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Optimization of Slow Sand Filtration for the Raw Municipal Wastewater Treatment by Using the Blood Cockle (*Anadara granosa*) Shell as an Alternative Filter Media through the Response Surface Methodology

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ABSTRACT

This research aimed to investigate the optimum conditions of slow sand filter (SSF) media modification by using ground *Anadara granosa* shell waste and the effect of the ripening period on the total coliform (TC) removal efficiency. The response surface methodology with the central composite design was conducted with three factors, namely, seeding time (2–3 weeks), running time (0–20 days), type of SSF media (i.e., river sand, *A. granosa* shell, and their combination), as independent variables. The results showed that the ripening period factor interacted insignificantly by improving the TC removal efficiency due to short ripening time ($p > 0.05$). The optimum conditions of the SSF to achieve maximum TC removal efficiency (99.70 ± 21.50%) were as follows: combination media of river sand and ground *A. granosa* shell waste, 2.8 weeks (20 days) of ripening period, and 20 days of operation. In conclusion, the optimum operating parameters of the slow sand filter revealed that the combination of river sand and *A. granosa* shell as well as prolonged ripening and running times could increase the removal efficiency of TC. Hence, the *A. granosa* shell has good application potential as filter media to remove TC from the municipal wastewater.

Keywords: domestic wastewater, response surface methodology, slow sand filter, total coliform.

INTRODUCTION

The access to proper sanitation and the sustainability of clean water needs are highly dependent on the availability of clean water from the ground table and the surface water sources. The increase in the urban area population has increased the municipal wastewater generation. The untreated municipal wastewater can cause surface and ground water pollution. The municipal wastewater discharge may pose a public health risk due to contamination with pathogens, especially coliforms. These pathogenic bacteria are assumed to be present in raw municipal wastewater at a high concentration and can end up in sewerage lines through toilet flushing, washing after defecation, and laundry activities. Some pathogenic bacteria may also enter the municipal wastewater system through raw food waste and washing from the kitchen (Morel and Diener, 2006). Coliforms are predominantly nonpathogenic, but indicate whether the water is contaminated by human or animal feces and its potential to transmit water-related diseases. Mugnai et al. (2015) reported that some lineages of coliforms, *Escherichia coli*, and *Salmonella spp.* may have acquired specific virulence attributes that allow them to cause various clinical manifestations, including diarrhea, urinary tract infections, meningitis, and septicemia. Coliforms are broadly used as indicators of fecal contamination because of their large quantity in human feces (around 10^{10}–10^{11} cells/day per excreta) and slightly greater resistance to pollutants than other enteric pathogenic bacteria (Von, 2007).

Nowadays, 52 heavily contaminated rivers are present across Indonesia. The Indonesian Government Act No. 82/2001 has regulated that all water sources for potable water treatment must not contain more than 10 000 CFU of total coliform (TC)/100 mL. Unfortunately, the Surabaya River has become one of the heavily polluted rivers in Surabaya City with more than 90 000 CFU of TC/100 mL (Widhana, 2017; Maryani et al., 2014; Kurniawan and Imron, 2019a). Moreover, Nilandita et al. (2019) reported that 76% of the river contamination in Surabaya City is caused by improper municipal wastewater discharge and debris from households and apartments. The groundwater is also widely used by most Surabaya City citizens as a source of clean water. Unfortunately, most well water in Surabaya City has also been polluted by the river water infiltration process. According to Ariseno et al. (2018), the groundwater in Surabaya City is contaminated by more than 1600 CFU of TC/100 mL, exceeding quality standards. The deterioration in groundwater quality is also exacerbated by the number of people who are unaware of the importance of the regular cleaning of fecal sludge and impervious septic tank usage (The World Bank, 2017). Thus, most raw water sources in Surabaya City are unsafe for daily use and drinking. An effective and efficient municipal wastewater treatment technology is needed to eliminate the TC for safe discharge into surface water bodies.

The slow sand filter (SSF) is traditionally designed as the most suitable potable water treatment unit in rural regions and equipped with a sand bed initially about 1 m deep and about 1 m of supernatant water (Logsdon et al., 2002). The effective size of sand grain may vary from 0.15 mm to 0.35 mm, and the uniformity coefficient should be less than 5 (preferably below 3). Filtration rates typically range from 0.1 m/h to 0.3 m/h (Galvis et al., 1998). Compared with the rapid sand filter, the biological process occurs in the upper layer of SSF bed and plays the most important function. The removal of biological contaminants, especially coliforms, occurs in the *schmutzdecke*, which is an active biofilm layer formed at the surface of the sand filter bed (Campos, 2002). The main limitation of operating SSF is the requirement of a long ripening period at the beginning of the filter run. The ripening period is necessary to grow the population of bacteria on the filter media (Ranjan and Prem, 2018). As the filter runs, this biological layer keeps on developing and contributes to the removal of water impurities (Dizer et al., 2004). The SSF is easy to operate, has a simple design, low construction cost, and high purification efficiency. The operation does not require any electric equipment or chemical addition and special cognitive skills for workers. All materials needed for the whole construction are widely available at a low price. No specific guidance related to any particular type of filter media is required, enabling the utilization of any existing nearby local material resource (Khudair, 2018).

One of the potential materials that may act as filter media is clamshell waste. Some popular types of clamshells, especially blood cockle (*Anadara granosa*), exist in Indonesia. This clamshell exists along the seashore and is economical in terms of price, because it only costs IDR 7000 per kg (Suwignyo, 2005). Unfortunately, the shell waste volume increases along with *A. granosa* production. Nowadays, this waste is mostly used as raw material for seashell craft, room decoration, and food for cattle, which is not enough to reduce the total number of the shell solid waste in the environment (Agustini et al., 2011). Awang-Hazmi (2007) has stated that the clamshell contains approximately 98% $CaCO_3$, which may be used in water filtration. Moreover, Surest et al. (2012) have found out that high calcite content is obtained from ground *A. granosa* shell waste. The *A. granosa* shell can be used as filter media to remove the BOD, COD, TSS, and turbidity of swamp water. The use of *A. granosa* as filter media on SSF remains lacking and has not been thoroughly investigated. Thus, these have the inevitably brought up to a new concept to take place waste of *A. granosa* shell as an alternative media of the SSF. Independent factors should be optimized using the response surface methodology (RSM) to achieve high TC removal efficiency by using the SSF. The RSM is conducted on the basis of the full factorial central composite design (CCD). This study aimed to investigate the best or optimum operating parameters. Therefore, this research can aid in the comparison of the biofilm growth in a conventional river sand and *A. granosa* shell filter media. The surface morphology and the chemical composition of every filter material were observed using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). The differences found from the SEM micrographs were identified and discussed on the basis of the microstructural changes in control samples.

MATERIALS AND METHODS

Wastewater collection

Around 2880 L raw municipal wastewater was collected from the wastewater treatment plant collecting chamber at Penjaringan Sari (Station 1) and Keputih (Station 2) Flats located in Surabaya City from November 2019 to February 2020. On the basis of preliminary turbidity test results, the Station 1 sample had a turbidity of 3 NTU, whereas the Station 2 sample had a turbidity of 120 NTU. Thus, the ratio of raw municipal wastewater demand required from Stations 1 and 2 was 7:3, providing a mixed raw municipal wastewater with a final turbidity of around 38.1 NTU. The wastewater was transported to the reservoir tank with 1100 L capacity located in the Environmental Laboratory, Universitas Airlangga. Wastewater collection was carried out every week to ensure that the volume of the raw municipal wastewater inside the reservoir would not be depleted. The characterization of wastewater was presented in Table 1.

Filter design and arrangement

The designed and constructed experimental laboratory-scale SSF reactors and the vertical roughing filter (VRF) and the horizontal roughing filter (HRF) used as pretreatment units are presented in Figure 1. These reactors were designed and constructed in accordance with the Indonesian standard design criteria described in the SNI 3981:2008 (Design of SSF Installation) (Table 2). On the basis of Table 2, VRF and HRF were assembled using a flat acrylic sheet with the thickness of 10 mm. The VRF and HRF were conducted in three compartments filled with 30, 20, and 10 mm of gravel diameter in compartment 1, 2, and 3, respectively. Meanwhile, SSF with 3 types of media was used in this study, which is SSF with sand media, SSF with *A. granosa* shell media, and SSF with a combination of sand and *A. granosa* shell media. Before being used as media, *A. granosa* shell media was mechanically crushed and sieved using mesh no. 40 and 60 to obtain an effective particle size of 0.25–0.42 mm, which is the same size as sand. These roughing filters were built to reduce the excessive load of turbidity and achieve the standard quality of the SSF intake water, which required turbidity as low as 5 NTU. The rate of filtration was controlled by adjusting the valve debit to reach 0.1 m/h. The raw

Table 1. Characterization of wastewater

Parameters	Unit	Concentration
pH	–	6–8
COD	mg/L	64.42
BOD	mg/L	19.96
NO_3-N	mg/L	21.07
NH_3-N	mg/L	0.037
PO_4-P	mg/L	49.06
TC	MPN/100 mL	920000

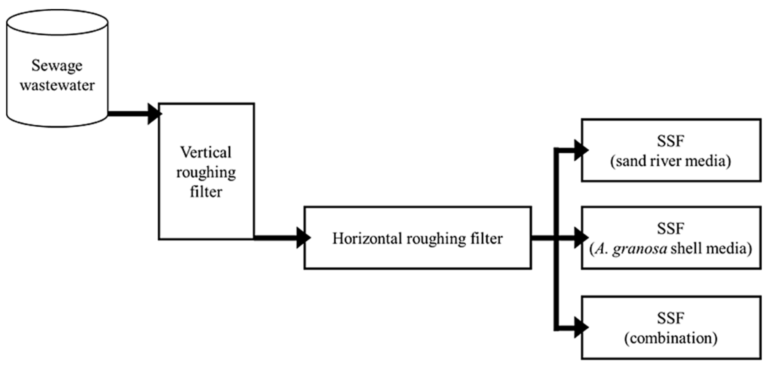

Figure 1. Experimental setup

Table 2. Design criteria of vertical roughing filter (VRF), horizontal roughing filter (HRF), and slow sand filter (SSF)

No.	Design criteria	VRF			HRF			SSF
		Compartments						
		1	2	3	1	2	3	1
1	Gravel diameter (mm)	30	20	10	30	20	10	-
2	*A. granosa* shell diameter (mm)	-			-			0.25–0.42
3	Length (cm)	22			95			32
4	Width (cm)	19			15			13
5	Height (cm)	95			25			110
6	Freeboard height (cm)	10			3			20
7	Supernatant height (cm)	10			3			10
8	Sand bed depth (cm)	-			-			60
9	Gravel bed depth (cm)	25			19			20

municipal wastewater was previously pretreated by flowing through the VRF, HRF, and SSFs in a one-way sequence. During this research, VRF and HRF were run merely in filtration stage.

Experimental design

Three independent factors (i.e., seeding time [A, 2–3 weeks], running time [B, 0–20 days], and type of SSF media [C; river sand, crushed *A. granosa* shell, and combination]) were used in this experiment. The optimum experimental factors were investigated using the CCD found in RSM through the Design-Expert software 11.0. Experimental runs were designed using the Design Expert 11.1.2.0 software. A total of 39 experimental runs were established using the program and used to test the validity of the independent factors. Five runs were suggested to confirm the effectiveness of the factors on the response (Al-Sahari, 2019). The TC removal efficiency was determined on the basis of the reduction in the most probable number (MPN) of TC. The second-order polynomial model of the TC removal efficiency was explained in accordance with Equation 1 (Hauwa et al., 2018; Thirugnanasambandham and Sivakumar, 2015a).

$$Y = \beta_0 + \sum_i^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^n \sum_{i<j}^n \beta_{ij} x_i x_j \quad (1)$$

where: Y is the predicted response for the removal percentage; β_0, β_i, β_{ii}, and β_{ij} represent the regression coefficients; x_i represent the coded variables; x_j represent the coded independent variables in form; k is the number of independent variables.

This quadratic equation was used to obtain the optimized responses as a function of independent process variables (Rakić et al., 2014; Thirugnanasambandham and Sivakumar, 2015b; Imron and Titah, 2018). For the optimization condition, the TC removal percentage was set to the maximum value, whereas the ripening and the operation conditions were set to the minimum value.

TC analysis

The water samples for TC analysis were collected from the inlet and the outlet valves of SSFs. The Method 9221: Multiple-Tube Fermentation Technique was used to determine the MPN of TC. Considering that the collected samples were highly polluted, the influent and the effluent samples were diluted to 10^{-2} and 10^{-3}, respectively. Three procedures adopted from Method 9221, namely, presumptive stage, confirmed stage, and completed test, were used to determine the TC on water samples.

Statistical analysis

Experimental runs were performed twice to increase the accuracy of results. The Design Expert 11.1.2.0 software was used to analyze the data and investigate the second-order polynomial model equations in terms of actual factors. The significance of the independent factors on the response was analyzed using analysis of variance (ANOVA, $p < 0.05$) (Kurniawan and Imron, 2019b). The adjusted coefficient of determination (adjusted R^2) represented the role of independent factors in the TC removal efficiency as a function of a quadratic model. The interaction among the independent factors and their role in the TC removal efficiency were presented using a 3-dimensional (3D) surface graphics.

SEM–EDX

The control and the postripened filter media samples (river sand and *A. granosa* shell) without lyophilization or chemical fixation were analyzed using SEM–EDX. A sterile spatula was used to scrape a small sample of filter grain with a depth of approximately 1 mm, which was equivalent to 1–2 g sample. Each sample was placed on standard double-sided carbon tape to ensure that all particles stick to the aluminum stubs. The samples were then coated with Au ion by using the DC *Sputter* JEOL JFC-1600. All samples were viewed under high vacuum with a field-emission SEM (FESEM) JEOL/JSM-7600F at 3–5 kV beam energy, 5–8 mm working distance, and 100×–30 000× magnification to obtain high-quality SEM micrographs. For the EDX analysis, the FESEM equipped with the INCA EDS System was used to determine the chemical composition, such as C, O, Na, Mg, Al, Si, K, Ca, and Fe elements.

RESULTS AND DISCUSSION

TC removal

The TC concentration before and after treatment as well as TC removal efficiency for the different experimental runs are shown in Tables 3 and 4, respectively. In turn, Table 3 shows the ANOVA result of the regression parameters of the predicted response surface quadratic model for the TC removal efficiency by using SSF. On the basis of Tables 3 and 4, SSF with different media shows different removal efficiency. This is because each medium has a different surface area and internal porosity where these two things are important in forming biofilms on the surface of the media (Wang et al., 2020). The larger the surface area, the more biofilm grows on the surface of the media, so that the biological, physical, and chemical processing will run well. According to Table 5, the p-value with 95% confidence level was used to indicate the significance of each independent factor. The model F-value of 1.07 implied that the model was not significant relative to the noise, but a 42.02% chance exists that an F-value this large could occur due to noise. $p > 0.05$ indicated that the model terms were not significant. In this case, all model terms except B^2 were found to be nonsignificant for the TC removal. On the basis of this result, the quadratic response surface model constructed in this study to predict the TC removal efficiency was unreasonable (Subari et al., 2018). This result revealed that the filter media type and the ripening period had no significant effect on the TC removal efficiency. Moreover, the difference between the 2- and the 3-week ripening periods were not significant. This study showed that only the type of filter media was quite responsible for improving the removal of TC regardless of the ripening period. On the basis of Table 3, the lack-of-fit test for the model was not significant ($p > 0.05$), indicating no lack of fit for the quadratic model. The lack-of-fit test was designed to determine whether the selected model was adequate to describe the observed data or whether a more complicated model than the current model should be used (Imron and Titah, 2018).

Table 5 also shows the fit summary for the TC removal efficiency in SSFs. The least square method is used to determine the quadratic regression coefficient and the interactions among independent factors (Suwignyo, 2005; Thirugnanasambandham, 2018a). The adjusted R^2 and the R^2 for the TC removal efficiency obtained were 0.0194 and 0.3033, respectively. The R^2 was recommended to be at least 0.80 to indicate a good fit model, illustrating good agreement between the calculated and the observed results within the range of the experiment (Subari et al., 2018). Unfortunately, the R^2 of 0.3033 interpreted a weak correlation between all independent factors and the response in this model. The adequate precision measured the signal-to-noise ratio. The adequate precision ratio should be greater than 4 to obtain an adequate signal for the model and was 4.034 in this study, indicating that the model might be used to navigate the design space. On the basis of the result, the SSF can remove the TC from the municipal wastewater. The removal of TC occurred because the TC strained by sand grains formed *schmutzdecke*. The adsorption mechanism in *schmutzdecke* played an important role on the TC removal (Bagundol et al., 2013). SEM was carried out to express the extracellular matrix and the products on the surface of *schmutzdecke* (Figure 4) and ensure results (Joubert and Pillay, 2008). The Design Expert program was used to identify the best operating parameters for the TC removal process. Therefore, the final equations presented were generated by the software to describe the second-order polynomial model in terms of actual factors. These equation models described the relationship among the modifications of SSF media, ripening period, and

Table 3. Concentration of TC before and after treatment

Ripening (week)	Running (day)	Type of media	Influent (MPN/100 mL)	Effluent (MPN/100 mL)
			Before pre-treatment	After pre-treatment and main treatment
1.8	10	Sand	1,700	680
1.8	10	Combination	1,700	200
1.8	10	Shell	1,700	780
2	0	Sand	920,000	1,700
2	0	Combination	920,000	1,400
2	0	Shell	920,000	800
2	20	Sand	14,000	1,100
2	20	Combination	14,000	450
2	20	Shell	14,000	2,000
2.5	0	Sand	11,000	200
2.5	0	Combination	11,000	1,700
2.5	0	Shell	11,000	1,700
2.5	10	Sand	1,800	1,700
2.5	10	Combination	1,800	170
2.5	10	Shell	1,800	450
2.5	10	Sand	4,500	170
2.5	10	Combination	4,500	170
2.5	10	Shell	4,500	170
2.5	10	Sand	1,800	1,100
2.5	10	Combination	1,800	170
2.5	10	Shell	1,800	450
2.5	10	Sand	4,500	170
2.5	10	Combination	4,500	170
2.5	10	Shell	4,500	170
2.5	10	Sand	1,800	1,400
2.5	10	Combination	1,800	170
2.5	10	Shell	1,800	450
2.5	24	Sand	1,300	170
2.5	24	Combination	1,300	400
2.5	24	Shell	1,300	1,500
3.0	0	Sand	11,000	200
3.0	0	Combination	11,000	1,700
3.0	0	Shell	11,000	1,700
3.0	20	Sand	57,000	180
3.0	20	Combination	57,000	170
3.0	20	Shell	57,000	1,100
3.2	10	Sand	4,000	1,400
3.2	10	Combination	4,000	820
3.2	10	Shell	4,000	830

operation time, as given by the final equation in terms of the following actual factors.

$$Y_{(SSF1)} = +\,97.45337 - 0.699559A - 6.25155B + 1.33517AB - 1.99355A^2 + 0.135222B^2 \quad (2)$$

$$Y_{(SSF2)} = +\,135.16967 - 10.29198A - 5.92019B + 1.33517AB - 1.99355A^2 + 0.135222B^2 \quad (3)$$

$$Y_{(SSF3)} = +\,101.56626 - 0.307640A - 5.97300B + 1.33517AB - 1.99355A^2 + 0.135222B^2 \quad (4)$$

where: Y is the TC removal efficiency percentage (%), A is the ripening period (week), and B is the operation time (day).

The equation in terms of actual factors can be used to make predictions about the response for the given levels of each factor (Thirugnanasambandham, 2018b). The levels should be specified in the original units for each factor. These equations were not used to determine the relative effect of each factor,

Table 4. Design matrix for the central composite design used for the total coliform removal and results of the total coliform removal efficiency

Ripening (week)	Running (day)	Type of media	TC removal efficiency (%)	
			Actual	Predicted
1.8	10	Sand	60.00	64.77
1.8	10	Combination	88.24	88.54
1.8	10	Shell	54.12	72.38
2	0	Sand	99.82	88.08
2	0	Combination	99.85	106.61
2	0	Shell	99.91	92.98
2	20	Sand	92.14	70.54
2	20	Combination	96.79	95.7
2	20	Shell	85.71	81.01
2.5	0	Sand	98.18	83.24
2.5	0	Combination	84.55	96.98
2.5	0	Shell	84.55	88.34
2.5	10	Sand	5.56	67.63
2.5	10	Combination	90.56	84.68
2.5	10	Shell	75.00	75.51
2.5	10	Sand	96.22	67.63
2.5	10	Combination	96.22	84.68
2.5	10	Shell	96.22	75.51
2.5	10	Sand	38.89	67.63
2.5	10	Combination	90.56	84.68
2.5	10	Shell	75.00	75.51
2.5	10	Sand	96.22	67.63
2.5	10	Combination	96.22	84.68
2.5	10	Shell	96.22	75.51
2.5	10	Sand	22.22	67.63
2.5	10	Combination	90.56	84.68
2.5	10	Shell	75.00	75.51
2.5	24	Sand	98.69	91.21
2.5	24	Combination	96.92	112.89
2.5	24	Shell	88.46	102.98
3.0	0	Sand	98.18	77.41
3.0	0	Combination	84.55	86.35
3.0	0	Shell	84.55	82.7
3.0	20	Sand	99.68	86.58
3.0	20	Combination	99.70	102.15
3.0	20	Shell	98.07	97.44
3.2	10	Sand	65.00	68.53
3.2	10	Combination	79.50	78.87
3.2	10	Shell	79.25	76.69

because the coefficients were scaled to accommodate the units of each factor, and the intercept was not at the center of the design space. The Design Expert program was used to illustrate these equations into 3D response surface plot graphics (Figure 2).

Validation of the optimal operating parameters

Validation experiments were carried out at the suggested optimized conditions, and the percentage of error from the expected result was around 5% on average (Subari et al., 2018). The optimum conditions were suggested using the Design Expert 11.1.2.0 software in accordance with the desirability results. The point optimization technique was used to seek the combination of factor levels that simultaneously satisfied the criteria placed on each factor and maximizing the desired TC removal response function of 95–100%. Desirability close to 1 indicates the settings achieve

Table 5. Analysis of variance (ANOVA) and fit statistics of the response surface quadratic model for the total coliform removal

Source	Sum of Squares	df	Mean Square	*F*-value	*p*-value	
Model	5435.13	11	494.10	1.07	0.4202	not significant
A-ripening period	0.8690	1	0.8690	0.0019	0.9657	
B-operation time	175.77	1	175.77	0.3801	0.5427	
C-type of media	1915.21	2	957.60	2.07	0.1456	
AB	534.80	1	534.80	1.16	0.2917	
AC	126.62	2	63.31	0.1369	0.8726	
BC	44.04	2	22.02	0.0476	0.9536	
A^2	5.10	1	5.10	0.0110	0.9171	
B^2	2618.45	1	2618.45	5.66	0.0247	
Residual	12485.50	27	462.43			
Lack of Fit	4780.66	15	318.71	0.4964	0.8996	not significant
Pure Error	7704.84	12	642.07			
Cor Total	17920.63	38				
Fit statistics						
Standard deviation			21.50	R^2		0.3033
Mean			82.87	Adjusted R^2		0.0194
Coefficient of variation, %			25.95	Predicted R^2		−0.1956
Adequate precision						4.0340

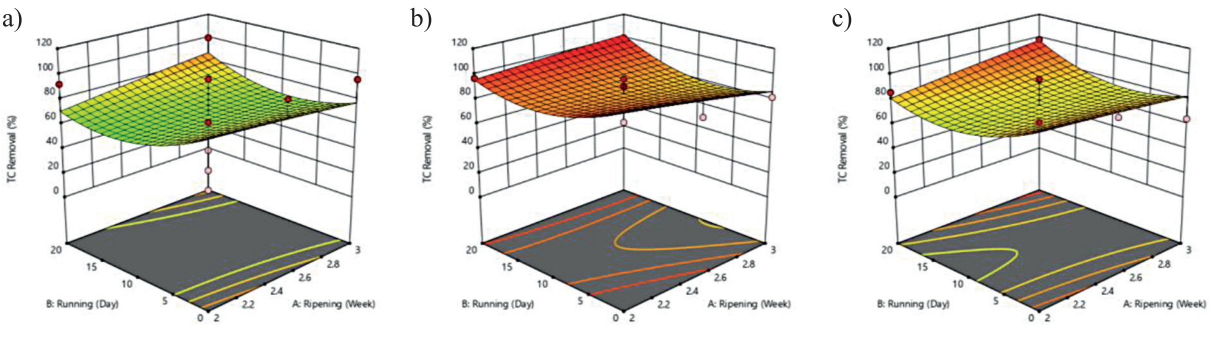

Figure 2. 3D response surface plot for total coliform removal efficiency by using slow sand filter with (a) river sand, (b) combination, and (c) *A. granosa* shell

favorable results for all responses (Thirugnanasambandham, 2017). Hence, the optimum point may vary in accordance with the criteria goal chosen before the optimization stage begins (Subari et al., 2018). The ripening and the operation times were set to the minimum as optimum conditions, and the TC removal percentage was set to the maximum as the response on the process.

The results showed that the optimal operating parameters of the SSF for 100% TC removal was recorded using the combination media of river sand and ground A. granosa shell waste, 2.8 weeks (20 days) of ripening period, and 20 days of operation. A 3D desirability graph and overlay plot were displayed to evaluate and prove each response and find the optimal operating parameter points (Figure 3). However, the validation experiment was carried out to determine the optimum conditions of the TC removal. The standard deviation and the percent error were determined to validate the experiments. The errors between the predicted and the actual values were calculated using the formula below:

$$Error = \frac{actual\ value - predicted\ value}{actual\ value} \times 100\% \quad (5)$$

The obtained results exhibited an actual (observed) response value of 99.70 ± 21.50% compared with the highest predicted response value of point optimization (100%) with a desirability value of 1. This finding showed that the predicted value of the point optimization was quite accurate

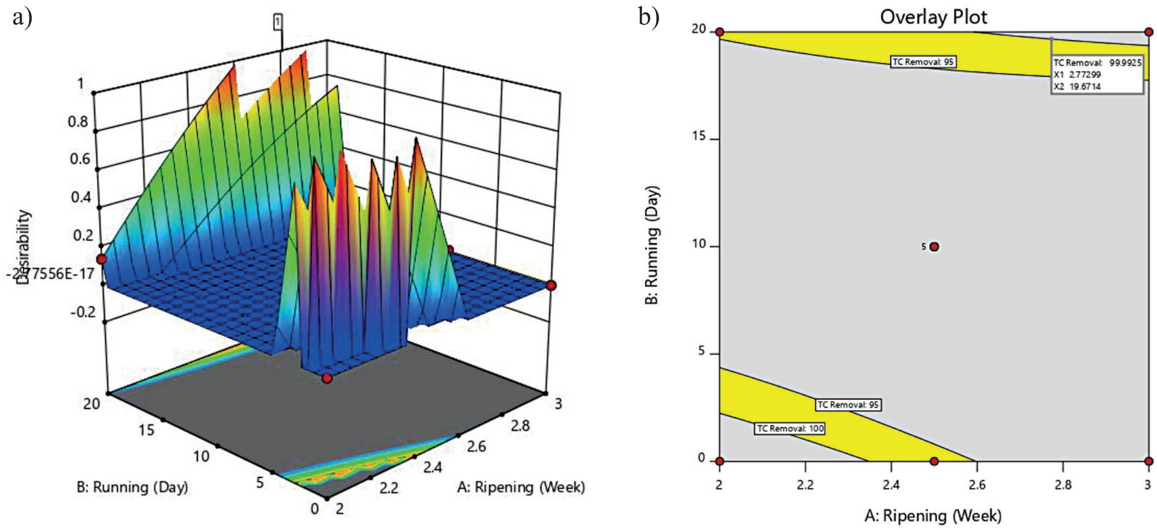

Figure 3. (a) 3D desirability graph and (b) overlay plot of the best operating parameters of slow sand filter with a combination media of river sand and Anadara granosa shell in removing coliforms

(Imron and Titah, 2018). On the basis of Equation (5), the percentage error was 0.3%, clearly indicating no significant difference (Olawale et al., 2015).

SEM–EDX

SEM–EDX was conducted on every filter medium at the end of the ripening stage and on the control filter media of SSF to analyze the physical appearance of the schmutzdecke layer on filter media. The results are presented in Figure 4. On the basis of the SEM micrographs in Figures 4 (a–b), the morphological samples of the control river sand media had bumpy and irregular edges and a porosity size of 0.1–10 µm. In contrast to that of river sand media samples, the surface of

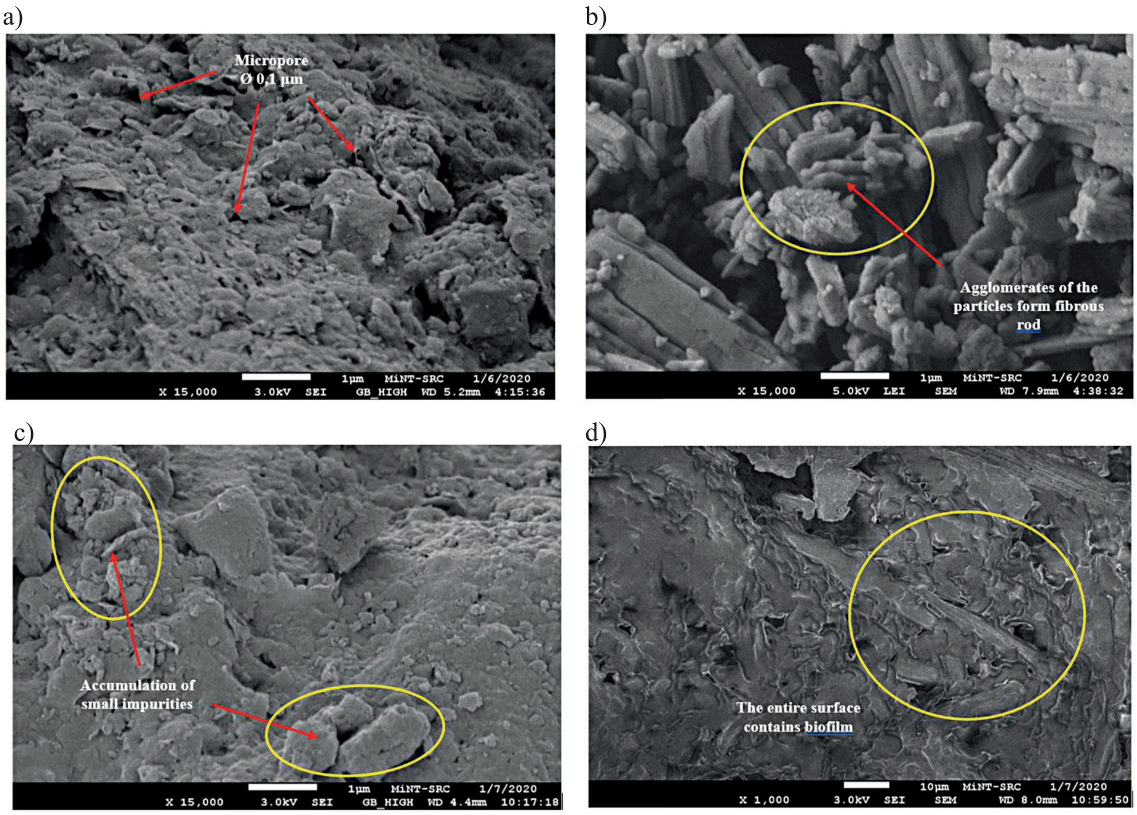

Figure 4. SEM micrographs of media before (a–b) and after (c–d) ripening.
a) and c) river sand media. b) and d) *A. granosa* shell media

the control A. granosa shell media sample had a sharp tip and trunk shaped-like appearance and contained many cavities and empty gaps. Both samples were confirmed to still be in clean condition. The filter media with clean conditions, one intact particle, no particle attached to the surface of the media, and the surface of the specimen holder or carbon tape that could be seen clearly were observed (Joubert and Pillay, 2008).

SEM was resumed with the sample of river sand media and the A. granosa shell after the ripening period. On the basis of the SEM micrographs in Figures 4 (c–d), the appearance and porosity of the two media filters changed. Significant changes occurred in the A. granosa shell media samples, which had A. granosa shell with increased cavities and pointed ends. Something similar was also observed in existing media samples, where pores in the media were rarely seen and the surface of the media was increasingly covered by a layer of mucus and impurities that began to stick to the mucus layer.

According to Joubert and Pillay (2008), the microenvironment in the filter media at the beginning of the biofilm development contains a low amount of nutrients. The observation of the biofilm development after two weeks showed the primary colonization (proliferation) of media particles and bacterial extracellular mucus production. This slime works as a place for attaching microorganisms on the surface of the media (Law et al., 2001). The structure of the this biofilm layer consists of mass deposits formed by several groups of microbes (Ni'matuzahroh et al., 2020). In this study, the biofilms developed at three weeks of ripening were not much different from those developed at two weeks of ripening. The physical structure of the surface of filter media changed a little due to the existence of the microorganisms associated with extracellular products and their breakdown products. This accumulation of material placed emphasis on physical screening, such as tension and sifting.

CONCLUSIONS

This research was designed to determine the TC removal and its optimization using the RSM. The obtained results showed that the modification of the SSF media using ground *A. granosa* shell waste resulted in higher TC removal efficiency (85% to 100%) compared with the conventional river sand media. RSM with CCD was a reliable and powerful tool for modeling and optimizing processes. The second-order quadratic regression model for the TC removal with R^2 of 0.3033 was obtained. The predicted optimum conditions of SSF to remove TC were as follows: combination of river sand and ground *A. granosa* shell waste, 2.8 weeks (20 days) of ripening period, and 20 days of operation. The desirability value was 1 with predicted value of 100% TC removal efficiency. After validation, the actual value of TC removal efficiency was 99.70 ± 21.50%. The percentage error between the predicted and the actual values was 0.3%. The results clearly indicated no significant difference.

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