Applied Environmental Research
- RESEARCH FOR SUSTAINABLE PLANET -

Research Article

The Combination of Activated Sludge and a Constructed Wetland for Treating Soybean Wastewater

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Abstract

Soybean is a nutritious and popular material in the Vietnamese food industry. Soybean processing wastewater contains a significant amount of organic compounds, which have a negative impact on aquatic life due to light penetration and oxygen consumption limitations. Therefore, this study was carried out to determine whether the combination of activated sludge and a constructed wetland using VA06 grass could be effective in treating soybean wastewater. Various amounts of activated sludge were investigated. To be specific, a 20% additional sludge percentage resulted in the shortest treatment time for chemical oxygen demand (COD), total nitrogen (TN), and phosphorus (TP). Additionally, the treatment time was gradually increased by adding smaller volumes of sludge (5% and 10%). Furthermore, the experimental model combining activated sludge and the constructed wetland showed a high treatment efficiency of 97.29% for COD removal, 67.52% for TN removal, and 91.61% for TP removal. Finally, the growth of VA06 grass rose from 41 cm to 80 cm, demonstrating that VA06 grass was adaptable and could be applied to treat soybean wastewater.

ARTICLE HISTORY

Received: 7 Mar. 2022
Accepted: 15 Jun. 2023
Published: 29 Jun. 2023

KEYWORDS

Soybean;
Wastewater treatment;
Activated sludge;
Constructed wetlands

Introduction

Soybean, also known as *Glycine max*, is a kind of annual plant grown in every country [1]. Because of the high protein content and stable composition of amino acids in soy products, they have a long tradition of use as foods that are both nutritious and popular [2]. However, the environment is being significantly impacted by a large amount of released wastewater from the soybean manufacturing process. Soybean wastewater is normally characterized by concentrations of chemical oxygen demand (COD), total nitrogen (TN), total suspended solids (TSS), and low pH [3–5]. As a result, if these contaminants are released without being treated, they cause a serious concern for both humans and the environment [6].

Hue is known as Vietnam's Buddhist capital, with a large number of disciples. Therefore, it has a high consumption rate of soybean-based products such as tofu and soy milk. According to collected data of authors, Hue city has approximately 50 - 80 soybean processing households, each of which consumes approximately 100 to 200 kg of raw materials and discharges about 3-5 m^3 d^{-1} of wastewater without any treatment system. Not only does this causes serious environmental pollution, but it also has an impact on the lives of those who live nearby. Because of the increased public awareness about the environment, soybean wastewater treatment has always attracted the attention of the world in general, and Vietnam in particular.

Many various methods are conducted for treating soybean wastewater including membrane technology [7], biological treatment like activated sludge [8], micro-organism strains [9-10], physico-chemical treatment such as the combination of coagulants with synthetic cationic polyelectrolyte [11], anaerobic reactor [2, 4], or

https://doi.org/10.35762/AER.2023011

constructed wetlands [12-13]. Despite having contributions to pollutant removal, these methods have their own certain drawbacks. Membrane technology requires significant construction and operating costs, as well as the ability of pore blockage. Physical-chemical treatment challenges the management of the potential for secondary contaminations, which requires further treatment [14]. Furthermore, the use of activated sludge and constructed wetlands have been separately investigated. However, treatment efficiencies are not high. Thus, developing another approach that can be modified as an alternative to the conventional method of removing contaminants is critical.

Activated sludge is a microbial community consisting of biological flocs that are microorganism matrix, non-living organic debris, and inorganic elements. The species in these communities are an important indicator of the performance of the wastewater treatment process. The microorganisms include bacteria, unicellular, fungi, protozoa, rotifers, insect larvae, and worms of different types [15]. The activated sludge process is the most commonly applied biological wastewater treatment technology.

Constructed wetlands (CWs) are the treatment system that is engineered to imitate the properties of a natural wetland. It is regarded as a promising technique for pollutant removal from wastewater due to its low cost and low energy consumption [16]. However, many problems occur in the practical application of constructed wetlands, such as vulnerability to changes in climatic conditions and temperature, substrates that are easily saturated and plugged, being easily affected by plant species, occupying large areas, and other issues such as irrational management, non-standard design, and a single function of ecological service [17]. One critical aspect of phytoremediation is the selection of appropriate plants [13]. Some plant species have been investigated for CWs including water hyacinth and water lettuces [18], water spinach [19], or vetiver grass [13]. The grass VA06 is a hybrid between *Pennisetum purpureum* and *Pennisetum mericanum*. This is a highly productive grass that can grow in a variety of soil types. Furthermore, it is not only resistant to cold and drought conditions, but it can also grow quickly and strongly in tropical climates. VA06 grass could be used to feed livestock such as buffaloes, or cows [20-21]. However, VA06 grass had not been widely investigated for the treatment of soybean wastewater.

The treatment of organic contaminants in wastewater has always been a major challenge in terms of efficiencies and costs. To address the drawbacks of the previous studies, the current research introduces a method of treating soybean wastewater in Hue, Vietnam, using a combination of activated sludge and CWs.

Materials and methods

1) Materials

1.1) The soybean wastewater and VA06 grass

Soybean wastewater was collected from five households in Hue, Vietnam. Before being used for experiments, the wastewater samples were filtered to remove sediments. The preservation method chosen is determined by the type of analytical parameters according to the standard method.

VA06 grass was obtained from the Hue University of Sciences campus. Before being grown in the experiment system, the VA06 grass was cut into 25 cm long pieces that included both roots.

1.2) Activated sludge

The sludge used in the experiment obtained from a brewer’s return sludge secondary sedimentation tank. The sludge has weak activity, a dark brown color, and a high amount of water that cannot be used directly. Therefore, the sludge will be provided nutrients for the growth of microorganisms. The nutrient solution is prepared in the following ratio: COD: N: P = 100: 5: 1, in which COD is supplied by glucose ($C_6H_{12}O_6$), N by ammonium NH4Cl, and P by KH_2PO_4 [22]. During the process of cultivating microorganisms, an air flow rate of 3.6 m^3 d^{-1} is maintained throughout the tank. Sludge formation was observed by using a microscope (Olympus CX31, Japan) at Ч 400 magnification. Then, the clear top water would be removed after three days, and the activated sludge would be collected. The number of microorganisms was determined by using the colony counting method and a colony counter (FUNKE GERBER Colony Star 8500).

2) Methods

2.1) Constructed wetland system designing

Figure 1 illustrates a pilot-scale CW model for soybean wastewater treatment, with length (cm), width (cm), and height (cm) of 50, 37, and 37, respectively. The system was supplemented with gravel and sand. The gravel layer was at the bottom of the system, and the sand layer was on top of it. A PVC (polyvinyl chloride) pipe was placed at the system's bottom to allow wastewater to pass through filter material layers. The VA06 grass was cut into 25 cm long pieces before being grown in the experiment system. The distance between the saplings was 10 cm. The system was only supplied with clean water for the first 30 days to allow the grass to develop and adapt to the environment. The model was designed with a 3% slope to collect the treated wastewater completely.

2.2) Evaluating the effectiveness of wastewater treatment using activated sludge

The wastewater treatment efficiency of activated sludge was studied using various sludge percentages of 5%, 10%, and 20%. The wastewater sample used was 5 L in volume and was continuously supplied with oxygen during the experiment. COD, TN, and TP would be determined after 12 h. This experiment with activated sludge is considered as initial wastewater treatment.

The percentage of used activated sludge volume was determined by the following Eq. 1.

$$AS\ (\%) = \frac{100 \times V_S}{V_S + V_{WW}} \quad \text{(Eq.1)}$$

Where: AS is the amount of added activated sludge (%), VS is the volume of activated sludge (mL) (%) and VWW is the volume of wastewater (mL) (%).

2.3) The combination of activated sludge and the constructed wetland

To determine the effectiveness of wastewater treatment when combining activated sludge with the CWs model, the 5 L of influent wastewater would be treated primarily by the activated sludge before transferring it into the CWs model. After 1.25 d, the treated water would be collected, and the quality of the water would be evaluated by COD, TN, and TP.

3) Analytical method

Water quality was measured by parameters including color (Pt-Co), COD (mg L^{-1}), TN (mg L^{-1}), TP (mg L^{-1}), and TSS (mg L^{-1}). The color was assessed through a UV-vis spectrophotometer (wavelength of 410 nm, UV-1800, Shimadzu, Japan). The TSS, COD, and TP of the samples were determined based on the Standard Method for Water and Wastewater examination [23]. COD and TP absorbances were read at 604 nm and 880 nm, respectively. TN was determined by using TOC-L laboratory total organic carbon analyzers (H544353, Japan). The pH was measured using the pH Meter MT 2310. Sobo Air Pump (SB-648A) 2 Outlets supplied the oxygen.

Treatment efficiency was determined by the following Eq. 2 [24].

$$H\ (\%) = \frac{a-b}{a} \times 100 \quad \text{(Eq. 2)}$$

Where; H is the removal efficiency, a is the influent value of the wastewater (mg L^{-1}), b is the effluent value of wastewater (mg L^{-1}).

Each experiment was repeated 3 times. Data were analyzed by descriptive statistics method using IBM® SPSS® Statistics software (version 20). All the graphs were constructed by Microsoft® Office Excel software (2013).

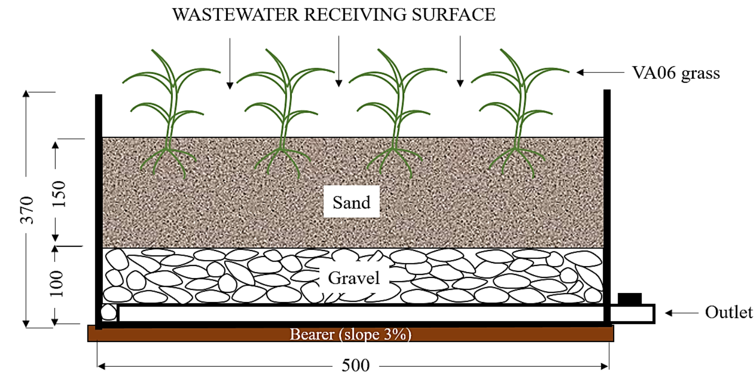

Figure 1 The design of the constructed wetland. From top to bottom, the system is made up of two layers of filter material: sand (particle size 1-1.5 mm) and gravel (particle size 15-20 mm). The sand and gravel layers are 150 mm and 100 mm in height, respectively. VA06 grass was planted into the system after the filter material layers were filled. The PVC pipe (∅ 10 mm) was placed into the model's bottom. All measurements were given in millimeters.

Results and discussion

1) The process of activated sludge formation

The formation of activated sludge was continually monitored during the experiment. Elements including floc size, cohesiveness, and distribution had changed significantly throughout time. To be specific, after 24 h of aeration, small flocs were formed and scattered in the solution. There were increases in the density, size, and cohesive capacity of flocs after 48 h of aeration. The high density of flocs prompted large blocks to form after 72 h of aeration, and the sludge biomass was collected for further treatment (Figure 2).

The composition and quantity of microorganisms in sludge were determined since they played a crucial role in wastewater treatment. The results indicated that the composition of microorganisms was extremely diverse including bacteria, yeast, actinomycetes and mold. In particular, the quantities of these microorganisms that were counted were 2.4×10^8 cells mL^{-1}, 2×10^8 cells mL^{-1}, 9×10^7 cells mL^{-1} and 1.3×10^5 cells mL^{-1}, respectively.

2) The characteristics of soybean wastewater

The characteristics of soybean wastewater were evaluated for COD, TN, TP, and TSS concentrations. The majority of the wastewater parameters were highly high, as illustrated by Table 1. The pH of the wastewater ranged from 5.4 to 5.6 with an average of 5.5. COD, TN, and TP mean concentrations were 5,982.40 mg L^{-1}, 92.40 mg L^{-1} and 63.20 mg L^{-1}, respectively. Additionally, soybean wastewater contains suspended substances, which cause considerable turbidity, as determined by a TSS value of around 2,000 mg L^{-1}. These results were higher than the Vietnamese wastewater standard, which was COD (150 mg L^{-1}), TN (40 mg L^{-1}), TP (6 mg L^{-1}), and TSS (100 mg L^{-1}).

The results obtained in this work are similar to what has been found in previous literature. According to Faisal et al. [25], the COD of tofu wastewater ranged from 5,000 – 8,500 mg L^{-1}. Additionally, the research of Satyanarayan et al. [11] reported that the COD value was between 4,260 and 7,200 mg L^{-1}. The high COD concentration indicated that the wastewater had a significant amount of organic compounds, which was the cause of the decrease in dissolved oxygen concentration in the water. The excessive release of nitrogen (N) and phosphorus (P) into the aquatic environment may result in eutrophication [26].

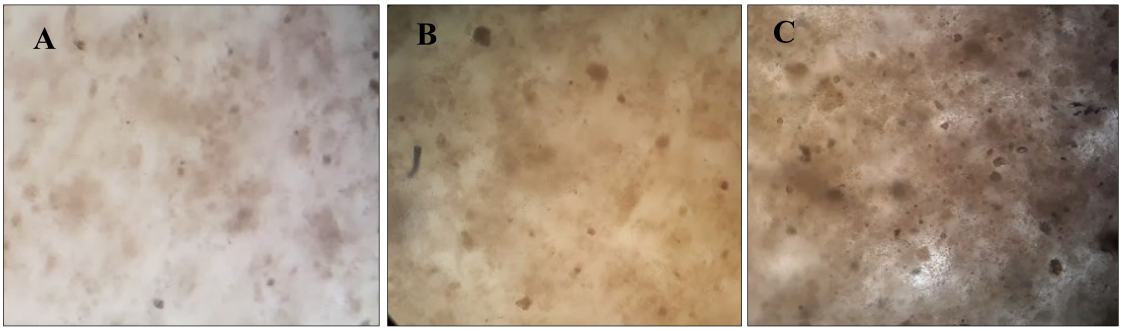

Figure 2 The formation of activated sludge was monitored over time (x 400 magnification). The sample of activated sludge after 24 h of aeration (A), after 48 h of aeration (B) and after 72 hof aeration (C).

Table 1 Characteristics of soybean wastewater

Parameter	Values[a]	Limit values (class B)[b]	
		Class A	Class B
Color	Daffodil	-	-
Odor	Stink	-	-
pH	5.5 (0.070)	5.5 -9	6-9
COD	5982.40 (205.805)	75	150
TN	92.40 (2.302)	20	40
TP	63.20 (3.633)	4	6
TSS	2000 (158.113)	50	100

Remark:
[a] All values are expressed in mg L^{-1} except from pH, color and smell. The values in the table are mean (SD).
[b] National Technical Regulation on Industrial Wastewater (Decree of the Minister of Natural Resources and Environment of Vietnam. No. 40, 2011), class B (Industrial wastewater is discharged into the water sources not serving tap water supply).

3) Evaluating the efficiency of soybean wastewater treatment by activated sludge

Figures 3 to 5 clearly indicate that various percentages of activated sludge had different treatment efficiencies and treatment times. It is possible that 20% activated sludge could remove COD faster than 10% and 5% activated sludge, with the times required for treating wastewater being 48, 72, and 84 h, respectively. The COD treatment efficiencies of the different ratios of activated sludge (5%, 10%, and 20%) were not significantly different from each other and, and the removal efficiencies were 97.38%, 97.42%, and 97.28%, respectively (Figure 3). In addition, the treatment of TN and TP using 20% activated sludge was

https://doi.org/10.35762/AER.2023011

demonstrated in Figures 4 and 5, with treatment efficiencies of 65.48% and 92.01%, respectively, in 48 h. TN and TP treatment times were similar to COD. The high concentration of microorganisms in 20% activated sludge might explain why this concentration of sludge can reduce treatment time to a value lower than that of 5% and 10% sludge.

The use of the activated sludge in treating soybean wastewater was demonstrated by Tay (1990) who concluded that the wastewater could be treated by activated sludge and COD removal efficiency was over 90% [8]. Moreover, TN removal efficiency ranged from 57% to 80%, whereas TP removal efficiency was at 57%. Additionally, in the study of Sakinah et al. [27], the author used various sources of activated sludge to treat tofu wastewater, and the results indicated that activated sludge obtained from a polluted river had the highest COD removal effectiveness with 81% on the 13th day. Thus, the findings demonstrate that the effectiveness of activated sludge in reducing the mentioned three main parameters is greater compared to that of previous research.

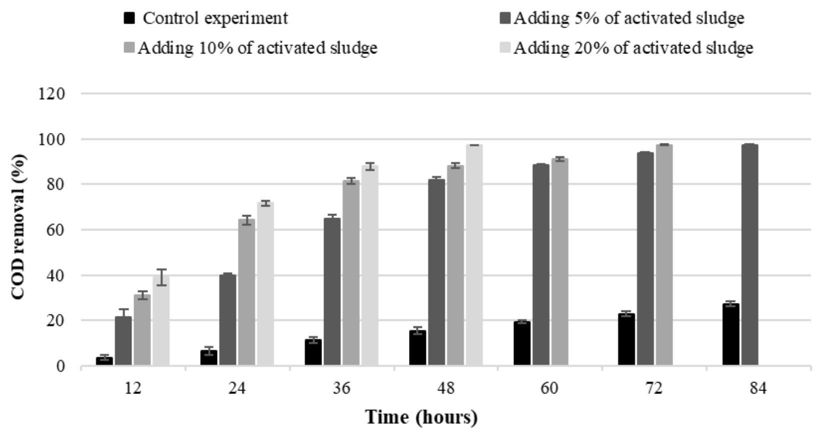

Figure 3 The treatment efficiency of COD of the soybean wastewater with different proportions of activated sludge. When 5%, 10%, and 20% activated sludge were added, the final treatment efficiencies were 97.38%, 97.42%, and 97.28%, respectively. Furthermore, after 84 h of treatment, the control group's efficiency was 27.24%.

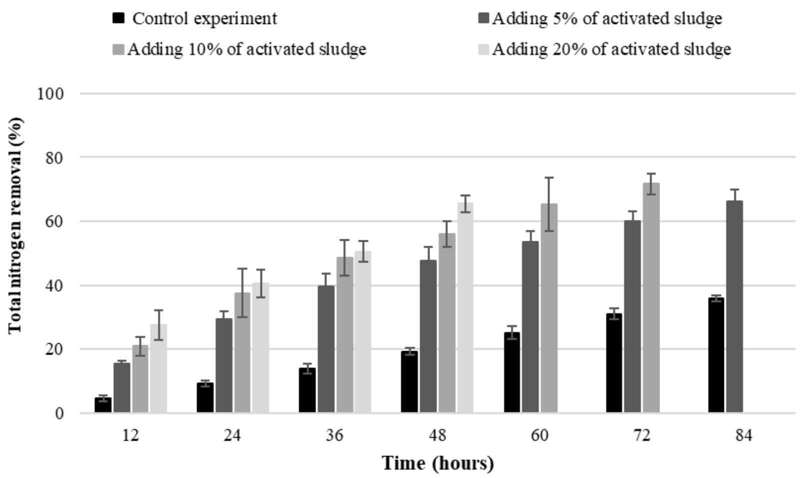

Figure 4 The treatment efficiency of TN of the soybean wastewater with different proportions of activated sludge. The treatment efficiencies of the three sludge proportions described above were 66.06%, 71.73%, and 65.48%, respectively. Meanwhile, the control group's efficiency was 35.96%.

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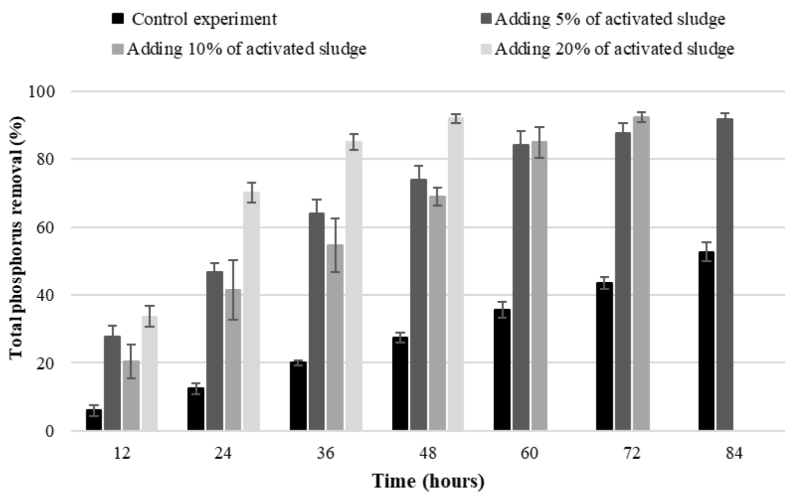

Figure 5 The treatment efficiency of TP of the soybean wastewater with different proportions of activated sludge. The final treatment efficiencies were 91.68%, 92.41%, and 92.01% when 5%, 10%, and 20% sludge were applied, respectively. Furthermore, the efficiency of the control experiment was 52.66%.

4) Evaluating the ability of treating soybean wastewater by using activated sludge combined with a constructed wetland

The ability of treating soybean wastewater when combining activated sludge with a constructed wetland was evaluated (Table 2). After pre-treating the wastewater with activated sludge for 24 hours, the COD of the water sample reduced dramatically from 5214.0 mg L^{-1} to 1,930 mg L^{-1}. After being transferred to the filtering system, the COD reached 141 mg L^{-1}. This value was lower than the Vietnamese standard (150 mg L^{-1}, in TCVN 40:2011, class B). In addition, the constructed wetland system's multiple layers structure significantly reduced TN and TP concentrations in treated effluent.

Through absorption with a well-developed root system, sorption, and precipitation with released biomolecules, biological intervention in the constructed wetland by growing plants decreases the pollutants [28]. Plants and substrates have a significant influence on the performance of constructed wetlands for sewage soybean wastewater treatment, with nitrogen removal mechanisms and processes carried out by nitrifying and denitrifying bacteria being one of the most important [29]. Additionally, The adsorption capability of filler layers, microorganisms, and plants also has a significant impact on the concentration of TP in constructed wetlands [30]. Therefore, after passing through the constructed wetland system, the concentrations of contaminants were identified to have been reduced by a significant amount.

The growth of VA06 grass was also observed and presented in Table 3. After one month of adaptation, the length of VA06 grass increased from 20 cm to 41 cm. Then, after one month of treating soybean wastewater, the length of VA06 grass rose from 41 cm to 80 cm. This demonstrated that soybean wastewater contains a high concentration of nutrients that enhance grass growth.

The effect of using plants on treating tofu wastewater has also been demonstrated in the research of Seroja et al. [13], who used vetiver grass (Vetiveria zizanioides) without combining with activated sludge to treat tofu wastewater. According to the author, the removal effectiveness of COD, BOD (biochemical oxygen demand), and TSS was 76%, 71.78%, and 75.28%, respectively. However, the treatment time was relatively long, with the best results obtained on the 15th day. Additionally, in the study of Oktorina et al. [31], the author applied a method called phytoremediation (Eichhornia crassipes) treatment variations used anaeration and aeration to treat tofu wastewater. The results indicated that anaeration treatment reduced BOD, COD, TSS, ammonia about 59.84%, 58.95%, 86.79% and 25.43%, respectively. Meanwhile, aeration treatment could reduce BOD by 80.67%, COD by 78.28%, TSS by 65.79%, and ammonia by 49.79%. The treatment time of this study was 10 days. These findings of this study demonstrated that the combination of activated sludge and a constructed wetland produced significant treatment efficiency for soybean wastewater treatment.

https://doi.org/10.35762/AER.2023011

Table 2 The ability of treating soybean wastewater by activated sludge combined with a plant filtration system

Parameter	Influent[a] (mg L^{-1})	Effluent 1[b] (mg L^{-1})	Effluent 2[c] (mg L^{-1})
COD	5,214.0 (91.337)	1,930.6 (88.384)	141.00 (9.618)
TN	87.80 (6.017)	62.00 (4.848)	28.60 (4.615)
TP	64.10 (4.980)	23.00 (4.472)	5.40 (1.140)

Remark:
[a] Raw wastewater
[b] Effluent had been treated with 20% activated sludge for 24 h.
[c] Effluent had been treated by combining 20 % activated sludge and plant filtration system.
The values in the table are mean (SD).

Table 3 The growth of VA06 grass during the treatment time

Days	The height of grass (cm)
0	41
5	49.25
10	54
15	60
20	65.5
25	71
30	80

Conclusion

The process of enriching the biomass of activated sludge has proven to be adaptable to the medium used. The addition of 20% sludge reduced the COD, TN, and TP treatment time. The addition of lower proportions of sludge significantly increased the treatment time. The treatment efficiency of the pilot-scale experimental system combining activated sludge and a constructed wetland was higher than that of activated sludge separately. After 1.25 days of treatment, the optimal condition of soybean wastewater was obtained. With COD, TN, and TP, the ultimate treatment efficiencies were 97.29%, 67.52%, and 91.61%, respectively. The present research supports that the combined use of activated sludge, and the constructed wetland is more efficient and economical than applied separately. Furthermore, the harvested grass biomass can be used for a variety of purposes and is environmentally friendly. Therefore, combining these two technologies can result in a significant improvement in wastewater treatment. However, this research has some limitations in terms of facilities, which causes difficulties in scaling up the research. Thus, it needs to be improved in the future.

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