# Microbial Community in Constructed Wetland during the Treatment of Domestic Wastewater

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**Abstract**. Constructed wetland (CW) was operated for treating domestic wastewater for 60 days. Water hyacinth (*Eichornia crassipes*) and lotus plant (*Pistia stratiotes.L*) were used for domestic wastewater treatment as vegetation in the CW system. Microbial community existed in the CW was investigated for studying their potential roles in the treatment processes; Samples for microbial composition were collected from the wastewater influent, sediment and the roots of the plants in the CW. During the operational days, the bacterial isolates were morphologically and physiologically characterized; phyto- and zoo-planktons were visualized and identified. The system was able to remove BOD up to 85%, and reducing the level of N up to 78%. There were identified 37 bacterial isolates comprising of 11 isolates from water, 17 isolates from sediment and 9 isolates from the roots. The identified bacterial isolates belong to groups *Acinetobacter, Aeromonas, Flavobacterium, Lucibacterium, Paracoccus, Proteus, Pseudomonas*, and *Vibrio*. These following species were also identified during the system was running; they were *Pandorina morum, Phacus sp, Euglena acus, Lepocinclis cudata, Scenedesmus acuminatus, Raphidonema spiculiforme, Euglena sanguinea,* and *Eudorina sp*.

## 1. Introduction

Water and sanitation specifically stated in sustainable development goals (SDGs) number 6 that is to ensure access to water and sanitation for all, The United Nation (UN) clearly demonstrated that "*Clean water is a basic human need, and one that should be easily accessible to all. There is sufficient fresh water on the planet to achieve this. However, due to poor infrastructure, investment and planning, every year millions of people — most of them children — die from diseases associated with inadequate water supply, sanitation and hygiene*." This has emphasized the important of water resources protection actions no matter how difficult and how big the challenges are faced [1].

Wastewater actually is valuable resource that still do not widely re-used and the only current practice is just by thrown them away and then it just become wastes that polluted and caused problems to the environment [2], especially in developing countries where wastewater treatment facilities are limited [3]. Technologies for wastewater treatment such as constructed wetlands have been developed for long time ago; application of constructed wetland for domestic wastewater treatment are widely reported, one of them was outlined by Brix and Carlos, 2005 in Denmark. Their study summarized and provided guidelines for several vertical constructed wetland systems practises.

Wetlands are defined in many literatures as a transitional between terrestrial and aquatic ecosystems that have common characteristics of each other [4]. They have been intensively studied in the Czech Republic for more than 30 years, but the first full-scale CW for wastewater treatment was built in the Czech Republic in 1989 [5–7]. It was defined as an artificial habitat that most visibly made up of vascular plants and algal colonies, which also provide a structural and nutritional support for an associated, highly heterogeneous microbial community [8]. The use of CWs for improving water quality is a relatively new; that was suggested it was started in the last two decades [9]. Truu et al., [10] summarized that in the CW, wastewater is treated by several mechanisms and steps including by the combination between existing microbial and filter media that are used and also by the plants that are grown in the systems. Studies reported, nitrogen removal in CW is occurred trough the processes of nitrification–denitrification in combination [11], however it also could be due to anaerobic oxidation of ammonium (ANAMMOX) as well [12]. Phosphorus is removed through biological mineralization by microorganisms and biochemical Mineralization [13].

Good CW performances need supporting conditions such as appropriate system capacity, types of plants used in the process, the features of the microbial colony, the interaction of biogenic and filterer material of the system [14]. According to Scholz and Lee [15], a diverse microbial community will act differently and specifically in a different biochemical conditions. Understanding the microbial structure is important for intensifying the system's performance [16]. The presence of plants in CW increases diversity and activity of microorganisms, specifically in the plant's root, the growth and diversity of plant species which is a key factor in improving interaction among microbial plants [17]. The dynamic of microbial population during the waste treatment process is important for determining their role in the system, and also the dynamic of decreasing and increasing trends of their population are useful information for operating and designing the system. Identification of dominance microorganisms is important for the strategy development for increasing its population and optimizes its performance [18].

Vymazal, [19] determined microbial community existed in constructed wetlands, in their study it was suggested the microbial community consisted of indigenous and foreign microorganisms that have adaptive features, survive and grow in wetland systems participating in purification processes. There are varies types of microorganisms in many types and locations across the globe existed in CWs reported in many studies that suggested the uniqueness and complexity of the systems. Considering the diversity of microbial composition in the CWs, this study was aimed to investigate the microbial community exist in CW treating domestic wastewater.

## 2. Material and Methods

### *2.1. Domestic wastewater used Domestic wastewater (collection and characteristics), and Constructed wetland (design and operation)*

Domestic wastewater used in this study was collected from two residential locations in Palembang. The domestic wastewater was collected every week and left for 24 hours at room temperature prior to be pumped into storage tank and continuously pumped into the CW with flow of 10 mL/min. The CW was operated for 60 days in batch mode with 72 hours residence time per cycle. Samples characteristics such as pH and temperature were directly measured by using Water-meter (Lutron YK-2005WA), the biochemical oxygen demand ($BOD_5$), total suspended solids (TSS), total nitrogen and total phosphorus were measured according to Indonesian National Standard Method (SNI) [20]. A constructed wetland with dimension of 20 x 12 x 100 cm similar to the Verawaty et al., [21] was operated for 60 days; Filter materials were consisted of stones, sands and sludge from the bottom to the top as follows: the bottom layer was lyme stone with diameter of 3-5 cm, the second layer from the bottom was lyme stone with diameter of 2-3 cm, followed by lyme stone with diameter of 0.5 cm, and then find sands and sludge that was collected from a pond in Palembang city, the plants that used in this study were water hyacinth (*Eichhornia crassipes*) and lotus flower (*Pistia stratiotes.L*). The

system was adapted for three weeks before the experimental testing. The image and diagrammatic design of the CW used in this experiment is presented in Figure 1.

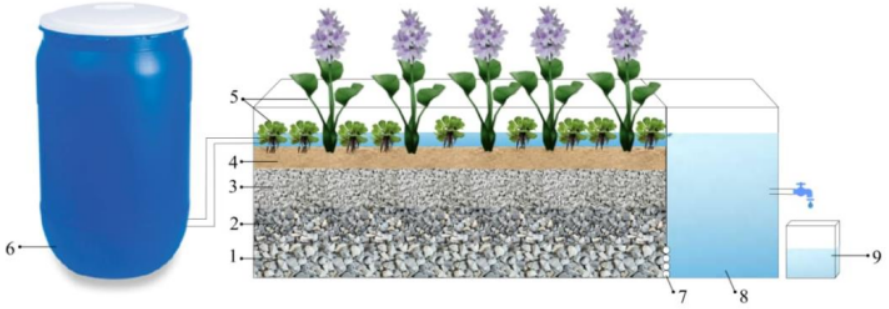


**Figure 1.** Constructed wetland design; filter materials consisted of lyme stone (Ls) diameter 3-5 cm (1), the second layer from the bottom was Ls with diameter 2-3 cm (2), Ls with diameter 0.5 cm (3), find sands and sludge (4), *Eichhornia crassipes* and *Pistia stratiotes L* (5), storage tank (6), holes (7), sedimentation chamber (8) and effluent (9).

### *2.2. Microorganism counting, isolation and identification (bacteria, algae and protozoa) during the bioreactor operational period*

Fluctuations of microbial type and number (bacterial, algae and protozoan) were monitored; identification of algae and protozoa species found in CW was carried out by observation using a light microscope with a magnification of 400 times. Sampling from sediments and water were done randomly. Samples that were collected from plants' roots were done by cutting the roots and putted them into sterile bottles with sterile Phosphate Buffer Saline (PBS). Growth media such as Nutrient Agar (NA) and Luria Bertani Broth (LB) were prepared for bacterial counting. The bacterial count was calculated by dilution in Plate Count Agar (PCA). A single colony of purified bacteria was isolated and identified based on their morphology and physiology characteristics. Bacterial identification was conducted according to *Bergey's Manual of Determinative Bacteriology 8th edition* dan *Bergey's Manual of Determinative Bacteriology 9th edition*. Protozoa and Algae were visualized by using microscope with 400x magnification. Total cells of algae and protozoa were counted according to Lackey drop microstransect counting (APHA, 2005).

## 3. Results and Discusions

This study showed during the operational periods, the domestic wastewater characteristics by the $BOD_5$ of 243 ± 4.9 mg/L; pH of 7.7 ± 0.13; and temperature of 26.8 ± 0.21. This system was able to remove BOD up to 90%, and can N and P removal for 78% and 67% respectively. The inlet TSS was 230 ± 42 mg /L in average, during the treatment process was decreased to 12.87 ± 5.5 mg /L.

Table 1. Number and codes of bacterial isolates purified from the sediments, plant's roots and domestic water samples during the operational periods.

| Sample Origin | Number of Isolates | Isolates codes |
|---|---|---|
| Water (W) | 11 | W1 - W11 |
| Sediments (S) | 17 | S1 – S17 |
| Roots (R) | 9 | R1 – R9 |
| Total | 37 | |

There were 37 bacterial isolates were isolated from water, sediments and plant's roots samples (Table 1); those bacteria consisted of 11 isolates from water sample, 17 isolates from sediments and 9 isolates from plants' roots. Based on the morphology and physiology characteristics of those bacteria (Table 2); according to *Bergey's Manual of Determinative Bacteriology 8th edition* dan *Bergey's Manual of Determinative Bacteriology 9th edition*, those bacteria belong to the families of *Acinetobacter, Aeromonas, Flavobacterium, Lucibacterium, Paracoccus, Proteus, Pseudomonas,* and *Vibrio*.

Table 2. Morphological and physiological characteristics of bacterial isolates purified from the sediments, plant's roots and domestic water samples during the treatment of domestic wastewater by using constructed wetland system.

| Isolates Code | Morphological and Physiological Characteristics | Phylum*) |
|---|---|---|
| W1, W4, S1, S2, S9, S10, S13, S14, S16, R1, and R3 | Rod-shaped, Gram negative, aerobe, motile, no endospore, it hydrolyzes starch and gelatine (+), catalase (+), Voges Proskouer (+), $H_2S$ (+), glucose and sucrose (acid) fermentation (+). | *Pseudomonas*. |
| W5, S15 and S7 | Comma/curve-shaped cell, Gram negative, facultative anaerobe, motile, no endospore, hydrolyzes starch and gelatine (+), catalase (+), methyl red and Voges Proskouer (+), $H_2S$ (+), glucose and sucrose (acid) fermentation (+). | *Vibrio* |
| W7, S4, S5, S11 and R4 | Rod-shaped cell, Gram negative, no endospore, facultative anaerobe, motile, hydrolyzes gelatine (+), catalase (+), methyl red (+), $H_2S$ (+), Glucose and sucrose (acid) fermentation (+). | *Aeromonas* |
| R5, R6 and R7, S12 and S17 | Cocci-shaped cell, Gram Negative, non-motile, endospore (+), facultative anaerobe, oxidase (+), catalase (+), urease (+), no glucose and sucrose (acid) fermentation (-). | *Paracoccus* |
| S3, S6, and R9 | Rod-shaped cell, Gram negative, facultative anaerobe, motile, do not have endospore, hydrolyze gelatine, catalase (+), $H_2S$ (+), urease (+), Glucose and sucrose (acid) fermentation. | *Proteus* |
| W6 and W9 | Rod-shaped cell, non motile, Gram negative, facultative anaerobes, no endospore, motile, hydrolyze gelatine, catalase (+), $H_2S$ (+), Glucose and sucrose (acid) fermentation. | *Flavobacterium* |
| R2, W3, R8, W10, W11, and S8 | Cocci/Coccobacil-shaped cell, non motile, Gram negative, strictly aerobe, motile, methyl red (+), did not hydrolyze gelatine (-), Citrate (+), catalase (+), oxydase (-). | *Acinetobacter* |
| W2 and W8 | Rod-shaped cell, Gram negative, facultative anaerobes, motile, indole (+), methyl red (+), it hydrolyzes gelatine (+), catalase (+), Glucose and sucrose (acid) fermentation (+). | *Lucibacterium* |

*) Identity based on characteristics suggested by The *Bergey's Manual of Determinative Bacteriology 8th edition* dan *Bergey's Manual of Determinative Bacteriology 9th edition*.

Microorganisms (protozoa, algae, and bacteria) found in CW for six periods (60 days) of domestic wastewater treatment were monitored periodically. The number of bacterial cells for 60 days with 15-day sampling intervals is shown in Figure 2. This study indicated that bacteria cells density obtained from plant root samples was higher compared to samples derived from sediments and water body (Figure 2). There was no significant change in the cells density trend of the samples collected from the

roots (only a slight fluctuation occurred between days 1 to 15). The cells density of samples collected from the sediment showed fluctuation trend (it showed a slight increasing at day 10 but significantly decreased after day 45, and then increased slightly to day 60) while samples collected from water showed increasing trend with a slight decreasing after day 45 to day 60. The bacterial cells densities in samples isolated from sediment and water also fluctuated slightly; the densities of bacterial cells of the sediment and water samples were lower than those were isolated from plant's roots.

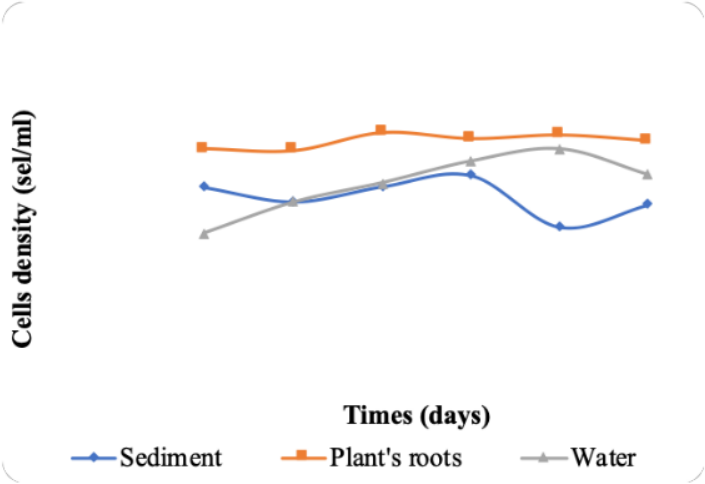


**Figure 2.** Bacterial cells densities during the 60 days of the CW operational periods for domestic wastewater treatment.

Studies showed there were some reasons as the possible cause of why the density of bacterial cells obtained from plant root samples was higher compared to samples derived from sediments and water; it including the plant' roots are more suitable habitats for bacterial growth, where it provides places for bacteria to attach and colonize and protect them from pressure due to water flow in the CW system. Plant roots also produce oxygen and nutrients needed for bacterial growth so that the population is higher when compared to sediments and water bodies. Free-living microorganisms must adapt to turbulence and water flow so that they are easily carried away and wasted when water flow leaves CW. The plant roots and rhizomes play an important role in wastewater treatment [14,22]. Plants also play a role as a stabilizer for microorganism community in the CW system [23]. According to Ibekwe et al., [9], the diversity of microbial communities inhabiting CW is very important to improve and produce an efficient nitrification and denitrification process during wastewater treatment. Furthermore, Gagnon et al., [17], explains there was high microbial respiration around the surface of plant roots that release oxygen which affected the microbial population. Hatano et al., [24] suggested there was a significant influence of the plant to microbial population that contribute to organic material decomposition in marsh ecosystem. Ibekwe et al., [9], emphasized the important role of microorganism diversity in obtaining an optimal wastewater treatment process. In associate to that, Mitchell, [25] explaineds six major biological reactions contribute to CW for wastewater treatment, those include photosynthesis, respiration, fermentation, nitrification, denitrification and phosphorus reduction; photosynthesis is done by algae and aquatic plants; they produce carbon and oxygen to CW, both of them are important for nitrification. The aquatic plants also transfer oxygen to surround the the roots and rhizosphere [25]. The process of respiration involves the oxidation of organic carbon and produces carbon dioxide and water. Fermentation causes an anaerobe decomposition of organic carbon and produces high-energy products such as methane, alcohol, volatile fatty acids [25]. While the

reduction of N occurs through nitrification and denitrification by the microorganisms. All processes that occur, causing fluctuations in the type and number of microorganisms found in the CW ecosystem [25].

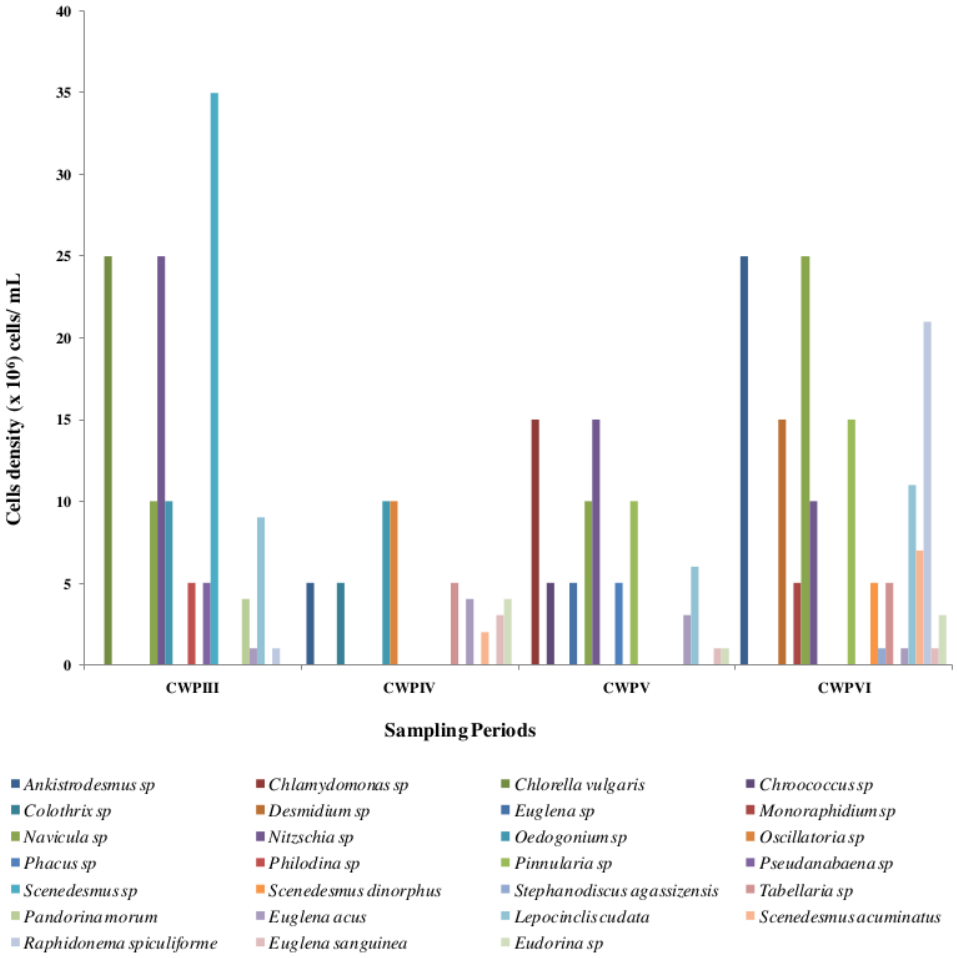


**Figure 3.** The dynamics of microorganism's thought during the period of four (3rd to 6th) of domestic wastewater treatment using CW

There were fluctuations in the types and number of protozoa, algae and bacteria, occurred during the 60 days operational periods of the domestic wastewater treatment. Population dynamics and diversity of algae species represented in the 3rd to 6th period (45 days) are shown in Figure 3. There were 20 species in total were identified in this study as shown in Figure 3. Algae species and population composition changes were identified in CW, the third period (CWPIII) was dominated by *Scenedesmus sp, Nitzscia sp, Chlorella vulgaris*; at the fourth period (CWPIV) the change was occurred, it was dominated by *Oedogonium sp and Oscillatoria sp*; then at the fifth period (CWPV) the CW was dominated by *Nitzschia sp, Navicula sp* and *Pinnularia sp*. Furthermore, at the sixth

period, it was dominated by *Navicula sp*, and also *Desmidium sp* and *Navicula sp*. Other species only have a small percentage of the total population.

Fluctuations in the type and number of protozoa, algae and bacteria occurred during the experimental periods of domestic wastewater treatment in CW system. The dynamics characteristics of wastewater that occur during each wastewater sampling period affect the substances of organic material and nutrients present in the wastewater treatment system; resulting in changes in Microbial Community composition of microorganisms. Palmer (1974) in Abdel-Raouf et al., [26] conducted a study and survey of microalgae genera in wastewater stabilization ponds.

Types of algae that are commonly found include *Chlorella, Ankistrodesmus, Scenedesmus, Euglena, Chlamydomonas, Oscillatoria, Micractinium* and *Golenkinia*. A number of similar groups of microorganisms are also identified in this study, including *Pseudomonas*, this group of microorganisms is reported to play a major role in the process of reducing BOD and COD in the treatment of domestic wastewater, especially *Pseudomonas aeruginosa* [27]. Likewise with *Aeromonas,* spesifically the *Aeromonas hydrophila* species [28]. In addition, several studies report that heterotrophic bacteria that play a major role in the BOD oxidation process include *Pseudomonas, Flavobacterium, Archromobacter* and *Alcaligenes spp* [26].

## 4. Conclusions

The result of the study showed *Constructed Wetlands* for domestic wastewater treatment achieved BOD up to 90% removal and remove N and P up to 78% and 67% respectively. TSS of influent wastewater can be decreased from 230,22 ± 2,95 mg/L to 12.87 ± 5.5 mg /L. Bacterial cell density obtained from plant root samples was higher compared to samples derived from sediments and water. Fluctuations in the type and number of protozoa, algae, and bacteria occurred during 60 days of the operational period. There were 20 algae species were identified in the system including *Pandorina morum, Phacus sp, Euglena acus, Lepocinclis cudata, Scenedesmus acuminatus, Raphidonema spiculiforme, Euglena sanguinea,* and *Eudorina sp.,* and there were 37 bacterial isolates of which seven isolated from water, eleven isolated from sediments, and nine isolated from plant's roots; the isolates were belongs to families of *Acinetobacter, Aeromonas, Flavobacterium, Lucibacterium, Paracoccus, Proteus, Pseudomonas, dan Vibrio* classification.

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