CHAPTER 2

EXPERIMENTAL

2.1 Chemicals and material

2.1.1 Standard chemicals

- Stock solution (1,000 mg L^{-1}) of Iron (SCP Science, Canada)
- Stock Magnesium Nitrate (10,000 mg L^{-1}) (SCP Science, Canada)

2.1.2 General chemical and solvent

- Iron (III) Chloride anhydrous sublimed for analysis (purity 98.0 %, MW 162.21 $gmol^{-1}$, AR Grade: Fluka, Switzerland)
- Potassium Dichromate (purity 99.9%, MW 294.22 $gmol^{-1}$: Ajax, Australia)
- Sodium Hydroxide (purity 99.0%, MW 40 $gmol^{-1}$: Lab-Scan, Ajax, Australia)
- Nitric Acid (purity 65%, AR Grade: Carlo Erba Reagent, USA)
- Ultra pure water (18.2 MΩ) (Maxima, ELGA, England)

2.1.3 Samples

Wastewater samples were collected from four ponds (facultative pond, final polishing pond 1, final polishing pond 2 and constructed wetland) in feedmill manufactory located in Banpru sub-district, Hat Yai city municipality, Songkhla province on 16 April 2007.

2.2 Instrument and apparatus

2.2.1 Graphite furnance atomic absorption spectrometry (GFAAS)

- Dell mornitor, syndrome UPS and hp laserJet 2200 printer (Perkin- Elmer, USA)
- Analyst 800 atomic absorption spectrophotometer and software Winlab 32^{TM} for AA (Perkin-Elmer, USA)
- THGA Perkin - Elmer graphite furnace and pyrolytically coated graphite tubes (Perkin-Elmer, USA)
- Hollow cathode lamp (Perkin-Elmer, USA)
- AS-800 autosample (Perkin-Elmer, USA)
- Natta automic voltage stabilizer (Perkin-Elmer, USA)
- The instrument parameters were as follows: wavelength: 357.9 nm; slit width: 0.7 nm; lamp intensity: 25 mA

The graphite furnace program is summarized in Table 2-1 shown below

Table 2-1 Graphite furnace program

Step	Temperature (^{o}C)	Ramp time (s)	Hold time (s)	Internal flow (ml/min)
Dry	110	1	30	250
Dry	130	15	30	250
Pyrolysis	Variable	10	20	250
Atomization	Variable	0	5	0
Cleaning	2450	1	3	250

2.2.2 Apparatus

- Laboratory test sieve mesh size 20 (0.800 mm), 30 (0.600 mm), 40 (0.425 mm), 50 (0.330mm) (Endecotts Ltd., England)
- pH meter model 15 (Denver Instrument, USA)
- Microliter auto pipette 1000 µL (Mettler Toledo, Germany)

- Microliter auto pipette 20, 100 and 1000 µL (Gilson ,France)
- Hotplate and stirrer
- General glassware such as volumetric 10, 25, 50, 100, 250, 500 mL; beaker 50, 100, 500 mL
- Glass column, 40 cm. x 1.7 cm. (I.D.) (Pyrex, USA)
- Polyethylene bottle 30.0, 60.0, 250.0 mL
- Polyethylene buckets 20 × 20 cm.
- Polyethylene bag

2.3 Methodology

2.3.1 Preparation of sand

The river sand was sieved to a geometric mean size, that was 0.600-0.850 mm (30-20 mesh), 0.425-0.600 mm (40-30 mesh) and 0.330-0.425 mm (50-40 mesh), distill water washed thoroughly to remove dirt, acid washed (pH 1; 24 h), rinsed with distilled water three times and dried at 110 ^{o}C for 10 h before use.

2.3.2 Preparation of 1.00 M $FeCl_3$

Working solution of 1.00 M $FeCl_3$ was prepared by dissolving a calculated amount of Iron (III) in deionized water.

2.3.3 Preparation of stock solution of chromium (VI)

Potassium dichromate had been dried for 2 h at 105 ^{o}C and was cooled before using. The stock solution of Cr (VI) was prepared by dissolving a calculated amount of potassium dichromate in deionized water (Aoki and Munemori, 1982). Working solution was prepared by diluting stock solution with deionized water to the desired concentration.

2.3.4 Preparation of working $Mg(NO_3)_2$ (matrix modifier) for determination Cr (VI) by GFAAS

Stock matrix modifier solutions are high concentrations of standard solution, working solution was prepared by diluting standard solution. The required concentration of the matrix modifier solution was calculated from the following formula

$$\% \text{ concentration of modifier solution} = \frac{\text{mass of modifier (mg) X 100}}{\text{injection volume (µL)}}$$

For example, 0.015 mg of $Mg(NO_3)_2$ of 5 µL, the concentration of modifier will be as shown

$$\% \text{ of modifier solution} = \frac{0.015 \text{ mg x } 100}{5 \text{ (µL)}}$$

$$= 0.3 \%$$

$$= 0.3 \text{ g}100 \text{ mL}^{-1}$$

$$\text{concentration of modifier solution} = 3 \text{ gL}^{-1}$$

2.3.5 Optimization of GFAAS conditions for chromium (VI) analysis

A standard furnace program usually includes the following steps:

- Drying: The solvent is vaporized during the drying steps.
- Pyrolysis: The pyrolysis step is used to remove as many matrix components as possible. A matrix modifier can be used to stabilize the analyte or aid in removal of matrix components.
- Atomization: The sample is atomized to form ground state atoms in the path of the radiation beam during this step.
- Clean-out: A high temperature clean-out step after atomization prepared the furnace for subsequent samples (AAnalyst 800, Perkin-Elmer).

The following parameters for GFAAS were studied: pyrolysis temperature and atomization temperature.

2.3.5.1 Optimization of pyrolysis temperature

The pyrolysis step is used to remove as many matrix components as possible. A matrix modifier can be used to stabilize the analyte or aid in removal of matrix components. The purpose of this topic, to optimize the pyrolysis temperature for determination Cr (VI), 20 µL of 30 µg L^{-1} Cr (VI) and 5 µL of $Mg(NO_3)_2$ were injected to the GFAAS, Perkin Elmer, AAnalyst 800 with Winlab 32 ^{TM} for AA data processor. The analysis was done by setting the atomization temperature at 2300 ^{o}C and using the temperature program obtained from Table 2-1. This was the recommended atomization temperature for Perkin Elmer, AAnalyst 800 (Perkin Elmer, 1997).

The optimum pyrolysis temperature was determined by varying pyrolysis temperature at 1100, 1200, 1300, 1400, 1500, 1600, 1650 and 1700 ^{o}C. Three replications of analysis were performed for each temperature. Measure the integrate absorbance and plotted the integrated absorbance versus the pyrolysis temperature. The optimum pyrolysis temperature was as high as possible without loss of the integrated absorbance signal (Perkin Elmer, 1997).

2.3.5.2 Optimization of atomization temperature

The atomization step is to produce an atomic vapor of the analyte elements, there by allowing atomic absorption to be measured. The GFAAS analysis was carried out on a Perkin Elmer, AAnalyst 800 (Perkin Elmer, 1997). The 20 µL of 30 µg L^{-1} Cr (VI) solution and 5 µL of Mg $(NO_3)_2$ were injected to the GFAAS. The pyrolysis temperature that used in this experimental was 1500 ^{o}C (the obtained from 2.3.5.1) and used the temperature program obtained from Table 2-1.

The optimize atomization temperature was determined by varying atomization temperature from 1900 to 2400 ^{o}C with increment of 100 ^{o}C. Three replications of analysis were performed for each temperature. Measure the integrate absorbance and plotted the integrated absorbance versus the atomization temperature. The optimum atomization temperature collected from a point where maximum absorbance occurs with minimum temperature (Perkin Elmer, 1997).

2.3.5.3 Detection limit

The detection limit is defined by the IUPAC as the concentration which will give an absorbance signal three times the magnitude of the baseline noise. The baseline noise may be statistically quantitated typically by making 10 or more replicate measurement of the baseline absorbance signal observed for an analytical blank, the determining the standard deviation of the measurement. The detection limit is then defined as the concentration which will produce on absorbance signal three times the standard deviation of blank (Beaty and Kerber, 1993).

The calibration curve of Cr (VI) was determined by GFAAS. The absorption of blank (10 replicates) was measured. The detection limit was defined by equation below (Ingle and Crouch, 1988).

$$\text{Detection limit} = (3*SD)/m$$

When, SD = standard deviation of blank

m = slope of calibration graph

2.3.5.4 Linear dynamic range (Linearity)

A series of Cr(VI) standard solution, 1-300 µg L^{-1} were prepared by diluting the stock solution Cr(VI) of 1.0 mg L^{-1} and 10.0 mg L^{-1} with deionized water. Each standard solution, 20 µL of standard solution and 5 µL of 0.015 mg $Mg(NO_3)_2$ were injected to the GFAAS by using the optimum pyrolysis temperature, atomization temperature and temperature program that were obtained from 2.3.5.1, 2.3.5.2 and Table 2-1, respectively.

Linear dynamic range was investigated by plotting the integrated absorbance of the signal versus the concentration of standard. The linearity of signal was determined by considering the correlation coefficient of the curve.

2.3.5.5 The accuracy and precision

The accuracy and precision of analytical method can be evaluated from % recovery and % RSD, respectively (EPA method 7010). The percentage of the concentration of 30 μg L^{-1} Cr (VI) solution was evaluated by using calibration graph and then % RSD and % recovery were calculated.

2.4 Chromium (VI) removal by using iron oxide-coated sand (IOCS)

The removal efficiency of Cr (VI) spiked in wastewater by IOCS depends on several parameters. These parameters are size of sand, pH of Cr (VI) solution, concentration of $FeCl_3$, flow rate, time for coating $FeCl_3$ on sand and weight of sand.

2.4.1 IOCS preparation

IOCS was prepared by adding 10 ml of 1.00 M $FeCl_3$ solution per 20 g of sand and the mixture was allowed to remain in the glass column (40 cm. x 1.7 cm (I.D.)) for 12 h. The column was washed with 20 ml of deionized water before staring the run.

2.4.2 Removal of chromium (VI)

Chromium solution of 10 mg L^{-1} was adjusted to pH 7, and passing 10 mL of chromium solution into a cleaned IOCS column with the flow rate of 5.0 mL min^{-1}. Chromium in the filtrate was determined by GFAAS and using the optimum pyrolysis temperature, atomization temperature and temperature program that were obtained from 2.3.5.1, 2.3.5.2 and Table 2-1, respectively. Each determination used 0.015 mg $Mg(NO_3)_2$ as matrix modifier.

2.4.3 Optimization of sand size

The optimum size of sand packed in the column was investigated by varying the size of sand at 0.330-0.425 (mesh size 50-40), 0.425-0.600 (mesh size 40-30) and 0.600-0.850 mm (mesh size 30-20).

The procedures of column preparation and Cr (VI) removal were described in 2.4.1 and 2.4.2. Three replicates were performed for each size of sand. The optimum size of sand was chosen the highest percentage removal of Cr (VI).

2.4.4 The comparison uncoated and $FeCl_3$ coated sand for removal Cr (VI)

The uncoated and $FeCl_3$ coated sand of size 0.330-0.425 mm were used as packing material in the column.

For coating sand, only deionized water was added. The procedures of column preparation and Cr (VI) removal were described in 2.4.1 and 2.4.2. Three replicates were performed for each condition.

2.4.5 Optimization of pH of Cr (VI) solution

The optimum of pH of Cr (VI) solution was investigated by varying the pH at 3, 5, 7, 8 and 9 to get the desired pH by adjusted the pH with 0.1 N, 0.01 N NaOH and/or HNO_3.

The procedures of column preparation and Cr (VI) removal were described in 2.4.1 and 2.4.2 but the size of sand was set according to the results of the experiments 2.4.3. Three replicates were performed for each pH. The optimum pH was chosen the highest percentage removal of Cr (VI).

2.4.6 Optimization of concentration of $FeCl_3$

The optimum of concentration of $FeCl_3$ was investigated by varying the concentration of $FeCl_3$ by diluting 1.00 M $FeCl_3$ stock solution with deionized water to the final concentration that was 0.25, 0.50, 0.70, 0.90 and 1.00 M, respectively.

The procedures of column preparation and Cr (VI) removal were described in 2.4.1 and 2.4.2 but the size of sand and pH of Cr (VI) solution were set according to the results of the experiments 2.4.3, 2.4.5, respectively. Three replicates were performed for each concentration. The optimum concentration of $FeCl_3$ was chosen the highest percentage removal of Cr (VI).

2.4.7 Optimization of flow rate through IOCS

The optimum flow rate through IOCS was investigated by varying the flow rate at 1.0, 2.5, 5.0 and 7.5 mL min^{-1}.

The procedures of column preparation and Cr (VI) removal were described in 2.4.1 and 2.4.2 but the size of sand, pH of Cr (VI) solution and the concentration of $FeCl_3$ were set according to the results of the experiments 2.4.3, 2.4.5 and 2.4.6, respectively. Three replicates were performed for each flow rate. The optimum flow rate through IOCS was chosen the highest percentage removal of Cr (VI).

2.4.8 Optimization of time for coating $FeCl_3$ on sand

The optimum time for coating $FeCl_3$ on sand was investigated by varying time at 1, 3, 5, 7, 9 and 12 h.

The procedures of column preparation and Cr (VI) removal were described in 2.4.1 and 2.4.2 but the size of sand, pH of Cr (VI) solution, the concentration of $FeCl_3$ and flow rate through IOCS were set according to the results of the experiments 2.4.3, 2.4.5 to 2.4.7, respectively. Three replicates were performed for each time. The optimum time for coating $FeCl_3$ on sand was chosen the highest percentage removal of Cr (VI).

2.4.9 Optimization of weight of sand

The optimum weight of sand was investigated by varying weight at 10, 20 and 30 g.

The procedures of column preparation and Cr (VI) removal were described in 2.4.1 and 2.4.2 but the size of sand, pH of Cr (VI) solution, the concentration of $FeCl_3$, flow rate through IOCS and time for coating $FeCl_3$ on sand were set according to the results of the experiments 2.4.3, 2.4.5 to 2.4.8, respectively. Three replicates were performed for each weight. The optimum weight of sand was chosen the highest percentage removal of Cr (VI).

2.4.10 Effect of anion on removal Cr (VI) by IOCS

The presence of various anion; NO_3^-, SO_4^{2-} and PO_4^{3-} in chromium solution was investigated by varying 500 mg L^{-1} NO_3^-, 500 mg L^{-1} SO_4^{2-} and 500 mg L^{-1} PO_4^{3-}.

The procedures of column preparation and Cr (VI) removal were described in 2.4.1 and 2.4.2; adding 10 ml of 1.00 M $FeCl_3$ solution per 30 g of sand and the mixture was allowed to remain in the glass column (40 cm. x 1.7 cm (I.D.)) for 1 h. The column was washed with 20 ml of deionized water before staring the run. The mixture of 10 mg L^{-1} Cr (VI) and 500 mg L^{-1} NO_3^- was a djusted to pH 7 and passing 10 mL of this mixture solution into a cleaned IOCS column with the flow rate of 1.0 mL min^{-1}. Chromium in the filtrate was determined by GFAAS and using the optimum pyrolysis temperature, atomization temperature and temperature program that were obtained from 2.3.5.1, 2.3.5.2 and Table 2-1, respectively. Each determination used 0.015 mg $Mg(NO_3)_2$ as matrix modifier. Three replicates were performed for each anion. The minimum effect of anion on removal Cr (VI) by IOCS was chosen the highest percentage removal of Cr (VI).

2.4.11 The comparison between the calibration and standard addition method for determination of Cr (VI) spiked in wastewater samples

The aim of this experiment was to study the effect of matrix for determination of Cr (VI) spiked in wastewater samples. The procedure of removal Cr (VI) spiked in wastewater was the same as that 2.4.1 and 2.4.2 by using the optimum conditions that obtained from 2.4.3 and 2.4.5 to 2.4.9 and then Cr (VI) was determined by using calibration and standard addition method.

2.5 Removal of Cr (VI) spiked in wastewater samples by IOCS

2.5.1 Sampling

Wastewater samples were collected from feedmill manufactory located in Banpru sub-district, Hat Yai city municipality, Songkhla province. Polyethylene bottles were washed and soaked in 10 % nitric acid for 2 days and rinsed with ultra pure water. All cleaned containers should be sealed in clean polyethylene bags until required. As soon as the sample collection was completed, one small portion was transfered to a polyethylene bottles for pH measurement. The pH of wastewater samples is shown in Table 2-2. The remaining wastewater samples were filtered through with 0.45 μm membrane syringe filter and preservation of sample by adding 1 mL of 50 % NaOH per 125 mL of sample (Parks *et al*., 2004).

Table 2-2 The pH of wastewater samples

Location	pH $\pm$ SD*
Facultative pond	7.29 $\pm$ 0.06
Final polishing pond 1	6.62 $\pm$ 0.02
Final polishing pond 2	6.69 $\pm$ 0.01
Constructed wetland	7.00 $\pm$ 0.01

* 3 replications

2.5.2 Removal of Cr (VI) spiked in wastewater samples by IOCS

Cr (VI) spiked in wastewater samples were taken from 10 mg L^{-1} Cr (VI) fed on filtered wastewater. The procedure of removal Cr (VI) spiked in wastewater was the same as that 2.4.1 and 2.4.2 by using the optimum conditions that obtained from 2.4.3 and 2.4.5 to 2.4.9. The concentration of Cr (VI) was calculated by standard addition method.