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Identification and optimization of triphenylmethane dyes removal by *Streptomyces* sp. from forest soil

Nurul Hidayah Adenan^{1,2}, Yau Yan Lim¹ and Adeline Su Yien Ting^{1*} 

Abstract

This study identified a common *Streptomyces* sp. (MN262194) from forest soil as an efficient decolorizer of triphenylmethane (TPM) dyes. Partial 16S rRNA sequencing identified the isolate as possibly *Streptomyces bacillaris* (similarity 99.32%). Live and dead cells of *Streptomyces* sp. were applied to decolorize Malachite Green (MG), Methyl Violet (MV), Crystal Violet (CV), and Cotton Blue (CB). The decolorization efficacy for both cell types was further optimized based on One-Factor-At-A-Time (OFAT) method to determine the influence of pH, agitation speed (rpm), biomass (g), initial dye concentration (mg L^{-1}), and oxygen. Removal of TPM dyes was repeated for both live and dead cells using combined optimal conditions determined for each biomass type. Results revealed that optimum conditions for live cells were pH 7, 100 rpm agitation, 0.5 g cell biomass, initial dye concentration of 100 mg L^{-1} (50 mg L^{-1} for CB), and with the presence of oxygen. In contrast, pH 9 (MG, MV, CV) and pH 3 (CB), with 100 rpm agitation, 0.75 g cell biomass, and initial dye concentrations of 100 mg L^{-1} (50 mg L^{-1} for CB), were the optimum conditions for dead cells. At optimal conditions, live cells showed significantly higher decolorization activities for all dyes (MG 95%, MV 92%, CV 87%, CB 68%). Removal of TPM dyes was via biosorption and biodegradation, detected with changes of ultraviolet-visible spectra between the untreated dye and treated dye. Sorption by *Streptomyces* sp. conforms to the Langmuir isotherm model. *Streptomyces* sp. was established as an effective decolorizer for most TPM dyes with > 85% decolorization (with the exception for CB).

Keywords: Biodegradation, Bioremediation, Biosorption, *Streptomyces* sp., Triphenylmethane dyes

Introduction

Triphenylmethane (TPM) dyes are categorized as one of the larger groups of synthetic dyes after azo dyes [1]. They have three phenyl groups attached to a central carbon atom [1]. TPM dyes are used primarily in the following industries; textile, paper, leather, food, cosmetics, and the aquaculture industry [1]. The discharge of industrial dyes (levels exceeding 0.2 mg L^{-1}) pollutes the environment [2]. Dye pollution is rampant as approximately 20% of unused dye from the industries are

released into the environment without proper treatment [3]. According to Abbas et al. [4], dyes have high toxicity and their presence in the environment reduces water quality, and disrupts the water ecosystem [5]. Exposure of living organisms to untreated dyes results in health hazards as dyes are carcinogenic and mutagenic [6]. Any contact, ingestion, or inhalation of these dyes leads to severe complications such as nausea, hemorrhage, skin ulcer, kidney failure, and damage to the central nervous system in humans [5]. Subsequently, several countries, including the United States and European Union restricted the use of TPM dyes, i.e., malachite green (MG) in aquatic products for the treatment of infectious diseases in fish [7].

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The removal of dyes from the environment is typically through the employment of physical, chemical, or biological methods. Physical methods involve adsorption and membrane filtration, which have been found to be expensive [8]. On the other hand, chemical techniques like ozonation, photochemical, and the use of Fenton's reagent have been reported to generate toxic sludge [9]. In recent years, biological approaches using microorganisms have received considerable attention [10]. Microbial cells remove dyes via biosorption and/or biodegradation, and these approaches are highly favored as they are eco-friendly, cost-effective, and efficient [11]. The efficacy of dye removal by microbial cells is however, dependent on various factors such as temperature, pH, and dye concentrations [11].

Actinobacteria are Gram-positive bacteria found ubiquitously in the environment [12]. Soil actinobacteria have been documented to be diverse and able to adapt to harsh environmental conditions [12]. Their potential to remove TPM dyes are however not well understood, although in recent years they have been found to remove azo dyes, heavy metals, and pesticides [12]. *Streptomyces* is the largest genus of actinobacteria with more than 800 species [13]. They are predominantly isolated from environmental samples and are also the most studied genus of actinobacteria. Among the many species of *Streptomyces*, several have been reported to have the potential to remove dyes. This includes *Streptomyces chromofuscus* on Acid Yellow 9 via biodegradation [14]; *Streptomyces coelicolor* on Acid Blue 74, Direct Sky Blue 6b, Reactive Black 5, Reactive Blue 19, and Congo Red via biodegradation [15]; *Streptomyces krainskii* on Reactive Blue-59 via biodegradation [16]; *Streptomyces globosus*, *Streptomyces alanosinicus*, *Streptomyces ruber*, *Streptomyces gancidicus* on Acid Fast Red and Congo Red via biosorption [17]; and *Streptomyces microflavus* on Crystal Violet (CV) and Safranin T (ST) via biodegradation [11]. These common soil species of actinobacteria [18] have also been reported to have roles in the bioremediation of hydrocarbons [19], biosorption of heavy metals [20], and as biocontrol agents [21]. There is, however, a gap in the knowledge on their potential to remove various TPM dyes.

This study aims to discover the potential of *Streptomyces* sp. in removing TPM dyes and to establish the optimum conditions (i.e., pH, agitation speed (rpm), biomass (g), initial dye concentration (mg L^{-1}), and presence of oxygen) for the decolorization of Malachite Green (MG), Methyl Violet (MV), Crystal Violet (CV) and Cotton Blue (CB). This study has elements of novelty in that the actinobacterial isolate was isolated from a pristine environment rather than a contaminated environment. Actinobacteria are known to inhabit soil naturally, but less so in polluted environments such as

effluents. The focus of TPM dyes is also significant as these dyes are rarely studied, hence the documentation on removal of MG, CB, MV and CV, would be highly beneficial.

Materials and methods

Preparation of TPM dyes

The TPM dyes, i.e., MG ($\text{C}_{52}\text{H}_{54}\text{N}_4\text{O}_{12}$, basic dye, 60 g L^{-1} water solubility at 20°C , 98% purity) (Riendemann Schmidt, Malaysia), MV ($\text{C}_{24}\text{H}_{28}\text{ClN}_3$, basic dye, 30 g L^{-1} water solubility at 20°C , 98% purity) (Sigma-Aldrich, Malaysia), CV ($\text{C}_{25}\text{H}_{30}\text{ClN}_3$, basic dye, 50 g L^{-1} water solubility at 20°C , 98% purity) (Merck, Malaysia), and CB ($\text{C}_{37}\text{H}_{27}\text{N}_3\text{Na}_2\text{O}_9\text{S}_3$, acid dye, 70 g L^{-1} water solubility at 20°C , 99% purity) (Sigma-Aldrich, Malaysia) were weighed and dissolved in autoclaved MilliQ water (Sartorius, Malaysia) to a concentration of 100 mg L^{-1} (CB at 50 mg L^{-1}) [22].

Isolation and molecular typing of actinobacterial isolate

Soil samples were collected at random from Kuala Lumpur Eco Forest Park, Malaysia (N3.1499235, E 101.7023324). The isolated colony was obtained using streak plate technique and maintained on Actinomycetes Isolation Agar (Difco, USA), containing Nystatin (Sigma-Aldrich, Malaysia; $\geq 95\%$ purity) and Nalidixic acid (Sigma-Aldrich, Malaysia; $\geq 98\%$ purity) to prevent the growth of fungi and non-actinobacteria, respectively. The incubation was carried out for 7 d ($30 \pm 2^\circ\text{C}$). For molecular typing, the isolate was first inoculated into 100 mL of International Streptomyces Project Medium (Difco, USA) and incubated (100 rpm, 7 d) at $30 \pm 2^\circ\text{C}$. The genomic DNA was extracted (GF-1 Bacterial DNA Extraction Kit, Vivantis Technologies, USA) and amplified (MJ Mini Thermal Cycler, Biorad, USA) using primers specific for actinobacteria; S-C-Act-878-a-A-19 (CCGTACTCCC CAGGCGGGG) and S-C-Act-235-a-S-20 (CGCGGC CTATCAGCTTGTTG). DNA purification was performed using MEGAquick-spin Total Fragment DNA Purification Kit (iNtRON Biotechnology, Korea) and sent for sequencing to Apical Scientific Sequencing (Singapore). The sequences were analyzed using Basic Local Alignment Search Tool (<http://blast.ncbi.nlm.nih.gov/>). A phylogenetic tree was generated using the Molecular Evolutionary Genetics Analysis (Version 10.0.5) [23].

To generate biomass, the culture was first inoculated into the International Streptomyces Project Medium-1 (ISP-1) (Difco, USA) and incubated ($30 \pm 2^\circ\text{C}$) for 7 d at 100 rpm. To obtain dead cells, the broth cultures were centrifuged (7000 rpm, 20 min), autoclaved (121°C , 20 min), and filtered through Whatman filter paper No. 1. The live cells were obtained similarly, with the exclusion

of autoclaving [24]. Both types of biomass (fresh weight) were then weighed to the required weight (e.g., 0.5, 1.0 g) and used for decolorization and optimization studies of TPM dyes.

Dye decolorization studies

Live cells (1.0 ± 0.1 g) were introduced into 100 mL of MG, MV, CV, and CB dye solutions, and the mixture was incubated (100 rpm, 30 ± 2 °C) for 14 d. A separate set was prepared similarly using dead cells. Throughout the incubation period, 3 mL of aliquot was withdrawn and centrifuged (10,000 rpm) at every 24 h interval. The supernatant obtained was read using Spark 10 M multi-mode microplate reader (Tecan, USA) at λ max of 617, 584, 590, and 599 nm for MG, MV, CV, and CB, respectively. The decolorization efficiency (DE) was calculated according to Eq. (1) [22].

$$DE = \frac{\text{initial absorbance} - \text{observed absorbance}}{\text{initial absorbance}} \times 100 \quad (1)$$

Optimization studies for decolorization of TPM dyes by live and dead cells of *Streptomyces* sp.

The One-Factor-At-A-Time (OFAT) approach was adopted to optimize the following parameters; pH, agitation speed (rpm), biomass (g), initial dye concentration (mg L^{-1}), and oxygen. Conditions were optimized by modifying the respective parameters from the standard protocol of OFAT, i.e., examining one parameter while keeping the other parameters at a pre-determined level [25]. Both live and dead cells of *Streptomyces* sp. were tested, with the exception of the influence of oxygen, where only live cells were used.

To determine the optimum pH, the initial pH of TPM dyes (using concentration MG, MV, CV at 100 mg L^{-1} , while CB at 50 mg L^{-1}) was adjusted to 3, 5, 7, and 9. The optimization test was performed, as defined previously. To determine optimum agitation speed, dye solutions were treated with cell biomass and agitated at 50, 100, 150 rpm, while other factors remained constant. To determine the optimum biomass, 0.25, 0.5, 0.75, and 1.0 g was introduced into the dye solutions while keeping the other factors constant. To determine the optimum initial dye concentration, dye solutions ($50, 75, 100,$ and 125 mg L^{-1} of MG, MV, and CV; $10, 25, 50,$ and 75 mg L^{-1} of CB) were tested, and other factors were kept constant. To examine the influence of the absence of oxygen, 100 mL dye solutions (100 mg L^{-1} of MG, MV, CV, and 50 mg L^{-1} of CB) were inoculated with 1.0 ± 0.1 g of cell biomass and overlaid with 2 mL of paraffin oil. The mixture was incubated as a standing culture. A separate set of flasks was prepared similarly, with the exclusion of

paraffin overlay, to determine the decolorization of TPM dyes with the presence of oxygen.

Removal of TPM dyes by *Streptomyces* sp. (live and dead cells) using optimized conditions

The optimized conditions from the OFAT test for live cells were combined and used to decolorize all TPM dyes, as described in previous section. The dye supernatant was sampled at 2, 4, 6, 8, and 24 h. The DE was determined using Eq. (1) [22]. This was repeated for MG, MV, CV, and CB using dead cells under the same experimental conditions.

Adsorption isotherm models

Two common isotherm models, i.e., Langmuir and Freundlich, were employed to determine the relationship between the biosorption of live cells with TPM dyes at an equilibrium state [26].

The Langmuir isotherm equation is shown in Eq. (2):

$$\frac{C_e}{q_e} = \left(\frac{1}{K_L q_m} \right) + \left(\frac{1}{q_m} \right) C_e \quad (2)$$

where q_e , C_e , K_L , and q_m indicate the quantity of dye absorbed (mg g^{-1}), equilibrium dye concentration (mg L^{-1}), Langmuir constant (L mg^{-1}), and biosorption capacity (mg g^{-1}), respectively. K_L and q_m were calculated from the intercept and slope of the linear plot of C_e/q_e against C_e . The characteristics of Langmuir isotherm is expressed by a separation factor, R_L , as written in Eq. (3):

$$R_L = \frac{1}{(1 + K_L C_0)} \quad (3)$$

where C_0 is the initial dye concentration (mg L^{-1}).

The Freundlich isotherm is given in Eq. (4):

$$\ln q_e = \ln K_F + \left(\frac{1}{n} \right) \ln C_e \quad (4)$$

where K_F and n refer to biosorption capacity (mg g^{-1}), and biosorption intensity, respectively. K_F and n were determined from the intercept and slope of the linear plot of $\ln q_e$ against $\ln C_e$.

Ultraviolet-visible (UV-vis) analysis for TPM dyes treated using optimized conditions

UV-vis analysis was performed to determine the underlying mechanisms for the removal of TPM dyes by *Streptomyces* sp., conducted at optimized conditions. The dye solutions were examined by comparing the changes in the spectra between the untreated and treated dye. The untreated dye (non-inoculated with biomass) (0 h) and the treated dye (24 h) were collected and centrifuged (10,000 rpm, 10 min). The supernatant was

collected and absorption spectra were scanned at wavelengths of 200 to 800 nm using a LAMBDA 365 UV-Vis spectrophotometer (Perkin Elmer, USA) [24].

Statistical analysis

All decolorization assays were conducted in triplicates, and untreated dyes (non-inoculated dye) was designed as a negative control. The data were analyzed using One-Way Analysis of Variance with statistical analysis performed with the Statistical Package for Social Sciences (Version 24.0). Means were compared using the Tukey-Kramer multiple comparison test (honestly significant difference (HSD), $P < 0.05$) or paired T -test ($P < 0.05$), when applicable.

Results and discussion

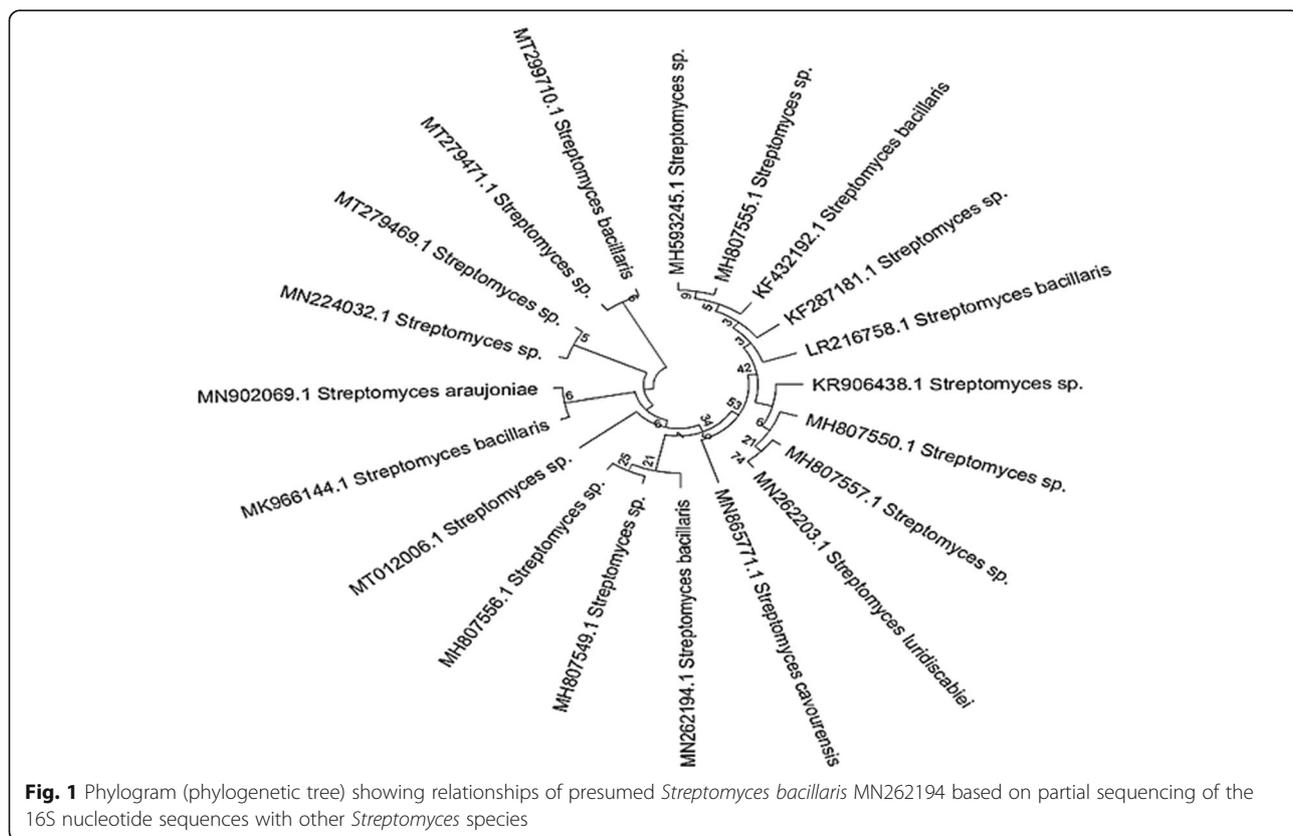
Identification of actinobacterial isolate

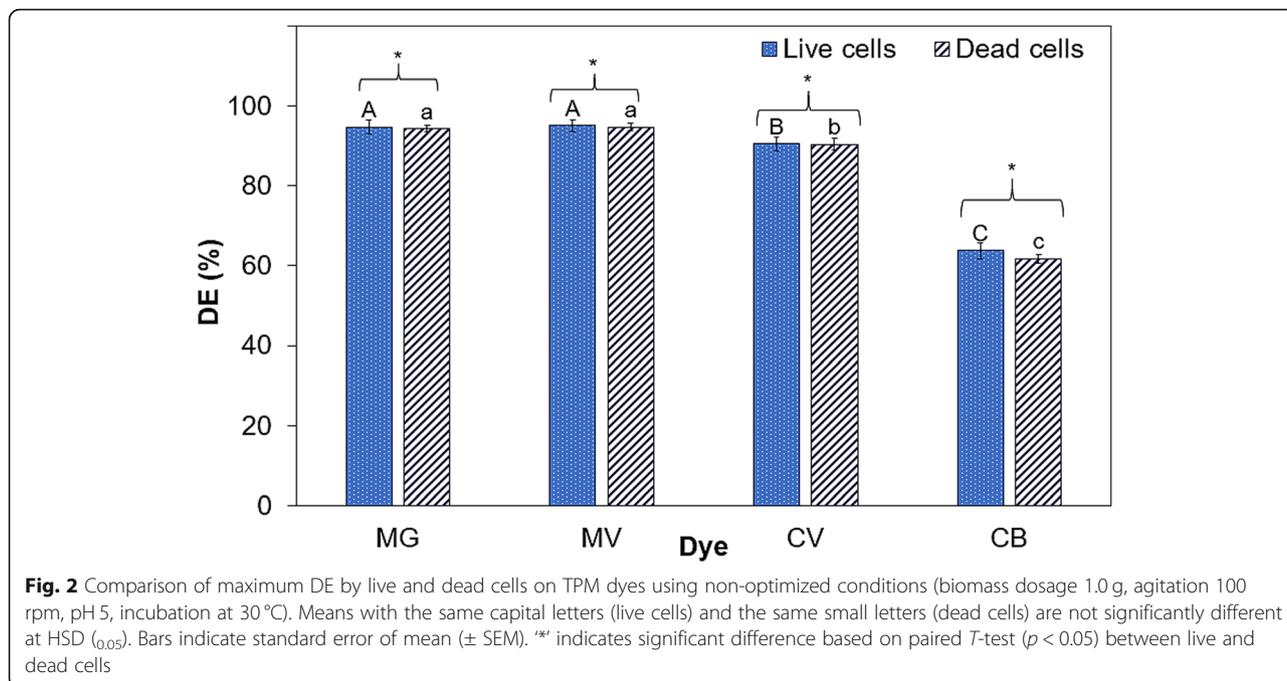
The isolated colony appeared powdery, yellowish-gray in color, and produced an earthy odor. The observed morphologies were aligned with species of *Streptomyces* described in other studies, where they appeared powdery [27], and colonies were of various pigmentation, e.g., white, gray, and yellow [27]. *Streptomyces* spp. were also reported to produce a distinct earthy odor, derived from a compound known as geosmin [28]. Results from 16S rRNA partial sequencing with

primer pairs of S-C-Act-878-a-A-19 (CCGTACTCCC CAGGCGGGG) and S-C-Act-235-a-S-20 (CGCGGC CTATCAGCTTGTTG) suggested that the isolate is presumably *Streptomyces bacillaris* (query cover 99%, similarity 99.32%). The query cover and similarity were however, not 100% suggesting the possibility in species variation, which can be further determined using whole-genome sequencing. In fact, the phylogenetic analysis revealed that *S. bacillaris* is closely related to other *Streptomyces* spp. such as *Streptomyces* sp. (MH807549), *Streptomyces* sp. (MH807556), *S. luridiscabiei* (MN262203), *S. cavourensis* (MN865771), and *Streptomyces* sp. (MH807557) (Fig. 1). Nevertheless, the analyzed partial sequence for the *Streptomyces* sp. was deposited in the GenBank, and the accession number MN262194 was assigned to *S. bacillaris*. This isolate (*S. bacillaris*.) is clustered with other *Streptomyces* isolates commonly found in soil environment (Fig. 1).

Decolorization of TPM dyes by Streptomyces sp.

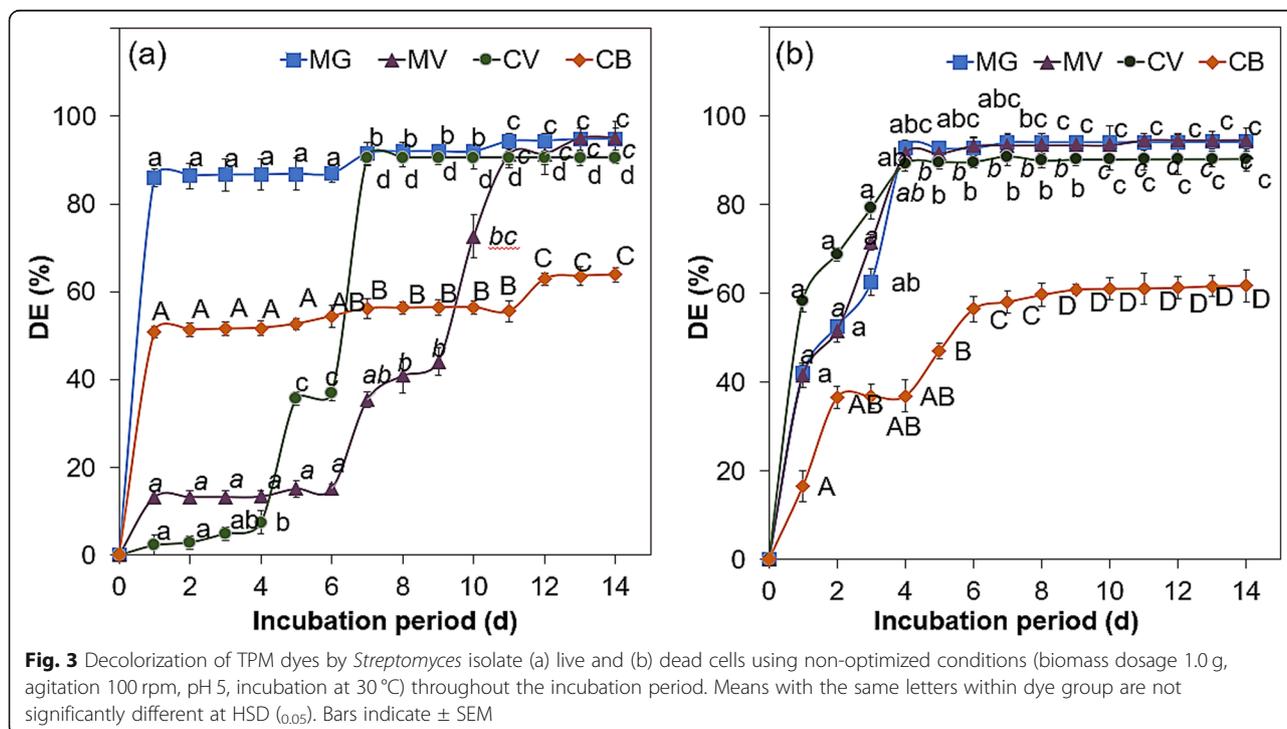
The *Streptomyces* isolate showed strong potential in decolorizing TPM dyes. The DE exhibited by live cells was significantly higher compared to dead cells (Fig. 2). Live cells decolorized 95, 95, 91, and 64% of MG, MV, CV, and CB, respectively (Fig. 2). Dead cells, on the other





hand, demonstrated a slightly lower efficiency for the removal of MG (94%), MV (95%), CV (91%), and CB (62%) (Fig. 2). The most rapid decolorization was performed by dead cells, where 93% (MG), 92% (MV), 89% (CV), and 37% (CB) were removed within 4 d (Fig. 3). This

rate of decolorization contrasted with the rate of decolorization by live cells, which were more gradual and took 8 d to achieve 929, 41, 91, and 56% of DE for MG, MV, CV, and CB, respectively (Fig. 3). This study has shown that live cells of *Streptomyces* sp. require a



longer incubation period despite their superiority in decolorizing TPM dyes. This may be attributed to the lag phase of cell growth, the adaptation to the presence of toxic dyes, and the period required for enzyme biodegradation [22, 24]. The rapid decolorization by dead cells compared to live cells was also demonstrated by *Diaporthe* sp., a fungal endophyte. Dead cells removed TPM dyes (MG, MV, CV, CB) within 3 h, whereas live cells decolorized the dye effectively by 24–120 h [24]. *Streptomyces* sp., notably in the form of live cells, has the potential for effective removal of TPM dyes.

Effect of different pH on decolorization of TPM dyes by live and dead cells

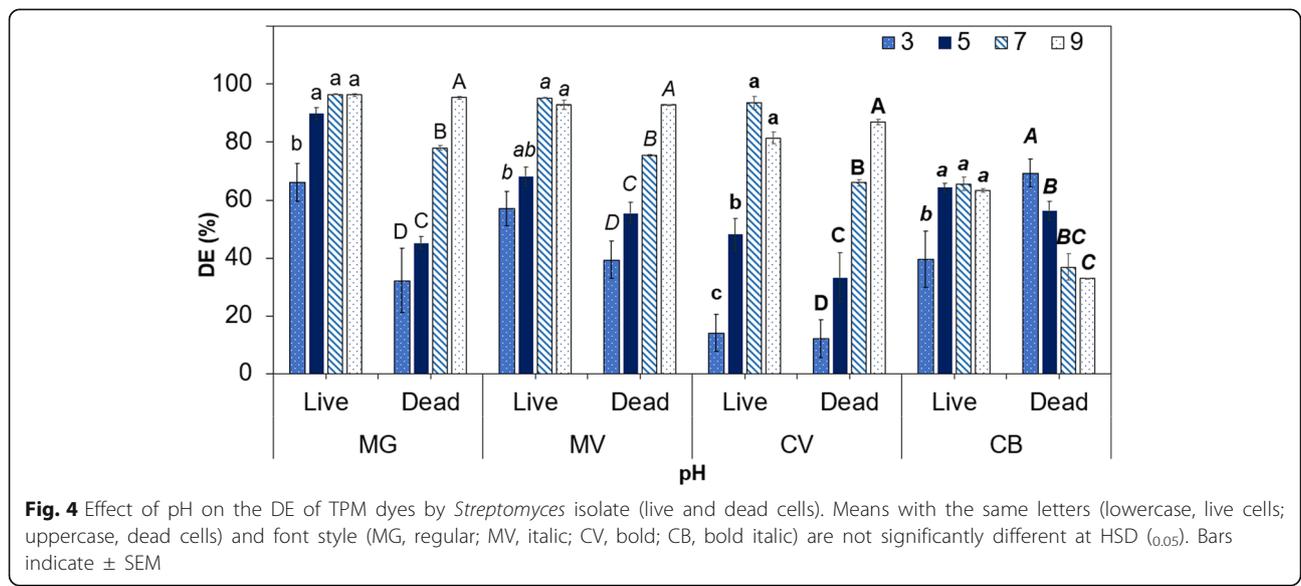
Decolorization of TPM dyes by live and dead cells of *Streptomyces* sp. was influenced by the changes in pH. For live cells, decolorization was optimum at pH 7 for all TPM dyes tested; MG (96%), MV (95%), CV (94%), and CB (66%) (Fig. 4). The decolorization at a lower or higher pH however, was less efficient than the efficacy achieved at pH7. The DE was significantly lower at a lower pH, with 14–66% and 48–89% at pH3 and 5, respectively (Fig. 4). At a higher pH, decolorization of TPM dyes varied, i.e., 63–96% of DE at pH9 (Fig. 4). The optimum pH (pH7) observed in this study was also the optimum pH for the removal of azo blue dye and CV by live cells of *Streptomyces* DJP15 [29] and *S. microflavus* CKS6 [11], respectively. This may be due to the fact that *Streptomyces* species prefer neutral pH for their growth and enzyme production [30], which perhaps leads to more efficient degradation of dyes at near-neutral pH [29]. The poor

removal of TPM dyes in pH3 and 5 may be attributed to their poor tolerance to grow in acidic pH [31]. According to Kontro et al. [30], the proliferation of *Streptomyces* occurs optimally within a pH range from neutral to slightly alkaline. Nevertheless, pH7 was documented as an optimum pH for their growth [29], as obtained in this study.

In contrast, the optimum pH for dead cells varied depending on the TPM dye. Removal of MG (95%), MV (95%), and CV (91%) was more efficient at a higher pH (pH9), while optimum pH for removal of CB (69%) was at lower pH (pH3) (Fig. 4). The DE of cationic TPM dyes (MG, MV, and CV) increased with pH due to the negatively charged surface of *Streptomyces* sp. [32]. As a result, the electrostatic interaction of dye molecules with functional groups on the biomass surface is enhanced [32]. CB however, is a type of anionic dye [33]. The negatively charged CB binds well to the biomass at low pH; as biomass surface is positively charged at lower pH, resulting in increased electrostatic forces with the negatively charged anionic dye [32]. This study showed that ionic activity between dead cells and dye ions has a profound effect on the decolorization of TPM dyes.

Effect of agitation speed on decolorization of TPM dyes by live and dead cells

The decolorization of TPM dyes was affected by agitation speed. For both types of cell biomass, the optimum agitation speed was 100 rpm. With 100 rpm, live and dead cells decolorized MG, MV, CV and CB at 97, 95, 92, 64% and 96, 95, 91, and 68%, respectively (Fig. 5). On the contrary, agitation at 50 and 150 rpm was less



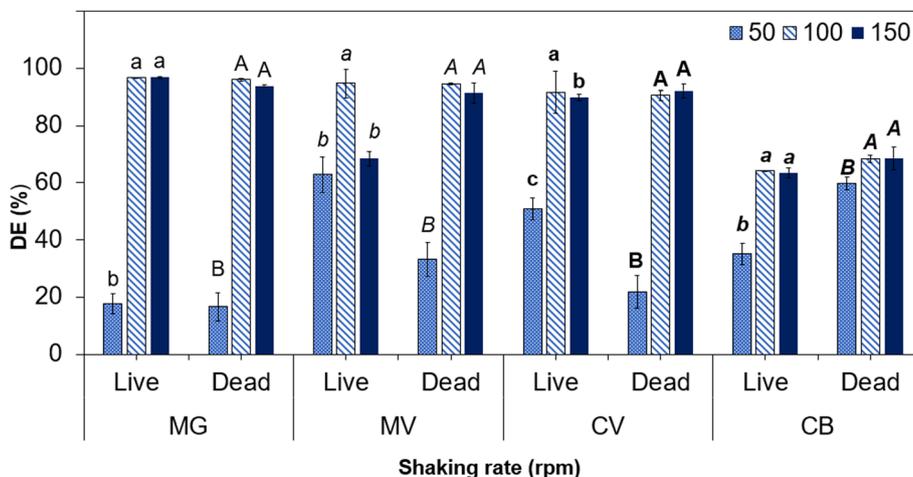


Fig. 5 Effect of agitation speed on the DE of TPM dyes by *Streptomyces* isolate (live and dead cells). Means with the same letters (lowercase, live cells; uppercase, dead cells) and font style (MG, regular; MV, italic; CV, bold; CB, bold italic) are not significantly different at HSD (0.05). Bars indicate ± SEM

effective than at 100 rpm, for all dyes treated with live and dead cells. At 50 rpm, the DE of TPM dyes was significantly lower, with 17–62% and 21–59% by live and dead cells, respectively (Fig. 5). At 150 rpm, there was no substantial difference observed for the removal of TPM dyes, i.e., 63–96% and 68–93% by live and dead cells, respectively (Fig. 5). It is postulated that the significant increase in DE at 100 rpm by live cells is associated with increased distribution of nutrients and oxygen transfer as a consequence of agitation [34]. Agitation at 100 rpm also enhanced decolorization by dead cells as there is better surface contact and distribution with dye molecules for biosorption [32].

At lower agitation speed (e.g., 50 rpm), the growth of live cells of *Streptomyces* may have been suppressed due to aeration and nutrient deficiency [34]. Lower agitation speed may also cause accumulation of particles [32], resulting in uneven dispersion of dead cells and dye molecules. By contrast, a higher agitation speed (150 rpm) results in constant contact of particulate with the surface of live cells [34] and biosorption rate of dyes [32], which is not significantly different from results derived from agitation speed of 100 rpm. As such, it is postulated that agitation with 150 rpm is similar to 100 rpm, primarily attributed to the availability of sorption sites. This study has revealed that 100 rpm agitation was sufficient for live

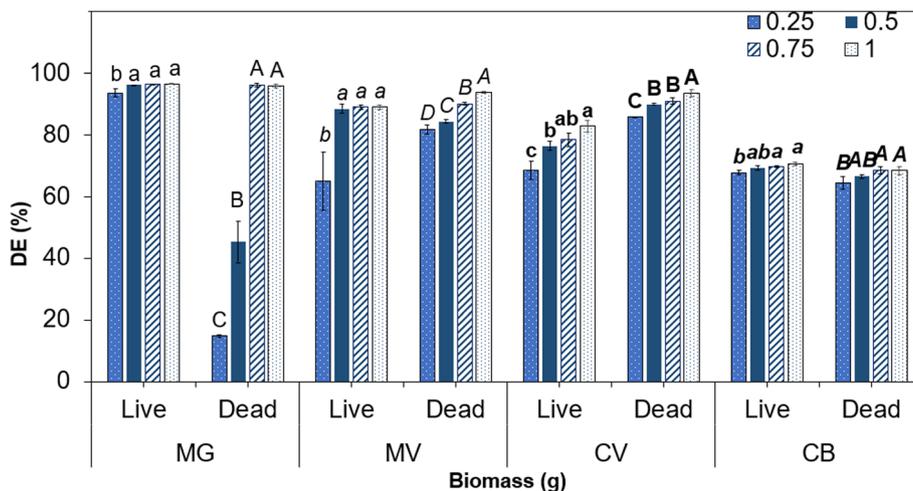


Fig. 6 Effect of biomass concentration of *Streptomyces* isolate (live and dead cells) on the DE of TPM dyes. Means with the same letters (lowercase, live cells; uppercase, dead cells) and font style (MG, regular; MV, italic; CV, bold; CB, bold italic) are not significantly different at HSD (0.05). Bars indicate ± SEM

and dead cells of *Streptomyces* to attain efficient removal of TPM dyes.

Effect of biomass on decolorization of TPM dyes by live and dead cells

Findings indicated that the optimum biomass for efficient removal of TPM dyes differed, depending on whether live or dead cells were used. For live cells, the minimum biomass required was 0.5 g, with a significant increase in DE of all TPM dyes, compared to the use of 0.25 g. With 0.5 g of cell biomass, the maximum DE achieved was generally high for MG (96%), MV (89%), CV (77%), and CB (69%) (Fig. 6). With the use of a higher amount of biomass (i.e., 0.75 and 1.0 g), a slight increase in DE was observed (Fig. 6). On the contrary, dead cells showed 0.75 g as the minimum biomass required for efficient decolorization of MG (96%), MV (90%), CV (91%), and CB (69%) (Fig. 6). When the amount of biomass was increased to 1.0 g, decolorization efficiency was improved for only two dyes; MV and CV, with 94% for each dye (Fig. 6). This test indicated that 0.5–0.75 g might be the optimum amount of biomass for the efficient removal of TPM dyes by the *Streptomyces* isolate. The use of less than 1.0 g of bacterial biomass was also documented in previous studies for the decolorization of azo dyes [35, 36]. Higher DE for all TPM dyes was observed when greater amounts of biomass were used. This may be attributed to higher secretion of enzymes by live cells [37] and greater sorption of dye molecules onto the dead cells surface [32]. This is definitely superior to the

use of lesser biomass as lesser biomass indicated lower DE, presumably due to the lower enzyme activity [37] and lesser availability of binding sites for dye molecules [35]. However, biomass exceeding 1.0 g is not considered for application as their production requires additional costs [32].

Effect of dye concentrations on decolorization of TPM dyes by live and dead cells

Results indicated that initial dye concentrations influenced the decolorization of TPM dyes. The optimum concentrations for the removal of TPM dyes by live and dead cells were 100 mg L^{-1} for MG, MV, CV, and 50 mg L^{-1} for CB. Decolorization achieved by live and dead cells at lower dye concentrations (i.e., 50 and 75 mg L^{-1} for MG, MV, CV; 10 and 25 mg L^{-1} for CB) did not differ significantly from DE at optimal dye concentrations, while removal at higher dye concentrations decreased considerably (i.e., 125 mg L^{-1} for MG, MV, CV; 75 mg L^{-1} for CB). It is evident that *Streptomyces* was capable of tolerating high concentrations of TPM dyes, which may be advantageous for the treatment of wastewaters from textile industries (with 10 to 50 mg L^{-1} dye concentrations) [38]. For live cells, decolorization efficiencies of 97, 89, 88, and 66% were achieved for MG, MV, CV, and CB, respectively (Fig. 7). Higher dye concentrations resulted in a reduction in DE, while lower dye concentrations led to an increase in DE (Fig. 7). Higher dye concentrations may inadvertently impose toxicity to live cells, implicating viability of cells and their enzyme activities, and subsequently the DE [37].

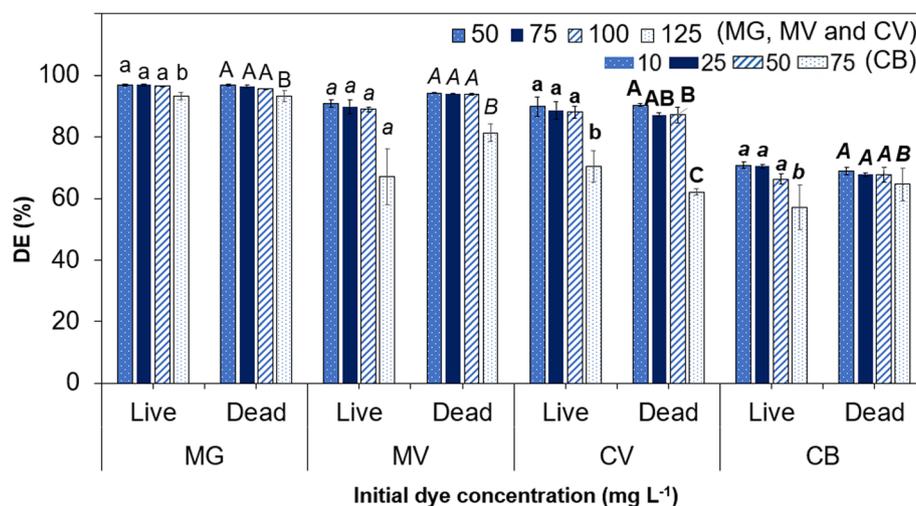
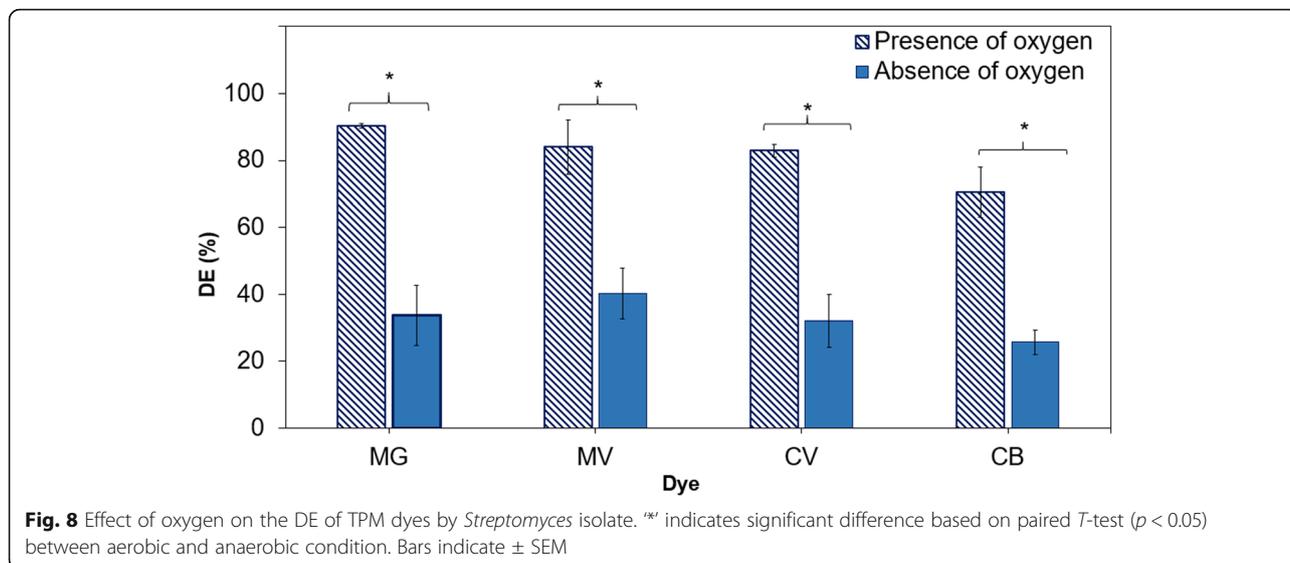


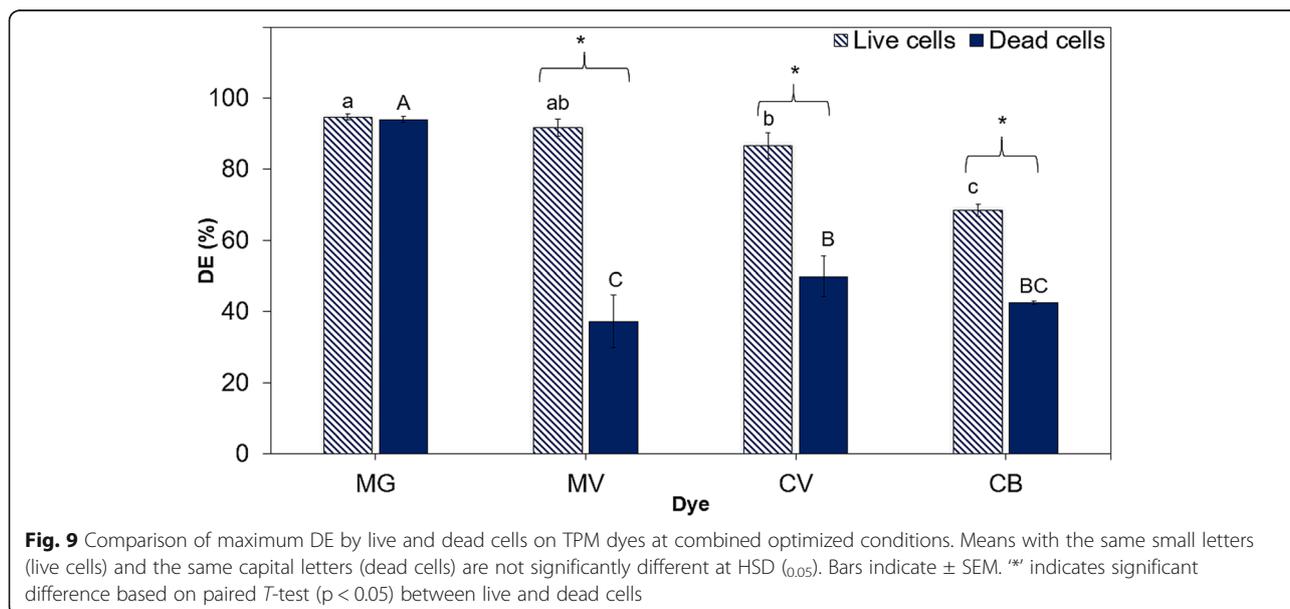
Fig. 7 Effect of initial dye concentrations on the DE of TPM dyes by *Streptomyces* isolate (live and dead cells). Means with the same letters (lowercase, live cells; uppercase, dead cells) and font style (MG, regular; MV, italic; CV, bold; CB, bold italic) are not significantly different at HSD (0.05). Bars indicate \pm SEM



Similarly, dead cells also recorded optimum initial dye concentration at 100 mg L^{-1} (MG, MV, CV) and 50 mg L^{-1} (CB). Dye removal efficacy for MG, MV, CV, and CB was 96, 94, 87, and 68%, respectively (Fig. 7). When higher initial dye concentrations (i.e., 125 mg L^{-1} for MG, MV, CV, and 75 mg L^{-1} for CB) were used, the DE for dead cells was significantly lower with 93, 81, 62, and 65% (Fig. 7). When higher dye concentrations were applied, saturation of dye molecules may have occurred, limiting electrostatic interaction between dye molecules and functional groups on the surface of dead cells [32]. As a result, the removal of TPM dyes decreased.

Influence of oxygen on the decolorization of TPM dyes by live cells

Results showed that live cells achieved higher DE in the presence of oxygen. The presence of oxygen enhanced the decolorization of MG (90%), followed by MV, CV, and CB at 84, 83, and 71% (Fig. 8). By contrast, the decolorization activities for the same dyes in the absence of oxygen were inferior, with significantly lower DE for MG (34%), MV (40%), CV (32%), and CB (26%) (Fig. 8). Similar observations were reported by Mane et al. [16] in which *S. krainskii* demonstrated higher decolorization activity (95%) on Reactive Blue-59 in the presence of



oxygen. The influence of oxygen and the enhanced decolorization efficiency by live cells is anticipated as *Streptomyces* are aerobic bacteria [39]. Species of *Streptomyces* require oxygen for their growth and the production of enzymes [39]. This includes oxidative enzymes, e.g., laccase, lignin peroxidase, and manganese peroxidase [39]. Studies [37, 40] reported oxidative enzymes are involved in the degradation of TPM dyes by breaking the double bond of the chromophore. As a consequence, efficient decolorization was achieved by this *Streptomyces* isolate in the presence of oxygen. On the contrary, anaerobic conditions may have caused poor survivability for *Streptomyces* and affected their metabolism [41]. Lower enzyme activity may have been produced [42], resulting in poor DE of TPM dyes. In short, this *Streptomyces* isolate decolorized the dyes better in the presence of oxygen.

Removal of TPM dyes by live and dead cells using optimized conditions

Both live and dead cells showed better removal for TPM dyes when applied for dye treatment using optimized

conditions. At optimized conditions, live cells demonstrated higher decolorization for MG (95%), MV (92%), CV (87%), and CB (68%) (Fig. 9) compared to DE achieved using non-optimized conditions, i.e., 86 (MG), 13 (MV), 2 (CV), and 51% (CB) (Fig. 3). Rapid decolorization was also observed when incubated at optimized conditions, in which 89 (MG), 88 (MV), 71 (CV), and 65% (CB) were successfully removed by live cells within the first 2 h (Fig. 10). On the other hand, dead cells demonstrated 94 (MG), 37 (MV), 50 (CV), and 43% (CB) of DE at optimized conditions (Fig. 9). Removal of TPM dyes by dead cells at optimized conditions was substantially higher for MG and CB, compared to only 42 and 17% of DE using non-optimized conditions (Fig. 3). This may be related to properties of dyes, e.g., molecular structure and type, number or position of substituents in the dye molecule, which also play a role in decolorizing TPM dyes [43].

The efficient decolorization for all TPM dyes by live cells could be due to the higher production of enzymes [24], implying the established optimal conditions were favored by this *Streptomyces* isolate. Higher dye removal

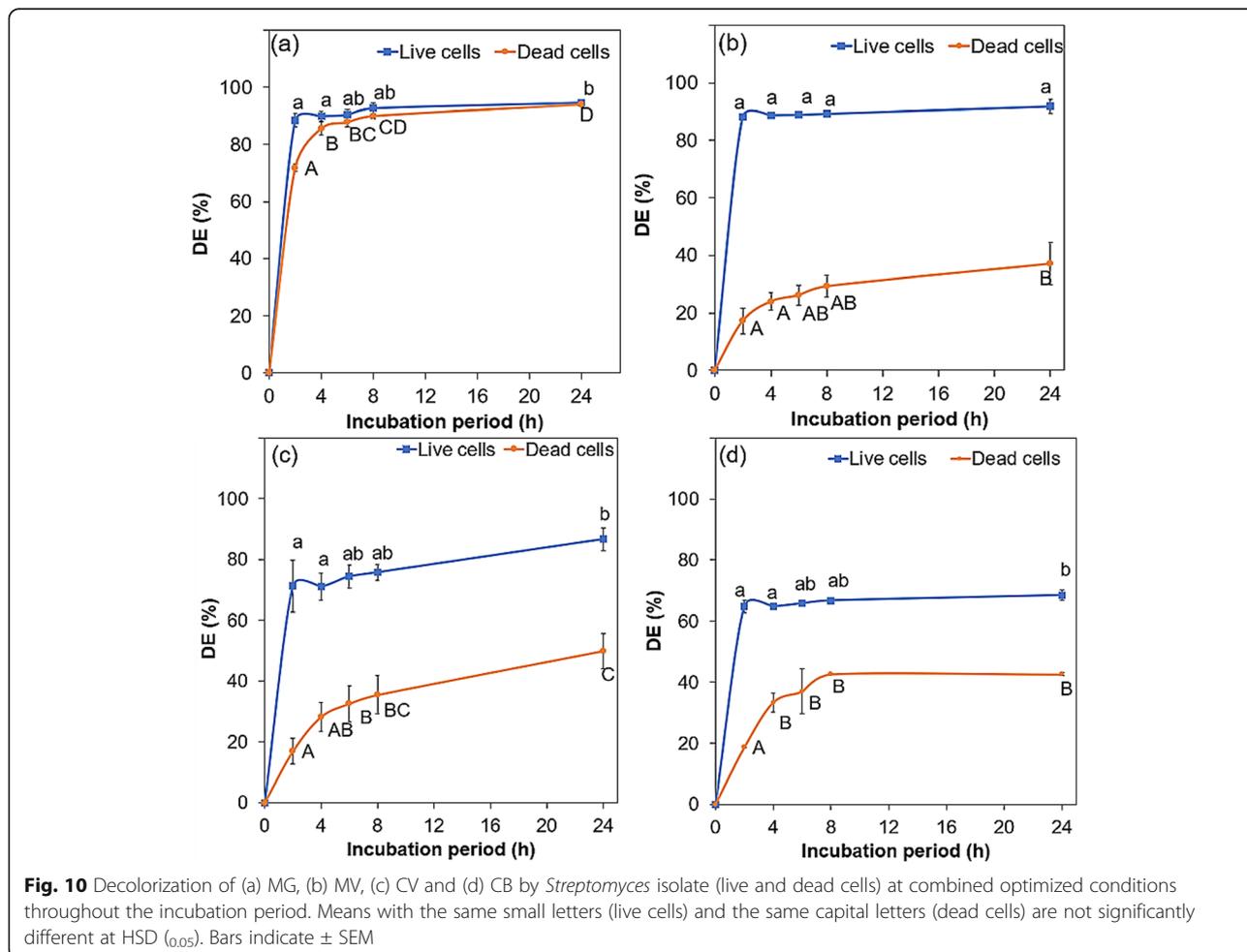


Table 1 Adsorption isotherm models for the sorption of TPM dyes by live and dead cells of *Streptomyces* sp.

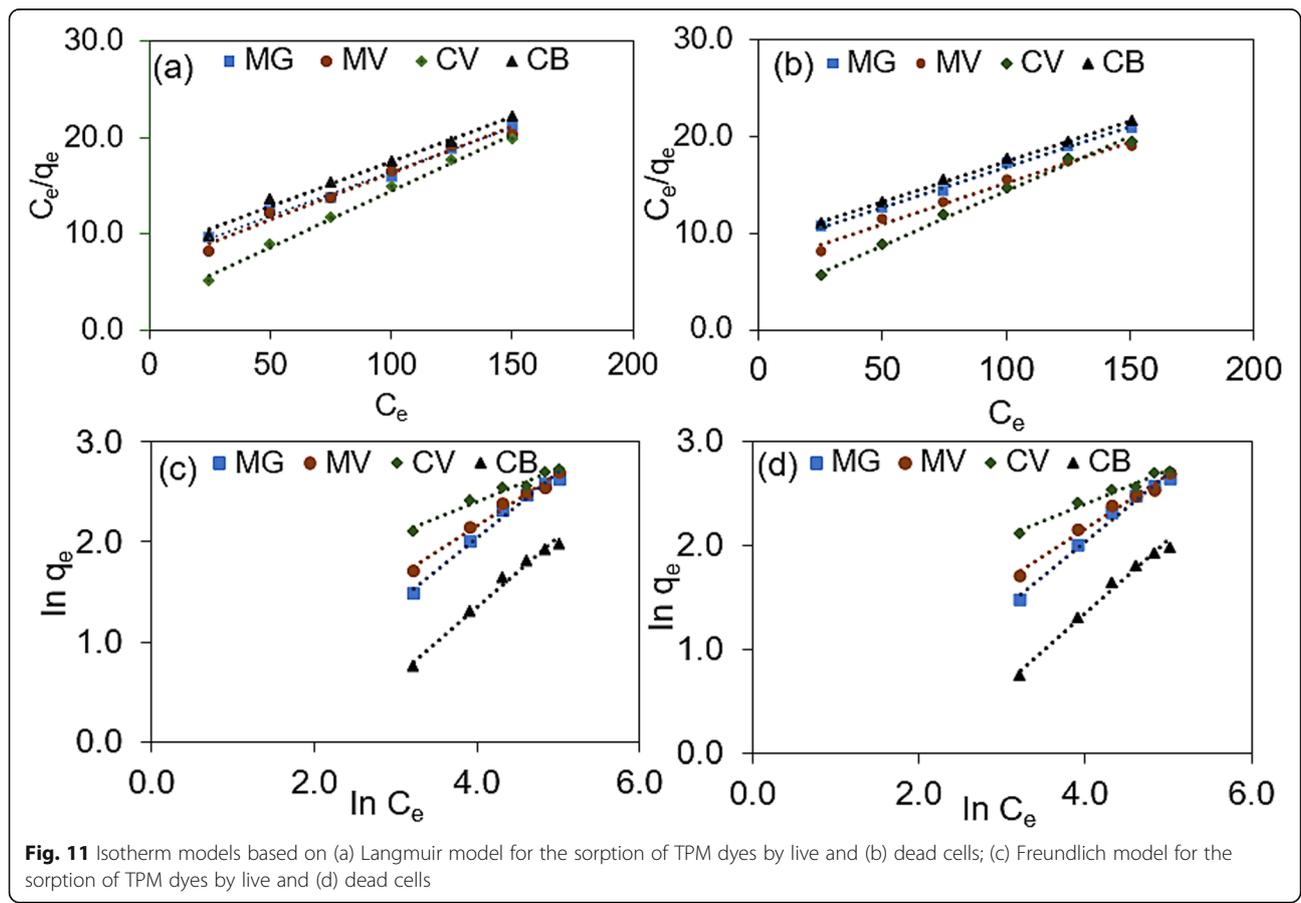
Cells	Dyes	Langmuir			Freundlich		
		q_m	K_L	R^2	n	K_F	R^2
Live	MG	12.6	0.011	0.999	1.64	1.78	0.986
	MV	11.7	0.013	0.991	1.90	1.05	0.987
	CV	9.4	0.038	0.997	3.04	2.98	0.972
	CB	10.9	0.011	0.997	1.43	4.36	0.987
Dead	MG	11.9	0.010	0.998	1.67	1.43	0.977
	MV	11.7	0.013	0.988	1.81	1.02	0.987
	CV	8.9	0.036	0.995	2.78	4.19	0.969
	CB	11.5	0.010	0.998	1.42	3.31	0.986

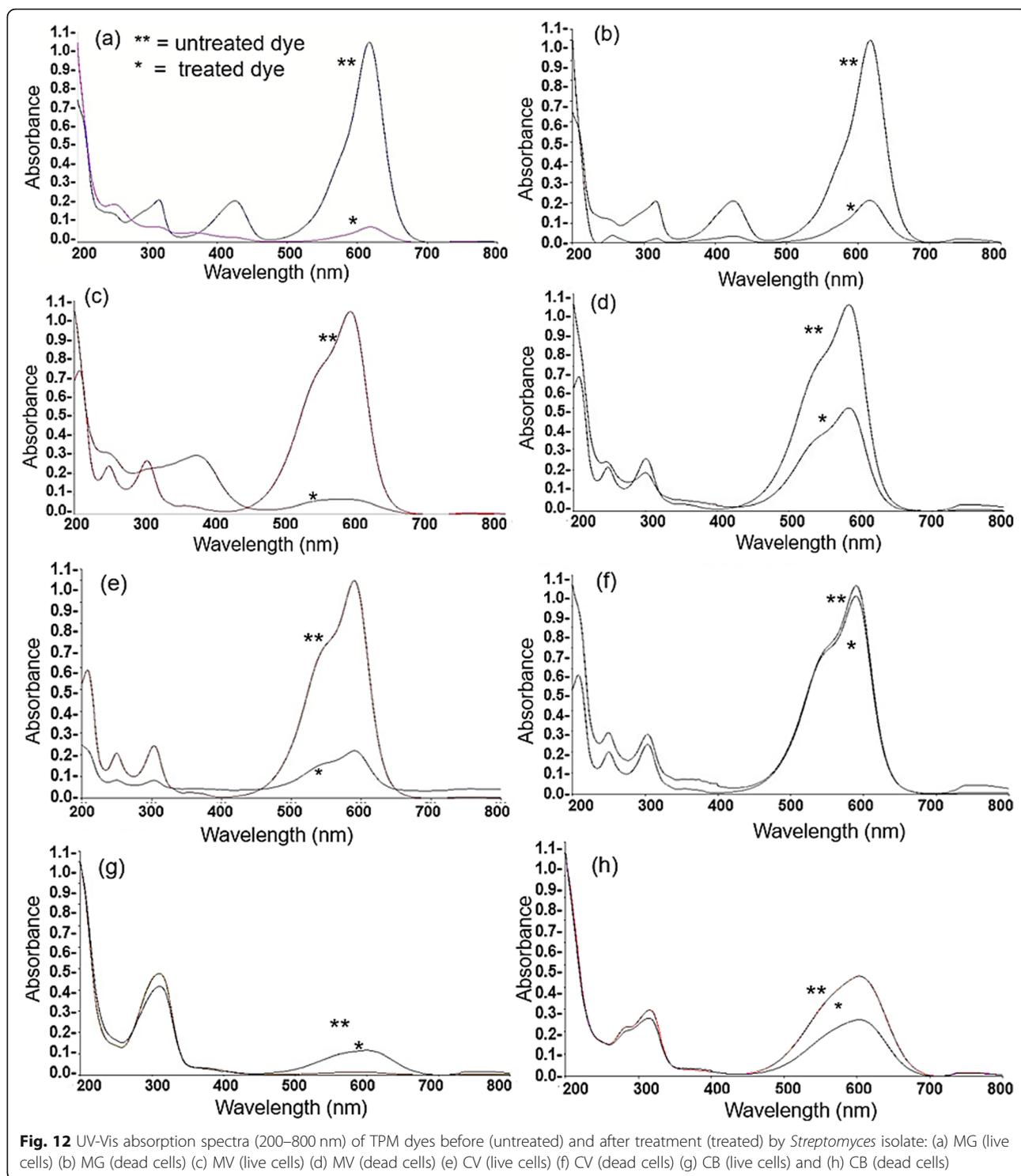
using optimized conditions was also reported when live cells of *Lysinibacillus fusiformis* and *Pseudomonas aeruginosa* were investigated for the removal of azo dyes; Methyl Red [44] and Remazol Black B [45], respectively. This study concluded that the optimized conditions have resulted in a rapid and efficient decolorization of TPM dyes, primarily for live cells. The rapid decolorization of dyes by *Streptomyces* sp. established the potential of *Streptomyces* sp. for use in TPM dye removal. This

contributes immensely to the existing knowledge of dye removal by *Streptomyces* sp., which have mostly focused on azo dyes to date [15–17]. *Streptomyces* sp. are now discovered to have the ability to biodegrade TPM dyes (i.e., MG, MV, CV, CB) and possibly to assimilate their biodegraded compounds, just as their utilization of azo dyes as carbon source [46]. This significant discovery further expounds the dye-degrading potential of *Streptomyces* sp. on various dye pollutants.

Adsorption isotherm kinetics

Langmuir isotherm is revealed to have a better fit to describe the sorption of TPM dyes, with higher R^2 values for both live ($R^2 = 0.991–0.999$) and dead cells ($R^2 = 0.988–0.998$) (Table 1) (Fig. 11). The highest maximum biosorption capacities ($q_m = 9.4–12.6 \text{ mg g}^{-1}$) were achieved by live cells for MG, followed by MV, CB, and CV (Table 1). For the Langmuir isotherm model, the R_L values of 0.15–0.37 and 0.16–0.40 for live and dead cells, respectively, were achieved, indicating a favorable characteristic of the Langmuir isotherm model for this study (Table 1). The fit to Langmuir isotherm model also infers that the biosorption of TPM dyes to live and dead cells of *Streptomyces* sp. was likely a monolayer sorption





process. The surface area of live and dead cells of *Streptomyces* sp. is therefore critical as dye sorption becomes limited when the active binding sites on the surface are saturated with dye molecules [26].

On the contrary, the lower R^2 values for the Freundlich isotherm model for both live and dead cells

suggested that the sorption was not to a heterogenous surface but a monolayer sorption as evidence in the Langmuir isotherm. Nevertheless, higher K_F (1.05–4.36) and n (1.43–3.04) values obtained for live cells suggested that greater sorption capacity and interaction with the dye molecules may have been observed compared to

dead cells ($K_F = 1.02\text{--}4.19$; $n = 1.42\text{--}2.78$) (Table 1). This suggested that live cells may have a different degree of sorption capacity compared to dead cells. Similar observations on isotherms have been reported by the fungus *Haematonectria haematococca* for the removal of anthraquinone dye [43]. This study has therefore suggested the benefit of using live cells for the sorption of TPM dyes.

Biosorption and biodegradation potential of TPM dyes by *Streptomyces* sp.

The removal of TPM dyes by live and dead cells of *Streptomyces* sp. at optimum conditions indicated the occurrence of biodegradation and biosorption. In this study, changes in the UV-Vis absorption spectra (200–800 nm) between untreated and treated dyes with live cells were observed. The occurrence of biodegradation is marked by the absence of the dye absorption peak [2]. Spectra of untreated dye (0 h) revealed peaks at 617, 584, 590, and 599 nm, which identifies with the typical peaks of MG, MV, CV, and CB, respectively (Fig. 12). This suggested that for untreated dyes, the dye chromophores were intact and detectable, as no biosorption and biodegradation has occurred.

Upon treatment with live cells, the absorption peaks were observed to have either reduced in intensity (MG, MV, CV) or the peaks have almost disappeared (CB) (Fig. 12). This implies that a certain degree of degradation of TPM dyes has occurred due to the breakdown of the dye chromophore [22]. The association of dye biodegradation with absence of peaks is an approach used by many to describe biodegradation of dyes by *Aspergillus niger* [34], *Penicillium simplicissimum* [37], and *Lysinibacillus fusiformis* [44]. Although enzymes responsible for biodegradation of dyes by *Streptomyces* sp. were not investigated in this study, it is suggested that it may be attributed to peroxidases and laccases, capable of breaking dye molecules into smaller and less toxic compounds [47]. The toxicity of the treated dyes was not determined in this study, but can be confirmed via phytotoxicity tests using seed germination [44], including a separate study by Buntic et al. [11] on phytotoxicity of CV treated with *S. microflavus*.

For TPM dyes treated with dead cells, all absorption peaks remained visible (Fig. 12). No major changes in the absorption peaks indicated that the original structure of TPM dyes remained intact, and dye chromophores were not degraded [24]. It was however observed that the creamy-white pellets (dead cells) turned into dye-colored biomass upon introduction into dye solutions. Colored-biomass is likely the result of the sorption of TPM dyes onto the biomass surface [24]. The biosorption is said to have occurred and may have been

attributed to the functional groups (e.g., hydroxyl, carbonyl, and amino) present on the surface of biomass with dye molecules, verified via Fourier transform infrared spectroscopy [32]. The dominance of either mechanism was not distinguished as both mechanisms usually occur together, except for dead cells where biosorption is the only mechanism involved.

Conclusions

This study revealed the decolorization potential of *Streptomyces* sp. (presumably *S. bacillaris* MN262194 based on 16S partial sequencing) on TPM dyes. It was established that the decolorization efficiency of the live and dead cells was influenced by pH, agitation speed, biomass, initial dye concentration, and oxygen. The removal of TPM dyes by live and dead cells was better when optimized conditions were implemented. It was also revealed that the application of live cells was more effective than the use of dead cells, where almost complete decolorization of MG and MV was observed. The efficient decolorization activities by live cells have been attributed to the degradation of dyes. This study proposed *Streptomyces* sp. (*S. bacillaris* MN262194) as an effective dye degrader of carcinogenic TPM dyes. Future explorations may include immobilization and transfer of technology for bioremediation of other TPM dyes.

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Authors' contributions

NHA carried out the experiments, analyzed the findings and prepared the initial draft of the manuscript. ASYT conceptualized the project and advised on methodology and data interpretation. ASYT and YYL supervised the project and were involved in the drafting and editing of the manuscript. All authors have read and approved the final draft of the manuscript prior to submission.

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NHA is completing her PhD and will return to her lectureship position upon graduation. Her focus is on microbiology and is interested to solve issues related to environmental microbiology. YYL is a senior academic in School of Science who specializes in chemistry. ASYT is currently the Head of Discipline (Biological Science) and her interest is in Applied Microbiology. She has published many papers pertaining to the beneficial use of microbes for sustainability.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon request.

Competing interests

The authors declare that they have no conflict of interest arising from the publication of the findings.

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