

APPLICATION OF PHYTOREMEDIATION FOR HERBAL MEDICINE WASTE AND ITS UTILIZATION FOR PROTEIN PRODUCTION

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Abstract

Herbal industry in Indonesia is progressing very rapidly. Increasing number of herbal medicine industries lead to an increase of the waste which are normally processed in anaerobic ponds by using chemical and biological process. However this process are not economical feasible and therefore an alternative method by using natural resource is required. Phytoremediation is an environmental friendly method to reduce contaminant using aquatic plant. This method uses water plant to reduce COD and nutrients content in the waste. Since the waste still high content of nutrient, therefore it is potential for medium growth of algae Spirulina. This study was aimed to evaluate the use of various plant species (water hyacinth and lotus) in decreasing contaminant and to determine optimal nutrient composition of the growth media. The phytoremediation was performed in 3-8 days and height of liquid in the tank was maintained constant at 5 cm. The effluent of first phytoremediation was transferred to second stage for cultivation of Spirulina with 15 days of cultivation time. The external nutrients were added each 2 days and the concentration of biomass was measured for its optical density. Spirulina grow well in herbal medicine waste that has been phytoremediation with lotus for 3 days and had a C:N:P ratio amounted to 57.790 : 9.281 : 1 with a growth rate of 0.271/day.

Keywords: biomass; herbal medicine waste; phytoremediation; spirulina; water plants

Abstrak

APLIKASI FITOREMEDIASI UNTUK LIMBAH JAMU DAN PEMANFAATANNYA UNTUK PRODUKSI PROTEIN. *Industri obat di Indonesia tumbuh sangat cepat. Pertumbuhan ini mengakibatkan meningkatnya limbah yang umumnya dilakukan dalam kolam anaerobik dengan menggunakan proses kimia dan biologi. Namun demikian, proses tersebut belum menunjukkan hasil ekonomis sehingga diperlukan metode lain yang relatif aman dan ekonomis. Salah satu cara yang dapat digunakan adalah phytoremediasi menggunakan tanaman air untuk mengurangi kandungan COD dalam limbah dan nutrient. Dikarenakan limbah masih mengandung nutrient yang cukup banyak, maka limbah tersebut juga sangat potensial untuk digunakan sebagai medium mikroalga. Penelitian ini bertujuan untuk mengevaluasi penggunaan tanaman air (enceng gondok dan teratai) untuk mereduksi kontaminan dalam limbah obat jamu. Phytoremediasi dilakukan selama 4-8 hari dan tinggi cairan dalam reaktor dijaga pada 5 cm. Keluaran dari phytoremediasi pertama menggunakan tanaman air digunakan sebagai medium di phytoremediasi menggunakan mikroalga Spirulina. Untuk mendapatkan pertumbuhan yang optimum, maka ditambahkan juga nutrient dan menunjukkan bahwa Spirulina tumbuh dengan sangat baik dalam medium ini. Pertumbuhan terbaik diperoleh dari phytoremediasi menggunakan teratai selama 3 hari dan kecepatan pertumbuhan 0,271/hari dengan perbandingan C:N:P = 57,790:9,28:1.*

Kata kunci: biomasa; limbah jamu; phytoremediasi; spirulina; tanaman air

INTRODUCTION

Indonesia is an agricultural country, where many plants grow very diverse. Among of them have function to cure various types of diseases in humans. For this purpose, then people use this plant into herbal medicine which made from a mixture of extracts of plants. Herbal medicine consists of two types, traditional herbal medicine and fitofarmaka or herbal medicine packs. Fitofarmaka is a traditional herbal medicine that uses natural ingredients but under modern processed (Lestari, 2007). Herbal medicine was commercialized in Indonesia since 1658 (Widiyanti, 2005).

Development of herbal medicine industries has proved that human consciousness to the use of natural ingredients for treating the disease. Synthetic drugs are mostly avoided due to its side effects. The main problem of herbal medicine industry is waste produced from its process. Most of herbal medicine waste contain phenol and its derivatives that have a serious effect to the environment (Kibret *et al.*, 2000; Chung *et al.*, 2003; Kumar *et al.*, 1995). An herbal medicine industry normally produces wastewater with COD content of 200-20000 ppm and 9.8 ppm phenol.

Herbal medicine waste has a high COD content that must be processed before discharged into the river body. Usually herbal medicine waste is processed using biological and chemical process such as coagulation, sedimentation, aeration, and using activated sludge (Milasari and Ariyani, 2010). But these methods are not economically feasible. Phytoremediation is a remediation method by using green plants especially aquatic plants such as water hyacinth, lotus, etc and their associated microbiota, enzymes, water consumption, soil amendments, and agronomic techniques to remove, contain, or render harmless environmental contaminants such as heavy metal, pesticides and xenobiotics, organic compound, toxic aromatic pollutants, and acid mine drainage (Dordio and Carvalho, 2011; Suresh and Ravishankar, 2004; Newman and Reynolds, 2004; Singh and Jain, 2003; Archer and Caldwell, 2004). Due to nutrient content in the effluent, the waste can be further process as medium for microalgae growth. The consumption by microalgae is then considered as the second phytoremediation process.

The phytoremediation of herbal medicine waste had not been studied till today. The application of phytoremediation has been applied to palm oil mill effluent (POME) by using *apu-apu* (*Pistia stratiotes*) (Ahdia and Krismawati, 2012). In another study, water hyacinth has been used to remediate the active ingredient carbofuran phytoremediation (Karyadi and Istiono, 2008). In this research, phytoremediation was able to reduce the contaminants up 82.21%. Microalgae require nutrient for their growth. The source of nutrient has been explored through the waste to cultivate *Spirulina* sp (Hadiyanto *et al.*, 2012;

Suryajaya and Sari, 2012). In another research, Markou *et al.* (2012) cultivated *Spirulina* sp in Olive-oil Mill Waste Water (OMWW) medium which pretreated with sodium hypochlorite (NaClO).

Since herbal waste load is enormous in Indoensia and has high conten of COD, N and P, therefore, in this research phytoremediation were used as the chosen method. The nutrient in the phytoremediation by aqua plants were used as medium growth of microalgae. The aim of this research was to evaluate the use of various plant species that are water hyacinth (*Eichhornia crssipes*) and lotus (*Nymphaea sp*) in decreasing contaminant and to determine optimal nutrient composition of the algae growth media.

MATERIAL AND METHODS

Herbal Medicine Waste

Herbal medicine waste was collected from one of herbal medicine industries in Semarang. The waste then was filtered to separate impurities. The wastewater characteristic can be described in Table 1.

Table 1. Characteristic Herbal Medicine Waste

Parameter	Content
pH	6.75
COD	200 ppm
BOD	277 ppm
TSS	66 ppm
Total N	0.704 ppm
Total P	0.549 ppm
Phenol	0.39 ppm

Spirulina sp

Spirulina sp was collected from Balai Besar Pengembangan Budidaya Air Payau (BBPBAP) Jepara and cultivated in Laboratory of Bioprocess, Diponegoro University. *Spirulina* sp was used as inoculums at OD680=0.5.

Aquatic Plants

Water hyacinth was collected from Rawapening Lake, Ambarawa. It was acclimated in 20% Herbal Medicine Waste medium for 5 days, while Lotus plant was collected from flower market in Surakarta and Semarang. It was acclimated in 20% Herbal Medicine Waste medium for 5 days.

First Stage of Phytoremediation

The first remediation was purposed to reduce levels of COD and other nutrients by using phytoremediation under different aquatic plants (water hyacinth and lotus). Here, the height of liquid in the tank was also set at 5 cm. Phytoremediation was done in different residence time (3, 6, 8 days).

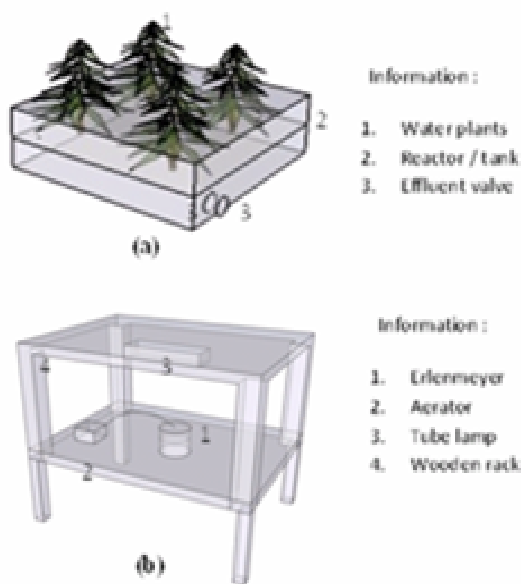


Figure 1. Experimental set up for phytoremediation

Second Stage of Phytoremediation

The second experiment of remediation was purposed to cultivate *Spirulina* sp using 80% herbal medicine waste that has been phytoremediated by different plants, retention time and using some nutrients (baking soda, TSP, and urea). The research was done in 15 days.

Measurement

Measurement of COD, N, and P concentration in herbal medicine waste done before and after phytoremediation according to set of residence time (day 3,6,8). Measurement of Optical Density (OD) was started from day 0 using spectrophotometer SP-300 wave length 680 nm. Medium was measured every day. Biomass was collected every two days from 2nd days until 10th days by using filter cloth. Wet Biomass was recorded as 90% moisture content (10% Total Suspended Solid). Carbon, Nitrogen, and Phosphor was measured by Benfield and Randal method.

RESULT AND DISCUSSION

Evaluation from Type of Plants to Decrease COD and Nutrient

COD reduction

The initial concentration of COD was 200 ppm and it decreases to 55 ppm (72%) after 8 days remediation using hyacinth, while 59 ppm (70%) by using lotus (Figure 2). It is known that water hyacinth which covering 50% of total area could reduce suspended residues up to 75-85%, while COD 5.52-76.83% (Rossiana *et al.*, 2007). Water hyacinth shows the second highest of TSS and COD reduction after *Cyperus papyrus* (Irawanto, 2010).

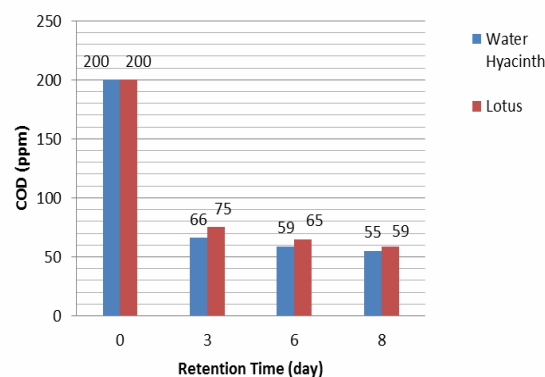


Figure 2. COD Reduction using Different Plants

Nitrogen reduction

Figure 3 shows the decrease of nitrogen content in the herbal medicine waste. The initial concentration of nitrogen is 0.704 ppm. After 8 days phytoremediation using water hyacinth, nitrogen concentration decrease to 0.222 ppm (68%). While phytoremediation using lotus can reduce the concentration of nitrogen to 0.366 ppm (48%).

Nitrogen is one of element needed for photosynthetic and it commonly consists of 3 forms such as organic nitrogen compounds, ammonium (NH_4^+) ions, and nitrate (NO_3^-) ions (Nasholm *et al.* 2008). The plant is able to reduce the concentration of ammonia by 81% within 10 days (Kutty *et al.*, 2009). The enormous absorption of nitrogen from herbal medicine waste by using water hyacinth because of the availability of stomata (the stomata frequency on the upper epidermis was 2.83 mm^2 and 3.32 mm^2 on the lower epidermis) and its size ($4 \mu\text{m} \times 4 \mu\text{m}$) greater than lotus. Water hyacinth has a hollow and porous stem causing the process of photosynthesis not only takes place in the leaves but also in stems (Mahmood *et al.*, 2005). This phenomena proves that water hyacinth need more nitrogen for photosynthesis than the lotus.

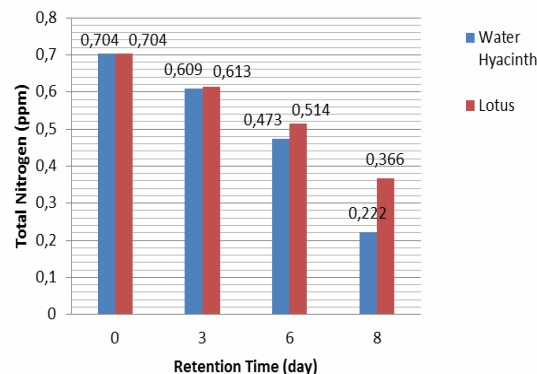


Figure 3. Total Nitrogen reduction using different aquatic plants

Phosphorus reduction

Phosphorus is also one of the essential nutrients for plant photosynthetic. Phosphorus contributes to the growth of the seeds, roots, flowers, and fruit. By improving the structure of root, the absorptive root would be better (Purwaningsih, 2009). The initial concentration of phosphorus in herbal medicine waste is 0.549 ppm and after 8 days phytoremediation using water hyacinth, the concentration decrease to 0.3132 ppm. In other hand, phytoremediation using lotus, phosphorous concentration decrease to 0.3144 ppm. Lotus also shows the capability to reduce the concentration of ammonia by 67% (Kutty *et al.*, 2009).

Figure 4 shows the decreased of phosphorus content in herbal medicine waste. Inline to absorption of nitrogen, phosphorus absorption using water hyacinth is higher than the lotus. It is because the water hyacinths have more stomata and its size is large. Water hyacinth also has a hollow stem and porous that support the absorption of minerals (especially phosphorus) more rapidly (Mahmood *et al.*, 2005). In other hand, water hyacinth has also the ability to reduce phosphorus in pesticides (Xia and Ma, 2006).

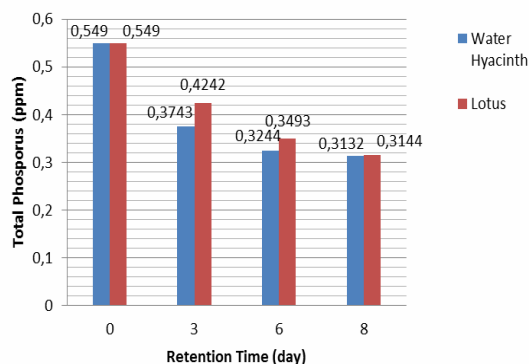


Figure 4. Total Phosphorus reduction using different plants

Growth Rate Analysis

The remediated herbal medicine waste used as cultivation medium of alga *Spirulina* with the composition of 80%. The addition of nutrients was the same i.e. 1000 ppm baking soda, urea 60 ppm, and 10 ppm triple super phosphate. Control medium was medium I, using fresh water without any mixture of herbal medicine waste. Medium was measured using a spectrophotometer with an optical density OD680. The increase of OD in the medium can be seen in Figure 5. Optimum growth occurred in medium III (phytoremediation with lotus for 3 days), followed by medium II (water hyacinth-3 days), medium IV (water hyacinth-6 days), medium VI (water hyacinth-8 days), medium V (lotus-6 days), and the last medium VII (lotus-8 days). Growth rate and CNP ratio can be depicted in Table 2.

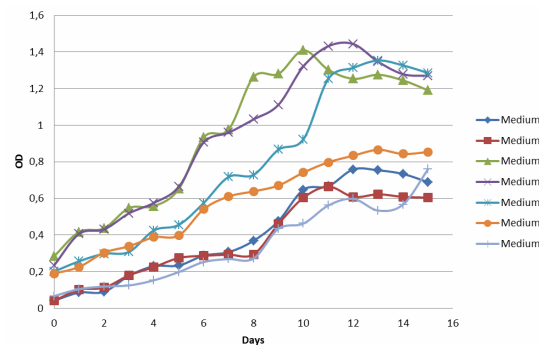


Figure 5. Increasing OD of *Spirulina* during cultivation using remediated medium

Table 2. Result of Growth Rate and CNP Ratio

No	Media (After remediation) plant	time (days)	CNP Ratio	Growth Rate/day
I	-	-	58.547 : 10.586 : 1	0.197
II	Water hyacinth	3	57.853 : 9.436 : 1	0.181
III	Lotus	3	57.790 : 9.283 : 1	0.271
IV	Water hyacinth	6	58.120 : 9.558 : 1	0.154
V	Lotus	6	58.235 : 9.489 : 1	0.115
VI	Water hyacinth	8	57.930 : 9.526 : 1	0.136
VII	Lotus	8	58.316 : 9.562 : 1	0.097

The recommended C:N:P ratio for algae growth was around 56:9:1 based on photosynthetic stoichiometry (Phang and Ong, 1988). In medium III, C:N:P ratio reached 57.790: 9.283: 1. The right content of the nutrients especially N and P influences on the growth rate of *Spirulina* (Hadiyanto *et al.*, 2012). Nitrogen is used to help produce proteins when amount of carbohydrates are limited (Goksan *et al.*, 2007). The main function of Carbon is as builder substances for *Spirulina* and only used a little to the process of photosynthesis. Excess of Carbon will not affect the rate of growth of *Spirulina* because it is an inhibition. While excess of minerals such as N and P will be converted as toxic and affect the growth rate of *Spirulina* (Mun *et al.*, 1989). The other factors that affect the growth rate of *Spirulina* is the adaptation time. *Spirulina* in medium IV had a higher growth rate than the medium V, while the content of N in medium IV is more than the medium V. This shows that time adaptation of *Spirulina* in medium IV is better than *Spirulina* in the medium V (Hadiyanto *et al.*, 2012).

CONCLUSION

From the result of experiment, it can be concluded that phytoremediation by using Water Hyacinth is more effective than using lotus for contaminant reduction. In the second stage of phytoremediation, microalgae *Spirulina* can grow well

in effluent of the first phytoremediation which has been remediated by lotus and 3 days of retention time.

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