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Lactic Acid Bacteria (LAB) from Fermented Milk Leftover for Wastewater Treatment of Soybean-Based Industries

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Abstract

Tempeh and tofu are widely consumed by Indonesians and easily made using simple tools and method, thus numerous home industries producing are emerging. However, soybean processing in these industries produces high volume of wastewater. This study provides a simple wastewater treatment instrument design that could be used by soybean-based home industries by applying a series of physical, chemical, and biological treatment. Physical treatment was performed by filtration, chemical by addition of alum salts, while biological treatment was done by supplementing with lactic acid bacteria (LAB) from leftover container of fermented milk. Wastewater was recorded by its parameters (temperature, pH, dissolved oxygen/DO, biological oxygen demand/BOD, turbidity) at every phase of treatment after incubation for two various of periods; 1 hour and 5 days. Results showed that wastewater treated using instrument design had changes in all of its parameters observed during the course of treatment. Wastewater treated in all phase of instrument incubated for 1 hour showed parameters approaching wastewater quality standards, thus it was safer to be disposed to environment compared to wastewater incubated for 5 days. Wastewater treatment as designed in the current study can be applied by soybean-based home industries with short incubation time to remediate wastewater produced during soybean processing before disposal.

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Keyword
Wastewater Treatment; Lactic Acid Bacteria; Soybean-Based Home Industry.

Introduction

Tempeh and tofu are popular food widely consumed by the Indonesian community. Both can be made from soybeans using easy method and simple tools. Because of that, local home industries focusing on both have products become numerous. However, soybean processing into tempeh and tofu produces high volume of wastewater.

Wastewater from tempeh and tofu production contains various type of organic materials. Wastewater produced by soybean-based home industry was found to contain 34.8% carbohydrate, 34.9% protein, 18.1% fat, and traces of salt, minerals, and other chemicals (Utami et al. 2013). Various parameters of soybean-based industrial wastewater were found to be above quality standards (Purwanti et al. 2018). However, because of their minimum cost and limited tools, soybean-based home industries hardly perform any

treatment to their wastewater before they dispose it to environment. Disposal of untreated wastewater with high organic content can induce eutrophication of water body. Continuous eutrophication leads to algal bloom that caused a lot of negative effects towards various species composing aquatic ecosystem (FAO & IWMI 2017).

Simple wastewater treatment can be performed by physical, chemical, and biological treatment. Physical treatment can be conducted via filtration using various substrate, such as fabrics, sands, or palm fibers, while chemical treatment by administer alum salts. Filtration of wastewater can effectively remove particles and suspended solids (Crini & Lichtfouse 2019), while alum salts can induce coagulation, causing lower turbidity and total dissolved solid (TDS) as performed previously performed in wastewater produced by blueberry and carrot processing (Ardley et al. 2019). Meanwhile, biological treatment can be conducted by adding microbe culture which is able to degrade organic materials, such as lactic acid bacteria (LAB).

Lactic Acid Bacteria (LAB) is a type of bacteria used widely in fermentation process of various foodstuffs, mainly dairy products, for example yoghurt, cheese, or ya kult. Lactic acid bacteria are able to metabolize carbohydrate into lactic acid, acetic acid, and ethanol (Ganzle 2015). Respiration of LAB can occur at minimal level of oxygen. In addition, LAB is also able to suppress the growth of other microorganisms by mean of its rapid metabolism speed and accumulation of lactic acid and acetic acid which their metabolism produces (Ganzle 2015).

In wastewater of tempeh and tofu industries, various organic materials are dissolved from raw material soybeans into water during their processing, such as protein and carbohydrate during boiling and soaking process, resulting in high level of total suspended solid (TSS) (Purwanti et al. 2018). Organic materials contained in wastewater from soybean-based home industries can be used by LAB as materials for their metabolism. Lowering organic materials contained in wastewater can neutralize thus it will be safer to be disposed to environment. One of LAB species, *Lactobacillus plantarum* had been studied its ability to remediate wastewater of dairy industry. Addition of *L. plantarum* culture under optimum condition was found to lower sugar level, total protein content, and chemical oxygen demand (COD) significantly (Golalikhani & Razavi 2015).

This study was conducted to design and subsequently test a simple wastewater treatment instrument that could be applied by soybean-based home industries using a series of physical, chemical, and biological treatment using LAB culture from leftover container of fermented milk.

Materials and Methods

Sample wastewater used in this study was wastewater produced by tempeh home industries located in Tembarak Village, Kertosono, East Java. Wastewater was measured of its parameters (pH, DO, temperature, and turbidity) before treatment was performed.

Simple wastewater treatment instrument was made by combining physical, chemical, and biological treatment into a series of equipment we called IPAL-LAB (*instrumen pengolahan air limbah dengan bakteri asam laktat*/wastewater treatment instrument using lactic acid bacteria). First step of treatment was physical treatment which was filtration

through cotton fabric. Second step of treatment was chemical, in which 10% alum salts was added into wastewater. The last step of treatment was biological, in which LAB culture from leftover container of fermented milk was added. The series of treatment was designed as schemed in Fig. 1. Sample wastewater was treated in volume 20 L for each respective replication, for four times replication. After each step of treatment, about 10 mL of wastewater sample was taken and then incubated for 2 various periods; 1 hour and 5 days to see whether incubation time affected parameters of treated wastewater and to measure BOD level. Water samples from pre-treatment, physical, and chemical step were stored in refrigerator, while samples after biological treatment was incubated in room temperature to let culture active. Water parameters (temperature, pH, DO, BOD, and turbidity) was recorded after samples were incubated and then described quantitatively.

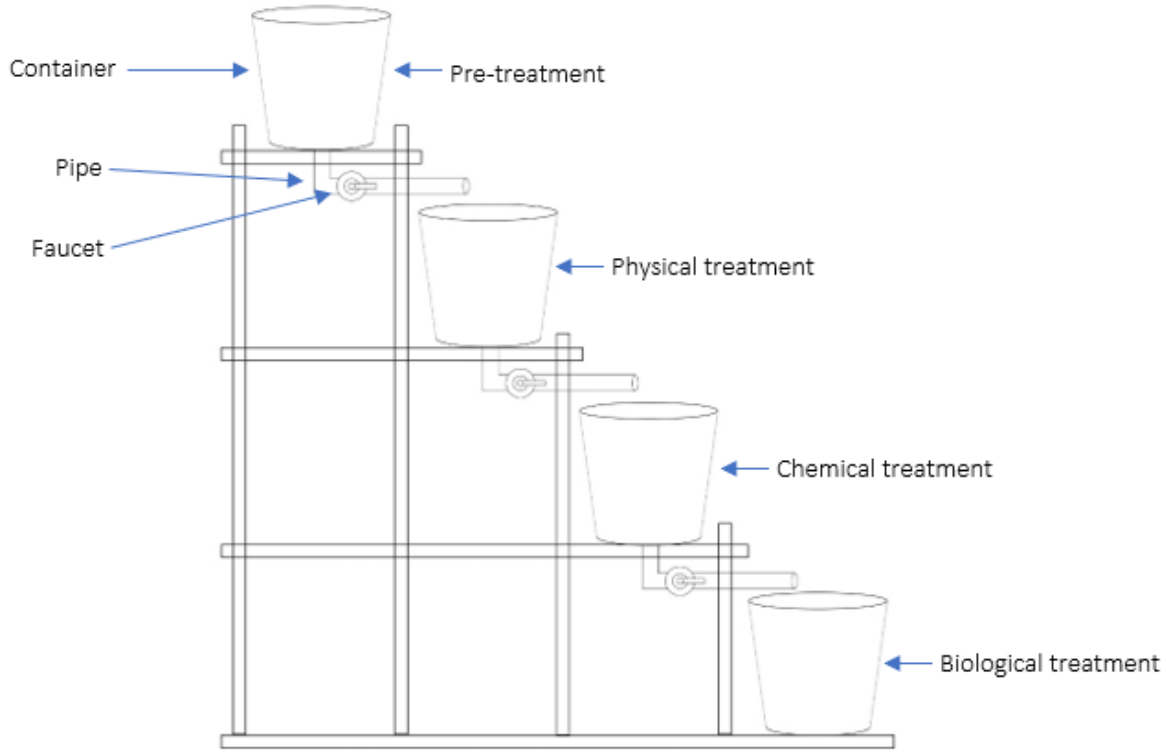

Figure 1. Series of Wastewater Instrument Using Lactic Acid Bacteria (LAB) for Wastewater Treatment of Soybean-Based Home Industries.

Results and Discussion

Comparation of various water parameters recorded from wastewater of tempeh industries from pre-treatment step up to last step of biological treatment with different incubation time is presented in Table 1.

Table 1. Parameters of Wastewater from Tempeh Home Industries Recorded After Treatment Steps with Different Incubation Time.

Parameter	Incubated 1-hour after treatment step-				Incubated 5-days after treatment step-			
	Pre-treatment	Physical	Chemical	Biological	Pre-treatment	Physical	Chemical	Biological
Temperature (°C)	28.38±0.25	28.45±0.10	28.18±0.24	28.28±0.22	29.80±0.00	29.80±0.00	29.80±0.00	29.80±0.00
pH	6.73±0.42	7.07±0.16	7.02±0.08	6.31±0.88	5.09±0.00	5.00±0.01	4.22±0.03	4.20±0.01
DO (mg/L)	2.80±0.53	3.73±0.10	5.80±1.44	5.18±0.91	1.50±0.08	1.53±0.05	2.20±0.12	2.30±0.08
BOD5 (mg/L)	n/a	n/a	n/a	n/a	1.30±0.45	2.20±0.14	3.60±1.50	4.88±0.83
Turbidity (NTUs)	182.00±21.95	133.50±30.65	67.25±15.65	43.35±4.82	157.25±2.22	141.95±6.32	41.32±5.44	57.75±3.40

Result of wastewater parameter recording from pre-treatment up to last step of treatment showed changes in some parameters during the course of treatment, both in 1-hour and 5-days incubation time. Acidity level did not change significantly in 1-hour incubation time, but in 5-days, pH was lowered up to acid level (4.20±0.01). Dissolved oxygen level was increased from pre-treatment until last step of treatment. Higher increase was found in 1-hour incubation time (from 2.80±0.53 to 5.18±0.91) compared to 5-days incubation time (from 1.50±0.08 to 2.30±0.08). Along with DO raise, BOD was also increased along with course of treatment, from 1.30±0.45 mg/mL during pre-treatment up to 4.88±0.83 mg/mL at the last step of treatment. Prominent change was observed in turbidity; in 1-hour incubation time, wastewater turbidity lowered as much as 139.65 NTU, while in 5-days incubation time, turbidity lowered as much as 99.50 NTU.

The series of wastewater treatment performed in the current study to remediate tempeh wastewater was based on commonly used process to treat wastewater before being disposed to environment, including physical, chemical, and biological process. Water parameters was recorded from pre-treatment and after steps of IPAL-LAB up to the last step treatment. From various water parameters recorded, all parameter except temperature showed changes. Temperature did not alter because treatment steps applied in the instrument did not involve process which could cause temperature change.

Acidity level of wastewater did not change much in wastewater incubated for 1 hour, however it was decreased up to pH acid level (5.09±0.00 to 4.20±0.01) in wastewater incubated for 5 days. Lowest pH was shown by wastewater after LAB biological treatment which incubated for 5 days (4.20±0.01).

Decrease of acidity level was related to LAB culture addition that was able to metabolize carbohydrate and other organic materials contained in tempeh industry wastewater into lactic acid (Ganzle 2015). The longer wastewater incubated, LAB activity in it was also higher, resulting in higher level of lactic acid produced. Increasing level of lactic acid cause wastewater pH to lower up to acidic range. However, this range of pH caused wastewater less than safe to be disposed of, due to unsuitable range as required by water organisms. Most water organisms require pH at normal range at around 7.4 to live.

Wastewater pH level after 5 days incubation also did not meet quality standards as established by local government at range of 6-9 (Permen LHK No. 68 Year 2016), in opposite to pH of wastewater incubated for 1 hour (6.31±0.88).

Level of DO was significantly raised in treated wastewater incubated for 1 hour. Dissolved oxygen level after last treatment and incubated for 1 hour was 5.18±0.91 mg/L. This level of DO is still in optimal DO range that indicate good water quality for most water organisms, at range of 4-6 mg/L (Shah & Joshi 2017).

In the other hand, DO level from treated wastewater incubated for 5 days had insignificant change. Level of DO after last treatment was 2.30±0.08 mg/L. This DO level is not suitable to meet oxygen demand of common water organisms and can induce hypoxia in them. Only certain organism with specific tolerance for low level of oxygen can survive such level of DO, such as bottom-dweller bacteria found in the river floor (Connolly et al. 2004).

Lowering DO level of wastewater after biological treatment incubated for 5 days compared to wastewater incubated for 1 hour also indicated microorganism activity in the wastewater. The longer wastewater incubated, both naturally occurred microorganism and LAB culture added to it also increased, resulting in lowering DO level.

Level of treated wastewater BOD after 5 days incubation was raised from pre-treatment step (1.30±0.45 mg/L) up to last step using LAB (4.88±0.83 mg/L). Although this level was below quality standards (Permen LHK No. 68 year 2016), increase of BOD level indicated that wastewater treated using instrument in the current study after 5 days incubation become more difficult to be degraded when disposed to environment.

The most prominent change in parameters observed was found in turbidity. Turbidity had linear relationship with total suspended solid (TSS) contained in the water (Hannouche et al. 2011), thus can be used as a proxy to measure particles contained in the wastewater (Rugner et al. 2013). Treated wastewater sample incubated for both 1 hour and 5 days had significant decrease of turbidity (182.00±21.95 to 43.35±4.82 NTUs and 157.25±2.22 to 57.75±3.40 NTUs, respectively). The most significant decrease occurred during chemical treatment, after alum salts was added into wastewater. Alum salts induce coagulation and flocculation of organic materials contained in the waste by forming aluminum hydroxide when it contacts water. Aluminum hydroxide is able to remove suspended materials via charge neutralization, sweep floc, dan adsorption mechanism (Ahmad et al. 2016), resulting in reduced turbidity.

Treatment using LAB culture from leftover container of fermented milk was able to lower turbidity further in wastewater incubated for 1 hour, but on the contrary, it caused turbidity to raise after chemical treatment in sample incubated for 5 days. Duration of incubation correlates to LAB population in the wastewater. The longer wastewater was incubated, the higher LAB population in it, causing turbidity to raise.

The utilization of LAB culture to remediate waste has been extensively studied, but mostly was in the reuse of solid waste with high content of organic material. *Lactobacillus amylovorus* had been applied to produce lactate from bread wastes (Adessi et al. 2018). *Lactobacillus casei* Shirota had also been studied for producing lactic acid from food waste

with high level of glucose, fructose, and amino acid (Kwan et al. 2016). In matter of remediation, previous study showed that *L. casei* was able to reduce COD level of wastewater from dairy industry up to 60% in 25 days (Keffala et al. 2017).

The series of IPAL-LAB simple wastewater treatment as designed and described above can be applied to neutralize parameters of wastewater produced by soybean-based home industries because the instrument was easy to be compiled with simple tool and materials. Wastewater incubated for 1 hour showed parameters that meet quality standards more than wastewater incubated for 5 days; thus, it was recommended to perform short-period incubation for this treatment.

Conclusion

Simple wastewater treatment instrument as designed in the current study can be applied by soybean-based home industries to remediate wastewater produced during soybean processing. Wastewater should be incubated in short amount of time (1 hour) to obtain parameters closer to government wastewater quality standards.

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