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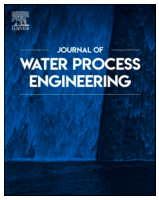
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Integrated membrane packed enzymatic column for the removal of atrazine in wastewater: Experimental, kinetics, breakthrough curves analysis and applicability in real wastewater

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ABSTRACT

The current study explored the performance of a packed bed biocatalytic column equipped with polyethersulfone (PES) hollow fiber membranes and Novoprime base 268 laccase (Lac) for the biotransformation of the recalcitrant herbicide, atrazine (ATZ). Column design parameters: feed concentration (5 mg L^{-1}), flow rate (2 mL min^{-1}) and bed height (14 cm) generated optimum membrane retention (MR) in the fixed bed. A standalone MR system attained >50 % of ATZ retention with a maximum capacity of 170.2 mg g^{-1} at bed service time of 559 min. Under optimized conditions (50 mg dosage, 5 mg L^{-1} solution concentration at 25 °C) 66 % degradation efficiency was observed in the laccase-assisted conversion within 24 h. PES-Laccase (PES-Lac) biocatalyst improved the overall degradation efficiency to 86 %, exhibited simple regeneration and satisfactory operational reusability, retaining 46 % activity in the sixth cycle. Overall, the work demonstrated the potential of PES-Lac biocatalytic system to selectively bioremediate ATZ and provided a detailed understanding of the factors influencing its efficiency for applicability in water reclamation.

1. Introduction

Continuous increase in agricultural productivity have intensified the environmental impacts of pesticides, is raising concerns around the world [1,2]. Atrazine (ATZ) (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a selective triazine herbicide frequently used for eradicating gramineous and broadleaf weeds in essential crops [3]. Residual amounts of ATZ permeate the environment through several non-point sources, which drive the widespread contamination of receiving water bodies, soil, and sediments [4–6]. Due to its relative stability, resistance to abiotic hydrolysis (stable at pH 5–9) and direct aqueous photolysis [7–10] the ATZ's persistent environmental detection continually threatens the aquatic ecosystems and human health [11,12]. Ephemeral, sub-lethal exposure of residual ATZ and its chlorinated metabolites have been documented to disrupt key endocrine signaling pathways [13–15], resulting in several pathological alterations and malignancies in the reproductive system [16–18]. Consequently, alternative water treatments strategies are necessitated for efficient eradication of ATZ in water sources.

Biocatalytic remediation affords an environmentally benign alternative that operates at lower energy requirements, under moderate operational conditions [19,20] and reduced unfavorable side reactions thus less-toxic byproducts [21]. Laccase-mediated treatment for waste streams consumes ambient oxygen which greatly reduces the need for enzyme-destabilizing agents such as H_2O_2 (an oxidant required by peroxidases) [20–24].

Laccases [*p*-diphenol: dioxygen oxidoreductases (EC 1.10.3.2.)] are a class of extracellular multi-copper containing oxidoreductases catalyzing the oxidation-reduction-assisted biodegradation various substrates to less toxic derivatives [25–27]. Lac-catalyzed degradation of ATZ promotes hydrolytic dechlorination producing hydroxyatrazine (HA) which is later converted to N-isopropylammelide or N-ethylammelide intermediates (hydrolytic deamination reactions) then finally to cyanuric acid [28]. Another pathway proceeds through N-dealkylation of the ethyl and isopropyl moieties which generates less toxic ATZ metabolites (deethylatrazine, deethyldeisopropylatrazine and deisopropylatrazine) which are used as fuel for growth and reproduction of microorganisms [29,30].

Due to the impeded application of pristine laccases [30], mitigation support onto stable carrier matrices [26] or coupling of enzymatic systems with polymer-based membranes [31] such as Polyethersulfone (PES). PES exhibits porous, continuously spatial structures, wide surface

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area, and superior thermal and chemical resilience [32] which have led to its integration in enzymatic membrane systems [33,34]. Extensive literature review revealed successful applications of integrated oxido-reductase systems for the remediation of micropollutants including ATZ [26–30] with improved efficiencies, long term operational stability, reusability, and simple product separation [35,36]. However majority of the studies primarily focused on the bioconversion of pollutants in batch mode processes [37–39]. For instance, three azo dyes were degraded with a reduction in the effluent's toxicity was reported in nano and ultrafiltration biosystems, enzyme deactivation and probable occlusion of active laccase sites of laccase during immobilization could not be avoided [38]. Notwithstanding the benefits and contributions of these studies in estimating the effectiveness of such systems; findings lack accurate predictions on the dynamic nature of adsorption for the treatment of wastewater on a large scale (i.e., industrial scale) [40–42]. Hence, the application of enzymatic-based separation processes in continuous systems that can be used in actual industrial settings remain scarce.

Moreover, to date, literature reports on the practical biotransformation of pesticides in real water samples remain limited. Therefore, it is essential to investigate the degradation of other emerging pollutants (i.e., triazine herbicides) at their environmentally relevant concentrations in biosystems. This will encourage more feasible developments in the comprehension of the fate of pesticides in enzymatic treatments and increase scientific understanding of their removal mechanisms thus creating an extensive database for future predictions.

Herein, our study explored a hybrid catalytically active packed-column reactor which coupled Novoprime® base 268 laccase with PES based hollow-fiber membranes for the bioremediation of atrazine in wastewater. This single unit integrated molecular retention with catalysis, two crucial components of wastewater purification.

2. Chemicals and materials

Atrazine (ATZ) was selected as a relevant pesticide pollutant based on its widespread occurrence in wastewater and various water sources. An analytical standard (Pestanal grade, > 99 % purity), ATZ (6-chloro-N-ethyl-N′-(propan-2-yl)-1,3,5-triazine-2,4-diamine) was purchased from Sigma-Aldrich (Johannesburg, South Africa). Acetonitrile (ACN), (HPLC)-grade, 2,2azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), sodium citrate dihydrate, citric acid, sodium dihydrogen phosphate and disodium hydrogen phosphate of analytical grade were also obtained from Sigma-Aldrich (Johannesburg, South Africa). Novoprime® base 268 was supplied by Novozymes (Denmark) while commercial PES hollow-fiber membranes used were kindly provided by Inge®, BASF, Greifenberg, Germany). All solutions were prepared using ultrapure water obtained from Millipore, Direct-Q® 3UV water purification system (Merck, Germany).

3. Experimental

3.1. ATZ removal: membrane packed column studies

PES hollow fiber membranes were evaluated for the adsorption of atrazine in a fixed-bed system according to a previously reported method [43]. A cylindrical glass tube column packed with PES was fed with the influent (ATZ) at a fixed flow rate. Pre-determined volumes of effluent samples were collected consecutively at varying experimental parameters: initial analyte concentration (5 to 15 mg L^{-1}), flow rate of 2 to 6 mL min^{-1} and bed height (3.5 to 14 cm). Each parameter was varied at a time and the breakthrough curves were then constructed by plotting C_t/C_o against time.

3.2. Prediction of column performance indicators

The dynamic performance of packed-bed adsorption is influenced by experimental conditions, significantly affects mass transfer resistance and hence adsorption parameters. This is based on the permeate concentration-profile or breakthrough curves [44] which express the point of breakthrough from the plot of C_t/C_o over time, empty bed contact time (EBCT), the number of processed bed volumes, (BV) and the adsorbate exhaustion rate (AER) [42]. The capacity at breakthrough (q_b) in mg g^{-1} at a given flow rate was calculated using the Eq. (1). [45].

$$q_b = Q \cdot \frac{C_0}{m} \int_0^b \left(1 - \frac{C_t}{C_o}\right) dt \quad (1)$$

where Q represents the volumetric flow rate (mL min^{-1}), m is the PES mass (g), C_o and C_t are the concentration (mg L^{-1}) of the influent and effluent, respectively at time (t).

3.3. Breakthrough modelling

The design and industrial-scale implementation of a successful fixed-bed column is dependent on the prediction of dynamic nature of the column. For this purpose, the well-known mathematical models i.e., Thomas, Bohart-Adams and Yoon Nelson were used to describe the lab-scale column setup. The general basis of the Thomas model involves an adsorption process which neglects internal or external diffusion restrictions [46]. The postulation here is that pollutant adsorption process follows the Langmuir isotherm and second-order reversible reaction kinetics without axial dispersion. Bohart-Adams model posits a surface reaction theory and suggests that equilibrium is instantaneous [47,48] while Yoon-Nelson developed a model in which it is presupposed that the probability of an adsorbate being absorbed is directly correlated with the likelihood that its concentration will exceed the breakthrough concentration on the adsorbent [49].

3.4. Enzymatic activity assay

Laccase activity was measured by observing the change in absorbance due to the oxidation of 1.0 mM ABTS recorded at 420 nm using UV–vis spectroscopy (Agilent Technologies, Cary Series 1000, USA). Known portions of laccase were agitated in a glass vials with 50 mM citrate-phosphate buffer (CPB, 5 mL, pH 5.0) followed by an addition of 1 mL of ABTS (5 mM). Enzymatic activity was computed using the molar extinction coefficient of ABTS ($\varepsilon_{420} = 36{,}000\ M^{-1}\ cm^{-1}$) where one unit of laccase activity is defined as the amount of enzyme that oxidized 1 μ mol of ABTS min^{-1}. This was expressed as units mg^{-1} [25].

3.4.1. Kinetic analysis of Novoprime base 268 laccase

The kinetic experiments were performed in 5.0 mL CPB (pH 5.0) consisting of laccase and ABTS concentrations in the range 0.1 to 5 mM and also at varied ATZ concentrations (1.0 to 20 mg L^{-1}). The rate of enzymatic reaction was described by estimation of the K_m and V_{max} parameters given by the Michaelis-Menten equation [50,51]:

$$V = \frac{V_{max}[S]}{K_m + [S]} \quad (2)$$

where V (mM min^{-1}) and S (mM) represent the velocity of the initial reaction rate and substrate concentration, respectively. V_{max} is the maximum reaction velocity in mM min^{-1} and K_m (mM) is the Michaelis-Menten constant. V_{max} and K_{max} values were predicted according to the fitting curve of 1/[V] versus 1/[S] [52,53] following the Lineweaver-Burk plot.

3.5. Optimization of ATZ removal by laccase

Experimental parameters affecting biodegradation efficiency viz. initial solution pH (pH 1–7), concentration (1.0–20 mg L^{-1}), enzyme mass (10–50 mg) and reaction time (1 - 24 h) were investigated in batch mode. A high-performance liquid chromatography (BISCHOFF,

Labexchange, Deutschland) was employed for analysis using an Ascentis® C18, 25 cm × 4.6 mm, 5 µm (SUPELCO®, Bellefonte, PA, USA) operated at a column temperature of 25 °C and a detection wavelength of 223 nm. A constant flow rate of 1.0 mL min^{-1} was maintained under isocratic elution with an eluent composition of acetonitrile: water (70:30, *v*/v). Calibration standards were prepared daily by serial dilution of the stock solution (1000 mg L^{-1}) which were kept in the dark at 4 °C when not in use.

3.6. Fabrication of PES-Lac fixed-bed column

The biocatalytic remediation of ATZ was systematically determined using the PES-Lac packed bed in continuous mode. Optimum membrane retention and degradation conditions were used as described in Sections 3.2 and 3.5. Removal efficiencies of ATZ by stand-alone PES system and PES-Lac treatment were determined by Eq.:

$$\%\boldsymbol{R} = \left(\frac{C_o - C_f}{C_o}\right) \times 100\% \quad (3)$$

where % R is the overall membrane removal and degradation efficiency (%), accordingly, C_0 and C_f are the pollutant's initial and residual concentrations (mg L^{-1}), respectively.

The applicability of the biocatalytic system in the presence of matrix components was tested using real water samples collected from the Crocodile River, South Africa. The river emerges from Constantia Kloof, in Gauteng province, and flows into the Witwatersrand Mountain range. Its confluence with the Juskei River is located to the north and the stream passes downstream Hartbeespoort area, used by residents for recreational fishing. A Thermo Scientific Dionex Ultimate 3000 high performance liquid chromatography (HPLC) system (ThermoFisher Scientific, Waltham, MA, USA) coupled to an HPG-3200RS pump and a WPS-3000TRS autosampler was utlilized for analysis. The HPLC was connected to a Bruker Daltonics Inc. compact quadrupole time-of-flight mass spectrometer system (Billerica, MA, USA). Data was collected and processed using Bruker Compass DataAnalysis version 4.3 and chromatographic elution was conducted in a positive ionization mode using formic acid (FA) in two solvent compositions constituting of (H_2O + 0.1 % FA) and (ACN +0.1 % FA), respectively.

3.7. Cost analysis study

Cost estimates were developed to evaluate the economic viability of the small-scale biocatalytic system designed for domestic duty. Each process configuration was designed and sized using the previously reported data then fully costed using actual construction prices from past and recent projects. Individual cost contributions were collated to generate the overall capital expenditure (CAPEX) i.e. direct or indirect cost and operating and maintenance (OPEX) costs (fixed and variable costs) of an agricultural wastewater reclamation facility for treatment capacities of 1000 m3/day as previously detailed [54]. CAPEX comprised of the equipment, instrumentation, piping, electrical and piping costs while OPEX covered the membrane and enzyme replacements, maintenance, chemicals and total energy costs [55–57]. The economic analysis was executed as using previous estimates described where 2, 10 and 40 % of the total equipment costs were assumed for maintenance, equipment installations and piping costs, respectively with 25 % estimated for electrical and control systems [54]. Membrane suppliers were also consulted for their valuable design input.

4. Results and discussions

4.1. Effect of operating conditions on column adsorption of ATZ by PES membranes

4.1.1. Effect of initial ATZ feed concentration (C_o)

The influence of ATZ concentration (5 to 20 mg L^{-1}) on the column performance was examined at constant feed flow rate (2 mL min^{-1}) and bed height (7.0 cm). As shown in Fig. 1, the column BT curves became sharper as the influent concentration increased. Meanwhile, the breakthrough time (t_b) and exhausted time (t_e) both decreased from 441 to 225 min and 730 to 550 min, respectively resulting in lower quantities of water treated.

Increasing the initial ATZ concentration prompted increased adsorption capacities (q_e) from 139.6, 129.9 and 95.68 mg g^{-1} (Table 1) corresponding to 5.0, 10 and 20 mg L^{-1}. This is attributable to accelerated concentration gradient between adsorbent and adsorbate, which provides the driving force between the solid and liquid phases and thus increases the adsorbent exhaustion rate (high ATZ loading rate onto PES binding sites) [58]. In other words, at lower influent concentrations, both the concentration gradient and the driving force between the solid and liquid phases are reduced, leading to a longer time required for PES saturation [43]. Similar trends were documented in the literature on fixed bed studies of phenol adsorption using neem leaves [59], diclofenac [60] and a number of other studies [61].

4.1.2. Effect of influent solution flow rate (Q)

Fig. 2 illustrates the effect of different feed solution flow rates (2.0, 4.0 and 6.0-mL min^{-1}) on the efficiency of the packed column. As the inlet flow rate rise from 2.0 to 6.0 mL min^{-1}, the EBCT decreased from 7.07 min to 2.34 min and the saturation time from 980 min to 405 min. This was qualified by migration of the adsorption zone leading to a gradual increase of the pollutant in the effluent until breakthrough is reached [42–62].

According to Gupta and Garg (2019) [45], higher volumetric flow rates tend to reduce the thickness and resistance of the external mass transfer layer while increasing the overall mass transfer coefficient and flux. Additionally, the axial dispersion and overall mass transfer coefficient rises as the inlet flow rate increases, resulting in an increase in adsorption rate and faster saturation of the fixed bed [43]. Evidently, lower feed flow rates yield higher pollutant removal. The trend agrees

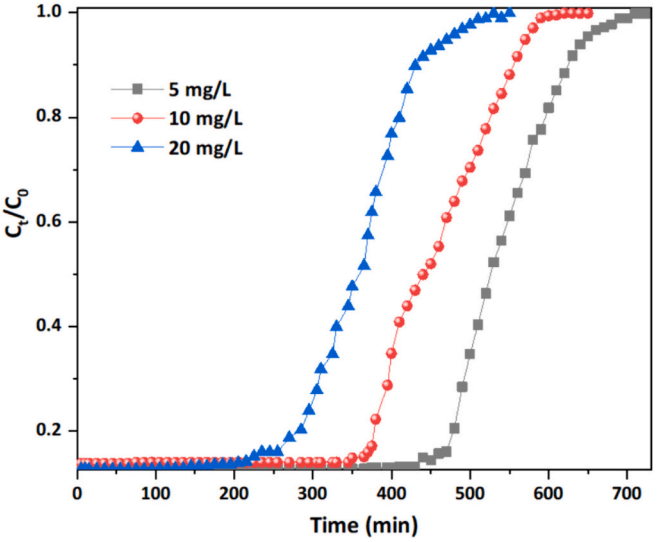


Fig. 1. Effect of influent concentration on the breakthrough curve for ATZ adsorption on PES membranes (conditions: flow rate = 2 mL min^{-1}, bed height = 7.0 cm).

Table 1
Estimation of breakthrough point parameters for ATZ adsorption in a fixed bed column using PES membranes as an adsorbent.

Process parameters	Service time, t_b (min)	Capacity, q_e (mg g^{-1})	Volume treated V_b, (mL)	AER $\times 10^3$ (g mL^{-1})	ECBT (min)
Influent concentration (mg L^{-1})					
5.0	470	139.6	940	1.33	7.04
10.0	364	177.3	728	1.72	7.04
20.0	271	203.6	542	2.31	7.04
Influent solution flow rate (mL min^{-1})					
2.0	470	139.6	940	1.33	7.04
4.0	254	129.9	1016	1.24	3.51
6.0	441	95.68	2646	0.47	2.35
Bed height (cm)					
3.5 (0.65 g)	226	73.72	452	1.43	3.52
7.0 (1.26 g)	470	139.6	940	1.33	7.04
14.0 (2.35 g)	559	170.2	1118	2.99	14.1

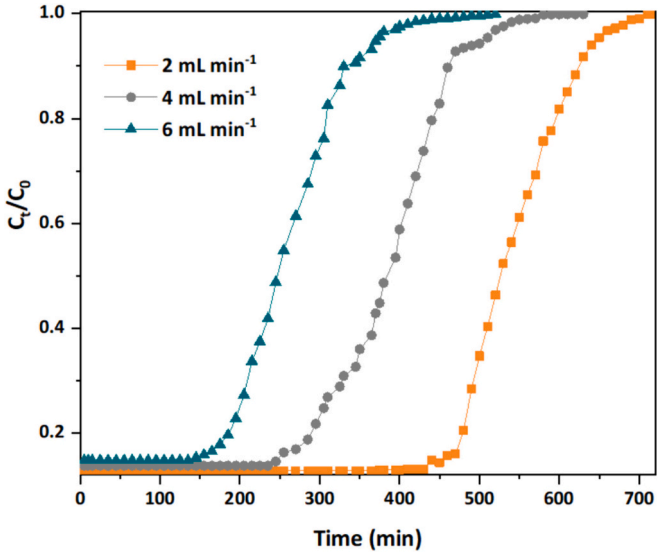


Fig. 2. Effect of inflow flow rate on the adsorption of ATZ in a packed bed column at bed height 7 cm and feed concentration of 5 mg L^{-1}.

with other published studies on breakthrough curves at different flow rates [44], [70–73].

4.1.3. Effect of PES bed height (H_b)

Pollutant accumulation in a fixed bed is largely dependent on the quantity of the packing material. The column was packed with different PES bed heights (3.5–14 cm). For this experiment, the inlet flow rate and concentration were kept constant. Fig. 3 demonstrates slower breakthrough time from 66.3 to 175.9 min with an increase in bed depth from 3.5 to 7.0 cm and subsequently increasing the adsorbent exhaustion time.

This observation may be associated with longer pollutant residence time flowing within the column resulting decreased residual effluent concentration and thus, allowing the treatment of larger volumes of ATZ. Other researchers reported similar observations that have been ascribed to predominance of axial dispersion phenomenon of mass transfer at diminished bed heights thus, restricting the permeability of ATZ into PES membranes [65,66].

Table 1 summarizes the process parameters at varying experimental conditions. The bed capacity (q_e) increased from 73.7 to 170.2 mg g^{-1}

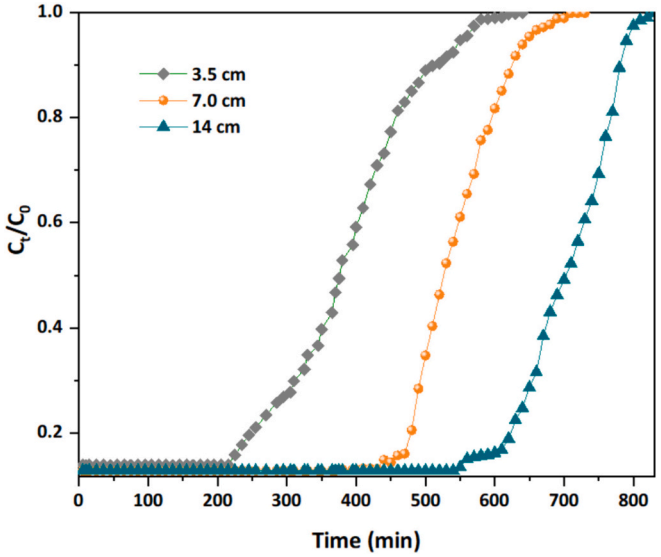


Fig. 3. Column breakthrough curves for the effect of bed mass at constant volumetric flow rate (2 mL min^{-1}) and feed concentration (5 mg L^{-1}).

with a rise in the column bed length from 3.5 to 14 cm resulting from increased surface area and availability of sorption sites on the PES [66]. Song et al. (2016) also reported an extended breakthrough point (from 15 to 120 min) with an increased height of modified rice straw from 1.5 to 4.5 cm and related the behaviour to longer mass transfer zones at higher bed heights yielding larger treated volumes. Notably, the quantities of solution treated at breakthrough (V_b) also increased 452, 940 and 1118 mL corresponding to 3.5, 7.0 and 14 cm, respectively which also led to improved EBCT permitting sufficient time for the solute to diffuse into the PES [44]. Consequently, a greater volume of the herbicide is removed. These findings suggest that the optimal performance of the bed can be attained using larger quantities of adsorbent mass.

4.2. Breakthrough model analysis of ATZ on PES membranes

The performance nature of the breakthrough curves on an industrial scale was assessed using breakthrough curve kinetic plots. The experimental conditions and model parameters are summarized in Table 1.

4.2.1. Thomas model

Model constants derived from the linear graphs (Table 1), indicate increasing k_{TH} values with increasing bed height, initial ATZ concentration and decreasing adsorption capacities (q_o). This observation was related to a decrease in mass transport resistance. These findings are comparable to those of other studies [45,68]. The maximum sorption capacity ($q_o = 298.9$ mg g^{-1}) was attained at 5 mg L^{-1}, feed flow of 2.0 mL min^{-1} and bed height of 14 cm.

4.2.2. Bohart-Adams

A summary of the computed Bohart Adams constants, k_{BA} and N_o values with their respective correlation coefficients is given in Table 2. The k_{BA} constant increased as all parameters increased. However, the R^2 for the model were relatively low (0.82 to 0.98) signifying that the Bohart-Adams model does not properly depict the equilibrium dynamics of ATZ adsorption utilizing PES membranes.

4.2.3. Yoon-Nelson

Yoon-Nelson developed a model in which it is presupposed that the probability of an adsorbate being absorbed is directly correlated with the likelihood that its concentration will exceed the breakthrough concentration on the adsorbent [49].

R^2 values ranged between 0.83 and 0.99 (Table 2) and the k_{YN}

Table 2
Column adsorption parameters under varying operational conditions in a packed-bed column.

Parameters	Thomas model				Bohart-Adams				Yoon-Nelson			
	$K_{TH} \times 10^{-2}$ (mL min^{-1} mg)	q_0 (mg g^{-1})	R^2	RMSE	$K_{BA} \times 10^{-3}$ (L mg^{-1} min^{-1})	N_0 (mg L^{-1})	R^2	RMSE	$K_{YN} \times 10^{-2}$	τ (min)	R^2	RMSE
Influent concentration (mg L^{-1})												
5	42.4	179.5	0.991	0.004	1.48	438.6	0.819	0.019	2.35	229.6	0.99	0.031
10	24.2	198.1	0.908	0.044	3.34	814.1	0.886	0.023	2.84	207.8	0.94	0.083
20	12.2	209.9	0.923	0.072	5.18	1335.0	0.935	0.062	2.66	175.7	0.90	0.079
Flow rate (mL min^{-1})												
2	12.1	248.2	0.990	0.037	1.73	269.8	0.904	0.011	2.25	245.0	0.99	0.010
4	11.6	242.9	0.962	0.089	1.35	656.7	0.924	0.068	2.28	180.3	0.95	0.013
6	10.4	179.3	0.993	0.091	2.22	691.4	0.977	0.036	2.20	103.1	0.97	0.067
Bed height (cm)												
3.5	178.6	135.1	0.914	0.063	1.05	268.5	0.913	0.028	0.54	65.64	0.93	0.013
7.0	231.8	206.9	0.823	0.036	1.52	444.1	0.829	0.101	0.16	260.1	0.99	0.084
14	286.9	298.9	0.857	0.012	1.71	751.05	0.923	0.093	0.24	324.5	0.83	0.103

constant did not uniformly follow any trend with varying parameters. Hence, this model proved to be unsatisfactory in the experimental working range. Similar trends were documented [59]. Indeed, it is worth noting that the criterion for assessing goodness of fit for the models predominantly relies on statistical evaluation indicators such including the chi-squared value (χ^2) and root of means square error (RMSE) [69,70]. Moreover, the coefficient of k_{YN} was changed as a function of bed height. The model's RMSE values were 0.010–0.103 which were relatively higher than those recorded for the other models. The results in Table 2 also revealed lower RSME values for the Thomas model suggesting better model fitting and similarity with the experimental data [71].

The viability of a PES hollow fiber membranes as an alternative sorption media for ATZ removal was compared with equilibrium adsorption capabilities of other adsorbents applied in packed-bed studies (Table 3). Although it was discovered that the current sorption medium was comparable to other previously documented adsorbents, there are limited research reports on the removal of ATZ in continuous fixed bed flow experiments.

4.3. Enzymatic degradation

4.3.1. Substrate specificity and kinetic parameters

The biotransformation of ABTS and ATZ at varying concentration is presented by the Michaelis-Menten plot in Fig. 4. The kinetic parameters (K_m and V_{max}) were also defined [51]. As illustrated, there was a general increase in the velocities with increasing substrate concentrations however, these considerably decreased when ATZ was used as an additional substrate in the ABTS solution, K_m (0.22 mM) and V_{max} (0.53 μM min^{-1}). (See Table 4.)

The V_{max} of ATZ + ABTS decreased two folds from the V_{max} (1.26 μM min^{-1}) of ABTS alone. This observation is prevalent in the oxidation of substrates substituted EWGs [74,75]. Similarly, the -NH_2 moieties and a halogen ring on the ATZ core, instigate substrate inhibition to the active catalytic sites of the enzyme [75,76] and thus slower oxidation of ATZ

Table 3
Comparison of the ATZ column equilibrium adsorption capacity of different adsorbents with PES membranes.

Adsorbent (s)	Capacity (mg g^{-1})	Reference (s)
Microwave irradiated *Aegle marmelos Correa* fruit shell	1230	[43]
Poly(divinylbenzene) sorbent	26.1	[41]
Waste activated carbon	17.2	[72]
Organic biomixture	95.3	[62]
Ceramic ultrafiltration membrane	NR	
Hollow fiber PES membranes	289.9	This study

*NR – not reported.

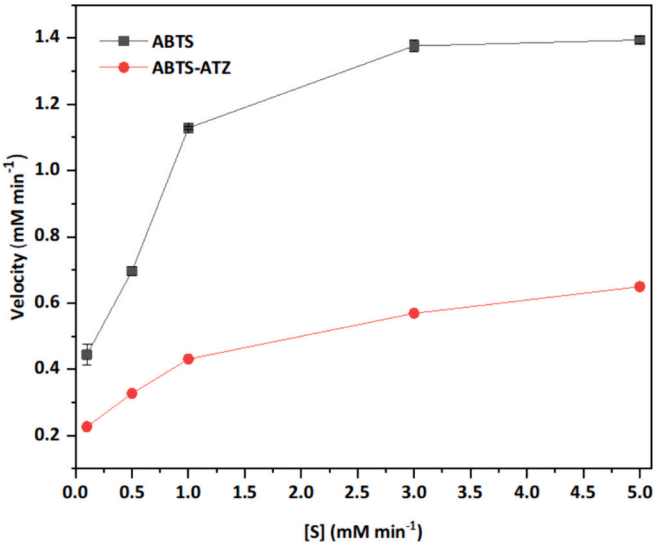


Fig. 4. Michaelis-Menten plots for Lac using ABTS and ABTS+ATZ as substrates for kinetic parameters.

Table 4
Kinetic parameters of Lac with substrates: ABTS and ABTS + ATZ.

Substrate	Kinetic parameters	
	K_m (mM)	V_{max} (μM min^{-1})
ABTS	0.19	1.26
ABTS + ATZ	0.22	0.53

compared to ABTS [36]. An increase in K_{max} from 0.19 mM (ABTS) to 0.22 mM (ABTS+ATZ) was avowed by lower affinity between ATZ and laccase however, enhanced ATZ removal was facilitated by the synergistic effect of integrating membrane retention into the enzymatic system.

4.3.2. pH and temperature profile on laccase degradation efficiency

Fig. 5 displays the temperature and pH profile on the conversion of ATZ. The enzyme showed inactivity at pH levels below optimal (pH 1–3) and a sharp reduction in activity at pH levels 7–10, retaining about 46 % and 35 % efficiency at pH 7 and 10, respectively. The diminished catalytic activity at non-optimum pH levels may be explained by the slower rate of substrate interaction and/or disruption of active catalytic sites [77] leading to irreparable denaturation [78]. Ideal fungal laccase activity is reportedly in the range pH 4–5 which tends to affect the electrostatic characteristics of the active site [79]. In essence, alkaline pH

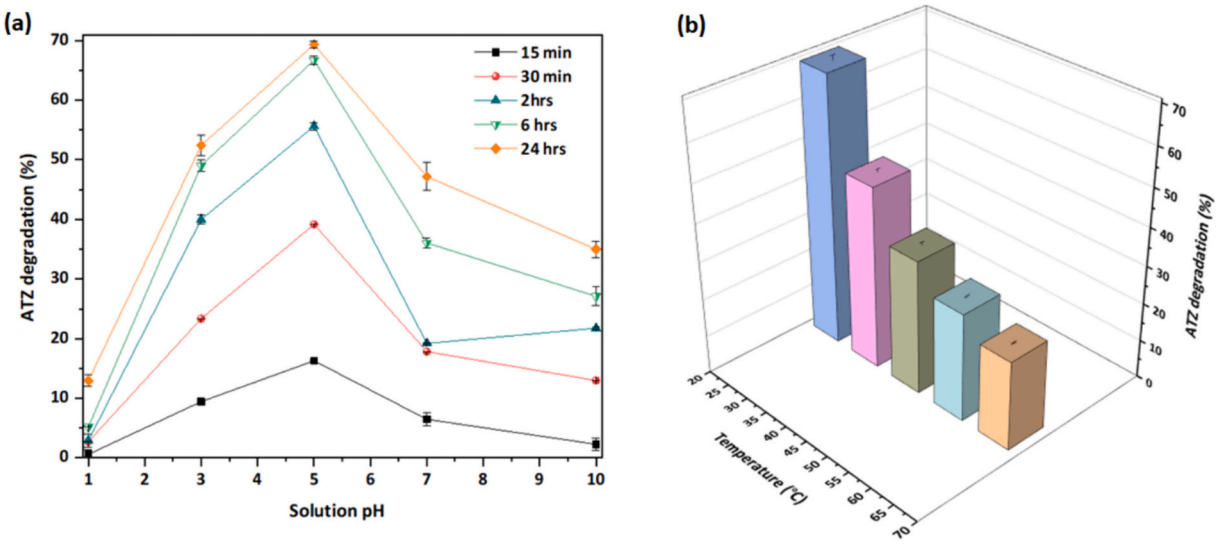


Fig. 5. (a) Influence of solution pH (b) and temperature on degradation of ATZ by laccase.

levels facilitate the binding of OH^- onto the copper ion at the active site, inhibiting the internal electron transfer pathway thereby decreasing laccase activity [76–80]. Margot et al. (2015) described a laccase-catalyzed mediated detoxification sulfamethoxazole and isoproturon at pH range 5–6.

Additionally, the enzyme showed substantially increasing activity from 15 min to 6 h of the reaction time maintaining approximately 55 % activity at the optimum pH. Following which the kinetics increased to 66 then 69 % from 8rs to 24 h, respectively. These high removal efficiencies may be due to the prolonged interactions of the substrate with the enzyme at longer reaction times subsequently, 24 h was used as the optimum time in successive batch enzymatic studies. From Fig. 5(b) the thermostability profile illustrated a decrease in efficiency from 25 to 70 °C, with maxima attained at 25 °C. A further increase in temperature (45–65 °C) led to diminishing ATZ degradation. This trend may be ascribed to permanent denaturation from thermal-induced conformational changes arising from irreparable unfolding of the protein structure as a result of enhanced chemical bond vibrations and linkage cleavage hence lower efficiencies [76–82].

4.3.3. Enzyme mass and stability tests

The quantity of enzyme required for the biocatalytic system's optimum performance was investigated at varying parameters. As shown in Fig. 6(a) the enzymatic efficiency improved from 25.6 % to 61.4 % with increasing lac mass from 10 mg to 40 mg, respectively. Higher doses increase the availability of catalytic active sites thus reducing enzyme-substrate bulk- interactions, driven by multiple surrounding layers thus creating steric hindrance and restrained dispersion of substrate [75]. These findings are consistent with previous reports [83,84]. The optimum dosage for ATZ degradation was 50 mg, which exhibits highest maximum activity (63.1 %) with a 1.7 % increment from the 40 mg enzyme mass.

The durability of Lac during storage was tested by submerging the enzyme in CPB buffer (pH 5) at 4 °C for 30 days. From Fig. 6 (b) the enzyme retained its relative activity until day 15 (56.2 %) with a slight decrease from 67.6 %, to 66.4 % then 62.1 % and from day 1to 5 to day 10, respectively.

The initial activity drastically dropped by close to three folds on day 25 (69 to 25 %), corresponding to degradation efficiency of 26.3 % which further decreased to 11.6 % on the last day of preservation. This observation is related to the stability in the enzyme's three-dimensional structure and its relative resistance to conformational changes under severe reaction conditions [75–85]. The use of carrier bound enzymes leads to the dilution of catalytic activity and, hence, reduced volumetric and space-time yields and lower catalyst efficiency [89]. Therefore, the Novoprime base 258 laccase demonstrated operational stability with efficiencies that are comparable to other immobilized laccases.

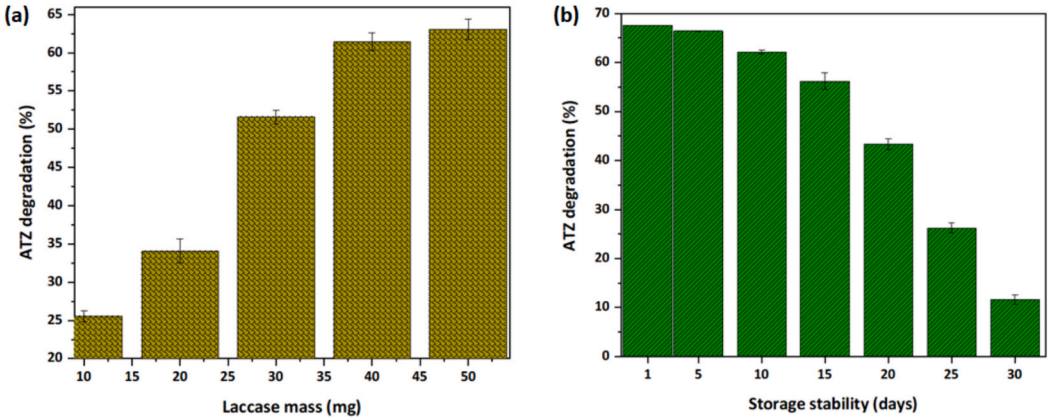


Fig. 6. (a) Effect of enzyme load on ATZ degradation and (b) storage stability tests over 30 days.

4.4. Fabrication of PES-Lac biocatalytic coulumn and operational reusability

The fabricated PES-laccase based column was applied for the bioremediation of ATZ using the previously optimized conditions. The effluent solution from the membrane packed-bed reactor subsequent to column exhaustion from 800 to 1190 min (corresponding to 10 min and 24 h degradation time) was used as the initial and final period for batch-mode enzymatic degradation, respectively. The overall performance of the biocatalytic system was then assessed by measuring ATZ concentration in samples collected at 10, 30, 60, 120, 240, 480, 960 and 1440 min corresponding to 880, 830, 890, 950, 1010, 1070, 1130 and 1190 min. Fig. 7b displays the combined retention bioconversion degree of ATZ in the packed bed column as a function of time. The enzymatic degradation increased from 37.3 to 69.2 % while the membrane efficiency decreased when the reaction time was increased from 800 min (55.9 %) to 1190 min (12.2 %). This was ascribed to membrane exhaustion (Fig. 3).

Coupling membrane retention with enzymatic degradation improved the overall removal of ATZ. The biocatalytic system attained 93.2 % removal efficiency with 37.3 and 55.9 % enzymatic degradation and membrane retention at 800 min operational time, respectively. After 24 h of operation, 81.3 % degradation was observed with notable a plateau in enzyme efficiency (69.1 %) and diminished membrane performance (12.2 %).

The effectiveness of a hybrid PES-Lac fixed bed column to effectively eradicate ATZ was highlighted with considerable improvement compared to other previously reported studies where <5 % removal was attained in an UF-EMBR [31]. Despite, laccase's ability to oxidize non-phenolic substances including those with EWGs, it is hampered by kinetic restrictions which are predominant in many enzyme-catalyzed reactions [86,87]. Nonetheless, this study demonstrated that ATZ conversion significantly improved following retention by PES membrane in the packed-bed column.

In practical terms, the enzyme's operational stability plays a vital role in establishing a continuous treatment process. The operational durability of the biocatalytic system was assessed over six successive cycles of the degradation of ATZ (Fig. 8). The reusability tests showed that the biocatalytic column's catalytic efficiency was maintained at 76 % after two cycles and at 50 % at cycle 5.

This favorable recyclability may be ascribed capability of the membrane support to preserve the enzyme's catalytic conformation under harsh conditions [88,89]. This observation suggests that Novoprime base 268 laccases may be suitable for applications in water reclamation.

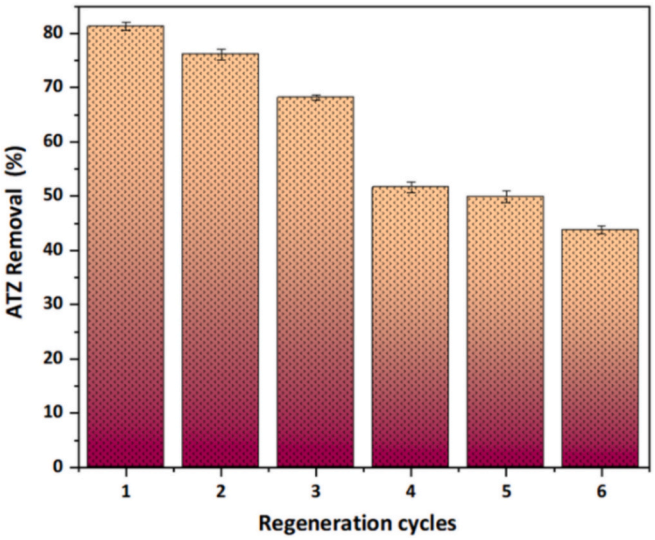


Fig. 8. Reusability tests for PES-Laccase reactor in triplicates.

4.5. Application of biocatalytic system in real water matrices

The applicability of the biocatalytic packed bed in real water samples was conducted using a water sample collected at a local river stream in Johannesburg. At the time of sampling, in-situ measurements of physicochemical properties of the sample included pH (7.2), conductivity (9 μS cm^{-1}), total dissolved solids (389 mg L^{-1}), temperature (27 °C) and dissolved oxygen of 8 mg L^{-1}. The sample was spiked with ATZ then fed into the packed-bed column under optimized conditions. The overall conversion degree was found to be 82 %, which outperformed the efficiency of the standalone membrane reactor at 52 %. The improved efficiency could be related to the synergistic effect offered by enzymatic degradation. In this sense the availability of PES as a support may have afforded retention of other competing substrates and thus allowing better accessibility of the catalytic active sites by the target analyte.

4.6. Economic assessments

The cost parameters of the proposed full-scale PBEMBR are summarized in Table 5. The system design was based on a treatment capacity of 1200 m^3/d. As shown in Table 5, the estimated total capital cost for full-scale PBEMBR was R7.036 mil at R2.38 and R1.31 mil of CAPEX and OPEX, respectively. Compared to the other studies, the system was established at a much lower cost and the latter is because the tool

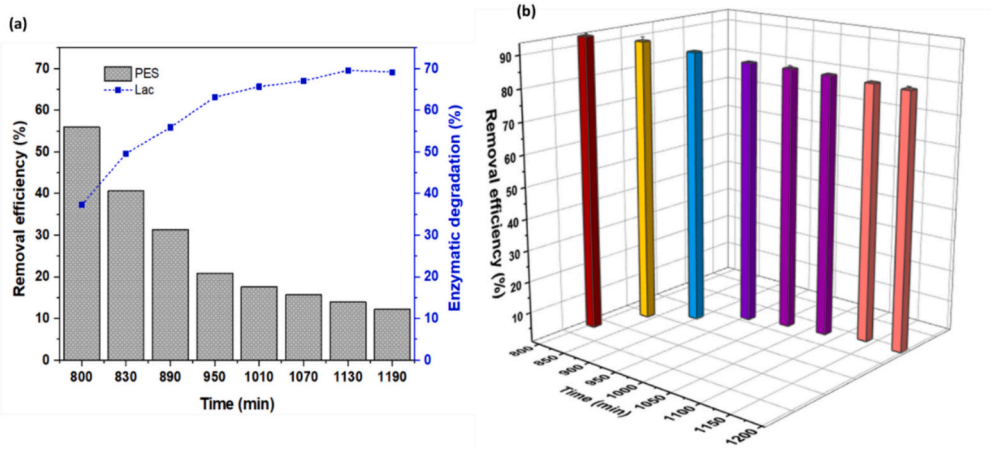


Fig. 7. (a) Enzymatic degradation and retention of ATZ (b) Overall removal efficiency of ATZ in the membrane packed enzymatic system.

Table 5
Total cost comparison of membrane-based water reuse plant systems in South Africa.

Plant	Process configuration	Year of costing	Capacity	Total Costing			Source
				Total Capital Cost (Rm)	CAPEX Rm/ML/d	O&M Cost (R/kL)	
Stellenbosch DPR modelling	Reverse osmosis (RO)	2019	10 ML/d	346	34.6	Not specified	[55]
	RO	2019	20 ML/d	504.21	25.21		[55]
Beaufort West	Sedimentation-media UF-RO-UVAOP		2 ML/d	23.7	19.23	38.5	[55]
Kubota FS	Membrane bioreactor	2020	50 ML/d	208.3	64.53	143.79	[91]
Paarl WRP feasibility study	UF-RO UV/H_2O_2	2019	10 ML/d	102.8	10.2	9.73	[55]
Zeeweed feasibility study	MBR	2020	50 ML/d	107.8	54.18	53.71	[91]
*USA Comparative costing of MBRs	MBRs	2021	1000 ML/d		0.84–1.71[a]	0.62–1.21[a]	[90]
Packed-bed enzymatic membrane system	UF-biocatalysis	2024	1200 ML/d	7.036	2.28	1.31	This study

[a] Currency in USD.

projected for development and testing of a pilot plant before upscaling. In addition, the listed literature reports [54,55] were based on fully functional facilities operated for treatment of larger quantities of water (ML/d) [90]. Moreover, the cost analysis method used in this study was developed specifically for this research topic and was confined to the research scope. The costing tool established is not generic and more comprehensive considerations should be considered for engineering decisions.

5. Conclusions

The removal of atrazine, a chloro-*s*-triazine herbicide was investigated in an integrated biocatalytic system (membrane retention + biodegradation). The overall eradication of the ATZ in the biocatalytic packed-bed enzymatic system varied from 93.2 to 81.3 %, while the standalone membrane retention studies achieved 55.9 % pollutant removal after 24 h reaction time. Breakthrough modelling error statistics (R^2 and RMSE) at optimum conditions revealed that the equilibrium dynamics of ATZ adsorption utilizing PES membranes followed the Thomas Model. Notably, the incorporation of biocatalyst (Novoprime base 268 laccase) improved ATZ conversion compared to separate membrane system and unsupported enzymatic tests. In addition, the column performance showed higher stability and suitable biocatalyst regeneration, retaining activity after six successive operational cycles. The capability of the fabricated PES-Lac enzymatic column to selectively remove ATZ present in a complex real matrix (river water) was demonstrated and, hence, the potential of a PES packed bed laccase column for enzymatic conversion of chloro-*s*-triazines in water treatment. In addition, economic estimates provided the comparable projections of developing a pilot scale of the system for treatment of 1200 ML/d with an estimated total cost of R7.036 mil.

CRediT authorship contribution statement

Mahadi Lesaoana: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Mbongiseni Dlamini:** Investigation, Methodology. **Dean Brady:** Conceptualization, Supervision, Validation, Writing – review & editing. **Roger Sheldon:** Supervision, Writing – review & editing. **Heidi Richards:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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