CHAPTER 3 EXPERIMENTAL SETUP

3.1 Overview

The methods of experimental are described in this chapter 3. The experiments can be divided into three parts that consist of physical and chemical properties analysis, batch test and mechanism test. Firstly, the properties of sewage sludge and alkaline are analysed to modify as biosoil under USEPA standard. Secondly the phosphorous and cadmium adsorptive capacity are examined by adsorption isotherm analysis. Moreover, the influence of phosphorus on cadmium sorption onto biosoil is also tested in this part. Thirdly, the mechanism adsorption and storage of cadmium and phosphorous in biosoils are examined by FT-IR spectrophotometer, sequential extraction procedure (SEP) and X-ray diffraction (XRD). The whole experimental setup is overviewed in Figure 3.1.

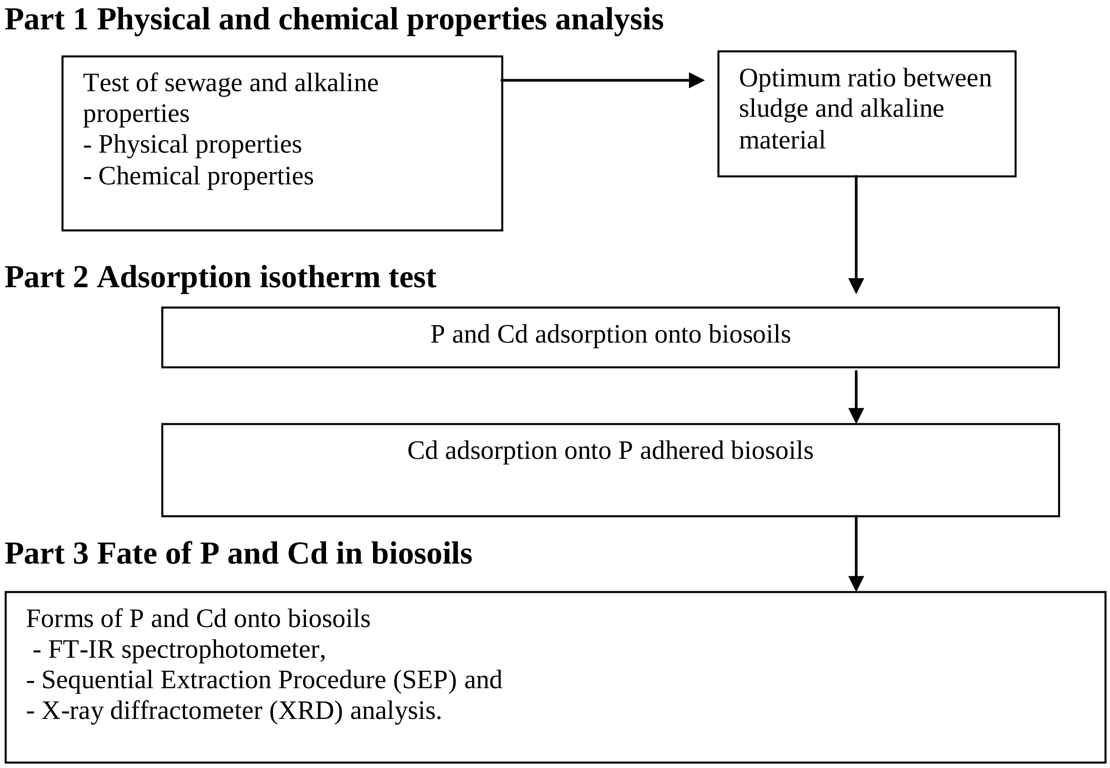

Figure 3.1 Overview of experimental set up

3.2 Materials

3.2.1 Sewage sludge

The sewage sludge is sampled from wastewater treatment plant of Sitthinan Co. Ltd., Pathumthani, Thailand, which is a glass noodle factory. The activated sludge (AS) system is served to treat the wastewater of glass noodle factory. The sludge sample is obtained at the outlet of sedimentation, before adding the polymer. The characteristics of sludge were examined by ASTM standard. The raw sludge has low solids content. Therefore, it is heated in the oven at temperature of 105 °C until the solids content is ranged 15-60% of total weight. After sewage sludge dried, it was sieved with standard sieve at a diameter 2 mm due to remove impurities. Sludge and biosoil physical and chemical properties were analysed in accordance with the ASTM standards and the Agronomy no.9 standards method. The parameters and analytical methods are given in Table 3.1.

Table 3.1 Parameters and analytical methods for testing of sludge and biosoil properties

Parameter	**Analytical method**	**Reference**
Physical properties		
Bulk density	Bulk density compact test	ASTM (1997)
Water content	Oven drying method	ASTM (1997)
Chemical properties		
Organic matter (%)	Walkley-Black procedure	Schnitzer (1982)
pH	Electrode pH meter method	Peech (1965)
Electrical conductivity, EC	Conductivity meter method	Jackson (1967)
Cation exchange capacity, CEC	Ammonium saturation	Rhoades (1982)
Total phosphorus	Perchloric acid digestion and Stannous chloride method	Olsen and Sommers (1982)
Soluble phosphorus	Stannous chloride method	Olsen and Sommers (1982)
Olsen-P	Extract by 0.5 M $NaHCO_3$ pH = 8.5 and Stannous chloride method	Olsen and Sommers (1982)
Soluble Cd	ICP- OES	

3.2.2 Fertiliser

The fertiliser used in this study was chemical fertiliser and organic fertiliser. The monopotassium phosphate (MKP) fertiliser with a chemical formulation of N-P-K at 0-52-34 was selected as single P fertiliser. This chemical was the commercial grade and widely used for cultivation. The organic fertiliser was the dried cattle manure. Properties of fertilisers were analysed using the Agronomy no.9 standards. The parameters and analytical methods are given in Table 3.2.

Table 3.2 Parameters and analytical methods for testing of fertilisers properties

Parameter	Analytical method	Reference
Organic matter (%)	Walkley-Black procedure	Schnitzer (1982)
pH	Electrode pH meter method	Peech (1965)
Electrical conductivity, EC	Conductivity meter method	Jackson (1967)
Cation exchange capacity, CEC	Ammonium saturation	Rhoades (1982)
Total phosphorus	Perchloric acid digestion and Stannous chloride method	Olsen and Sommers (1982)
Soluble Phosphorus	Stannous chloride method	Olsen and Sommers (1982)
Olsen-P	Extract by 0.5 M $NaHCO_3$ pH = 8.5 and Stannous chloride method	Olsen and Sommers (1982)

3.2.3 Alkaline additive

The alkaline reagents used in this study were the commercial grade quicklime and the fly ash. The fly ash is also obtained from Sitthinan Co. Ltd, which is residue of biomass burning boiler. The fly ash was dumped outside the factory, it was exploded to the environment. The quicklime had a general chemical formula of CaO, the commercial grade quicklime is collected from cement plant at Saraburi, Thailand. The preparation of alkaline materials is provided by mixing between quicklime and fly ash. The alkaline material is put into the sewage sludge and mixing until the biosoil reaches the USEPA criteria. The parameters and analytical methods are given in Table 3.3.

Table 3.3 Parameters and analytical methods for testing of alkaline properties

Parameter	**Analytical method**	**Reference**
Organic matter (%)	Walkley-Black procedure	Schnitzer (1982)
pH	Electrode pH meter method	Peech (1965)
Electrical conductivity, EC	Conductivity meter method	Jackson (1967)
Cation Exchange Capacity, CEC	Ammonium saturation	Rhoades (1982)
Total Phosphorus	Perchloric acid digestion and Stannous chloride method	Olsen and Sommers (1982)
Soluble Phosphorus	Stannous chloride method	Olsen and Sommers (1982)
Olsen-P	Extract by 0.5 M $NaHCO_3$ pH = 8.5 and Stannous chloride method	Olsen and Sommers (1982)
Mineral compounds	X-ray fluorescence (XRF)	

3.2.4 Synthesis cadmium wastewater

Analytical grade of $Cd(NO_3)_2$ $4H_2O$ is diluted to prepare the Cd(II) solution. The stock solutions of Cd(II) is prepared by dissolving the $Cd(NO_3)_2$ $4H_2O$ into the demineralised water and the concentration of stock solution is at 100 mg Cd/L.

3.3 Alkaline stabilised sewage sludge preparation

The alkaline material is provided by mixing between quicklime (CaO) and fly ash. The biosoil from the mixing is dried until achieving 60% of solid content. The pH value was above 12 for 12 hours that it can decrease pathogen as well as odour. The pH is maintained for 72 hours. The methods that are used to analyse biosoil properties are same as given in Table 3.1. The alkaline stabilisation is involved to support the phosphorous adsorption capacity. The samples that pass the class A criteria of USEPA standard are continuously tested the maximum phosphorous and cadmium adsorption capacity. The ratio of biosolids and alkaline materials are assigned as shown in Table 3.4.

Table 3.4 Ratios of biosolids and alkaline materials used in biosoil preparation

Ratio	Sludge (g)	Cao:FA	CaO (g)	FA (g)
70:30 (Sludge:Alkaline)	20.0	2:5	2.44	6.12
	20.0	2:6	2.14	6.43
	20.0	2:7	1.90	6.66
	20.0	2:8	1.71	6.86
50:50 (Sludge:Alkaline)	20.0	2:5	5.71	14.28
	20.0	2:6	5.00	15.00
	20.0	2:7	4.44	15.55
	20.0	2:8	4.00	16.00
60:40 (Sludge:Alkaline)	20.0	2:5	3.81	9.52
	20.0	2:6	3.33	9.99
	20.0	2:7	2.96	10.37
	20.0	2:8	2.67	10.66

3.4 Adsorption Isotherms

3.4.1 Phosphorus adsorption isotherm

Firstly, the equilibrium time of phosphorous is examined by batch experiment. In case of MKP fertiliser applying, the preparation in this study can provide by mixing 5 g of biosoil sample with 100 mL of chemical fertiliser solution. For blank preparation, the 100 mL of deionised water was used replace of chemical fertiliser solution. Another is organic manure application, the 3 g of biosoil samples were mixed with 50 mL of manure solution. For blank preparation, the 50 mL of deionised water was used instead of organic manure solution. Then the solution is shaken with the incubation shaker model LAB-Line 2528 at a speed level of 250 rpm under room temperature (25 °C)**.** The solution samples were grabbed at the periods of 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90 and 120 minutes. When the concentration of phosphorous was stable, the reaction achieves the equilibrium condition. The concentrations of phosphorous was analysed by the soluble phosphorous method (Table 3.2). All of bioslurry samples were filtrated through Whatman paper number 42. The detection of phosphorous concentration was analysed by using a UV-Visible spectrophotometer at wavelength of 470 nm. Secondly, the phosphorous adsorption capacity was examined by adsorption isotherm. The biosoil at the weights of 1, 2, 3, 4 and 5 g were mixed with 100 mL of MKP-fertiliser solution. The 1.0, 1.5, 2.0, 2.5 and 3.0 g of biosoil samples were mixed with 50 mL of organic manure solutions. These biosoil slurry samples were shaken until achieving equilibrium time. The concentrations of soluble P of every effluent samples were determined. The data was modeled with isotherm equations.

3.4.2 Cadmium adsorption isotherm

The equilibrium time was examined as same manner as the previous isotherm test. A set of 250 mL-Erlenmeyer flasks were filled with 10 g of biosoil and Cd(II) concentration 100 ppm of 100 mL under uncontrolling pH. The sludge solutions were stirred with the incubation shaker model LAB-Line 2528 with a speed of 250 rpm under room temperature (25 ^{o}C). All samples solutions were analysed at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90 and 120 minutes of continuous mixing period. The concentrations of cadmium were determined by ICP-OES. When the solution samples were constant, this means the reaction reached to the equilibrium time.

The dosage of adsorbent may influence the Cd adsorption capacity of biosoils. The testing procedure was varied for biosoil weights 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 g with 100 mL of 50.0 mg/L cadmium solutions. The samples of bioslurry were shaken for 60 minutes (equilibrium time) in the incubation shaker model LAB-Line 2528 with a speed of 250 rpm. After filtration, the supernatant solutions were analysed by ICP-OES.

The standard deviation of ICP-OES to analyse cadmium concentration was prepared in ranging of 0.01-100 mg/L.

The effect of pH was investigated in pH range 1.0-10.0. The solutions of 0.1-6.0 M HCl and solutions of 0.1-3 M NaOH were used to adjust the initial pH of Cd synthesis wastewater. The 10 g of biosoil put into100 mL of cadmium solutions containing 50 ppm in each pH value (1.0-11.0). Then all flasks are agitated on the incubation shaker model LAB-Line 2528 with a speed of 250 rpm since equilibrium time.

The adsorption isotherm of Cd onto biosoil was examined. A 10 g of biosolids is mixed with a various concentration of Cd(II) solution ranging from 10 to 90 mg/L, at controlling pH of 3.0 to prevent the Cd precipitation. The biosoil slurry samples are shaken until achieving equilibrium time. The bioslurry samples are grabbed and filtered, the effluent samples are analysed for Cd(II) concentration by ICP-OES.

3.5 Influence of phosphorus on cadmium adsorption

The examination of Cd(II) adsorption onto the biosoil that saturated with P can be prepared by each flasks are varied 100 mL aqueous solution of Cd(II) with concentration adjustment 10-90 mg/L respectively. All of flasks contain with the optimum equilibrium time, amount of biosoil and pH value that obtain from the testing of 3.6. The residual biosoils which is used in this step obtained from adsorption isotherm of P from the step of 3.5. The wet biosoil from P adsorption isotherm can be dried by freeze dryer for 6 hours until constant weight. Then all flasks are agitated on the incubation shaker model LAB-Line 2528 with a speed of 250 rpm since equilibrium time and concentrations of cadmium which are remained in the solutions are analysed by ICP-OES.

3.6 Species of sorbed cadmium and phosphorous

3.6.1 Sequential extraction

The sequential extraction procedure is used to determine the nature of any given system in terms of chemical species present and their relative mobilisations. The various selected phases consist of water soluble, exchangeable, carbonate and sulfide bound, reducible (Fe and Mn oxide bound), oxidisable (organic matter bound), and residual (lattice bound). To estimate the amount of cadmium bound into different phases in soils and sludge can conduct as follows.

Water soluble fraction step: A 1 g of dry samples with deionised are taken into 50 mL polypropylene centrifuge tube and then capped centrifuge bottles are shaken for 30 minutes. After that, it was centrifuged for 30 minutes at 2750 rpm. The next step, five drops of 1:10 HNO_3 are added in the samples and keep in refrigerator until ICP analysis. The residue was washed with 8 mL of deionised water and shaking by hand shaking followed by 30 minutes in the centrifuge at 2750 rpm. The supernatant was discarded and the residue is saved to use in exchangeable fraction step.

Exchangeable fraction step: A 8 mL of 1 $MgCl_2$ $6H_2O$ at pH 7.0 to the residue from the previous step and mechanically shaken for 1 hour at room temperature. Then the samples were centrifuged for 30 minutes at 2750 rpm and decant the supernatant through the filter 0.2 micrometer. Then five drops of 1:10 HNO_3 are added in the samples and maintained in refrigerator until ICP analysis. The residue was washed with 8 mL of deionised water and shaking by hand shaking followed by 30 minutes in the centrifuge at 2750 rpm. The supernatant was discarded and the residue is continuously used in carbonate and sulfide bound fraction step.

Carbonate and sulfide bound fraction step: A 8 mL of 1 M NaOAc was adjusted to pH 5 with CH_3COOH and take into the residue that was collected from the previous process. Then the samples were extracted with shaker for 5 hours under room temperature. Then it was centrifuged for 30 minutes at 2750 rpm. The steps after centrifuge process can follow from the Exchangeable fraction step. The residue is continuously tested in Fe and Mn Oxide bound (reducible) fraction step.

Fe and Mn Oxide bound (reducible) fraction step: A 20 mL 0.04 M $NH_2OH{\bullet}HCl$ in 25% (v/v) CH_3COOH take in to the residue which is collected from carbonate and sulfide bound fraction step and mechanically shaken for 6 hours in a water filled beaker on a plot plate under temperature 96 °C. Then it was centrifuged for 30 minutes at 2750 rpm. The steps after centrifuge can follow in part of exchangeable fraction step. The residue was collected to test in the next step.

Organic matter bound (oxidisable) fraction step: A 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2 (adjusted to pH 2 with HNO_3) are added in the residue. Then it is heated in water bath at 85 °C for 2 hours. Then a 3 mL of 30% H_2O_2 are added and adjust the pH is 2 with HNO_3 again. The samples are heated in water bath at 85 °C again for 3 hours with intermittent agitation. Then the samples are cool down under room temperature. A 5 mL of 3.2 M CH_3COONH_4 in 20% (v/v) HNO_3 and then the samples are dilute with 20 mL of deionised water. Then it is continuously agitated by mechanically shaker for 30 minutes and it was centrifuged for 30 minutes at 2750 rpm. The next step after centrifuge can be followed in step of exchangeable fraction. The residue was collected to test in the next step.

Lattice/mineral matrix bound (residual) fraction step: The residual was digested by microwave. A 2 mL of 30% H_2O_2 (adjusted to pH 2 with HNO_3), 8 mL of 65% HNO_3 were added to the residue left after collection of oxidisable fraction and digestion for 40 minutes in MLS softWAVE. Then it was centrifuged for 30 minutes at 12000 rpm, and the mineral matrix phase was collected.

All samples were filtered through membrane filters (0.2 mm pore size) after sequential extraction and digestion. The samples were analysed by ICP-OES.

3.6.2 FT-IR spectroscopy

FTIR spectroscopy can provide the information about the natures of Cd and P. These species may form bonds with the various biopolymer at the specific functional sites, including carboxyl, sulfonate, hydroxyl and amino groups. These functional groups have been proposed to respond for metal and phosphorous uptake by biosoil. The important parameters that related in the phosphorous and cadmium biosorption consist of quantity and availability of the binding sites, chemical site and affinity between of metal or phosphorous and functional site. This technique is used to detect vibration frequency changes in the biosoil with or without of cadmium and phosphorous by using a Fourier transform infrared spectrometer (PerkinElmer-Spectrum One). Pressed pellets were provided by pulverising the powder specimens with IR grade KBr in an agate mortar.

3.6.3 X-ray diffraction

The Cd-P reactions and other particular compounds were identified by XRD diffraction technique. The diffraction patterns of random oriented samples were obtained from X-ray diffractometer; Bruker AXS model D8 discover by target Cu, voltage 40kV, current 40 mA, angle 10-65 degree, increment 0.02 degree/step and scan speed 0.3 sec/step.

3.7 Summary

The batch and laboratory scale soil batch tests could provide the observation. The experiments were carefully setup and the observed data were collected and analysed. The obtained data are interpreted via the Langmuir and Fruendlich adsorption models to describe the interactions among the biosolids, alkaline materials, Cd(II) and P, which is stated in the hypothesis of this research. These results were useful as they could justify the alternative solution for reducing the leaching of P and Cd into soil. Details of laboratory setup and analytical methods were presented. The observations governed from the series experiment would be described and discussed in the next chapter.