Coaction of bio-sorption and bio-filtration for the remediation of domestic and agricultural wastewater contaminated with heavy metal.

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Abstract. Most heavy metals are well known for their toxicity and carcinogenic properties and when disposed-off through wastewater from anthropological activities poses a serious threat to the human population and other living organisms of the receiving body. The presence of heavy metals also hampers biodegrading potential for microorganism to remediate wastewater due to theirs toxic-inhibition. In this study we combine the coaction of bio-sorption and bio-filtration with aim to improve the remediation efficiency of the Copper (Cu\(^{2+}\)) contaminated domestic and agricultural wastewater samples in Nakhon Pathom Province, Thailand. Rice husk, agricultural waste from rice farming abundant in the area, was used as natural bio-sorbent material and tested to identify its optimum adsorption conditions. The adsorbed wastewater after sorption was fed through bio-filtration tank (sand-filled and bio-augmented with Pseudomonas stutzeri DMST 28410) and underwent bio-filtration regimes to complete the coaction remediation. Results from the present study showed rice husk grounded to the size of 106µm powder at optimal sorption conditions (pH 7, dosage 10g/L, mixing rate 150rpm., sorption time 90min., initial Cu\(^{2+}\) concentration 12mg/L) was effective and removed more than 40% of the metal concentration from the waste water sample. For domestic wastewater, coaction with bio-filtration further improved the copper removal efficiency up to 96% when treated for 180min. Poultry farm wastewater yielded similar copper removal results, with sorption accounted for about 36% and coaction with bio-filtration totalling up to 97% copper removal. Wastewater parameter, Chemical Oxygen Demand (COD) from both sources after bio-sorption exhibited rises in concentration. This poses the need to further explore other pre-treatment methods for rice husk for it to fully be utilized as bio-sorbent in the removal of copper and other heavy metals from wastewater.

1. Introduction
Water demand generates wastewater, the characteristics of which depend on the end use of water [1]. The discharge of these wastewater into the environment, in addition to those discharged naturally, cause contamination of the natural water systems making it unsuitable for use. Ideally the wastewater requires to be treated prior to discharge into the receiving water bodies [2].

Contamination of ground and surface water by different organic and inorganic pollutants are a major factors of environmental problems. The generally uncontrolled nature of wastewater collection causes different sources of contaminants to be mixed in the process. Often one would find the wastewater to
contain organic contaminations causing high Chemically Oxygen Demand (COD) and also inorganic contaminants like toxic chemicals or heavy metal, further causing high toxicity in the water [3]. An example being agricultural wastewater with intensive farming and heavy use of chemical herbicides, pesticides and insecticides. Effluent with high COD and high metal ions if discharged into the natural water streams without the proper treatment processes could risk contaminating the groundwater.

Different remediation processes such as chemical oxidation, coagulation, solvent attraction, membrane permeation, and adsorption have previously been employed to remove pollutants in the wastewater [4], [5], [6]. These treatment processes however often involve high material and operating costs. The inhibitory effects of the heavy metal ion in wastewater contaminated with copper further complicated the treatment process [7]. It is the ability of damaging or suppressing microbial organelles by reducing bacterial membrane permeability and damaging nucleic acids and as the result, the presence of contaminant copper significantly impact the effectiveness of the conventional wastewater treatment [8]. This presents a significant challenge for the in-situ bioremediation of wastewater at contaminated sites.

Conventional bioremediation of the wastewater mainly uses naturally occurring microorganisms and other aspects of the natural environment to degrade the wastewater of its contaminants [9]. The remediation is deemed as an important approach to the environmentally and economically sustainable water management. The degrading potential thus the remediation, however, is hampered by the presence of the toxic heavy metal. Possible counter measure to the problem could be to add a remediate system in between wastewater discharge (high COD and high metal ions) and the treated effluent water (low COD and low metal ions) prior to the conventional biological wastewater treatment (bio-filtration) unit. An additional unit aims to reduce the concentration, hence its inhibitory effect, of the metal through adsorption process so the less toxic wastewater could be treated more effective biologically. The treatment system, utilizes metal adsorption unit, is to work in conjunction with biological filtration unit. These two in-between processes: - bio-sorption (using bio-sorbent materials) to reduce the toxicity of copper and bio-filtration to further remove COD summing up the term coaction in the title of this research.

The conversion of byproduct of natural materials especially from agricultural wastes into bio-sorbent material presents an attractive, environmental friendly and economical viable alternative to other sorbent materials. Rice husk, which is the low value waste from rice farming, highly abundant in Nakhon Pathom area makes them a good source of cheap raw material as natural adsorbent. Rice husk contains about 20% silica has been reported as a good adsorbent for many metals such as lead, cadmium, selenium, copper, zinc, and mercury [10].

Major components of rice husk which may contribute for the sorption of metal ions are carbon and silica. Silica composes of silicates in which each oxygen atom is shared between two adjacent tetrahedrons. The ionic nature of the Si-O bond due to the large different their electro-negativity and benefits chemical adsorption of the metal ions. The material physical characteristics especially the bulk density of about and solid density of 0.73 g/ml 1.5g/ml respectively [11], with high porosity and large surface area per unit gram of material make them a good natural source of both physical and chemical adsorbent.

The primary objective of this study was to combine the coaction of bio-sorption and bio-filtration with aim to improve the remediation efficiency of the Copper (Cu2+) contaminated domestic and agricultural wastewater samples in Nakhon Pathom Province, Thailand. Evaluation of how various sorption parameters such as pH, agitation, reaction time, sorbent dosage and initial sorbate concentration that could influence the biodegradation potential, both the COD removal and metal reduction, of the wastewater from contaminated sites were also investigated.

2. Materials
Investigation on the coaction of bio-sorption and bio-filtration for the remediation of domestic and agricultural wastewater contaminated with copper was set up at the Department of Biotechnology Laboratory, Faculty of Engineering and Industrial Technology, Silpakorn University, Sanam Chandra
Palace Campus. The scope was set to focus on domestic and agricultural discharges at low to moderate strength COD concentrations. The sources of wastewater were selected and obtained within the vicinity areas as follow,

- Cafeteria discharge: moderate strength COD of around 500-600mg/L for the domestic wastewater samples.
- Poultry farm discharge: low strength COD of around 100-200mg/L for the agricultural wastewater samples.

Rice husk as bio-sorbent material was sourced locally and prepared (washed/oven dried and sterilized) before any modification to be used in the bio-sorption process through size reduction. Contaminant copper was prepared from Copper (II) Sulphate (CuSO₄.5H₂O) solutions with the simulated concentration of 10mg/L as the initial wastewater copper contamination.

3. Methodology

3.1. Bio-sorption

Parametric studies were conducted to determine the optimum adsorption parameters. These included:

- Size (unmodified, ground to 106µm, 150µm, 212µm respectively) - tested at mixing/agitation speed of 150rpm. and 60minutes reaction (sorption) time.
- An initial 10g/L bio-sorbent dosage then varied from 0-20g/L at 2.5g/L increments.
- An initial 10mg/L Cu²⁺ contamination concentration then varied from 0-20mg/L at 2mg/L increments.
- Reaction time from 0-180minutes at 30minutes increments.

3.2. Bio-filtration

The copper tolerance bacteria used in the study was *Pseudomonas stutzeri* DMST 28410 obtained from the Department of Medical Sciences, Thailand. Cells were harvested in the exponential growth phase with the colony forming unit per millilitre (CFU/ml) measured against McFarland standards. For this study, the bacterial concentration was prepared so its visibility was matched to Scale 1 on the McFarland standard scales in which the number of cells of 300x10⁶ CFU/ml could be approximated. Bacterial dosage was a constant 10% volume by volume through all column experiments (figure 1).

![Figure 1. Pseudomonas stutzeri DMST 28410.](image)

All filtration experiments were conducted in glass columns at three different points as follow,

- Top section (wastewater inlet): 2cm. diameter, thickness 0.2cm.
- Bottom section (treated water outlet): 0.5cm. diameter, thickness 0.15cm.
- Columns height: 30cm.

The packed column was filled with autoclaved quartz sand with particle size <0.45 µm (washed with deionized water and heating in an oven at 110°C for 4 hours to remove any traces impurities.)
Figure 2. Set-up of apparatus for sand-filled bio-augmented columns.

The bio-filtration process was performed with 3 retention stages as follow,

1) flow-through: the control valves at column inlet and outlet were set to open fully so that the incoming wastewater flowed through column without being retained.

2) 3 hours retention/contact time: the inlet control valve open to let the incoming wastewater in while the outlet control valve at bottom, of the column remain shut to achieve retention time of 3 hours.

3) 6 hours retention/contact time: Similar to the 3 hours retention period but with outlet valve remained close for 6 hours to achieve the desired retention period.

The effluent water coming out from the bio-filtration column were measured for their standard water quality parameters, conducted in triplicate for the averaging value determination.

3.3. Measurement of Standard water quality parameters

3.3.1. pH and wastewater temperature.
The pH and the temperature of the wastewater samples (both raw and treated) were measured periodically in accordance with the treatment processes.

3.3.2. Chemical Oxygen Demand (COD)
The COD levels (measured in milligram per litre of wastewater discharge) for the initial untreated raw wastewater and the treated were measured before and after the remediated experiments (both biosorption and bio-filtration) following APHA, 1998, Standard Methods for the Examination of Water and Wastewater (Methods: 5220 B. Opened Reflux Titrimetric Method).

3.3.3. Copper contamination concentration
A quantitative analysis of copper in a soluble copper salt was performed by complexometric standard titration method.

4. Results and discussion

4.1. Control condition
Cafeteria initial COD concentration was 524mg/L and with the stimulated concentration of copper contamination yielded Cu²⁺ concentration of 10.9mg/L. The higher value than the added stimulated
concentration could be attributed to the small traces amount of copper present in the wastewater prior to the addition. The COD value for poultry farm discharge was 128mg/L with Cu\(^{2+}\) approximately 10mg/L. These initial COD and copper contamination in the wastewater remained unchanged for the non-treated samples (Control condition) and were above the standard limits set out by the governing body, Pollution Control Department of Thailand, of 60mg/L. Discharging wastewater without any treatments neither in the form of bio-sorption, bio-filtration nor both resulted in wastewater not passing water regulatory limits and risk damaging the natural water conditions.

4.2. **Bio-sorption**

The optimum adsorption conditions using rice husk as bio-sorbent tested on cafeteria discharge wastewater (initial COD of 524mg/L) can be concluded as follow,

- ground to powder with 106µm. particle size
- at 150rpm mixing rate
- with 90min sorption time
- with 12mg/L initial Cu\(^{2+}\) concentration
- at 10g/L sorbent

These conditions resulted in 42.1 % of the copper contaminants being effectively removed from 12mg/L to 6.95mg/L as seen in figure 3. Rice husk with minimal modification to the actual agricultural waste material i.e. simply washed, dried, and grounded to small powder could substantially reduce almost half the initial concentration of copper in the wastewater.

4.3. **Bio-filtration**

Tests for the influence of bio-filtration on the wastewater were conducted on both wastewater sources, cafeteria and pouty farm discharges with no copper contamination. Results showed that the flow-through system was most effective in removing the COD (both moderate and low strength) from the two wastewater sources. Flow-through system achieved the highest %COD removal with 41.2% (figure 4) for cafeteria discharge and 31.3% for poultry farm, respectively. Bio-filtration through sand-filled column bio-augmented with degrading bacterium *Pseudomonas stutzeri* DMST 28410 proves sufficient in removing nearly half COD contamination from the wastewater in relatively short retention time.

4.4. **Coaction**

4.4.1. **Copper removal**
Wastewater samples were treated under coaction of bio-sorption and bio-filtration to determine their copper removal efficiency. Copper contamination in the wastewater were at the concentration of 12mg/L and 50mg/L. According to 12mg/L was the optimum concentration from conditions optimization in Section 4.2, there was also reported from previous research that Pseudomonas stutzeri bacteria was able to withstand at this concentration and still yielded considerable copper reduction. Figure 5. shows coaction resulted in higher copper reduction efficiency with improved copper removal at 50mg/L initial Cu$^{2+}$ concentration compare to the less contaminated concentration.

When considering retention periods, flow-through, 3hr or 6hr retention yielded no significant improvement in the copper removal. The maximum copper reduction of 96.2% obtained with the 3hr retention.

Similar conclusion could be drawn from agricultural sample from poultry farm discharge where coaction resulted in better copper reduction efficiency. Improvement in copper reduction at 50mg/L Cu$^{2+}$-initial concentration also was shown in figure 6. Filtration retention parameters i.e. flow-through, 3hr or 6hr yielded no significant improvement in the copper removal with maximum copper reduction 97.4% obtained at 3hr retention.

4.4.2. **COD concentration**
Wastewater parameter, Chemical Oxygen Demand (COD) from both sources after bio-sorption exhibited slight rises in concentration as seen in figure 7 and figure 8. The wastewater COD from cafeteria discharge in figure 7 was around 524mg/L initially, with 10mg/L stimulated Cu²⁺ contamination, the value first dropped down to 392mg/L before raised up to 576mg/L for 3hr and 6hr retention times, respectively. This represents an increase in COD concentration of around 10% from the initial COD. Cafeteria discharge with the initial Cu²⁺ concentration of 12mg/L and 50mg/L yielded similar increase in COD concentration, 16% and 40% for 12mg/L and 50mg/L Cu²⁺, respectively. The rises in COD concentration could be attributed to the fact that during adsorption with rice husk biosorbent, some of the organic contents of the fine powder rice husk was leached out, darker colour adsorbed water discharged out of bio-sorption process, as seen from visual inspection, causing the rises in the COD concentration.

Similar results also appear with wastewater from poultry farm discharge, presented in figure 8. The initial COD concentration of 128mg/L recorded a raise in values for all three-copper contamination concentrations (10,12 and 50mg/L). Higher copper contamination, Cu²⁺ of 50mg/L posted the highest increase in COD of up to 50%, the same as from the moderated strength COD of cafeteria discharge.

These increases in COD concentration after bio-sorption with the treated rice husk poses the need to further explore alternative pre-treatment methods for it to fully be utilized as bio-sorbent in the removal of copper and other heavy metal from wastewater. Modification methods to the raw agricultural waste materials for example through strong acidic and alkali pre-treatment have been reported to further
improved sorption properties [12][13] may help lessen the wastewater COD discharging out from the biosorption process. Further investigation into thermal modification [14] for example microwaving and carbonisation[15] present possible pre-treatment methods that could potentially be integrated in bio-sorbent preparation with the aim to fully be utilized as bio-sorbent in the removal of copper and other heavy metals from wastewater.

5. Conclusion
Treated rice husk (grounded powder) is an effective bio-sorbent material for removing heavy metal ions Cu⁺ from domestic and agricultural wastewater contaminated with copper. Coaction of bio-sorption and bio-filtration through bio-augmentation of Pseudomonas stutzeri DMST 28410 further enhanced the removal of copper metal ions in both domestic and agricultural wastewater samples. This could be attributed the process of further bio-sorption through metal uptake and/or biomineralization by microbial cell of the bacteria. An increase of wastewater COD concentration after bio-sorption by rice husk poses the need to further explore alternative pre-treatment methods or additional measures (modification to the bio-sorbent) to fully utilize its bio-sorbent material prospect.

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