. The solution is then diluted by making up to 200 ml using distilled water.

4. Nessler reagent

   - Dissolve 100 g of Hg_2I and 70 g of KI in trace amount of distilled water while, in another beaker, 160 g of NaOH is dissolved in 500 ml of distilled water. The solution is let to cool down and slowly added with the solution

of Hg_2I and KI followed by making up to 1,000 ml. (keep this solution in dark bottle)

5. ammonium chloride stock solution

- Dissolve 3.819 g of NH_4Cl in 1 L of distilled water (1 ml = 1.22 mg NH_3-N = 1.00 mg N)

6. dechlorinating agents: sodium thiosulfate solution (0.0142 N $Na_2S_2O_3$)

- Dissolve 3.5 g of $Na_2S_2O_3$ in 1,000 ml of distilled water.

determination

1. One hundred ml of filtered water sample (diluted to 2 %) is put in 250 ml-erlenmeyer flask.
2. One ml of $ZnSO_4$ solution is added and the flask is well-shaked.
3. NaOH 6 N is added with the amount of 0.4 - 0.5 ml to obtain pH 10.5 in the solution. (measured by pH meter)
4. Shake the flask and leave it for few minutes until the presence of white precipitate occurs.
5. The solution is then filtered to separate the supernatant. Do not rinse the precipitate with distilled water.
6. One drop of EDTA reagent is added to the obtained supernatant
7. Two ml of Nessler reagent is then added and the flask is well-shaked.
8. Percentage transmittance at the wavelength of 430 nm is then measured using spectrophotometer (Spectronic 21) provided with 1 cm light path.

9. A series of standard stock solutions of ammonium chloride (NH_4CL) are prepared and measured percentage transmittance at the same wavelength (430 nm) in order to get data for making standard curve. It should be noticed that all standard NH_4Cl solution with known concentration must be prepared and treated under and with the same condition with water sample being analysed.

10. NH_3-N as ppm (mg/l) is then calculated using formula:

$$NH_3\text{-N (mg/l)} = \frac{\text{mg } NH_3\text{-N}^* \times 100}{\text{ml of sample}}$$

* obtained by compared with standard curve

NO_3-N analysis method (ศิริเพ็ญ, 2530 and Traichaiyaporn, 1985)

chemical preparation

1. standard 0.02 N H_2SO_4

- Prepare 0.02 N H_2SO_4 by diluting conc. H_2SO_4 with distilled water and make up to 1,000 ml. The volume of conc. H_2SO_4 can be calculated from the formula:

$$\text{ml of stock } H_2SO_4 = \frac{20}{\text{Normality of stock } H_2SO_4}$$

2. aluminium hydroxide

- Dissolve 125 g of pure $Al_2(SO_4)_3$ in 500 ml of distilled water and add NH_4OH until being completely precipitated. Filter to separate supernatant by

rinsing several times with distilled water. Stir the solution well and then precipitate to eliminate chlorides, nitrate ans ammonia.

3. standard silver sulfate solution

- Dissolve 4.397 g of Ag_2SO_4 (CP) in distilled water 1 L

(1 ml = 1 mg Cl)

4. phenoldisulfonic acid solution

- Dissolve 25 g of pure phenol in 150 ml of conc. H_2SO_4 and add 75 ml of fuming H_2SO_4. Then the solution is heated for 2 hours in the water bath.

5. 12 N NaOH

- Dissolve 480 g of NaOH in distilled water and make up to 1,000 ml.

6. standard nitrate solution

- Dissolve 0.7216 g of KNO_3 (AR) in 1,000 ml of distilled water

- Pipette 50 ml nitrate standard solution and evaporate to dry on water bath. Then moisten the residue thoroughly with 2 ml of phenoldisulfonic acid and rub it well with a glass rod to ensure the well contact of the residue and acid before making up with distilled water to 500 ml.

(1 ml = 0.01 mg N = 0.04426 mg NO_3^-)

determination

1. Place 50 ml of water sample (diluted to 50 %) in a 50-ml beaker and evaporate to dryness (on water bath or hot plate.)
2. Moisten the residue with 2 ml phenoldisulfonic acid and rub it well with a glass rod to ensure contact of the acid with the residue.

3. Dilute to about 20 ml with nitrate-free distilled water.

4. Add 12 N NaOH, until the maximum yellow color is developed. (not more than 6 ml of NaOH should be used)

5. Filter the solution, rinse the beaker and filter paper with nitrate-free distilled water, make up to 100 ml using distilled water.

6. Percentage transmittance at the wavelength of 425 nm is then measured using spectrophotometer (Spectronic 21) provided with 1 cm light path.

7. A series of standard stock solutions of potassiun nitrate (KNO_3) are prepared and measured percentage transmittance at the same wavelength (425 nm) in order to get data for making standard curve. It should be noticed that all standard (KNO3) solution with known concentration must be prepared and treated under and with the same condition with water sample being analysed.

8. NO_3-N as ppm (mg/l) is then calculated using formula:

$$NO_3\text{-N (mg/l)} = \frac{\text{mg } NO_3\text{-N}^* \times 10}{\text{ml of sample}}$$

* obtained by compared with standard curve

PO_4-P analysis method (ศิริเพ็ญ, 2530)

chemical preparation

1. phenolphthalein indicator

- Dissolve 5 g of phenolphthalein disodium salt in 1 L of distilled water.

2. strong acid solution

- Slowly pore 300 ml of conc. H_2SO_4 into 600 ml of distilled water and let to cool down before adding 4.0 ml of conc. HNO_3 and make up with distilled water to 1,000 ml.

3. ammonium molybdate reagent (I)

- Dissolve 25 g of $(NH_4)_6Mo_7.O_{24}.4H_2O$ in 175 ml of distilled water. Slowly pore 280 ml of conc. H_2SO_4 into 400 ml of distilled water in another beaker. Then mix the two solution by adding molybdate solution to the solution of conc. sulfuric acid and make up with distilled water to 1,000 ml.

4. stannous chloride reagent (I)

- Dissolve 2.5 g of $SnCl_2.2H_2O$ in 100 ml of glycerol. Heat the solution on water bath and rub with a glass rod until completely dissolved.

5. standard solution

- Dissolve 219.5 g of anhydrous KH_2PO_4 in distilled water and make up to 1,000 ml.

determination

1. Obtain 100 ml of filtered water sample. (diluted to 2 %)
2. color development : Add 4.0 ml of molybdate reagent (I) and shake well before apply 0.5 ml of stannous chloride reagent (I.) It shouldd be noticed here that color and concentration vary with temperature of the solution, 1°C increase 1 % color intensity increase, so temperature of blank, standard and reagent should be in between 20 - 30 °C.

3. color measuremnt : measure the percentage transmittance at the wavelength of 690 nm with spectrophotometer (Spectronic 21) provided with 1 cm light path.

4. A series of standard stock solutions of anhydrous KH_2PO_4 are prepared and measured percentage transmittance at the same wavelength (690 nm) in order to get data for making standard curve.

5. PO_4-P as ppm (mg/l) is then calculated using formula:

$$PO_4\text{-P (mg/l)} = \frac{\text{mg } PO_4\text{-P} * \times 100}{\text{ml of sample}}$$

* obtained by compared with standard curve

Additionally, the statistic method of ANOVA/ CRD (completely randomized design) was adopted to be used in this study in order to (i) determine if there is any differences of all chemical parameters among sampling sites lining along the estimated flowing path-way, which can then indicate the efficiency of the wetland for wastewater treatment, and (ii) confirm the differences of data from before and after the physical change of the studied wetland in April. This statistic method was chosen because of the limit of the data gained from the laboratory.