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Influence of powdered *Moringa oleifera* seeds and natural filter media on the characteristics of tapioca starch wastewater

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Abstract

Background: *Moringa oleifera* tree is indigenous and highly abundant in Indonesia and its seeds are widely known to be used as a natural coagulant for treating wastewater. Wastewater from tapioca starch industry, particularly in Indonesia, which contains high organic matters, was revealed by high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) value. In this study the combination of powdered *M. oleifera* seed and natural filter media was studied. A laboratory scale of tapioca starch wastewater (TSW) treatment was designed using a continuous two-stage clarifier tanks filled with sand or coconut fibre combined with the addition of powdered *M. oleifera* seed.

Results: A significant improvement on both the physical and chemical characteristics of the effluent quality, showing a clearer colour and a greater reduction in BOD, COD and total suspended solid values; while pH was in the acceptable range for effluent disposal. In terms of the microbial characteristics, all treatments gave slightly higher counts of total coliform in the range of 26 to 40 MPN 100 ml^{-1}.

Conclusions: The combination of two-stage clarifier tank with natural filter media and the use of *M. oleifera* as natural coagulant gave a significant improvement in the quality and appearance of TSW final effluent.

Keywords: Tapioca starch wastewater; Natural coagulant; *Moringa oleifera* seed; Natural filters; Total *coliform*

Introduction

In Indonesia, tapioca starch industries provide significant contribution to economic and job opportunity. However, many of these industries have problems with their waste because of improper and inadequate waste treatment facilities. Tapioca starch wastewater (TSW) is one of the problems faced by tapioca starch industry due to a large amount of water consumption in the production process (Suprapti 2005; Setyawaty et al. 2011). Tapioca starch industry in Indonesia generated TSW at approximately 12 to 15 times of the volume of the processed cassava (Suprapti 2005) or about 10 to 30 m^3 $tonne^{-1}$ of tapioca produced (Hidayat et al. 2011) with annual TSW generation of 2,400 million m^3 (Setyawaty et al. 2011). Most of the tapioca starch industries directly discharged TSW to water streams without proper treatment. Indeed, TSW contains high value of biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total solids (TS) (Ukita et al. 2006; Sun et al. 2012) and cyanide (Kaewkannetra et al. 2009), leading to a serious hazard to the environment and the aquatic life in the receiving watercourse (Siregar 2006; Chavalparit and Ongwandee 2009; Kaewkannetra et al. 2009, 2011).

Wastewater treatment of TSW has been the subject of many studies, and many treatment technologies have been implemented such as a modified rotating biological contactor (Radwan and Ramanujam 1996), anaerobic pond system (Rajbhandari and Annachhatre 2004), upflow anaerobic sludge blanket technology (Annachhatre and Amatya 2000; Chavalparit and Ongwandee 2009), anaerobic pond with bamboo filter (Colin et al. 2007), anaerobic sequencing batch reactors (Sreethawong et al. 2010), microbial fuel cells (Kaewkannetra et al. 2011), upflow

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multistage anaerobic reactor (Sun et al. 2012) and anaerobic baffled reactor (Thanwised et al. 2012). In order to be used in small and medium-sized enterprises of tapioca industry, wastewater treatment technology should be based on a simple system, a high efficiency process, and a low-cost construction (Sutherland et al. 1994; Yan et al. 2011; Racho et al. 2012). An alternative is to apply an aerobic biological treatment combined with the addition of chemical coagulants to remove the fine starch particles from the water, thereby significantly reducing COD and BOD from wastewater. Chemical coagulants, such as iron salt, lime and polymers, are widely used to enhance the effluent quality wastewater treatment and even treatment of TSW, but these tend to be expensive or difficult to obtain as well as having toxicological and harmful effects (Yongabi 2010). The use of natural coagulants to replace chemicals may be advantageous since they can be environmentally friendly and economically feasible (Nkhata 2001).

Moringa oleifera tree is indigenous and highly abundant in Indonesia. It has been widely used for many purposes, including water purification by activated carbon from its seed husks (Pollard et al. 1995); heavy metal removal such as Cd(II), Cr(III) and Ni(II) (Sharma et al. 2006, 2007); nutrition sources and medicinal purposes (Fahey 2005) and antibacterial agents (Ferreira et al. 2011; Mangale et al. 2012). Moreover, several studies observed that *M. oleifera* seeds contain coagulant molecules such as proteins which showed good flocculating effects (Ndabigengesere and Narasiah 1998; Okuda et al. 1999; Ghebremichael et al. 2005; Kwaambwa and Maikokera 2007; Santos et al. 2009; García-Fayos et al. 2010).

M. oleifera seed can be used as a coagulant in water or wastewater treatment (Ndabigengesere et al. 1995; Nkurunziza et al. 2009; Vieira et al. 2010; Ubuoh et al. 2013). A protein extracted from *M. oleifera* seed has demonstrated its effectiveness in wastewater systems (Bhatia et al. 2007), removing 99% of suspended solids without changing the pH of the water (Katayon et al. 2006). Previous study by Sutherland et al. (1994) also demonstrated that *M. oleifera* seed coagulant was able to significantly improve the clarification of highly turbid river water, showing a high reduction in its turbidity from 270 to 380 NTU to below 4 NTU. Similarly, *M. oleifera* seeds coagulant increased the turbidity removal of the river water up to 96.23% at a dose of 0.4 mg l^{-1} (Ali et al. 2010). Another finding showed that *M. oleifera* in conjunction with aluminium sulfate as coagulants reduced turbidity by 85.9% to 98% and decreased the amount of *Escherichia coli* by 99.2% to 99.97% (Bina et al. 2010). Furthermore, according to Bhatia et al. (2007), the protein in the *M. oleifera* seed is a natural product, which can reduce hardness in water or wastewater with better performance and a low risk to environment, thus replacing the more costly chemical coagulants such as alum and powdered-activated carbon. However, several findings reported that the application of high quantity of *M. oleifera* seed powder leads to toxicity and mutagenic effect to, for example, male albino rats at doses higher than 2,000 mg l^{-1} (Oluduro and Aderiye 2009), the cell-free plasmid DNA and the *Salmonella typhimurium* (using Ames and Kado assays) at doses of more than 400 mg l^{-1} (Rolim et al. 2011), the Nile tilapia fish *Oreochromis niloticus* at doses above 200 mg l^{-1} (Ayotunde et al. 2011), and freshwater fish *Cyprinus carpio* at a dose of 124.0 mg l^{-1} or more (Kavitha et al. 2012). These findings therefore provide the safety thresholds for the dose of powdered *M. oleifera* seed that needs to be used for treating water or wastewater.

Filtration treatment is one of the promising methods to reduce dissolved and particulate matters in water or wastewater. A range of filter media has been used for water or wastewater purification purposes: granular activated carbon (Bansode et al. 2004; Gur-Reznik et al. 2008; Antony et al. 2012; Rigobello et al. 2013), zeolite (He et al. 2007; Caro and Noack 2008; Mažeikiene et al. 2010; Qiu et al. 2010), ceramic particle (Taslicukur et al. 2007; Oyanedel-Craver and Smith 2008; Yue et al. 2009; Qiu et al. 2010) and membrane (Gopal et al. 2007; Kim et al. 2007; Chiemchaisri et al. 2011; Li et al. 2012). Furthermore, several experimental results showed that natural filter media can be used in filtration process, such as sand (Hamoda et al. 2004; Kang et al. 2007; Gunes and Tuncsiper 2009; Hollender et al. 2009), gravel (Hatt et al. 2007; Nkwonta 2010; Wadi 2010), charcoal (Nkwonta and Ochieng 2010), coconut fibre (Frankel 1979; Sherman 2006; Nkwonta and Ochieng 2009), burnt rice husks (Frankel 1979) and date palm fibres (Riahi et al. 2009). Combining coagulation process with filtration process may result in excellent removal rates (Choi et al. 2008; Dialynas and Diamadopoulos 2008; Li et al. 2011). For instance, the combination of *M. oleifera* seed coagulant and alum with sand filtration process was able to improve COD removal (Bhuptawat et al. 2007). Furthermore, to the best of our knowledge, although the powder of *M. oleifera* seeds has been tested as a single primary coagulant for treating TSW, none has been tested in combination with two-stage filtration process. Thus, to assess the applicability of natural filter media combined with a natural coagulant (powdered *M. oleifera* seed), several experiments were performed at the laboratory scale. The main objective was to investigate the performance of TSW treatment system in the arrangement of two-stage filtration process combined with the addition of powdered *M. oleifera* seed in reducing BOD, COD, TSS and total *coliform*, as well as in increasing pH.

Methods

The experiments used laboratory-scale two-stage clarifier tanks working at aerobic condition in treating TSW with different doses of powdered *M. oleifera seed* and natural filter media. This experiment was conducted at the Laboratory of Bioindustry, University of Brawijaya, Indonesia.

Tapioca starch wastewater (TSW) and powdered *M. oleifera* seed

TSW was collected fresh from small-scale tapioca industry in Kediri city, East Java, Indonesia. It was packed and stored at approximately 26°C. *M. oleifera* seeds used were also collected from Kediri city. The seeds' wings and coat from the mature and selected good quality of seeds were manually peeled. The kernel of *M. oleifera* seeds was then dried at approximately 24 h and milled to a fine powder using a domestic blender. Fine powders that passed through a 50-mesh siever were then used as coagulant.

Natural filter media

Two natural filter media used in this experiment were sand (Ø = 1.54 mm) and coconut fibre. Prior placing in the clarifier tank, the sand was washed, dried and sieved to obtain homogeneous size, while no pre-treatment was required for coconut fibre. Each natural filter medium was laid at the second compartment of the clarifier tank with a height of 0.25 m. Sand was chosen because it is naturally available and abundant, has excellent absorption, drainage and water-holding ability as well as act as permeable reactive barrier to trap organic pollutants (Wang et al. 2001). Coconut fibre has similar characteristics to sand, but it needs to be used with care due to the possibility of flavouring the water throughout the filtration process (Nkwonta and Ochieng 2009).

Experimental set-up

A small-scale experiment was carried out in two open clarifier tanks with an effective working volume of 25 l, operating under aerobic condition at 26°C (Figure 1). The clarifier tank was made of stainless steel with dimensions of 0.3 m wide × 0.6 m long × 0.3 m high. Each clarifier tank has three connected compartments, with three different functions: influent collection, purification and effluent collection. The two-stage clarifier tank was designed in a continuous and series system. The primary influent collection tank (A) is where raw TSW was treated with different doses of powdered *M. oleifera* seeds. The coagulation process in this tank was conducted based on Jahn (1986) as follows: each dose of powdered *M. oleifera* seed was diluted in 1 l TSW then gradually agitated at 15 to 20 rpm for 5 min and incubated for 3 h for sedimentation. The influent then flowed directly to the first clarifier tank (B) and passed through the first filter media layer. The influent from the first clarifier tank continuously flowed into the second clarifier tank (C) and passed through the second filter media layer for enhanced the organic matters removal in treated TSW. The final effluent was then stored in the final effluent collection tank (D). The use of two-stage clarifier tank with different natural filter media aimed to prolong the settling or contact time to improve the removal efficiency of organic matters and

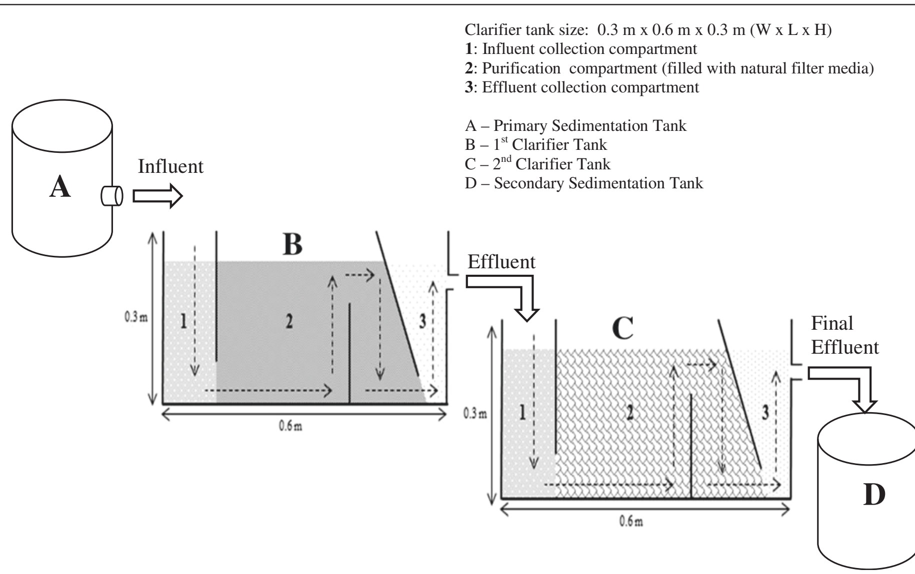

Figure 1 Model of clarifier tank and design of TSW treatment. The model of clarifier tank and design of TSW treatment with two-step clarifier tanks filled with natural filter media.

the effluent quality of TSW. Both clarifier tanks have the same settling time and flow rate, with total incubation time of 24 h.

The whole test was divided into two arrangements of clarifier tanks as follows: the first clarifier tank filled with sand followed by the second clarifier tank filled with coconut fibre (T_1) and first clarifier tank filled with coconut fibre followed by second clarifier tank filled with sand (T_2).

These two-stage clarifier tanks were combined with the addition of different doses of powdered *M. oleifera* seeds at 110 mg l^{-1} (D_1), 130 mg l^{-1} (D_2) and 150 mg l^{-1} (D_3). The doses were selected based on the safety threshold of less than 200 mg l^{-1} (Ayotunde et al. 2011; Rolim et al. 2011). Each experiment was carried out in duplicate with the TSW flow rate maintained at 5 l day^{-1} and retention time of 10 days. The BOD, COD, TSS, pH and the amount of total coliform were analysed on a 5-day basis.

Analytical methods

COD was determined by the dichromate closed reflux method and measured using a spectrophotometer (the Shimadzu UV-1601, Shimadzu Coorporation, Japan) at 444 nm. BOD was measured by the 5-day BOD test method where the sample was incubated at 20°C ± 1°C for 5 days and the oxygen consumed was measured. TSS was measured by a gravimetric measurement of the residue dried to a constant weight for at least 1 h at 103°C to 105°C. The effluent and influent pH values were measured using a pH metre and a combination glass electrode calibrated in buffers at pH 7 and 9. The multiple-tube fermentation method was used to determine the most probable number (MPN) of coliform presences in TSW. In this method, the coliform bacteria are detected and quantitated by their ability to grow and produce gas in lactose-containing liquid medium under specified incubation conditions (at 35°C for 48 h). The procedures for COD, BOD, TSS, pH and the multiple-tube fermentation methods were followed as described in the Standard Methods for the Examination of Water and Wastewater (APHA 2005). All these parameters were routinely monitored during the experimental period.

Statistical analysis

One-way analysis of variance (ANOVA) was employed to examine any difference between different groups of wastewater treatments. Duncan's multiple range test was performed in case of significant differences with the independent factor or with the interaction between the independent and repeated factors. Differences were considered to be statistically significant when $P < 0.05$. A statistical program (IBM SPSS Statistics 20) was used to compute the data.

Results and discussion

The initial physical and chemical characteristics of TSW

It can be seen from Table 1 that initial BOD, COD and TSS values (1702.1, 6370.4, and 206.6 mg l^{-1}, respectively) were far higher than the standard value for TSW discharge given by the government of East Java, Indonesia. It is obvious that typical BOD and COD are often associated with the biodegradability of wastewater (Chamarro et al. 2001) and the strength of organic contents in wastewater (Lee and Lin 2000; Penn et al. 2009). The high BOD and COD values in TSW therefore constitute a high concentration of organic matters and indicate that fresh TSW is relatively biodegradable as BOD/COD ratio was 0.3, which remained between the limits of 0.1 and 1.0 and which can be categorised as the biodegradable zone where organic matter can be decomposed by microbes under both natural and artificial treatment conditions

Table 1 Comparison of the characteristics of TSW and the water quality standards and classifications

Parameters	Units	Fresh TSW	Standard value[a]	Water quality standards and classifications[b]			
				Grade 1	Grade 2	Grade 3	Grade 4
BOD	mg l^{-1}	1,702.10	150	2	3	6	12
COD	mg l^{-1}	6,370.4	300	10	25	50	100
TSS	mg l^{-1}	206.6	100	50	50	400	400
pH	-	5.8	6 to 9	6 to 9	6 to 9	6 to 9	5 to 9
Cyanide	mg l^{-1}	4.16	0.2	-	-	-	-
Odour	-	Stink and typical cassava	-	-	-	-	-
Colour	-	Cloudy white	-	-	-	-	-
Turbidity	NTU	289	-	-	-	-	-
Total coliform	MPN 100 ml^{-1}	17	-	1,000	5,000	10,000	10,000
Maximum waste discharge	m^3 tonnes^{-1} tapioca	-	30	-	-	-	-

[a]The effluent quality standard of TSW (based on the Ministries of Environment Decree no. 51/1995) (Source: Ministry of Environment of the Republic of Indonesia 1995).
[b]Guidelines of water quality and its classification (based on Indonesian Government Regulation No.82/2001) (Source: the State Secretary of the Republic of Indonesia 2001, Secretary of the Republic of Indonesia 2001).

(Samudro and Mangkoedihardjo 2010). The high organic contents in fresh TSW in this study showed good agreement with those typically found in tapioca starch industries (Nandy et al. 1995; Colin et al. 2007), which indicates the higher polluting power to the ecosystem (Penn et al. 2009). Fresh TSW has low pH value (pH 5.8) which was in good agreement with the value found by other studies (Hien et al. 1999; Colin et al. 2007; Setyawaty et al. 2011). This acidic condition was possibly a result of lactic acid production derived from the acidification process of starch (Colin et al. 2007). Cyanide concentration of fresh TSW in this study was 4.16 mg l^{-1} higher than the standard value of 0.2 mg l^{-1}, indicating the possibility of toxic effects to the ecosystem (Balagopalan and Rajalaksmy 1998; Olukanni et al. 2013). The colour of fresh TSW was cloudy white since it contains residual dissolved organic materials derived from the residual cassava and it has a very strong typical cassava odour (Figure 2a). The turbidity of fresh TSW was 289 NTU, which was slightly higher than the value found by Colin et al. (2007) at 250 NTU. Furthermore, based on the water quality standards and classifications, although fresh TSW has a low amount of total coliform, the organic content values exceeded the required standard values; thus, none of the grade classification was fitted to fresh TSW, suggesting that fresh TSW was not even suitable to reuse for irrigation or agricultural purposes. These findings support that fresh TSW is not recommended to be discharged directly into water streams and needs to be treated properly to minimise a serious threat to the environment.

The TSW characteristics after treatments

After being treated with the combination of powdered *M. oleifera* seed and two-stage clarifier tanks, the final effluent of TSW showed a significant change in its odour and colour (Figure 2b,c). This finding is supported by Chaudhuri and Khairuldin (2009) and Wilson and Andrews (2011) who found that the addition of *M. oleifera* seed effectively improved the clarification and discolouring process of wastewater. In this process, *M. oleifera* seed acted as a coagulant to bind the pollutants in wastewater thus improving the removal efficiency of colour in wastewater (Madrona et al. 2012), while the arrangement of two different natural filter media was able to further enhance the removal of particulates and dissolved organic materials derived from the residual cassava that causes discolourisation and cassava-like odour in TSW. In general, a better performance in this regard was achieved by increasing the dose of *M. oleifera* seed coagulant. All the combination of the treatments resulted in a clearer colour and an odourless effluent, where adding 150 mg l^{-1} of powdered *M. oleifera* seed with the arrangement of the clarifier tank in the sequence of coconut fibre-sand (D_3T_2) gave the best result compared to other combinations. These findings confirmed that the two-stage process of clarifier tanks combined with *M. oleifera* seed as a natural coagulant is potential to be used to recovering TSW.

In T_1 treatment, the addition of 110 mg l^{-1} of powdered *M. oleifera* seed (D_1T_1) resulted in pH value of 7, and increasing the dose of powdered *M. oleifera* seed to 130 mg l^{-1} (D_2T_1) and 150 mg l^{-1} (D_3T_1) gave slightly less effect on increasing the pH value, giving an average pH value of 6.7 and 6.9, respectively (Table 2). In T_2 treatment, the combination of the highest dose of powdered *M. oleifera* seed (D_3T_2) gave the highest pH value of 7.8, opposite to that in the lowest dose (D_1T_2) at pH 7.4. The use of *M. oleifera* seed as natural coagulant, to a certain extent, influenced the pH in TSW effluent as Ndabigengesere et al. (1995) found that the protein

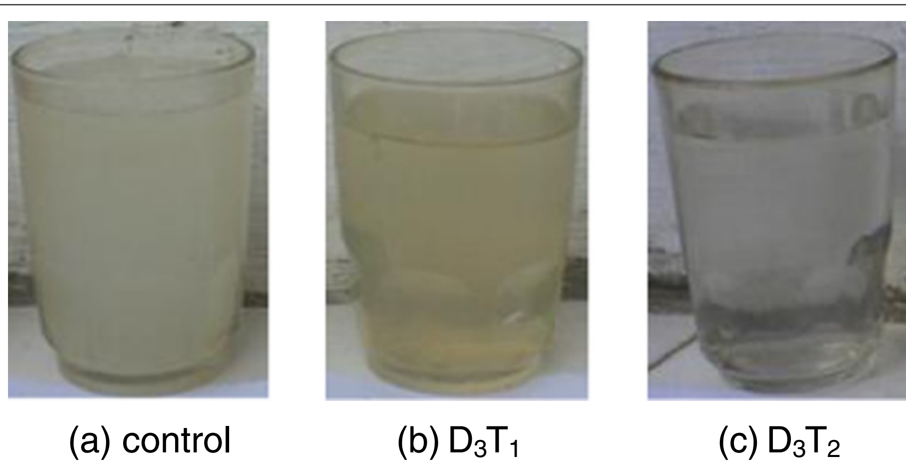

Figure 2 Comparison of the colour of TSW effluent. Before treatment **(a)** and after addition of *M. oleifera* seed coagulant combined with sand-coconut fibre clarification **(b)** or coconut fibre-sand clarification **(c)**.

Table 2 Performance indicators for the TSW final effluent at the end of experiment at day 10

Parameters	D_1T_1	D_2T_1	D_3T_1	D_1T_2	D_2T_2	D_3T_2
BOD (mg l^{-1})	14.30 a,b	20.30 a	13.05 a,b	7.80 b,c	10.55 b,c	6.50 b,c
COD (mg l^{-1})	45.11 a,b	63.14 a	54.93 a	29.74 b,c	35.18 a,b	21.47 c
TSS (mg l^{-1})	30.20 a	17.80 a	15.90 a	12.90 a	9.90 a	17.60 a
pH	7.00 a,b	6.70 a	6.90 a	7.40 b,c	7.50 c	7.80 c
Total coliform (MPN 100 ml^{-1})	34 a	40 a	30 a	26 a	40 a	40 a

D_1 = 110 mg l^{-1} of powdered *M. oleifera* seed; D_2 = 130 mg l^{-1} of powdered *M. oleifera* seed; D_3 = 150 mg l^{-1} of powdered *M. oleifera* seed; T_1 = clarifier tank (CT) filled with sand followed by CT filled with coconut fibre; T_2 = clarifier tank (CT) filled with coconut fibre followed by CT filled with sand. Results presented are the means of duplicate samples. Each treatment denoted by different lowercase letters differs significantly at $P < 0.05$ in one-way ANOVA.

component in *M. oleifera* seed that releases a hydroxyl (OH^-) group when intact with water caused an increase in the pH values. However, the one-way ANOVA analysis did not detect significant differences among the three dosages of *M. oleifera* seed in all treatments ($P >$ 0.05), but the effect due to the sequence of natural filter media was significant ($P < 0.05$). The findings in this study therefore indicated that the increasing pH values is not necessarily contributed by the doses of powdered *M. oleifera* seed added in TSW, in contrast to the results found in other studies (Amagloh and Benang 2009; Mangale et al. 2012), which confirmed that the pH increases by increasing the dose of *M. oleifera* seed coagulant. In addition, the microorganisms present in wastewater treatment systems use organic materials, such as BOD and COD, in the wastewater as an energy and carbon source for the synthesis of new cells, respiration and mobility; thus, lack or absence of organic materials gave a negative result as indicated by growth limitation or inhibition (Gray 2004). It is likely that since the natural filter media were able to enhance the reduction of the organic materials in TSW thus leading to a decrease in the availability of food resource, this resulted in an inhibition of the growth of microorganism such as the acid-producing bacteria which caused TSW to become neutral or more alkaline (Radojević and Bashkin 1999). Another possible factor was due to the production of CO_2 during aerobic degradation of organic matter causing a slight increase in the pH value (Hagman and Jansen 2007). In general, all the combinations in these treatments gave a significant improvement in pH value from 5.8 to the range of 6.7 to 7.8, which remained well within the standard value for TSW discharge (pH 6 to 9). The results indicated that the proposed system effectively increased the final pH values of treated TSW without any additional chemical additives.

Before treatment, the influent TSW has very high BOD values, which represents a high concentration of organic materials in TSW. However, as shown in Table 2 that after treatment, the BOD values were significantly degraded with the average value in the ranges of 6.50 to 20.30 mg l^{-1}; this remained well within the acceptable range for the standard value for TSW discharge (50 mg l^{-1}). In T_1 treatment, the results indicated that the highest dose of powdered *M. oleifera* seed at 150 mg l^{-1} (D_3) gave the highest BOD removal showing a greater reduction in BOD values from 1,702.1 to 13.05 mg l^{-1}, followed by a dose of 110 mg l^{-1} (D_1) at 14.30 mg l^{-1}, and a dose of 130 mg l^{-1} (D_2) at 20.30 mg l^{-1}, respectively. The same trends were observed in T_2 treatment, where adding powdered *M. oleifera* seed at 110 mg l^{-1} (D_1), 130 mg l^{-1} (D_2) and 150 mg l^{-1} (D_3) gave BOD values of 7.80, 10.55, and 6.50 mg l^{-1}, respectively. These findings suggested that BOD value decreases with increasing the dose of powdered *M. oleifera* seed and by applying two-step clarifier tanks (T_2) which resulted in an additional removal, thus, this combination was able to further improve the BOD removal. The one-way ANOVA analysis showed that the variation in the dose of *M. oleifera* seed coagulant did not have significant effect on BOD values of the TSW effluent ($P > 0.05$). However, the sequences of natural filter media used in two-stage clarifier tanks significantly influenced BOD values ($P < 0.05$). The results also demonstrated that all treated samples had almost the same BOD removal efficiency of approximately 99% after 10-day period (Figure 3); these were much higher than those found in other studies (Bhatia et al. 2006; Ashmawy et al. 2012). The higher BOD removal percentage in this experiment confirmed that *M. oleifera* performed very well as a primary coagulant to binding in particles or structural organic matter presences in TSW. The use of two-stage natural filter media, in sequence of coconut fibre-sand (T_2), certainly gave an extra improvement in reducing the BOD values. This is in a good agreement with the results from Luanmanee et al. (2002) who found that the use of multi-soil-layer with different organic materials (i.e. Japanese sawdust, rice straw, kenaf, and corncob) was able to achieve 88.0% to 99.8% of BOD removal. Similarly, Hamoda et al. (2004) also found that natural filter media, such as sand, removed BOD by 99%. Furthermore, in addition to improving the TSW final effluent quality, the filtration also played an important role in the stability of effluent quality as it significantly reduces the organic materials in water or wastewater (Stevika et al. 2004).

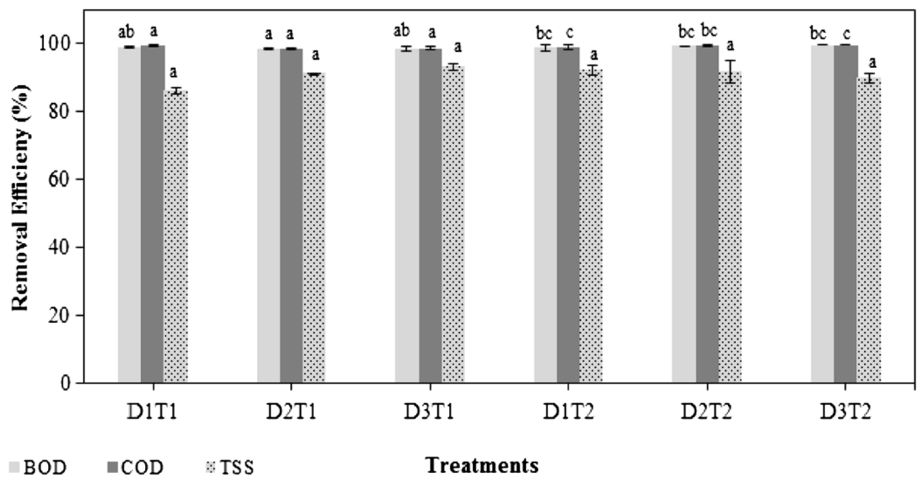

Figure 3 Removal efficiency of BOD, COD and TSS after treatments. Data are expressed as means of duplicate samples and bars represent standard error (SE). The absence of a bar means that the SE is smaller. Each treatment denoted by different lowercase letters differs significantly at $P < 0.05$ in one-way ANOVA.

These findings therefore suggested that the combination of coagulation and two-stage filtration provides an efficient process for the elimination of organic or biodegradable materials in TSW.

COD value indicated that the quality of the TSW final effluent was significantly improved after subjected to the treatments. Table 2 shows that COD values resulted from all treatments were within the range of 21.47 to 63.14 mg l^{-1}, lower than the required standard (100 mg l^{-1}). Again, D_3T_2 treatment showed the best result, giving the lowest COD value of 21.47 mg l^{-1} compared to other treatments. The results also suggested that COD removal is associated with the reduction of organic materials suspended in wastewater (Hamoda et al. 2004), which also indicated that powdered *M. oleifera* seed has a high ability to bind the organic matters suspended in TSW. In all treatments, however, the increase of the dose of powdered *M. oleifera* seed did not necessary attribute to COD degradation in TSW. The one-way ANOVA confirmed that an increase in doses of *M. oleifera* seed did not significantly differ from each other statistically in terms of COD ($P > 0.05$). The use of two-stage filtration system, such as sand-coconut fibre and vice versa, certainly enhanced the purification process, thus significantly decreasing COD values in the effluent TSW. Note that the main effect on COD removal due to the sequence of natural filter media used in the two-stage of clarifier tanks was statistically significant (one-way ANOVA; $P < 0.05$). The probable explanation for this phenomenon is due to the high absorption of the organic materials onto the filtering media (Al-Malah et al. 2000; Bansode et al. 2004). Furthermore, the natural filter medium itself, such as sand, has higher COD removal of 99% (Hamoda et al. 2004), reflecting its high possibility to provide positive input for the treatment of TSW. The combination of coagulation and two-stage filtration, proposed in this study, gave a COD removal efficiency of more than 99% in all treatments (Figure 3). These values were far higher than the values reported in previous studies (Bhuptawat et al. 2007; Othman et al. 2008), suggesting a potential application of this modification for treating TSW.

The TSS value was also significantly reduced after subjected to all treatments, giving an average value ranging from 9.90 to 30.20 mg l^{-1} (Table 2). In T_1 treatments, the highest dose gave the best performance in reducing the TSS value from 206.60 to 15.90 mg l^{-1}, reflecting that increasing the dose of powdered *M. oleifera* seed had a significant reduction of TSS in TSW. This value, however, is still much higher than that of achieved in T_2 treatment, where the combination with the addition of 130 mg l^{-1} of powdered *M. oleifera* seed (D_2T_2) resulted in the lowest reduction of TSS value at 9.90 mg l^{-1}. While the addition of 110 mg l^{-1} (D_1T_2) and 150 mg l^{-1} (D_3T_2) gave slightly higher TSS values at 12.90 and 17.60, and 6.50 mg l^{-1}, respectively. Nonetheless, the TSS performances due to the dose of powdered *M. oleifera* and the sequences of natural filter media were not significant (one-way ANOVA; $P > 0.05$). Yet, the experimental results suggested that two-stage filtration in the sequence of coconut fibre-sand provides a better performance in purifying TSW. The reason is that sand has greater ability to capture remained particles that pass through coconut-fibre filter media. According to Bhuptawat et al. (2007), particles captured in the bed of sand filter media serve as additional collectors for incoming particles resulting in an improvement in the filter effluent quality. Furthermore, the removal efficiency of TSS was within the range of 85% to 95% (Figure 3), indicating the success of this proposed system. These values were in a good agreement with the values found in other studies (Bhatia et al. 2007; Othman et al. 2008; Ashmawy et al. 2012). These findings demonstrated that the use of *M. oleifera* seed coagulant combined with two-stage filtration process improved the effluent quality of TSW to meet with the given standard values.

From Table 2, it can be seen that after treatment, the number of total coliform in effluent TSW was within the range of 26 to 40 MPN per 100 ml; however, it was higher than the initial value of 17 MPN per 100 ml. In T_1 treatments, increasing the dose of powdered *M. oleifera* seed coagulant did not significantly reduce the total coliform in TSW. For instance, at a dose of 110 mg l^{-1}, there was an increase in the total coliform from 17 to 34 MPN per 100 ml, and adding 130 and 150 mg l^{-1} of powdered *M. oleifera* seed coagulant increased the number of total coliform to 40 and 30 MPN per 100 ml, respectively. Similarly, the use of two-stage filtration combined with the sequence of coconut fibre-sand (T_2 treatment) also showed that increasing the dose of powdered *M. oleifera* seed coagulant had no effect on total coliform, as there was a gradual increase during the experimental period, giving the value from 26 to 40 MPN per 100 ml. This suggested that the addition of powdered *M. oleifera* seed was not be able to reduce the growth of coliform in TSW. The one-way ANOVA test found that the reduction of total coliform was not significantly influenced either by the dose of powdered *M. oleifera* seed coagulant or by the sequences of natural filter media used in the two-stage clarifier tank ($P > 0.05$). In contrast, other findings found that *M. oleifera* seeds have a degree of antibacterial properties thus reducing the number of microbial species, such as total coliform in the water or wastewater (Ghebremichael et al. 2005; Kawo and Daneji 2011; Walter et al. 2011). It is possible that failure in reducing the number of total coliform in TSW was due to the powdered *M. oleifera* seed coagulant that releases its other water-soluble proteins and organic matters, thus increasing the concentration of dissolved organic matter in water or wastewater during treatment (Okuda et al. 2001; García-Fayos et al. 2010; Jerri et al. 2012), which facilitates the growth of microorganism during storage in the final collection tank (Broin et al. 2002). A further combination with two-step clarifier tanks filled with two different natural filter media did not have any apparent effects on the reduction of total coliform, opposite to the finding found in another study (Jerri et al. 2012). The findings from this study demonstrated that the use of *M. oleifera* seed coagulant combined with two-stage filtration process has no significant effect on reducing the amount of total coliform presences in TSW. Although there was a slight increase in the amount of total coliform, this still remained well within the acceptable value for disposal or other uses (Table 1), indicating that the proposed system has a potential to produce water that can be recycled back the processing plant such as for washing purposes. However, further chlorination is still needed for safety reason and to meet the standard value of clean water (0 MPN per 100 ml) (Ministry of Health of the Republic of Indonesia 1990).

The selection of the best treatment to the standard of TSW final effluent

All key performance parameters above were compared with the standard values of TSW effluent for disposal (Table 1) to select the best treatment in treating TSW. Based on the above findings, it was confirmed that the combination treatment of adding 150 mg l^{-1} of powdered *M. oleifera* seed coagulant with two-stage filtration process in the sequence of coconut fibre followed by sand filter media (D_3T_2) was selected due to higher quality of the TSW final effluent and best appearance in terms of colour and odour. Furthermore, D_3T_2 has a higher removal percentage of BOD, COD and TSS compared to other treatments, giving average values of 99.6%, 99.7% and 91.5%, respectively.

Placing the clarifier tanks in the sequence filter media of coconut fibre-sand contributed to improve the quality of effluent TSW due to the differences in their characteristics, such as particle size distribution, size of porosity and specific surface area. It is obvious that coconut fibre has higher particle size and larger porosity compared to sand filter media; thus, it cannot capture a large amount of organic materials in TSW. Therefore, placing sand after coconut fibre is the best option since sand has smaller particle size, larger pores fraction and higher specific surface area, reflecting a greater potency in reducing organic material. This is supported by Chang et al. (2010), who claim that filter media with higher porosity need to be placed at the first stage of filtration and followed by the filter media with a larger pore fraction at the second stage, as it will give better performance. They added that filter media with a high porosity allow the sufficient oxygen transfer throughout the filter bed, while filter media with a larger fraction of pores provide more surface area for microorganism for growth, resulting in the higher removal of organic materials.

Issues with the use of natural filter media and their potential solution

Indeed, in the practical application, the proposed system will require a large amount of natural filter media, such as sand and coconut fibre, to treat a large amount of TSW a day. After several filtering cycles of purification process, consideration on how these filter media need to be further treated is crucial as discharging them after usage is not the best option and will lead to another waste problem. Reusing these filter media is one of the options; however, it is only suitable if the filter media still meet the required specification and be able to perform satisfactorily in terms of the quality of filtered water (Environmental Protection Agency 1995; Logsdon 2002). Thus, it is important to find alternative solutions to overcome these problems in a sustainable way. One of the solutions is to combine the proposed system in this study, for instance, with constructed wetland, such as the buried subsurface flow constructed wetland systems which have been successfully adopted by the village of Ileydagi, Turkey (Gunes and Tuncsiper 2009). With this combination, besides treating TSW, the filter media can also be used as media for growing vegetables such as water spinach, spinach, tomato or other easy growing vegetables, which will also generate additional source for food consumption or income. Adopting this system can be economically, environmentally, and socially feasible to address TSW problems, particularly in small-scale enterprises; however, further research is still needed.

Conclusions

Two-stage clarifier tanks with the arrangement of natural filter media: coconut fibre followed by sand media combined with the addition of powdered *M. oleifera* seed as natural coagulant was possible to be applied in wastewater treatment system for treating TSW. This system did appear to improve the appearance and the quality of TSW final effluent, with higher reduction of BOD, COD, TSS and stability in pH value, but failed to reduce the number of total coliform in the effluent TSW. The proposed system was able to produce effluent TSW that meet the standard value for TSW discharge, thus significantly reduce the potential hazard to the environment. Since the system studied is efficient and reliable, a further practical application in a full-scale is highly feasible.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ER carried out the experimental work and participated in the statistical analysis. SS participated in the design of the study, performed the statistical analysis, drafted the manuscript and revised it critically for important intellectual content. NH performed in the design of the study and participated in drafting the manuscript. All authors read and approved the final manuscript.

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