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Abstract

Wastewater from squid processing has high content of organic pollutants, but low fat oil and grease content (FOG). Wastewater of the company was found to have a COD of 800-2500mg/L depending on the time of the day. Ammonium, phosphate content were much higher the limit of TCVN 5945-2005 (type B). Anaerobic treatment in a batch reactor required long retention time. After 9 days, COD value reduced from 2546 to 1973 mg/L that didn’t meet requirement of constructed wetland (CW) input. Aerobic treatment in batch reactor quickly reduced COD value to 200-400mg/L in less than a day. In an activated sludge continuous reactor, COD value reduced more than 80% in 12.7 hours, longer retention time didn’t help to lower COD content. Ammonium, nitrate, nitrite contents in all set retention times were acceptable for CW.

Two species of Limnophila and Cyperus genera have potential of using in constructed wetland (CW). Results showed that they met the conditions of high organic matter and salt content of wastewater. Both systems using these plants were equivalent in reducing COD value and phosphorous, achieved percentage 60%, 68%, respectively. The species of Limnophila genus advantaged in treating ammonium, nitrite, nitrate ions, achieved 66.3%, 76.4%, 65.0%, respectively. Biomass of the selected plants could take into account as food for animal and materials of handicraft.

Constructed wetland (CW) was cultivated Cyperus Malaccensis Lam.. Hydraulic loading rate was controlled approximately 135mm/day. Percentage of nutrition conversion of ammonium, nitrite, nitrate, total phosphorous was stable according to the time. The system had high effect in removing ammonium, nitrite, nitrate, phosphorous, 80.3±15.8%, 93.2±7.2%, 72.8±25.0%, 73.1±26.6%, respectively. Output concentrations met requirements of the Vietnamese standard QCVN 11:2008. COD value was reduced from 300-400mg/L to 91.6±9.9 mg/L. The presence of anammox strain could cause reducing concentration of nitrite remarkably.
Acknowledgement

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Finally, I would like to express deep appreciation to my lovely family, my beloved family and relatives for their love, kind support, and encouragement for the success of this study.

This thesis is dedicated for you.
Abbreviations

ABS: Absorptance
ADP: Adenosine Di phosphate
AMP: Adenosine Mono Phosphate
ATP: Adenosine Tri Phosphate
CW: Constructed Wetland
DAAD: Deutscher Akademischer Austausch Dienst (German Academic Exchange Service)
COD: Chemical Oxygen Demand
FWS: Free Water Surface
HLR: Hydraulic loading rate
HUS: Hanoi University of Science
SF: Subsurface Flow
TSS: Total Suspended Solids
TCVN: Vietnamese standard
QCVN: Vietnamese guide
VNU: Vietnam National University, Hanoi
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Introduction

Currently, although Vietnam authorities and organizations have tried much in implementing the policies and legislations on the environmental protection, the situation of polluted environment is still a very worrying issue.

With rapid speed of industrialization and urbanization, the population growth has increasingly caused severe pressure on water resources in the territories. Water source in many urban areas, industrial zones and trade villages has been increasingly polluted. In big cities, hundreds of industrial production cause of the polluting of the water source as there is no waste treatment equipment or plant. Water pollution caused by industrial production is very serious.

With abundant marine resources, seafood industry plays an important role in the economy of Vietnam. But seafood processing factories are also the major sources of pollutant to surrounding environment especially to water and soil if the wastewater is not treated properly. Conventional wastewater treatment system with aero-tank, sedimentation, disinfection in almost seafood processing plants in south of Vietnam gives unstable output with BOD, COD, nitrogen-total many times higher than allowed values of Vietnamese Standards (Department of Natural resources and environment of Hochiminh City).

Therefore, with given reasons, using constructed wetlands for treatment of wastewater in seafood processing is realistic and necessary at the moment situation of Vietnam.

Objectives of Study

- Using constructed wetland to treat seafood processing wastewater,
- Optimization pretreatment system for constructed wetland
- Selecting suitable vegetation for local environment to plant in constructed wetland.
Chapter 1: Review of the literature

The most common treatment process consists of chemical physical treatment step, and biological treatment step depending on the composition of the wastewater. Biological wastewater treatment process is more commonly used because of its high efficiency in organic matter removal. Constructed wetland system relies on the biodiversity process due to the plant and microorganisms.

1.1. Wastewater from food processing factory

Seafood processing wastewater contains highly concentrated pollutants, including suspended solids, organics and nutrients. These may deteriorate the quality of the aquatic environments into which they are discharged (Sirianuntapiboon and Nimnu, 1999). To avoid this impact, treatment of seafood processing wastewater before discharge has been proposed. A candidate method of treatment is constructed wetland. Wetlands have significant merits of low capital and operating costs compare with conventional system as activated sludge, aerated lagoon system and so on (Hammer et al., 1993; Cronk, 1996; Kadlec and Knight, 1996; Hill and Sobesy, 1998; Humenik et al., 1999; Neralla et al., 2000; Szogy et al., 2000). And the growth of non-food crops in a closed hydroponic system, using wastewater as nutrient solution, could solve in an ecologically acceptable way the wastewater problem and in the meantime produce biofuels, or other products useful for industry (Mavrogianopoulos et al., 2002). Constructed wetlands have been widely used in treating different types of contaminant found in domestic sewage, storm water, various industrial wastewaters, agricultural runoff, acid mine drainage and landfill leachate (Green and Martin, 1996; Vrhovsek et al., 1996; Higgins et al., 1993; Karathanasis and Thompson, 1995; Bernard and Lauve, 1995). Natural treatment systems have been shown to have a significant capacity for both wastewater treatment and resource recovery (Hofmann, 1996; Ciria et al., 2005; Reed et al., 1988). The wetland system was usually applied as the tertiary treatment due to the high solids content and organic matter concentration of the raw wastewater (Kadlec and Knight, 1996).
1.2. Constructed wetlands

1.2.1. General information

Constructed wetlands are engineered systems that have been designed and constructed to utilize the natural processes involving wetland vegetation, soils, and their associated microbial assemblages to assist in treating wastewater (Vymazal, J., 2006). Constructed wetland technology is more widespread in industrialized countries due to more stringent discharge standards, finance availability, change in tendency to use on-site technologies instead of centralized systems, and the existing pool of experience and knowledge based on science and practical works (Korkusuz et al., 2005).

Constructed wetlands are becoming increasingly common features emerging in landscapes across the globe. Although similar in appearance to natural wetland systems (especially marsh ecosystems), they are usually created in areas that would not naturally support such systems to facilitate contaminant or pollution removal from wastewater or runoff (Hammer, 1992; and Mitsch and Gosselink, 2000). According to Lim et al. (2003), the constructed wetlands have higher tendency to remove pollutants such as organic matters, suspended solids, heavy metal and other pollutants simultaneously. Some of the studies show that the ability of wetland systems to effectively reduce total suspended solid, biochemical oxygen demand (Watson et al., 1990 and Rousseau, 2005) and fecal coliform (Nokes et al., 1999 and Nerall et al., 2000) are well established. Nitrogen (ammonia and total nitrogen) and phosphorus are processed with relatively low efficiency by most wetland systems (Steer et al., 2005). The constructed wetlands systems can have different flow formats, media and types of emergent vegetation planted. Constructed wetlands are classified into two types in general, namely free water surface systems (FWS) and subsurface flow systems (SF).
1.2.2. Classify and design

Constructed wetlands could be classified according to the various parameters but two most important criteria are water flow regime (surface and sub-surface) and the type of macrophytic growth. Different hybrid or combined systems in order to exploit the specific advantages of the different systems.

**Figure 1-1. Basic types of Constructed Wetlands**

Constructed wetlands with surface flow (= *free water surface*, FWS) consist of basins or channels, with soil or another suitable medium to support the rooted vegetation (if present) and water at a low flow velocity, and presence of the plant stalks and litter regulate water flow and, especially in long, narrow channels, ensure plug-flow conditions (Reed et al., 1988). One of their primary design purposes is to contact wastewater with reactive biological surfaces (Kadlec and Knight, 1996). The FWS CWs can be classified according to the type of macrophytes.

Subsurface flow constructed wetlands (SSF CWs) have two typical types: horizontal flow subsurface flow (HF-SSF) CWs; vertical flow subsurface flow (VF-SSF) CWs, besides two types a combination call hybrid systems with horizontal and vertical flow.
**Horizontal Flow (HF)**

Figure 1-2 shows schematic cross section of a horizontal flow constructed wetland. It is called HF wetland because the wastewater is fed in at the inlet and flow slowly through the porous substrate under the surface of the bed in a more or less horizontal path until it reaches the outlet zone. During this passage the wastewater will come into contact with a network of aerobic, anoxic and anaerobic zones. The aerobic zones will be around the roots and rhizomes of the wetland vegetation that leak oxygen into the substrate. During the passage of wastewater through the rhizosphere, the wastewater is cleaned by microbiological degradation and by physical and chemical processes (Cooper *et al.* 1996). HF wetland can effectively remove the organic pollutants (TSS, BOD5 and COD) from the wastewater. Due to the limited oxygen transfer inside the wetland, the removal of nutrients (especially nitrogen) is limited; however, HF wetlands remove the nitrates in the wastewater.
**Vertical flow (VF)**

VF constructed wetland comprises a flat bed of sand/gravel topped with sand/gravel and vegetation (Figure 1-3). Wastewater is fed from the top and then gradually percolates down through the bed and is collected by a drainage network at the base.

VF wetlands are fed intermittently in a large batch flooding the surface. The liquid gradually drains down through the bed and is collected by a drainage network at the base. The bed drains completely free and it allows air to refill the bed. The next dose of liquid traps this air and this together with aeration caused by the rapid dosing onto the bed leads to good oxygen transfer and hence the ability to nitrify. The oxygen diffusion from the air created by the intermittent dosing system contributes much more to the filtration bed oxygenation as compared to oxygen transfer through plant. Platzer (1998) showed that the intermittent dosing system has a potential oxygen transfer of 23 to 64 g O2.m-2.d-1 whereas Brix (1997) showed that the oxygen transfer through plant (common reed species) has a potential oxygen transfer of 2 g O2.m-2. d-1 to the root zone, which mainly is utilized by the roots and rhizomes themselves. The latest generation of constructed
wetlands has been developed as vertical flow system with intermittent loading. The reason for growing interest in using vertical flow systems are:

- They have much greater oxygen transfer capacity resulting in good nitrification;
- They are considerably smaller than HF system,
- They can efficiently remove BOD5, COD and pathogens.

Figure 1-3. Schematic cross-section of a vertical flow constructed wetland (Morel & Diener 2006).

**Treatment principles for different types of CWs**

Constructed wetlands are usually designed for removal of the following pollutants in wastewater:

- suspended solids;
- organic matter (measured as BOD and COD);
- nutrients (nitrogen and phosphorus).

Treatment processes occur in about eight compartments:

- Sediment /gravel bed
- Root zone/pore water
- Litter/detritus
- Water
- Air
- Plants
- Roots
- Bacteria growing in biofilms

The treatment in the CWs is the result of complex interactions between all these compartments. Due to these compartments a mosaic of sites with different redox conditions (anaerobic, aerobic and anoxic) exists in constructed wetlands, which triggers diverse degradation and removal processes.

The general prerequisites for being able to use constructed wetlands for wastewater treatment are:

- Availability of enough space because it is a “low-rate system” with a high space requirement,
- Organic loading not too high (expressed as gBOD/m²/day),
- Hydraulic loading not too high; detention time long enough,
- Sufficient incident light to allow photosynthesis,
- Temperature not too low (CWs still work in cold climates, but designs need to be adjusted (Jenssen et al., 2008)),
- Trained maintenance staff or committed users are available who carry out the (simple) maintenance tasks,
- Wastewater not too toxic for bacteria and plants,
- Adequate quantities of nutrients to support growth.

1.2.3. Microorganisms

Microorganisms play an important role in the removal of pollutants in constructed wetlands (CWs, Tietz et al., 2008; Ahn et al., 2007; Krasnits et al., 2009). Many microorganisms play different roles in mediating mineralization or in the transformation of pollutants, such as degradation of organic matter (i.e., organic
carbon compounds, proteins, organic phosphorus and sulfur compounds), nitrogen transformations (including ammonification, nitrification and denitrification), sulfate oxidation and reduction (Ahn et al., 2007; Calheiros et al., 2009; Faulwetter et al., 2009). The substratum provides the support and attachment surface for microorganisms able to anaerobically (and/or anoxically if nitrate is present) reduce the organic pollutants into CO$_2$, CH$_3$, H$_2$S, etc. Phosphorus is adsorbed and can be implanted in the plant growth of the CW. The substratum also acts as a simple filter for the retention of influent suspended solids and generated microbial solids, which are then themselves degraded and stabilized over an extended period within the bed.

Therefore, pollutant removal and microbial communities in CWs are closely tied to the cycling of carbon, nitrogen, phosphorus and sulfur.

1.2.4. Plants

Wetland plants are prolific plants growing in water bodies. The wetland plants intercepts overland water flow and remove some or most of its sediment and nutrients, and reduce the volume of runoff (Lim et al., 2002). Bacteria that attach to the surface of wetland plants plays important role in removing pollutants in wastewater (Cronk and Fennessy, 2001). 3 types of wetland plants, which are emergent plants, submerged plants and floating plants.

Emergent plants type where, shoots distinctly above the water surface and are attached to the soil by their roots such as cattail and bulrush as shown in Figure 1-4. These plants tend to have a higher potential in wastewater treatment, because can serve as a microbial habitat and filtering medium. They are typical plants using in SSF-CWs.
Figure 1-4. Emergent plants: (a) Bulrush, (b) Cattail, (c) Reeds Submerged

Establishing vegetation is probably the least familiar aspect of wetland construction. Vegetation can be introduced to a wetland by transplanting roots, rhizomes, tubers, seedlings, or mature plants; by broadcasting seeds obtained commercially or from other sites; by importing substrate and its seed bank from nearby wetlands; or by relying completely on the seed bank of the original site. Many of the wetlands are planted with clumps or sections of rhizomes dug from natural wetlands. Propagation from seed and planting of the established plantlets is gaining popularity.

Two main techniques for planting rhizomes are:

- Planting clumps
- Planting cuttings

Clumps of rhizome mat can be excavated from an existing stand of reeds whilst minimizing damage to the existing wetland and the rhizomes clump obtained. For the small scale wetland, it can be dug out with a spade but for large-scale projects the use of an excavator is required. When transporting or storing, clumps should not be stacked. In this way the aerial stems are not damaged. The spacing of planting depends on the size of the clumps obtained. Planting 1 m² clumps, at 10 m spacing
or smaller clumps 1 or 2 m$^2$ should achieve full cover within one year depending upon mortality (Cooper et. al., 1996).

Rhizome cuttings can be collected from the existing wetlands or from commercial nurseries. Sections of undamaged rhizome approximately 100 mm long with at least one internode, bearing either a lateral or terminal bud, should be used for planting. Rhizomes should be planted with one end about a half below the surface of the medium and other end exposed to the atmosphere at spacing of about 4 rhizomes per m$^2$.

1.3. Pretreatment system

Before the wastewater can be treated in CWs, suspended solids and larger particles as well as some organic matter need to be removed. This can be achieved by:

- Pre-treatment (screens)
- Primary treatment by septic tanks, settling tanks, Imhoff tanks or anaerobic baffled reactors (ABRs).

Adequate pre-treatment is extremely important to avoid clogging of subsurface flow CWs (clogging reduces the treatment efficiency drastically by reducing the free pore spaces due to accumulation of solids).

**Aeration tank**

The aeration tank in the wastewater treatment plant provides aerobic biological treatment. Microbes utilize the organic matter in the wastewater as a food/energy source, producing additional biomass, carbon dioxide and water. The process does not include biomass collection and recycling. Biomass accumulation occurs as a result of only a portion (i.e. 37%) of the tank’s contents being removed each cycle, and therefore a certain level of suspended growth biological treatment develops (Marsh, 2007).
The aeration tank is operated as a continuous mix reactor. The air for the diffusion system is supplied by a compressor, which results in elevated dissolved oxygen (DO) levels in the tank.

1.4. Wastewater treatment by constructed wetlands

1.4.1. Microorganisms role

Biological treatment using the aerobic method is based on aerobic microbial activity in wastewater. The result of treatment is the contaminated organic matter which is mineralized into inorganic, simple gases such as CO$_2$ and water.

The treatment process consists of three stages, indicated by the reaction:

- Oxidation of organic matter: $C_nH_yO_x + O_2 \xrightarrow{Enzyme} CO_2 + H_2O + \Delta H$

- General construction of the cell:

$C_nH_yO_z + O_2 \xrightarrow{Enzyme} Microbial + CO_2 + H_2O + C_xH_yNO_2 - \Delta H$

- Self-oxidation of cell material (biodegradable):

$C_xH_yNO_2 + 5O_2 \xrightarrow{Enzyme} 5CO_2 + H_2O + NH_3 + \Delta H$

In the process of aerobic biological treatment, if the wastewater contains NH$_4^+$, it may occur nitrification as follows:

$NH_4^+ + \frac{3}{2}O_2 \xrightarrow{Nitrosomonas} NO_2^- + H_2O + 2H^+ + 275\text{ kJ}$

$NO_2^- + \frac{1}{2}O_2 \xrightarrow{Nitrobacter} NO_3^- + 75\text{ kJ}$

$NO_3^- + CH_2O + 4H^+ \xrightarrow{Denitrification} N_2 + CO_2 + H_2O$

Wastewater containing phosphorus will occur phosphorus absorption process of microbial cells under molecules as AMP, ADP, ATP.
It is well known that the ability of CWs to purify wastewater is mainly achieved by microbes and plants, e.g., microbes remove pollutants from wastewater through decomposition of organic matter, transformation of inorganic compounds (such as ammonification, nitrification and denitrification) and uptake of nitrogen and other nutrients, whereas plants remove pollutants mainly through uptake of nutrients (Ahn et al., 2007; Tietz et al., 2008; Wang et al., 2010), but the frequently asked question whether plants have effects on the structure and activity of microbial communities in CW systems for wastewater treatment is debatable. Some studies reported that plants have a major effect on the size, structure and function of microbial communities in CW systems for wastewater treatment (Collins et al., 2004; Caravaca et al., 2005; Osem et al., 2007; Calheiros et al., 2009; Kantawanichkul et al., 2009), while others have demonstrated that plants appear to have little or no effect on the performance of CW for pollutant removal, the community structure or the abundance of one or several particular functional groups of microbial organisms such as the ammonia-oxidizing bacteria (Gorra et al., 2007), methanogens and methanotrophs (DeJournett et al., 2007), or the bacterial community (Ahn et al., 2007; Baptista et al., 2008; Tietz et al., 2007).

Although the magnitude of effects of plants on microbial communities in CWs is difficult to demonstrate due to inherent variations between studies or monitoring practices (Baptista et al., 2008), the diversity–ecosystem function relationship theory in ecology provides a theoretical framework to evaluate whether plants have a strong influence on microbial communities in CW systems. Some previous studies on terrestrial ecosystems have showed that plant functional group composition of a given community tends to have a greater impact on soil microbial communities than plant species richness (Spehn et al., 2000; Johnson et al., 2003; Milcu et al., 2006).

1.4.2. Plant role

Plants absorb nitrogen from the soil as both \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) ions, but because nitrification is so pervasive in agricultural soils, most of the nitrogen is taken up as
Nitrate moves freely toward plant roots as they absorb water. Once inside the plant \( \text{NO}_3^- \) is reduced to an \(-\text{NH}_2\) form and is assimilated to produce more complex compounds. Because plants require very large quantities of nitrogen, an extensive root system is essential to allowing unrestricted uptake. Plants with roots restricted by compaction may show signs of nitrogen deficiency even when adequate nitrogen is present in the soil.

Most plants take nitrogen from the soil continuously throughout their lives and nitrogen demand usually increases as plant size increases. A plant supplied with adequate nitrogen grows rapidly and produces large amounts of succulent, green foliage. Providing adequate nitrogen allows an annual crop, such as corn, to grow to full maturity, rather than delaying it. A nitrogen-deficient plant is generally small and develops slowly because it lacks the nitrogen necessary to manufacture adequate structural and genetic materials. It is usually pale green or yellowish, because it lacks adequate chlorophyll. Older leaves often become necrotic and die as the plant moves nitrogen from less important older tissues to more important younger ones.

On the other hand, some plants may grow so rapidly when supplied with excessive nitrogen that they develop protoplasm faster than they can build sufficient supporting material in cell walls (Don Eckert).

**1.4.3. Removing of organic materials**

Wetland systems have the capability to remove organic priority compounds in wastewater primarily by mechanisms including volatilization, adsorption, microbial degradation, and plant uptake. Bacterial degradation of organic priority pollutants under both aerobic and anaerobic conditions has been shown to be feasible but adsorption of the pollutants onto the biofilms must precede the acclimation and biodegradation processes. Organic priority pollutants can also be removed by physical adsorption onto settleable solids followed by sedimentation. This often occurs in the initial portion of the bed. Removal by plant uptake has been reported
but the significance of the pathway is relatively unknown and may be dependent on plant species and pollutant characteristics.

There is concern about the fate of many trace organic compounds in the environment. These include pesticides, fertilizers, process chemicals, and others that fall under the category of priority pollutants. The fate of these compounds in wetlands is dependent on the properties of the compound, the characteristics of the wetland, the species of plants, and other environmental factors. The most important separation and transformation mechanisms involved include volatilization, sedimentation/interception, biodegradation, adsorption, and uptake. These mechanisms have been discussed previously. Recalcitrant organics that have been separated may accumulate in the wetland sediments. Some may be taken up by plants and be returned to the system upon plant decomposition. Biodegradation of some organic compounds may result in completely mineralized end products, or the process may produce end products that may be more toxic than the parent compound. At this time, there is insufficient data available on full-scale wetland systems to evaluate how effective they are in the long-term removal and destruction of most priority pollutants. Based on pretreatment performance, oxidation or facultative lagoons remove a high percentage of volatile and semi-volatile organic compounds (Hannah et al., 1986), resulting in low influent concentrations to the FWS system that follows, while primary sedimentation is less effective and results in higher influent concentrations of both to subsequent VSB systems.

Table 1-1. Pollution Remove Mechanisms in constructed wetlands (Cooper et al...1997)

<table>
<thead>
<tr>
<th>Wastewater constituents</th>
<th>Removal mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspended solids</td>
<td>- Sedimentation</td>
</tr>
<tr>
<td>Soluble organics</td>
<td>- Filtration</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>- Aerobic microbial degradation</td>
</tr>
<tr>
<td></td>
<td>- Anaerobic microbial degradation</td>
</tr>
<tr>
<td></td>
<td>Matrix sorption</td>
</tr>
<tr>
<td></td>
<td>Plant uptake</td>
</tr>
</tbody>
</table>
1.4.4. Nitrogen removal

In wetlands systems, nitrogen transformations take place in the oxidized and reduced layers of soil, the root-soil interface and the submerged portions of the emergent plants. Removal of nitrogen in wetlands is achieved through three main mechanisms, which are nitrification/denitrification, volatilization of ammonia and uptake by plants.

Organic Nitrogen is mineralized to NH$_4^+$ in both oxidized and reduced soil layers. The oxidized layer and the submerged portions of plants are important sites for nitrification in which NH$_4^+$ is converted to NO$_2^-$ by *Nitrosomonas* and eventually to NO$_3^-$ by *Nitrobacter* bacteria. At higher pH, some NH$_4^+$ exists in the form of NH$_3$ and is lost to the atmosphere by the volatilization process. Figure 1-5 depicts the processes of nitrogen removal in the flooded soil environment. Nitrate in the reduced zone is depleted through denitrification, leaching and some plant uptake (Eng, 2002). Submerged plant provided more organic material of high quality to support heterotrophic organisms. It is also possible that the surfaces of submerged plant offered more suitable surfaces for bacterial growth and thereby increased the bacterial population (Bastviken *et al.*, 2005).

<table>
<thead>
<tr>
<th>Nitrogen</th>
<th>Ammonification followed by microbial nitrification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Denitrification</td>
</tr>
<tr>
<td></td>
<td>Plant uptake</td>
</tr>
<tr>
<td></td>
<td>Matrix absorption</td>
</tr>
<tr>
<td></td>
<td>Ammonia volatilization (mostly in SF system)</td>
</tr>
<tr>
<td>Metal</td>
<td>Absorption and cation exchange</td>
</tr>
<tr>
<td></td>
<td>Complexation</td>
</tr>
<tr>
<td></td>
<td>Precipitation</td>
</tr>
<tr>
<td></td>
<td>Plant uptake</td>
</tr>
<tr>
<td></td>
<td>Microbial Oxidation/ reduction</td>
</tr>
<tr>
<td>Pathogens</td>
<td>Sedimentation- Filtration- Natural die-off-</td>
</tr>
<tr>
<td></td>
<td>Predation- UV irradiation</td>
</tr>
<tr>
<td></td>
<td>(SF system)</td>
</tr>
<tr>
<td></td>
<td>Excetion of antibiotic from roots of macrophytes</td>
</tr>
</tbody>
</table>
As far as the root-soil interface is concerned, oxygen from the atmosphere diffuses into the rhizosphere through the leaves, stems, rhizomes and roots of the wetlands plants and creates anoxic layer similar to that existed at the soil-water interface (refer Figure 1-5) (Maehlum, 1999; Johnson et al., 1999). Nitrification takes place in the aerobic rhizosphere where $\text{NH}_4^+$ is oxidized to $\text{NO}_3^-$. The $\text{NO}_3^-$ not taken up by plants diffuses into the anoxic zone where it is reduced to $\text{N}_2$ and $\text{N}_2\text{O}$ by the denitrification process. Ammonium in the rhizosphere is replenished by $\text{NH}_4^+$ in the anoxic zone by diffusion.

### 1.4.5. Phosphorus removal

The phosphorus removal mechanisms in wetland systems include vegetation uptake (Fraser et al., 2004; Huett et al., 2005), microbial assimilation, adsorption onto soil and organic matter, and precipitation with $\text{Ca}^{2+}$, $\text{Mg}^{2+}$, $\text{Fe}^{3+}$ and $\text{Mn}^{2+}$. Adsorption and precipitation reactions are the major removal pathways when the hydraulic retention time is longer and finer-textured soils are being used, since this allows greater opportunity for phosphorus sorption and soil reactions to occur. Adsorption and precipitation reactions merely trap the phosphorus in the wetland.
soil. Once the storage capacity has been exceeded, the soil / sediment have to be dredged for ultimate disposal. The mechanisms for phosphorus removal in constructed wetlands are adsorption, complication and precipitation, storage, plant uptake and biotic assimilation (Watson et al., 1989).

**Figure 1-6. Phosphorus cycling in a FWS wetland (adapted from Twinch and Ashton, 1983)**

### 1.4.5. Pathogen removal

Pathogens are removed in wetland during the passage of wastewater through the system mainly by sedimentation, filtration and adsorption by biomass. Once these organisms are entrapped within the system, their numbers decrease rapidly, mainly by the processes of natural die-off and predation (Cooper et al., 1996)

### 1.4.6. Acidity - Alkalinity

pH affects the chemical nature of water and the creatures in the constructed wetlands reactions in the biological processes that occur in a limited range of pH, such as treatment by microorganisms will occur. In the pH range of 4.0 to 9.5 and the reaction is nitric filter applications. The creature is in the range pH 6.5 to 7.5. It does best in a pH of 7.2 or more, and so on.
Chapter 2: Materials and Method

2.1. Chemicals and equipment

- **Chemical:**
  Grade of all chemicals using in experiments was pure analysis, including:
  - Potassium dichromate (K₂Cr₂O₇)
  - Sulfuric acid (H₂SO₄ 98%)
  - Silver sulfate (Ag₂SO₄)
  - Salt Mohr
  - Mercury (II) sulfate (HgSO₄)
  - Ferroin indicator
  - KNaC₄H₄O₄.4H₂O, NaOH
  - HgI₂, KI, KOH
  - Sulfanilic acid(C₆H₇NO₃S)
  - α-naphtylamine (C₁₀H₇NH₂)

- **Equipment:**
  - Cone Can, spheres, electric stove, burette, pipette...
  - Spectrophotometer, pH meter,
  - Pilot treatment system: pretreatment system, CW.

2.2. Equipment design

2.2.1. Aeration tank design

Aeration was designed in form of parallelepiped have volume of 190L (Figure 2-1). There are three inlets at bottom for air, wastewater feeding, and recycling sludge.

The lab scale pilot as in the figure 2-1 had total capacity of 250L, wastewater was stored in the tank 1 then pulped through a sieve, solid part stored in the tank 3, liquid was stored in the tank 2, and here input concentration was control. Then wastewater went into the reactor tank 4 with manual pre-setted flow rate then
wastewater went pass settling tank. A part or all of sludge in the settling tank was recycling. The effluent of the system was analysis to ensure that it met the requirement of the influent of CW.

![Figure 2-1. Laboratory wastewater treatment systems](image)

### 2.2.2. CW design

Design of the CW pilot was based on CW manual (UN-HABITAT, 2008 and Suwasa Kantawanichkul, 2009). The CW was VF-SSF CW type, cultivated *Cyperus Malaccensis* Lam..

Dimension of the CW was height x width x depth \(= 1.5 \text{ m} \times 0.75 \text{ m} \times 1 \text{ m}\), had volume of 1.125m³.

Material layers: bottom layer (4) was 10 cm thickness, used gravel with 3 cm diameter, middle layer (3) was 10cm thickness, and used smaller gravel with 1cm diameter, top layer was 60 cm thickness, used sand. The CW had capacity of 250L of water.

The CW had one inlet and 4 outlets. The outlets had different depth to take wastewater sample: level 1 was 20cm depth, level 2 was 40cm depth, level 3 was 60cm depth, and level 4 was 80cm depth.

![Figure 2-2. Constructed wetland design](image)
2.3. Experiment design

2.3.1. Batch experiments

The real samples, wastewater, were taken from Quang Ninh Seafood Process and Export Plant No. 2. To evaluate effect of anaerobic and aerobic treatment to the samples two batch experiments were carried out in a 20L reactor without and with strong air supply. Concentrations of ammonia, nitrite, nitrate, phosphate, and pH value were monitored every day from May 13\textsuperscript{th} 2011 to May 28\textsuperscript{th} 2011.

Results of batch treatment were used to select using the continuous aeration tank (4).

2.3.2. Flow rate optimization of the pretreatment system

The pre-treatment system had the volume of 250L, and operated continuously, it needed a large volume of wastewater. Thus, artificial wastewater was made of squid rubbish with supplement substances to create a similar environment of wastewater from squid processing plant had provided for the systems. Continuous biological treatment had conducted by pumping the wastewater into the system. The process used settling tank to collect biomass for returning to the aeration tank and discharging when exceeding the demand. Settling tank is not only for deposition but also as an anaerobic tank, thus it also contributes to NO$_3^-$ on de-nitrate reaction. The system was operated at pre-setted retention time according to the table 2.
Table 2-1. Flow rate and corresponding retention time and continuous operation conditions

<table>
<thead>
<tr>
<th>Flow rate (L/h)</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time whole system (h)</td>
<td>83.3</td>
<td>41.7</td>
<td>27.8</td>
<td>20.8</td>
<td>16.7</td>
</tr>
<tr>
<td>Retention time of aeration tank (h)</td>
<td>63.3</td>
<td>31.7</td>
<td>21.1</td>
<td>15.8</td>
<td>12.7</td>
</tr>
<tr>
<td>Retention time of settling tank (h)</td>
<td>20.0</td>
<td>10.0</td>
<td>6.7</td>
<td>5.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

2.3.3. Plant selection

Several plants was considering in our research as elephant grass, reed, friendship bamboo (*Dracaena sanderiana* Hort.) two plants had advantage character are showed in figure 2-3.

![Figure 2-3. Two species of Limnophila (b) and Cyperus (a) genera](image)

Biomass of two plants are useable, *Cyperus* genus can feed animal, *Limnophila* genus (sedge) is used as material of weave craft. Plants were grown in 30 cm depth basins with sand layer was 15cm depth and 1000cm² area. At bottom of each bucket was water collecting system, wastewater was controlled 5-10cm above the sand surface. The volume of the bucket was 30L. Plants had a similar density in each basin. The growth of plants was monitored in two periods: adative period and stable period.
The adaptive period: at beginning plants were grown in wastewater medium then kept water level in basin by adding supply water. This period was about 30 days length, finished when COD value went down 80% and there were buds of new plants. The stable period: wastewater was delivered semi-continuous. Wastewater samples of top and bottom of basins were taken at the same time for determination of ammonium, nitrite, nitrate, phosphate concentration, COD, pH value.

2.3.4. CWs operation

Wastewater run out the pre-treatment system was fed CW at the hydraulic loading rate: 135mm/day (135 L.m⁻².day⁻¹). Samples were taken on surface and at four levels (figure 2-2) from June 25th, 2011 to September 24th, 2011.

2.4. Procedures and analysis method

Effect of treatment process was evaluated from concentrations of anions NO₂⁻, NO₃⁻ and PO₄³⁻, and COD, pH value of samples before and after treatment. Determination methods of pH, COD, NO₂⁻, NO₃⁻ and PO₄³⁻ were based on Vietnamese standards TCVN 6492: 1999, TCVN 6491: 1999, TCVN 6178:1996, TCVN 6180:1996, TCVN 6180:1996, respectively.

2.4.1. Determination of COD

Reagent preparation

Using K₂Cr₂O₇ solution as strong oxidizing agents to oxidize organic compounds in acidic medium of H₂SO₄ and using crystals Ag₂SO₄ to catalyze the reaction according to the equation:

\[
\text{Organic matter} + K_2Cr_2O_7 + 2H^+ \rightarrow CO_2 + H_2O + 2Cr^{3+} + 2K^+ 
\]

The remaining amount of K₂Cr₂O₇ is titrated with Mohr salt solution [Fe(NH₄)₂(SO₄)₂] using Ferroin indicator.

Cl⁻ is regularly presented in the water causing the error of analysis.

\[
Cr_2O_7^{2-} + 6 Cl^- + 14H^+ \rightarrow 3Cl_2 +2Cr^{3+} + 7H_2O
\]

So, Hg₂SO₄ solution is used to remove the effect of Cl⁻.
Procedure

Pour \( V_m = 5 \text{ mL} \) of sample into a reflux flask, add \( V_1 = 5 \text{ mL} \) of mixture solution of 0.25 N HgSO\(_4\) and K\(_2\)Cr\(_2\)O\(_7\), adding 10 ml mixture solution of H\(_2\)SO\(_4\) and Ag\(_2\)SO\(_4\) contents of 11 grams/liter Ag\(_2\)SO\(_4\) in concentrated H\(_2\)SO\(_4\) solution, adding 2 to 3 boiling stones. The mixture in the flask was well mixed. Apply heat to the flask and reflux for 2 hours. Allow the flask to cool and wash down the condenser with about 25 mL of distilled water. Add 1-2 drops of indicator Ferroin, and mixing and titrate the excess K\(_2\)Cr\(_2\)O\(_7\) with 0.1 N Mohr salt to the end point.

Calculation

The COD value is determined by the formula:

\[
\text{COD} = \frac{(V_1 \cdot N_1 - V_{\text{mohr}} \cdot N_2)}{V_m} \times 8 \times 1000
\]

In which: \( V_m \): volume of sample (ml).

\( V_1 \): K\(_2\)Cr\(_2\)O\(_7\) solution volume (ml)

\( V_{\text{mohr}} \): Mohr salt (ml).

\( N_1 \): Equivalent concentration of K\(_2\)Cr\(_2\)O\(_7\) (N)

\( N_2 \): The equivalent salt concentration Morh (N).

8: gram-equivalent of oxygen

1000: Conversion factor from liters to ml.

2.4.2. Determination of ammonium by colorimetric method with Nessler indicator

Reagent preparation

Principle of the method is in alkaline medium, NH\(^{4+}\) reacts with K\(_2\)HgI\(_4\) to form yellow-brown precipitate (NH\(_2\)HgI\(_3\)):

\[
\text{NH}_4^+ + \text{OH}^- \rightarrow \text{NH}_3 + \text{H}_2\text{O}
\]

\[
2\text{K}_2\text{HgI}_4 + \text{NH}_3 + \text{KOH} \rightarrow \text{NH}_2\text{HgI}_3 + 5\text{KI} + \text{H}_2\text{O}
\]
Depending on the concentration of NH₄⁺ in solution, the complex is from yellow to red brown and stable for about 1 hour.

Factors hinder the determination of ammonia by this method are water hardness, iron, sulphide, chlorine, turbidity of the water. Complexion III is used to overcome the hard water. The iron ion, sulfite, and the turbidity are eliminated by adding the zinc salt (add 1mL of 10% ZnSO₄.7H₂O to 100 mL water sample). Obstacle of chlorine at levels of 0.01 mg/l is eliminated by adding sodium thiosulfate or sodium arsenate.

**Procedure**

- Solution A: Dissolve 0.2965 g of dried NH₄Cl (dry at 105°C for 2 hours) with distilled water in a 1L flask. Obtained solution has concentration of NH₄⁺ 100 mg/L.

- Solution B: From Solution A we dilute into 10 times to obtain the solution of NH₄⁺ concentration of 10 mg/L.

Seignette and Nessler solution

- Seignette solution: Dissolve 50 g of potassium sodium tartrate in 10% NaOH solution.

- Nessler solution:
  - Mix 4.55 grams of HgI₂ with 3.49 grams of KI then dissolve with about 30 ml of distilled water. We obtained solution 1.
  
  + Mix 11.2 g of KOH with about 30 ml of distilled water (mix separately). We obtain solution 2. Let the solution1 and solution 2 until they are cool; pour both these two solutions into 100 ml volumetric flask. Store the solution into a dark vial. The new solution can be used after 3 to 5 days.

Dilute sample by distilled water so that its concentration is in the linear range. Take 5 ml sample into clean and dry test tube, add 0.2 ml of Seignette and 0.3 ml of Nessler, shake well then let stand for 10 minutes and measured absorbance at a
wavelength of 420 nm. Calculate the concentration of ammonium in the sample according to equation of standard curve.

**Standard curve**

**Table 2-2. Data of standard curve NH₄⁺**

<table>
<thead>
<tr>
<th></th>
<th>Solution B (mL)</th>
<th>Distilled water (mL)</th>
<th>Seignette (mL)</th>
<th>Nessler (mL)</th>
<th>[NH₄⁺] (M)</th>
<th>ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>5.0</td>
<td>0.2</td>
<td>0.3</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>4.8</td>
<td>0.2</td>
<td>0.3</td>
<td>0.04</td>
<td>0.036</td>
</tr>
<tr>
<td>3</td>
<td>0.4</td>
<td>4.6</td>
<td>0.2</td>
<td>0.3</td>
<td>0.08</td>
<td>0.097</td>
</tr>
<tr>
<td>4</td>
<td>0.6</td>
<td>4.4</td>
<td>0.2</td>
<td>0.3</td>
<td>0.12</td>
<td>0.157</td>
</tr>
<tr>
<td>5</td>
<td>0.8</td>
<td>4.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.16</td>
<td>0.20</td>
</tr>
<tr>
<td>6</td>
<td>1.0</td>
<td>4.0</td>
<td>0.2</td>
<td>0.3</td>
<td>0.20</td>
<td>0.253</td>
</tr>
<tr>
<td>7</td>
<td>1.2</td>
<td>3.8</td>
<td>0.2</td>
<td>0.3</td>
<td>0.24</td>
<td>0.306</td>
</tr>
<tr>
<td>8</td>
<td>1.4</td>
<td>3.6</td>
<td>0.2</td>
<td>0.3</td>
<td>0.28</td>
<td>0.352</td>
</tr>
<tr>
<td>9</td>
<td>1.6</td>
<td>3.4</td>
<td>0.2</td>
<td>0.3</td>
<td>0.32</td>
<td>0.406</td>
</tr>
<tr>
<td>10</td>
<td>1.8</td>
<td>3.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.36</td>
<td>0.455</td>
</tr>
<tr>
<td>11</td>
<td>2.0</td>
<td>3.0</td>
<td>0.2</td>
<td>0.3</td>
<td>0.40</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Figure 2-4. Standard curve of NH₄⁺**

\[ y = 1.272x - 0.003 \]

\[ R^2 = 0.999 \]
2.4.3. Determination of \( \text{NO}_2^- \) concentration in water by colorimetric method with Griss reagent

Reagent preparation

The general principle is that in acetic acid medium, \( \text{NO}_2^- \) ion reacts with acid sulfanilic and \( \alpha \)-naphtylamine to form a red compound

\[
\text{C}_6\text{H}_4(\text{NH}_2)\text{SO}_2^-\text{OH} + \text{HNO}_2 \rightarrow \text{C}_6\text{H}_4(\text{N} = \text{NOH})\text{SO}_2^-\text{OH}
\]

\[
\text{C}_6\text{H}_4(\text{N} = \text{NOH})\text{SO}_2^-\text{OH} + \text{C}_{10}\text{H}_7\text{NH}_2 \rightarrow \text{C}_6\text{H}_4(\text{N} = \text{N} - \text{C}_{10}\text{H}_6\text{NH}_2)\text{SO}_2^-\text{OH}
\]

Color intensity is proportional to the content of \( \text{NO}_2^- \) in water. Absorbance was measured at 520 nm wavelength.

Procedure

- Sulfanilic acid solution: Dissolve 0.5 g of sulfanilic acid in 150 ml of 12% \( \text{CH}_3\text{COOH} \). Keep the obtained solution in dark vial.

- \( \alpha \) – naphtylamine solution: Weigh 0.1 g \( \alpha \) - naphtylamine for a glass containing about 240 ml of distilled water; boil on electric stove until the volume to about 200 ml. Then move 200 ml solution above into a dark vial containing 150 ml of \( \text{CH}_3\text{COOH} \) acid 12%.

- To get 150 ml of 12 % \( \text{CH}_3\text{COOH} \) solution: mix 18.4 ml of \( \text{CH}_3 \text{COOH} \) (99.5%) with 131.6 ml of distilled water.

Standard curve

- Solution A: Dissolve 0.1497 g of pure \( \text{NaNO}_2 \) (dry at 105°C for 2 hours) into 1L flask; diluted to 1 liter, we obtained a 0.1 mg/ml solution of \( \text{NO}_2^- \).

- Solution B: Dilute 100 times solution A with distilled water, obtain a 0.001 mg/ml solution of \( \text{NO}_2^- \), using within the day.

- Analysis of samples: Take 5 ml sample into a clean and dry test tube, measure the absorbance (ABS) at the wavelength of 520 nm.
- Set up the standard curve: Take into clean, dry test tubes the volumes of solution standard B, distilled water and the reagents are as follows:

### Table 2-3. Data of NO\textsubscript{3}\textsuperscript{-} standard curve

<table>
<thead>
<tr>
<th></th>
<th>Solution B (ml)</th>
<th>Distilled water (ml)</th>
<th>Sulfanilic (ml)</th>
<th>α-naphtylamine (ml)</th>
<th>[NO\textsubscript{3}\textsuperscript{-}] (M)</th>
<th>ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>5.0</td>
<td>1</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>4.8</td>
<td>1</td>
<td>1</td>
<td>0.04</td>
<td>0.028</td>
</tr>
<tr>
<td>3</td>
<td>0.4</td>
<td>4.6</td>
<td>1</td>
<td>1</td>
<td>0.08</td>
<td>0.053</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>4.5</td>
<td>1</td>
<td>1</td>
<td>0.10</td>
<td>0.066</td>
</tr>
<tr>
<td>5</td>
<td>0.8</td>
<td>4.2</td>
<td>1</td>
<td>1</td>
<td>0.16</td>
<td>0.099</td>
</tr>
<tr>
<td>6</td>
<td>1.0</td>
<td>4.0</td>
<td>1</td>
<td>1</td>
<td>0.20</td>
<td>0.126</td>
</tr>
<tr>
<td>7</td>
<td>1.5</td>
<td>3.5</td>
<td>1</td>
<td>1</td>
<td>0.30</td>
<td>0.183</td>
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<tr>
<td>8</td>
<td>2.0</td>
<td>3.0</td>
<td>1</td>
<td>1</td>
<td>0.40</td>
<td>0.25</td>
</tr>
<tr>
<td>9</td>
<td>2.5</td>
<td>2.5</td>
<td>1</td>
<td>1</td>
<td>0.50</td>
<td>0.311</td>
</tr>
<tr>
<td>10</td>
<td>3.5</td>
<td>1.5</td>
<td>1</td>
<td>1</td>
<td>0.70</td>
<td>0.431</td>
</tr>
<tr>
<td>11</td>
<td>5.0</td>
<td>0.0</td>
<td>1</td>
<td>1</td>
<td>1.00</td>
<td>0.601</td>
</tr>
</tbody>
</table>

![Figure 2-5. Standard curve of NO\textsubscript{3}\textsuperscript{-}](image)

\[ y = 0.603x + 0.004 \]
\[ R^2 = 0.999 \]

#### 2.4.4. Determination of NO\textsubscript{3}\textsuperscript{-} concentration

NO\textsubscript{3}\textsuperscript{-} ion reacts with phenoldisulfonic form acidic nitro phenoldisulfonic, this acid give yellow color in the presence of NH\textsubscript{3}. Color intensity is proportional to the content of NO\textsubscript{3}\textsuperscript{-} in solution. Formed compounds absorb light at a wavelength of 410 nm.
Reagent

Take 50 ml liquefied phenol into 500 ml flask, and then adds 100 ml of H$_2$SO$_4$ 98%. Shake well then heat the mixture in 1 hour 30 minutes until the liquid turns brown.

Procedure

- Solution A: Dissolve 0.1631 g KNO$_3$ (dry at 105°C in 2 hours) in 1L flask. Obtained solution is NO$_3^-$ concentration of 0.1 mg/ml.
- Solution B: From the standard solution A, we dilute 10 times then obtain the 0.01 mg NO$_3^-$ solution.

Dilute the sample with distilled water so that sample concentration is in the linear range. Take 5 ml samples into the heat-resistant glass, dry on the electric stove covered with asbestos until the samples dry completely, and let for the glass cool then pour 70 to 10 ml of distilled water, add 0.5 ml phenoldisulfonic, add 5 ml solid NH$_3$, shake and transfer the entire solution in the glass into 25 ml volumetric flask. Let stand for 10 minutes then measure the absorbance at a wavelength 410 nm.

Standard curve

Take the 10 mg/L NO$_3^-$ solutions and distilled water into a glass and then dry on the electric stove covered with asbestos, adding more reagents and the solution of NH$_3$ as in the following table:

**Table 2-4. Results of standard NO$_3^-$**

<table>
<thead>
<tr>
<th></th>
<th>Solution B (ml)</th>
<th>Distilled water(ml)</th>
<th>Phenol disulfonic (ml)</th>
<th>Ammonium (ml)</th>
<th>[NO$_3^-$] (M)</th>
<th>ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>5.0</td>
<td>0.5</td>
<td>5.0</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>4.8</td>
<td>0.5</td>
<td>5.0</td>
<td>0.04</td>
<td>0.029</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>4.5</td>
<td>0.5</td>
<td>5.0</td>
<td>0.1</td>
<td>0.041</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>4.0</td>
<td>0.5</td>
<td>5.0</td>
<td>0.2</td>
<td>0.057</td>
</tr>
<tr>
<td>5</td>
<td>1.5</td>
<td>3.5</td>
<td>0.5</td>
<td>5.0</td>
<td>0.3</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
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</tr>
<tr>
<td>6</td>
<td>2.0</td>
<td>3.0</td>
<td>0.5</td>
<td>5.0</td>
<td>0.4</td>
<td>0.082</td>
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<td>5.0</td>
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<td>0.5</td>
<td>5.0</td>
<td>0.8</td>
<td>0.137</td>
</tr>
<tr>
<td>9</td>
<td>5.0</td>
<td>0.0</td>
<td>0.5</td>
<td>5.0</td>
<td>1.0</td>
<td>0.169</td>
</tr>
</tbody>
</table>

**Figure 2-6. Standard curve of NO₃⁻**

### 2.4.5. Determination of phosphorus by mean of optical measurement with reagents Ammonium molybdate-vanadate

In the acidic medium, ammonium molybdate reacts with octophosphate solution form acid acid molypdophosphoric. In the presence of vanadate, yellow vanadomolybdophosphoric acid is formed. Color intensity shows the concentration of phosphate presents in solution with minimum detectable concentration was 10⁻⁴ g/L. The hindered factors caused by the presence of silicon dioxide (SiO₂) and arsenate when samples are heated.

The impact of negative factors is due to the presence of the ion: AsO₃⁻, F⁻, Br²⁺, SO₃²⁻, S₂O₈²⁻, or excess amount of molybdate. The blue color of the solution may be formed due to the presence of iron but does not affect the analysis. The ions do not affect the analysis when the concentration exceeds 100 (mg/L) include: Al³⁺, Fe²⁺, Mg²⁺, Ca²⁺, Ba²⁺, Sr²⁺, Na⁺, K⁺, the sodium nitrate, nitrite, sulfate, tetra borate...
Reagent preparation

- Solution A: Weight exactly 25 g (NH₄)₂MoO₄ to the beaker. Then use distilled water to dilute into 300 ml.

- Solution B: Weigh exactly 1.25 g ammonium vanadate NH₄VO₃ to the beaker. Add 300 ml of distilled water, boiled on the electric stove covered with asbestos until it melt, cooling, and then put more 330 ml of concentrated hydrochloric acid, shake, let it is cool to the room temperature and then pour this solution and solution A into an 1L flask, let it is cool to room temperature then dilute to 1000mL.

- Prepare standard solution PO₄³⁻: Dissolve 0.1211 g NaH₂PO₄·H₂O (dried at 105°C in 2 hours) in 1L flask. Dilute to 1000mL we obtain 0.1 mg/ml PO₄³⁻ solution.

Standard curve

Add 17.5 ml of sample in 25 ml volumetric flask. Add 5 ml of vanadate molybdate reagent and dilute to the mark. Shake well and let it’s stand for 10 minutes and measure the absorbance at the wavelength of 470 nm.

Pour the 0.1 mg/ml solution of PO₄³⁻ and reagents into the 25 ml flask as follows:

Table 2-5. Results of standard PO₄³⁻

<table>
<thead>
<tr>
<th>Standard Solution(ml)</th>
<th>Vanadate molybdate (ml)</th>
<th>Water (ml)</th>
<th>[PO₄³⁻] (M)</th>
<th>ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>5.0</td>
<td>20</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.7</td>
<td>5.0</td>
<td>19.3</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
<td>5.0</td>
<td>18.6</td>
<td>0.08</td>
</tr>
<tr>
<td>4</td>
<td>2.1</td>
<td>5.0</td>
<td>17.9</td>
<td>0.12</td>
</tr>
<tr>
<td>5</td>
<td>2.8</td>
<td>5.0</td>
<td>17.2</td>
<td>0.16</td>
</tr>
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<td>3.5</td>
<td>5.0</td>
<td>16.5</td>
<td>0.20</td>
</tr>
<tr>
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<td>5.0</td>
<td>15.8</td>
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</tr>
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</tr>
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<td>7.0</td>
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<td>13.0</td>
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</tr>
<tr>
<td>10</td>
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<td>5.0</td>
<td>11.6</td>
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</tr>
<tr>
<td>11</td>
<td>10.5</td>
<td>5.0</td>
<td>09.5</td>
<td>0.60</td>
</tr>
</tbody>
</table>
Figure 2-7. Standard curve of PO$_4^{3-}$.
Chapter 3. Results and discussions

3.1. Batch treatment

3.1.1. Anaerobic process

Anaerobic treatment process was carried in two periods: the 1st run from May 13, 2011 to May 17, 2011 and the 2nd run from May 19, 2011 to May 28, 2011. The result was showed in the table 3-1.

Table 3-1. Anaerobic treatment from May 13th, 2011 to May 17th, 2011 and May 19th, 2011 to May 28th, 2011.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>COD (mg/L)</th>
<th>NH$_4^+$ (mg/L)</th>
<th>NO$_2^-$ (mg/L)</th>
<th>NO$_3^-$ (mg/L)</th>
<th>PO$_4^{3-}$ (mg/L)</th>
<th>pH</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st run</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1209</td>
<td>57.97</td>
<td>0.37</td>
<td>2.34</td>
<td>30.51</td>
<td>7</td>
<td>Initial</td>
</tr>
<tr>
<td>4</td>
<td>2727</td>
<td>60.08</td>
<td>0.32</td>
<td>0.07</td>
<td>15.86</td>
<td>7-8</td>
<td></td>
</tr>
<tr>
<td>2nd run</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2546</td>
<td>62.70</td>
<td>0.08</td>
<td>0.35</td>
<td>18.00</td>
<td>7</td>
<td>Initial</td>
</tr>
<tr>
<td>9</td>
<td>1973</td>
<td>279.95</td>
<td>0.34</td>
<td>0.14</td>
<td>25.87</td>
<td>7-8</td>
<td></td>
</tr>
</tbody>
</table>

From table 3-1, the original wastewater samples had low content of nitrite and nitrate. Ammonium concentration was much higher than industrial wastewater standard (TCVN 5945:2005 type B). The initial COD value of the second run was two times higher than that of the second run, but phosphate content was much lower. Squid processing wastewater normally contains small amount of FOG, but COD value is similar to other fish processing water in the range of 400-2000 mg/L (Carawan, 1979).

Nitrite and nitrate content was small change by anaerobic treatment, however phosphate content significantly reduce in the first run. After 5 days of treatment COD value and ammonia content were increased by activity of anaerobic bacteria cause hydrolysis and mineralization organic. After 9 days of anaerobic treatment the COD value reduced insignificant. However, the ammonia concentration was much
higher than the initial concentration. Ammonia came from the digestion of nitrogen content compounds as amino acid, and can reach 300mg/L (Carawan, 1979).

3.1.2. Aerobic process

Because anaerobic process requires long retention time, causes bad smell, so we was continue to investigate aerobic process. Aerobic treatment process (using aeration tank) was carried out from May 30, 2011 to June 25, 2011, with 5 runs

Changing in COD value

Figure 3-1 shows the trend of reducing COD according to the time. The COD value decreased quickly, some experiments achieved the COD limit of the influent for CW (400mg/L) in the first day. All are achieved requirement after three days. Active sludge increased and was stable at ratio 210ml/L.

![Figure 3-1. COD value changing in aeration tanks](image-url)
Figure 3-2: Changing trend of ammonia (a), nitrite (b), nitrat (c), and phosphorous equivalent (d) content.

Ammonia content was clearly lower initial concentration (figure 3-2a), other course the first 2 days the \( \text{NO}_2^- \) and \( \text{NO}_3^- \) concentration went up that means nitrosomonas, and nitrobacter were exist and kept a key role in the conversion. The results of investigation showed that the aerobic treatment was more feasible than the anaerobic treatment. The aerobic treatment shortened retention time and reduced COD value to the requirement value.
3.2. Continuous treatment – retention time optimization

The pretreatment system was operated at retention times of 3, 6, 9, 12, 15 hours. In an activated sludge continuous reactor, COD value reduced more than 80% in 12.7 hours (figure 3-3), longer retention time didn’t help to lower COD content. The COD value lies in the range of 70-80% (Figure 3-2), and no significant differences, which indicates that approximate 20% is more difficult to disintegrate. They could be needed very long retention time in CW. Activated sludge concentration standing for 30 minutes was in the range of 100-150ml/L, during the operation, equipment does not meet quality requirements because of small size tank. From these two reasons, the retention time in the reactor and the system could be shorten in full size system.

![Figure 3-3. Effect of retention time on the COD value of effluent.](image)

Figure 3-3. Effect of retention time on the COD value of effluent.
Figure 3-4. Effect of retention time on ammonium (a), nitrate (b), nitrite (c), phosphate (d) removal.

Ammonia concentration significantly reduced when the retention time was increased (Figure 3-4a), when the retention time was longer 15 hours, ammonium concentration dropped below the limit of TCVN 5945-2005 on industrial waste water- type B (10 mg/L). At the retention time in the reactor of 63.3h, ammonium concentration decreased to 99.8%, and there was no significant change after waste water went out of the settling tank (5). The trend of changing of nitrite and nitrate concentration showed in figure 3-4bc, the wide desperation of markers means that the concentration increased but wasn’t linear to retention time. Nitrite concentration increased when the retention time was less than 20 hours and decreased with longer
retention time, whereas nitrate concentration increased when the retention time increased (Figure 3-4b) and had high value when retention time set at 20 hours to 40 hours. Phosphate content in the artificial samples was lower than 8 mg/L, so, the equivalent concentration of phosphorus was below the TCVN 5945-2005 standard (5mg/L). Figure 3-4d showed that after the different retention times, phosphate concentrations fell below 5 mg/L (equivalent to 1.6 mg P/L).

While at these concentrations of ammonia, nitrate, nitrite, phosphorous don’t affect to the proper working of CWs, COD value need to be considered. When the retention time was 12.7 hours the COD value of effluence was acceptable for CW.

3.3. Plant selection

Two plants were grown in the same conditions. Figure 3-5 showed that in a cycle of 6 days the COD value reduced about 80% after 4 days. At beginning days (1, 2, 3), organic compounds were only mineralized apart, that limits using of plants, micro-organism and leading to COD value lowered insignificantly.

![Figure 3-5. Percentage of COD reduction in Limnophila basin and Cyperus basin.](image)

After 4 days, when organics were hydrolized and inorganized, micro-organism and plants easily used for development. The COD value lowered significant, reached to 85%. The ability in COD treatment of two genera were similar. However, percentages of ammonia, nitrite và nitrate removal of sedge were higher.
those of Limnophila genus about 10%, and error bar showed that sedge basin was more stable than Limnophila basin (Figure 3-6).

Figure 3-6. Amoni, nitrit, nitrat treatment of Cyperus (sedge) and Limnophila genera.

Phosphorous treatment of two basins were similar in every day. Average concentration of influence were 14.2 mg/L of the Limnophila basin and 17.7 mg/L of sedge basin and average percentage of phosphate removal was 68%.

Figure 3-7. Phosphorous treatment of Cyperus (sedge) and Limnophila genera
Sedge had the advantage of *Limnophila* in ammonium, nitrite, nitrate treatment and was suitable for growing in CW of local area.

### 3.4. Constructed wetland

Sedge was chosen to grow in the CW pilot. Wastewater from the pretreatment system was fed to the CW with the hydrolytic retention time 135L.m\(^{-2}\).day\(^{-1}\). The initial COD value was under 400mg/L, this was the harder digestion part of organics. Reduction of 70% COD was obtained, and the concentration decreased gradually from level 1 to level 4. That was the same way as ammonium concentration (figure 3-8abcd). It means that plants and bacterial were good cooperation in the CW (figure 3-8abcd).

COD removal effect of the SVF-CW in the experiments was close to the report of Vymazal, 2005; Kadlec et al., 2000. In this study, for the HLR of 3 cm.day\(^{-1}\), the maximum removal efficiency in terms of COD was 67% for an average inflow of 1966mgO\(_2\) L\(^{-1}\) (590 kg ha\(^{-1}\) d\(^{-1}\)). Concerning the HLR of 6cmd\(^{-1}\), the maximum removal efficiency in terms of COD was 73% for an average inflow of 2093mgO\(_2\) L\(^{-1}\) (1256 kg ha\(^{-1}\) d\(^{-1}\)). In another study, carried out with effluent of the manufacture of finished shoe upper leather from multisource wet blue with a typical COD inflow of 1160mg L\(^{-1}\), reductions of 85%, 82% and 70% COD were obtained, for CWs planted with two subspecies of *Glyceria maxima* and *Phragmites*, respectively, in a five day root–zone system. Both species proved to be extremely robust and survived shock dosing, long periods of drying out, total immersion and cold (Daniels, 1998).
Figure 3-8: Percentages of COD (a), ammonium (c), nitrite (e), nitrate (g), phosphate equivalent reduction; Column graphs b, d, f, h, i show average contents of these parameters according to 4 levels; the straight line scatter showed removal effect according to 4 levels.

NO$_2^-$ is the product of aerobic treatment process when oxygen is provided insufficiently for the demand. This easily happens because aerobic processing requires much energy for aeration. NO$_2^-$ is not useful even harmful for the development of plants but NO$_2^-$ in the experiment declined dramatically, reaching 90% at effluent of CW (Figure 3-8f, level 4). This maybe have two reasons: the first was the oxygen carried by roots oxidized nitrite to nitrate then NO$_3^-$ was absorbed by the plants, the second was anammox bacteria which had ability to transform 50% NH$_4^+$ and 50% NO$_2^-$ into nitrogen releasing from the filtering area with other gases was the product of nutritious metabolism process in general.
The more permanent removal of N in constructed wetlands is dependent on the N cycle. As part of the cycle, the various forms of N are converted into gaseous components that are expelled into the atmosphere as nitrogen gas ($N_2$) or nitrous oxide ($N_2O$). Key processes in the N cycle include ammonification, nitrification, and denitrification. Nitrification denitrification reactions are the dominant removal mechanisms in constructed wetlands (Benham and Mote 1999). Nitrification is the biological formation of nitrite-N ($NO_2^-$-N) or NO$_3^-$-N (Alexander 1977) from NH$_4^+$. Nitrification occurs in aerobic regions of the water column, soil-water interface, and root zone (Reddy and D’Angelo 1997). Dissolved oxygen levels < 1-2 mg/L in water substantially reduces nitrification (Hammer and Knight 1994; Lee et al. 1999). Denitrification is the biological process of reducing NO$_3^-$-N or NO$_2^-$-N, into $N_2$, $N_2O$, or nitric oxide (NO) (Kadlec and Knight 1996). Denitrification is a significant mechanism inequality degradation, reducing NH$_3$ concentrations drives the design process for many wetland treatment systems (Kadlec and Knight 1996). Thus, unsuitable conditions for nitrification can seriously limit the treatment potential of these systems. The use of supplemental aeration may enhance nitrification activity, due to the addition of dissolved oxygen into the wastewater which would induce a more aerobic environment for this reaction. Cottingham et al. (1999) found that aerating laboratory scale subsurface flow constructed wetlands promoted increased rates of nitrification. Surface flows, or free water surface constructed wetlands, however, are used for treating livestock wastewater in Nova Scotia due to their ability to handle relatively high solids content. Subsurface flow wetlands are generally not recommended for agricultural wastewater treatment with substantial solids content (NRCS 1991).

Percentage of treatment varies in a wide range from 30% to close 100%. Nitrate concentration seems to be instable that because of much changing the initial nitrate content and affected by many factors. In constructed wetlands, after NO$_3^-$ is formed under aerobic conditions; it diffuses down into the anaerobic portion of the soil, where it is denitrified (Patrick and Reddy 1976; Nichols 1983). Since ammonium
was being nitrified to $\text{NO}_3^-$ within the system, $\text{NO}_3^-$ increased at level 2 as a result. The accumulation of $\text{NO}_3^-$ at level 2 indicates that after $\text{NH}_3$ was nitrified, subsequent denitrification was limited (figure 3-8gh).

At level 4, nitrate and nitrite contents were low, possible factors that could promote denitrification include enough residence time for denitrification to remove $\text{NO}_3^-$-N, lack of DO, available carbon (C) within the system. Denitrification activity is reduced if available C supplies are low (Gersberg et al. 1983; Hammer and Knight 1994; Wood et al. 1999) and proceeds only when the oxygen supply is inadequate for microbial demand (Hammer and Knight 1994). However, limited denitrification activity has been observed in the presence of DO (Phipps and Crumpton 1994).

Phosphor at the top layer was absorbed strongly by the roots and microorganism, thus they declined significantly, reaching over 45%. At subsequent layer, due to not ingrained roots, only microorganism use them, $\text{PO}_4^{3-}$ declines much less, just reaching over 20%. Beside that $\text{PO}_4^{3-}$ could be participated with other cations.

**Conclusion**

Seafood processing wastewater treatment using the anaerobic method required long retention time, which didn’t meet the requirement of reducing COD down to 200-300mg/L even after 9 days.

The results of aerobic treatment in the continuous aeration tank using activated sludge indicated the possibility of applying for pre-treatment of wastewater containing high COD. When the retention time was 12.7 hours the COD value of effluence was acceptable for CW.

Sedge had the advantage of *Limnophila* in ammonium, nitrite, nitrate treatment and was suitable for growing in CW of local area.

Sedge should be grown in CWs because of the reduction of ammonium, nitrite, nitrate, total phosphorous was stable. The system had high effect in removing
ammonium, nitrite, nitrate, phosphorous, 80.3±15.8%, 93.2±7.2%, 72.8±25.0%, 73.1±26.6%, respectively which meet Vietnamese Guide QCVN 11:2008. COD value was reduced from 300-400mg/L to 91.6±9.9 mg/L.
Referents


Chong-Bang Zhang, Wen-Li Liu, Jiang Wang, Tong Chen, Qing-Qing Yuan, Cheng-Cai Huang, Ying Ge, Scott X. Chang, Jie Chang, 2011. Plant functional group richness-affected microbial community structure and function in a full-scale constructed wetland.


Vietnam economic time 2007

