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Removal of pollutants by mycelium-colonized sawdust

Osayomwanbo Osarenotor[a,b,*], Helen M. K. Essandoh[a,b] and Isoken Tito Aighewi[b]

[a] Department of Civil Engineering, College of Engineering, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[b] Regional Water and Environmental Sanitation Centre, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

*Corresponding author. E-mail: osayomwanbo.osarenotor@uniben.edu

Abstract

Slaughterhouses generate wastewater daily and often discharge it to the environment. Many lack wastewater treatment systems, due to such systems' typically high cost and technological demands. In this study, slaughterhouse wastewater was filtered through columns of mycelium – *Pleurotus ostreatus* – grown on sawdust substrates of different particle sizes. The columns' pollutant removal efficiencies were evaluated for color, turbidity, total suspended solids, total dissolved solids, electrical conductivity, dissolved oxygen, biological oxygen demand (BOD), chemical oxygen demand, total nitrogen and total phosphorus. The best color (75%), BOD (88%) and total nitrogen (86%) removal efficiencies were recorded with 2.36 mm particle size and 9 cm depth. Electrical conductivity removal efficiency was best with 2.36 mm particle size but 3 cm bed depth. The study showed that particle size has a significant effect on physiochemical pollutant removal by mycelium-colonized sawdust systems.

Key words: particle size, ramified, removal efficiency, slaughterhouse wastewater, spawn

Highlights

- Mycelium was grown on sawdust of varying particle sizes.
- The mycelium-colonized sawdust were evaluated for their removal efficiency at varying bed depths.
- Sawdust of particle size 2.36 mm and bed height 9 cm significantly removed colour, BOD and total nitrogen from slaughterhouse wastewater.
- Electrical conductivity was removed significantly by mycelium-colonized sawdust of particle size 2.36 mm and bed depth of 3 cm.

INTRODUCTION

Wastewater pollution is a serious issue in many developing countries due to lack of suitable treatment facilities. Many slaughterhouses in developing countries lack wastewater treatment facilities, and some sites discharge untreated wastewater to water bodies. It is hoped that an easily operated and cheap wastewater treatment technology will significantly reduce the pollution burden on nearby water bodies receiving such effluents (Mehta *et al.* 2017).

Most wastewater treatment plants involve advanced technology, and the unavailability or lack of a regular electricity supply has hampered their adoption in developing countries. Therefore, an eco-based wastewater treatment technology needs to be harnessed (Mehta *et al.* 2017).

Mycofiltration is an emerging eco-solution for wastewater treatment involving the application of macroscopic fungi mycelium grown on suitable substrates – for example, straw, wood chips and sawdust. Fungi mycelium can use the nutrients in the wastewater, as well as forming a network for trapping physical pollutants (Mehta *et al.* 2017). Mycofiltration systems have been studied extensively for pollutant removal from wastewater. Shekhar *et al.* (2017) showed that *Pleurotus ostreatus* grown on wheat straw reduced chemical oxygen demand (COD) and biological oxygen demand (BOD) in pre-treated sewage by 70 and 40% respectively. The relatively high surface area available for colonization makes sawdust an excellent substrate for growing mycelium for pollutant removal (Rogers 2012). Several studies have demonstrated the ability of mycelium-colonized to remove fecal coliforms and *Escherichia coli* from wastewaters (Rogers 2012).

The pollutant removal capacity of the mycelium-sawdust system depends largely on the extent of substrate colonization (Royse & Sanchez-Vazquez 2001). Their studies established the role of sawdust particle size in mycelium colonization, showing that smaller particle size gives greater colonization than larger particles due to the larger surface area available for mycelium biomass development. Column filter depth can also influence pollutant removal rate (Fletcher & Deletic 2007; Kandra *et al.* 2014; Nwankwo *et al.* 2018).

This study was aimed at gaining insight into the influence of mycelium growth substrate (sawdust) particle size in removing physiochemical pollutants from slaughterhouse wastewater. The interactive effect of varying filter column bed depth was also investigated.

MATERIALS AND METHODS

Substrate preparation

Sawdust from a local sawmill was sun-dried to reduce its moisture content and then sieved to 0.6, 1.18 and 2.36 mm particle sizes using Indian Standard sieves. The size-separated sawdust sets were then saturated before being autoclaved.

Sawdust inoculation with mushroom spawn

Pure *P. ostreatus spawn* was sourced commercially from Mycofarms and Synergy, Nig Ltd The autoclaved sawdust was aseptically inoculated with spawn in sterilized plastic buckets in the ratio 1:10 (1 part spawn to 10 sawdust), as proposed by Rogers (2012). Both inoculated and uninoculated control sets were kept in the dark, and incubated at room temperature and monitored for mycelium development.

Column treatment set-up

When mycelium growth in the sawdust was sufficient, about day 20, the mycelium-colonized sawdust composite was packed aseptically into sterilized cylindrical plastic containers 14 cm tall and with 28.3 cm^2 cross-section area. The plastic containers were sorted into treatment groups in duplicate, based on the three different particle sizes and bed depths of 3, 6 and 9 cm, to monitor variations caused by bed depth. Mesh was placed over the inlet and outlet pipes, and twenty-four experimental units were made and used.

To establish the removal ability of the mycelium-sawdust system, both mycelium-colonized and uncolonized sawdust were used. Unsorted sawdust was packed at 9 cm bed depth and the experimental setup was in duplicate.

Wastewater was collected from a slaughterhouse in a sterilized jar and taken immediately to the laboratory, where it was filtered using a mesh to remove physical impurities and particles that could cause clogging. The tank containing the effluent was placed above the trial filter set so that the wastewater could be introduced into the columns using gravity at constant flow rate. Effluent samples were collected as soon as outflow started in each treatment unit and physiochemical parameters determined, including color, turbidity, electrical conductivity (EC), dissolved oxygen, COD, BOD, total suspended solids (TSS), total dissolved solids (TDS), total nitrogen (TN), total phosphorus (TP) and pH. All analyses were done using standard methods prescribed by the American Public Health Association (APHA 2005).

Statistical analysis

The significant mean difference between pollutant levels in the inoculated and uninoculated substrates after filtration was checked using the independent sample t-test. Multiple analysis of variance (MANOVA) was used to check parameters that had significant mean differences with variable particle size and bed depth. Both analyses were performed using SPSS version 22 (IBM Corporation 2013).

RESULTS AND DISCUSSION

Pollutant removal capacity of mycelium-colonized sawdust systems

The system's ability to remove slaughterhouse wastewater pollutants was pre-determined. Relatively high removal rates were recorded for mycelium-colonized sawdust, which, compared to uncolonized sawdust, reduced color (1.2 times better), turbidity (6.9), TDS (1.8), EC (2.2), BOD (1.5), COD (1.46), TN (1.3) and TP (1.01) – see Tables 1 and 2.

Table 1 | Wastewater treatment comparison of mycelium-colonized and uncolonized sawdust

Pollutant (units)	Wastewater	Mycelium-colonized sawdust	Uncolonized sawdust
Color (Pt Co)	368 ± 62.6	284 ± 62.39	344 ± 79.45
Turbidity (NTU)	264 ± 66.2	39 ± 13.2	268 ± 68.9
TSS (mg/l)	301 ± 195.6	305 ± 42.03	336 ± 184.96
pH	7 ± 0.4	7 ± 0.22	7 ± 0.72
TDS (mg/l)	784 ± 542.4	430 ± 24.50	800 ± 582.75
EC (μs/cm)	1,618 ± 1,136.8	739 ± 130.5	1,688 ± 1,119.03
Dissolved oxygen (mg/l)	3 ± 1.3	3 ± 0.95	2 ± 1.2
BOD (mg/l)	194 ± 39.6	135 ± 21.2	213 ± 63.44
COD (mg/l)	509 ± 200	349 ± 25.47	513 ± 167.43
TN (mg-N/l)	6 ± 1.46	5 ± 1.04	6 ± 1.59
TP (mg/l)	6 ± 1.3	4 ± 1.17	4 ± 0.93

The results show that the fungal mycelium biomass in the sawdust contributed significantly to pollutant removal from the wastewater. Fungi are saprophytes and can degrade organic matter (Stamets 2005), which is reported as the major cause of color and turbidity in slaughterhouse wastewaters (Djonga *et al.* 2019). Nitrogen and phosphorus are also major nutrients for fungi, and are the building blocks for proteins, nucleic acids, coenzymes and chitin (Thomas *et al.* 2009).

Table 2 | Pollutant removal efficiency of mycelium-colonized and uncolonized sawdust from slaughterhouse wastewater

Removal efficiency (%)		
Pollutant	**Mycelium-colonized sawdust**	**Uncolonized sawdust**
Color	23	7
Turbidity	85	−1
TSS	−2	−12
EC	54	−4
BOD	30	−10
COD	31	−1
TN	23	1
TP	30	−7

Effect of colonized-sawdust particle size and bed depth on pollutant removal

Pollutant removal was recorded in all mycelium-colonized sawdust columns at different efficiency levels. Pollutant removal was relatively high in the biofilter with 2.36 mm particle size except for TSS, where the best removal was achieved with 0.6 mm particles in a 9 cm deep bed – see Table 5 – at 36.8% efficiency (Figure 1). High reduction in color (368–93 pt.co), turbidity (264–22 mg/l), BOD (193–32 mg/l), COD (509–62 mg/l) and TN (6–1 mg/l) representing 75, 92, 88, 88 and 86%, respectively, were recorded at the maximum bed height (9 cm) for this study (Table 5, and Figures 2–6).

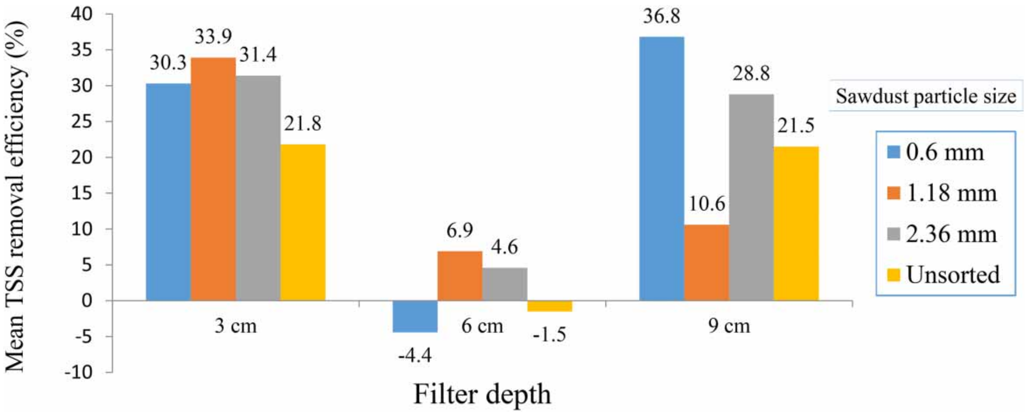

Figure 1 | Mean TSS removal efficiency for mycelium-colonized sawdust.

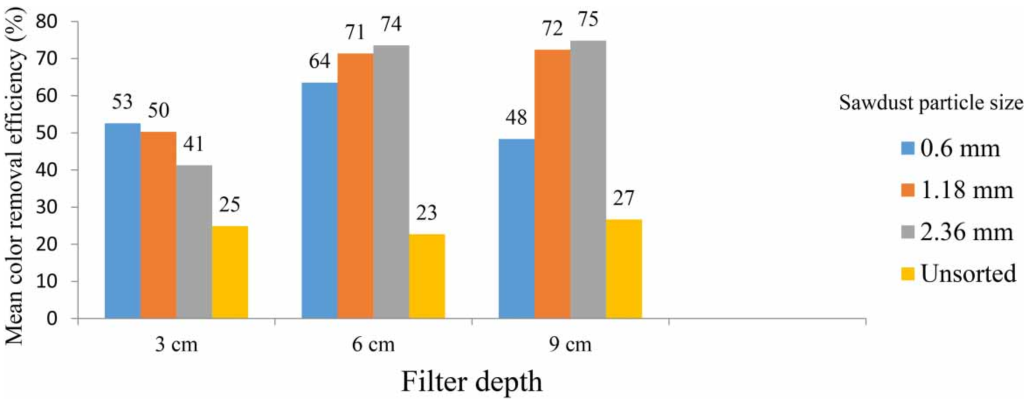

Figure 2 | Mean color removal efficiency for mycelium-colonized sawdust.

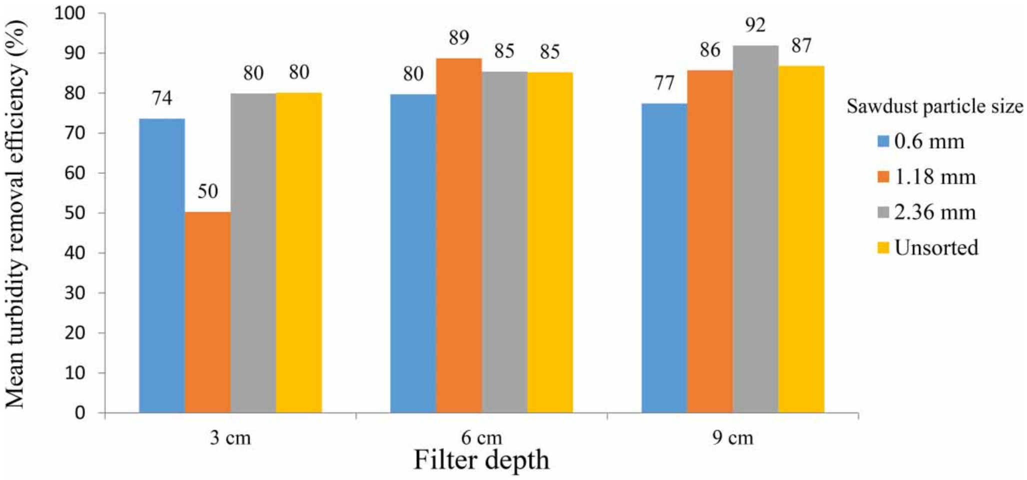

Figure 3 | Mean turbidity removal efficiency for mycelium-colonized sawdust.

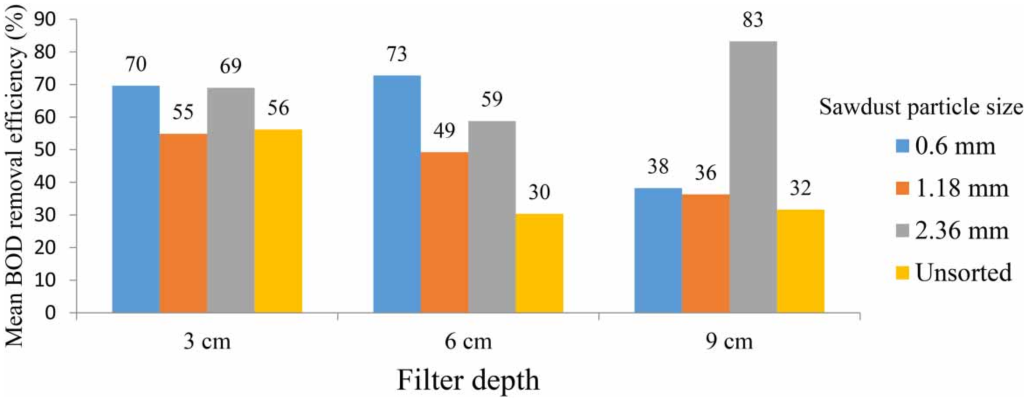

Figure 4 | Mean BOD reduction efficiency for mycelium-colonized sawdust.

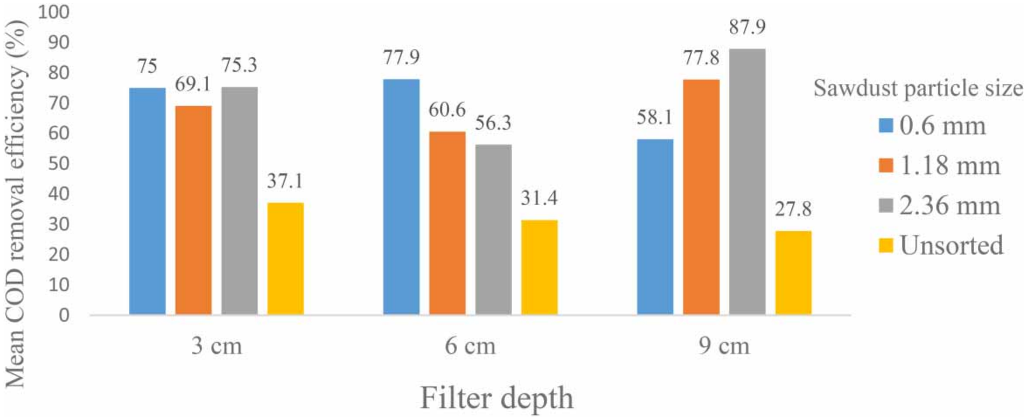

Figure 5 | Mean COD removal efficiency for mycelium-colonized sawdust.

Phosphorus was removed by the 2.36 mm particles at all three bed heights (6–0.2 mg/l = 96%) (Tables 3–5). The reductions in EC (1167–508 µs/cm) and in TDS concentration (783–250 mg/l) (Table 3), which represent 67 and 68% efficiency respectively (Figures 8 and 9), occurred at the minimum bed depth used (3 cm).

The removal of color, turbidity, BOD, COD and/or TN removal is an indication of organic matter decomposition. The greatest color removal observed in the trials arose from the high mycelium biomass

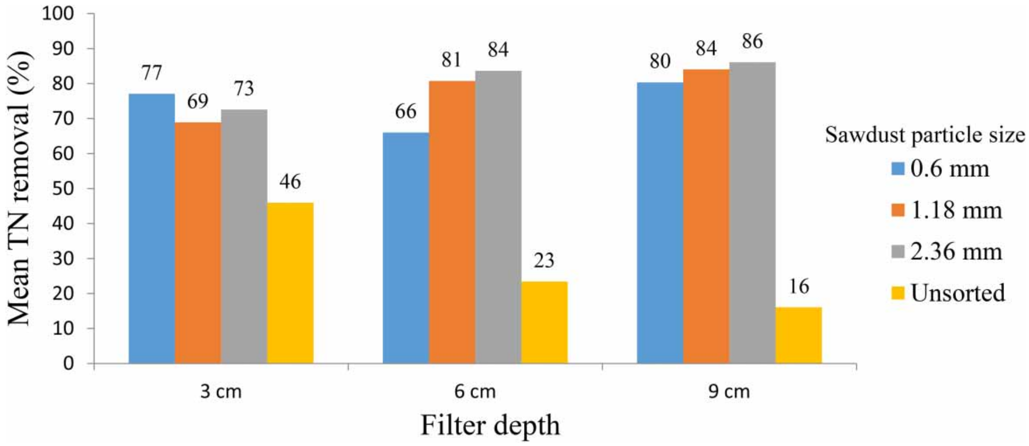

Figure 6 | Mean TN removal efficiency for mycelium-colonized sawdust.

in the 2.36 mm sawdust. As the mycelium degrades ligno-cellulose material, micro-cavities are formed and eventually filled with air (Stamet 2005). Increased biomass raises oxygen levels in the micro-cavities, thus improving mycelium metabolic activity (Royse & Sanchez-Vazquez 2001). It is because of this and the 9 cm bed depth that pollutant removal efficiency was high. Contact time is needed for fungi mycelium to remove biological pollutants (Thomas *et al.* 2009), and the 9 cm filter and slow infiltration rate provided relatively longer contact time for biological reactions to occur.

The best TN removal occurred in the 9 cm bed, indicating that the mycelium biomass had to convert the nitrogen into forms that it could use (nitrate and nitrite), and biological mineralization depends on contact time between the substrate and the biological organism carrying out the process (Thomas *et al.* 2009). Relatively greater bed depth gives more contact time between the biomass and the nitrogen species present.

TP was removed efficiently in all bed depths in the trial, at 2.36 mm particle size, showing clearly that phosphorus removal does not depend on bed depth (Figure 7). This may be a function of the form of phosphorus present in the wastewater used – for example, Thomas *et al.* (2009) state that fungi, just like plants, absorb orthophosphate readily. Radial mycelium extension can easily use this phosphorus species with no need for biomass enzymatic transformation, as is the case with organic phosphorus.

TDS and EC were removed most efficiently in 2.36 mm particle size mycofilters with 3 cm bed depth. EC is an indication of the presence of metals and inorganic salts. It shows that adsorption of the metals on the mycelium surface is sufficient to achieve maximum removal of metal ions. Fungi mycelium cell walls are made up of chitin, which carries anionic charges that attract the cationic metal ions by electrostatic forces, resulting in adsorption of the metal onto the mycelium surface (Stamets 2005; Thomas *et al.* 2009). Adsorption is a surface phenomenon, unlike biosorption, which requires intracellular uptake of metals (Djonga *et al.* 2019).

The maximum turbidity and COD removals were not statistically different at 2.36 mm particle size and 9 cm bed depth from their removals in other treatment combinations used. This suggests that other factors than organic matter may also be responsible for the turbidity found. Inorganic species like clay and silt are known to contribute turbidity to wastewaters (Elemile *et al.* 2019). COD is a measure of both biodegradable and non-biodegradable organic pollutants, the insignificant difference in COD removal efficiency could be caused by the presence of non-biodegradable organic compounds. Non-biodegradable organic species – for example, polycyclic aromatic hydrocarbons – have recently been detected in slaughterhouse wastewater (Olaniran *et al.* 2019). Non-biodegradable pollutants can be adsorbed onto the surface of mycelium-colonized substrates and also taken up into the cells (Stamets 2005).

Tukey's Honest Significant Difference (HSD) test revealed the major pollutant removal contributor. The removal of color, BOD, EC and TN was influenced mainly by sawdust particle size, with estimated size effects of 84, 33, 26 and 74% respectively (Table 6).

Table 3 | Mean wastewater treatment results from the 3 cm deep mycofilter (Mean ± SD)

	Color (PtCo)	Turbidity (NTU)	TSS (mg/l)	pH	TDS (mg/l)	EC (μs/cm)	DO (mg/l)	BOD (mg/l)	COD (mg/l)	TN (mg/l)	TP (mg/l)
Raw wastewater	368 ± 62.6^{a}	264 ± 66.2^{a}	300.5 ± 19.5^{a}	7.0 ± 0.4^{a}	784 ± 54^{a}	1,617 ± 113.6^{a}	3.0 ± 1.3^{a}	194 ± 39^{a}	509 ± 20^{a}	6.0 ± 1.5^{a}	6.0 ± 1.3^{a}
0.6 mm	175 ± 39.4^{a}	70 ± 30.3^{a}	210 ± 83^{a}	7.0 ± 0.5^{a}	313 ± 11.9^{a}	643 ± 19.6^{a}	5.0 ± 3.1^{a}	59 ± 2.9^{a}	127 ± 49.2^{a}	1.0 ± 0.32^{a}	0.3 ± 0.1^{a}
1.18 mm	183 ± 25^{a}	51 ± 27.3^{a}	199 ± 69^{a}	7.0 ± 0.1^{a}	280 ± 60^{a}	574 ± 13.7^{a}	3.0 ± 2.1^{a}	87 ± 7.4^{a}	157 ± 11^{a}	2.0 ± 0.9^{a}	0.3 ± 0.1^{a}
2.36 mm	216 ± 48.4^{a}	53 ± 24.6^{a}	206 ± 59.9^{a}	7.0 ± 0.5^{a}	250 ± 15^{a}	508 ± 37^{b}	2.0 ± 0.1^{a}	60 ± 6.6^{a}	126 ± 18^{a}	2.0 ± 1.2^{a}	0.2 ± 0.1^{a}
Unsorted	276 ± 12.5^{a}	53 ± 15^{a}	235 ± 50.7^{a}	6.0 ± 0.3^{a}	450 ± 28^{a}	813 ± 48.6^{a}	3.0 ± 1^{a}	88 ± 2.4^{a}	320 ± 67.8^{a}	3.0 ± 0.62^{a}	3.0 ± 1^{a}

*Values in any column with different superscripts – either ^{a} or ^{b} – are significantly different, $P < 0.05$.

Table 4 | Mean wastewater treatment results from the 6 cm deep mycofilter (Mean ± SD)

	Color (PtCo)	Turbidity (NTU)	TSS (mg/l)	pH	TDS (mg/l)	EC (μs/cm)	DO (mg/l)	BOD (mg/l)	COD (mg/l)	TN (mg/l)	TP (mg/l)
Raw wastewater	368 ± 62.6^{a}	264 ± 66^{a}	301 ± 19^{a}	7 ± 0.4^{a}	784 ± 54^{a}	1,617 ± 11.3^{a}	3.0 ± 1.3^{a}	193 ± 39^{a}	509 ± 20.1^{a}	6.0 ± 1.5^{a}	6.0 ± 1.1^{a}
0.6 mm	134 ± 15.5^{a}	54 ± 27^{a}.	314 ± 9.7^{a}	7 ± 0.5^{a}	540 ± 21^{a}	885 ± 18.8^{a}	1.0 ± 0.3^{a}	52 ± 20^{a}	113 ± 33.5^{a}	2.0 ± 1.2^{a}	0.4 ± 0.2^{a}
1.18 mm	105 ± 25.6^{a}	30 ± 3^{a}	280 ± 52.4^{a}	8.0 ± 0.9^{a}	342 ± 10^{a}	1,083 ± 43^{a}	2.0 ± 0.8^{a}	98 ± 50^{a}	201 ± 9.9^{a}	1.0 ± 0.2^{a}	0.4 ± 0.1^{a}
2.36 mm	97 ± 11.4^{a}	39 ± 5.1^{a}	287 ± 49.5^{a}	8.0 ± 0.6^{a}	302 ± 12.4^{a}	684 ± 20.3^{a}	4.0 ± 2.4^{a}	80 ± 34^{a}	222 ± 12.2^{a}	1.0 ± 0.18^{a}	0.2 ± 0.1^{a}
Unsorted	284 ± 62.4^{a}	39 ± 13.9^{a}	305 ± 42^{a}	7.0 ± 0.2^{a}	430 ± 24.5^{a}	739 ± 13^{a}	3.0 ± 0.9^{a}	132 ± 5.6^{a}	349 ± 25.5^{a}	5.0 ± 1.0^{a}	4.0 ± 1.2^{a}

*Values in any column with superscripts – either ^{a} or ^{b} – are not significantly different, $P > 0.05$.

Table 5 | Mean wastewater treatment results from the 9 cm deep mycofilter (Mean ± SD)

	Color (PtCo)	Turbidity (NTU)	TSS (mg/l)	pH	TDS (mg/l)	EC (μs/cm)	DO (mg/l)	BOD (mg/l)	COD (mg/l)	TN (mg/l)	TP (mg/l)
Raw wastewater	368 ± 62.6^{a}	264 ± 66.2^{a}	301 ± 19.5^{a}	7.0 ± 0.4^{a}	784 ± 542.4^{a}	$1{,}617 \pm 1{,}136.8^{a}$	3.0 ± 1.3^{a}	194 ± 39.6^{a}	509 ± 200^{a}	6.0 ± 1.5^{a}	6.0 ± 1.3^{a}
0.6 mm	190 ± 25.5^{a}	60 ± 23.8^{a}	190 ± 25.7^{a}	7.0 ± 0.7^{a}	302 ± 12.4^{a}	600 ± 21.6^{a}	2.0 ± 1.7^{a}	120 ± 20.9^{a}	213 ± 29.6^{a}	1.0 ± 0.25^{a}	0.2 ± 0.1^{a}
1.18 mm	102 ± 7.4^{a}	38 ± 6.6^{a}	269 ± 27.5^{a}	7.0 ± 0.1^{a}	415 ± 68.2^{a}	837 ± 135^{a}	1.0 ± 0.2^{a}	123 ± 15.8^{a}	133 ± 41^{a}	1.0 ± 0.45^{a}	0.2 ± 0.2^{a}
2.36 mm	93 ± 2.7^{b}	22 ± 12.4^{a}	214 ± 8.8^{a}	7.0 ± 0.6^{a}	379 ± 14.7^{a}	767 ± 25.9^{a}	3.0 ± 0.6^{a}	32 ± 7.4^{b}	62 ± 12.6^{a}	1.0 ± 0.2^{b}	0.2 ± 0.1^{a}
Unsorted	270 ± 7.3^{a}	35 ± 3.4^{a}	236 ± 26.3^{a}	6.0 ± 0.3^{a}	461 ± 135.6^{a}	707 ± 36.2^{a}	3.0 ± 1.2^{a}	132 ± 5.6^{a}	367 ± 56.9^{a}	5.0 ± 1.9^{a}	4.0 ± 0.9^{a}

*Values in any column with superscripts – either ^{a} or ^{b} – are significantly different, $P < 0.05$.

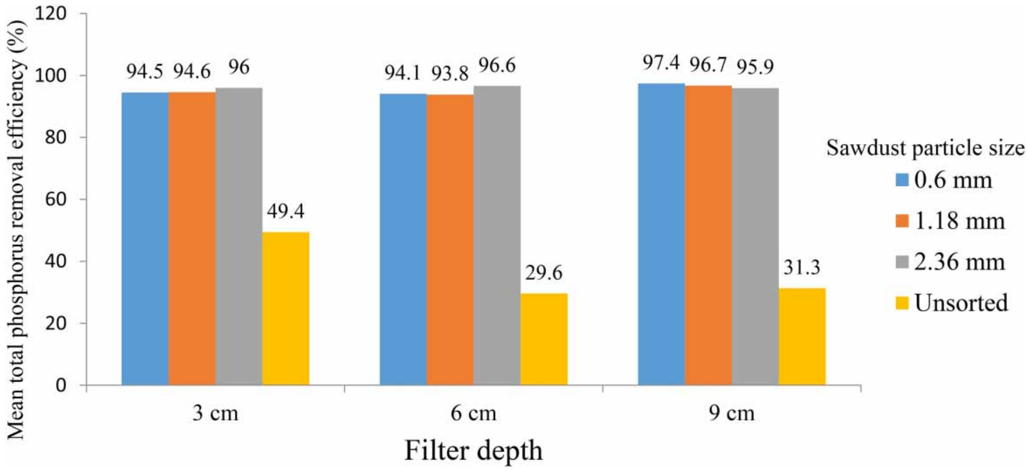

Figure 7 | Mean TP removal efficiency for mycelium-colonized sawdust.

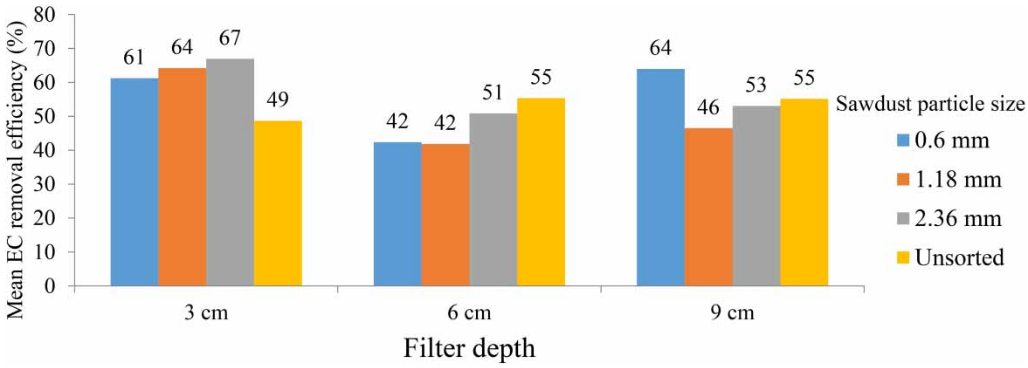

Figure 8 | Mean EC removal efficiency for mycelium-colonized sawdust.

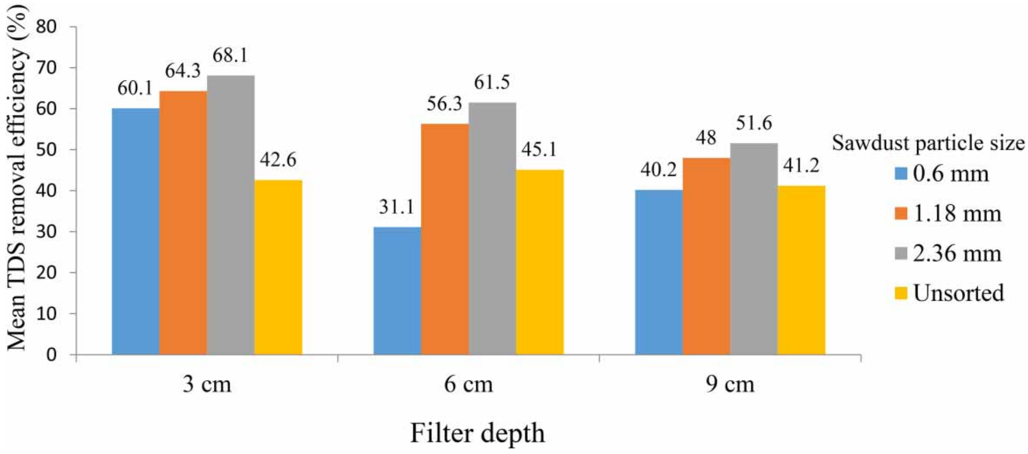

Figure 9 | Mean TDS removal efficiency for mycelium-colonized sawdust.

CONCLUSIONS

Removal of physiochemical pollutants from slaughterhouse wastewater by *Pleurotus ostreatus* mycelium-colonized sawdust was influenced by sawdust particle size and column depth. The study demonstrated that 2.36 mm particle size sawdust was a suitable substrate for efficient removal of

Table 6 | Estimated effect of the main variables on pollutant removal efficiency

Parameter	Overall	Particle size effect (%)	Bed depth
Color	51	84	50
BOD	36	33	15
EC	28	26	19
TN	29	74	1.2

color, BOD, TN and EC from the wastewater studied. However, while color, BOD and TN were removed significantly by the 9 cm bed, EC was removed best by the 3 cm bed. Statistical studies showed that, in this study, sawdust particle size had more influence on pollutant removal than bed depth. The study showed that the sawdust particle size and bed depth chosen should be based on the pollutant(s) targeted.

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CONFLICT OF INTEREST

We declare that we have no competing interest concerning this study.

AUTHORS CONTRIBUTION

Osayomwanbo Osarenotor conceived and designed the study, drafted the manuscript, and acquired, analyzed and interpreted the data, and Helen Essandoh and Isoken Tito Aighewi revised the manuscript for critical intellectual content and approved the final version for submission.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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