Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2019.24794

171 (2019) 356–368
December

Optimization of algicidal activity of indigenous strain *Schizophyllum commune* 104UTHM against *Microcystis aeruginosa* in freshwater using response surface methodology

Abdelrahman Hamed[a], Efaq Ali Noman[b], Adel Al-Gheethi[a,*], Mohammed Al-Sahari[a], Radin Maya Saphira Radin Mohamed[a], Balkis A Talip[c,*], Nur Adila Ab. Aziz[a], Amir Hashim Mohd Kassim[a]

[a]*Micro-pollutant Research Centre (MPRC), Department of Water and Environmental Engineering, Faculty of Civil & Environmental Engineering, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia, Tel. +607 456 4316; Fax: +607 453 6588; emails: adelalghithi@gmail.com/adel@uthm.edu.my (A. Al-Gheethi), abdokahkey@gmail.com (A. Hamed), mohammedalsahari@gmail.com (M. Al-Sahari), maya@uthm.edu.my (R.M.S.R. Mohamed), nuradila@uthm.edu.my (N.A.A. Aziz), amir@uthm.edu.my (A.H.M. Kassim)*
[b]*Department of Applied Microbiology, Faculty of Applied Sciences, Taiz University, Taiz Yemen, email: eanm1984@gmail.com*
[c]*Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM), KM11 Jalan Panchor, 84000 Pagoh, Muar, Johor, Malaysia, Tel. +606 9742172; Fax: +606 9742 190; email; balkis@uthm.edu.my*

Received 22 February 2019; Accepted 15 August 2019

ABSTRACT

The present study aims to optimize the algicidal activity of *Schizophyllum commune* against *Microcystis aeruginosa* in the freshwater through the bio-flocculation process. Four independent factors including fungal inoculum size, x_1 (10–100 mL [10^6 CFU mL^{-1}]), pH, x_2 (4–9), mixing rate, x_3 (50–150 rpm) and hydraulic retention time, x_4 (30–180 min) were optimized using response surface methodology. The effect of algicidal activity of *S. commune* 104UTHM on *M. aeruginosa* cell morphology was determined using scanning electron microscope. The optimal inactivation rate was 50.32 vs. 54.63% in Chl-a, (y_1), for actual and predicted percentage, respectively, achieved with 100 mL of the fungus inoculum size, pH 6, with 125 rpm of the mixing rate and within 135 min of HRT with 72% of the coefficient (R^2). A significant coefficient of the inactivation rate was recorded at 93.60% of confidence level. The algicidal activity of *S. commune* 104UTHM led to damage of *M. aeruginosa* cell and changes the cell shape.

Keywords: Response surface methodology; Bio-flocculation; Mechanism; Environmental factors

1. Introduction

Harmful algae bloom (HAB) is a natural phenomenon that occurs in the natural water system which receives high contents of nutrients and fertilizers from discharged wastewater or runoff water in the surrounded plantation. HABs are associated with the production of poisonous substance and high-biomass producers [1]. The relationship between HABs biotoxins, and negative impacts on human health has been reported several years ago [2]. Most HABs toxins are neurotoxins, while other toxins cause skin and liver damage and even cancer. The majority of human diseases associated with HABs toxins appear to be acute phenomena.

* Corresponding authors.

However, ciguatera fish poisoning causes prolonged sub/chronic disease [3,4].

Many of the chemical, physical, and biological methods have been applied for controlling HABs in fresh water. In the chemical methods, HABs are inactivated by using metal ions [5,6], herbicides [7] and photosensitizers [8]. The investigated physical methods included, membrane filtration technology [9,10], ultrasound techniques [11], UV irradiation [12], coagulation/flocculation [13,14] and adsorption [15]. Both physical and chemical methods have the ability to remove HABs efficiently. However, these methods have several disadvantages including high costs, secondary pollution and non-target toxicity to aquatic organisms and humans. These disadvantages encouraged the researchers to find an alternative method such as biological methods for inactivating HABs in fresh water. The most common biological methods used for inactivating HABs are using aquatic plants, animals, and algicidal microorganisms such as bacteria and fungi to limit the overgrowth of algae [16–18]. These methods gained much attention in the recent years and considered as an effective strategy due to their algicidal potential and environmental friendliness [19,20]. Microorganism-based methods using algicidal bacteria, fungi, viruses, and protozoa are considered as promising eco-friendly approaches, compared with the physical and chemical methods. Two inactivation methods of HABs have been reported using algicidal bacteria, which are classified as direct and indirect method. The direct inactivation method had been reported as a result of the interaction between the bacterial cell wall and the HABs wall leading to lose the HABs shape. Meanwhile the indirect method takes place as a result of algicidal substances produced by the algicidal bacteria which attacks the HABs and destroys the HABs cells [21,22]. The inactivation of HABs using fungi depends on the palletisation process, in which HABs are attracted by the fungal mycelium leading to form fungus–alga pellets. The size of fungus–alga pellets, spherical, oval masses or ellipsoidal intertwined hyphae varied from many hundred micrometres to many millimetres resulting in the formation of the pellets [23]. In the palletisation, the filamentous fungi cell wall containing polysaccharides with active sites will attract algal cells by changing their surface charge and consequent co-palletisation [20]. Nonetheless, many of the fungal species have been reported to have high potential to inactivate the HABs. Han et al. [24] reported that *Irpex lacteus*, *Trametes hirsuta*, *Tinea versicolor*, and *Bjerkandera adusta* degrade the HABs through direct attack. The fungi destroy the cell membranes and the nucleic acids of the algal cells, thereby leading to cell death [25]. The antagonistic activity of the fungi towards HABs had been observed in 1978. However, the preying ability of fungus on algae has not apparently been pursued in detail. Redhead and Wright [26] had claimed that the ability of fungus to inhibit and destroy live blue-green algae is associated with their production of diffusible extracellular substances. In contrast, many of the algae displayed the efficiency in inactivating fungal species. For instance, a study on the antifungal activity of algae against *Aspergillus flavus* and *Penicillium* sp. has been previously reported in the literature [23].

Several studies have shown the uses of fungal pellets as flocculants for treatment of wastewater [20]. However, the ability of some fungal species to produce toxins and spores is a limitation in their use. The application of WRF for the treatment of wastewater has been introduced by Stamets in the late 1980s [27]. In a report published by USEPA, Stamets [27] claimed that there is a substantial evidence showing the ability of fungi to consume bacteria and secrete antibacterial metabolites. However, only few applications of WRF in HABs inactivation for freshwater treatment have been reported. Malaysia has been listed as one of the world's 12-mega biodiversity countries. It has a large area of forests that has favourable conditions for fungal diversity [28]. However, the isolation and identification of potential fungi in water treatment are still minimal and require extensive exploration.

The fungal bio-flocculation was influenced by several factors such as pH, agitation, fungal species and density. The optimum pH value (acidic) of fungal bio-flocculant is non-existent in treating HABs in rivers and reservoirs [20,29]. Therefore, pH of the wastewater should be adjusted before the flocculation process. The compounds produced by algicidal fungi are also affected by the temperature and acidic conditions. In order to achieve high removal of HABs, the factors that affect the HABs inactivation should be optimized. Response surface methodology (RSM) is a statistical programme that is used to determine the best operating parameters for many biotechnology processes. It is a computerized software that has been designed to investigate the optimal parameters required to achieve the best results. RSM experimental runs are designed based on the selection of independent factors which might have a positive or negative effect on the production process, as well as the interaction between the factors. The optimization of fungal growth and their application in biotechnology by RSM have been reported by several authors in the literature [30–32]. Several studies have reported that the algicidal bacterium was used against HABs [33]. However, only few studies were conducted on inactivation of HABs using WRF. Meanwhile, studies on the optimization of algicidal activity against HABs by *Schizophyllum commune* have never been reported before. Therefore, this present student aims to optimize the environmental factors of bio-flocculation process for improving the algicidal activity of *S. commune* 104UTHM against HABs in fresh water. Addition to that, the inactivation mechanism of HABs by *S. commune* 104UTHM was investigated based on the scanning electron microscope (SEM) images analysis.

2. Materials and methods

2.1. Isolation of algicidal fungal strain

S. commune 104UTHM was isolated and cultivated from the peat soil sample using standard serial dilution spread plate method on potato dextrose agar medium (PDA, Himedia, India) [34]. The fungal strain was purified using single spore isolation technique [35].

2.2. Identification of fungal strain

The identification of the fungal strain was conducted using colony morphological and molecular analysis techniques.

2.2.1. Colony morphological analysis

The colonies morphology of the fungal strain was determined by culturing the isolates on the PDA, V8 juice agar medium (V8A) (Hi media, India), *Sabouraud Dextrose* Agar supplemented with 4% sucrose (SDA, Hi Media, India), Czapek Yeast Extract Agar (CYA, Oxoid, UK), Czapek-Dox *Agar* (CZ, R&M Marketing, UK) and Malt Extract Agar (MEA, Merck, Germany). The colony morphology characteristics were recorded after the incubation period of 7 d at 28°C based on the colony size (diameter, mm), surface and texture as described by Promputtha et al. [36].

The microscopic analysis for the fungal isolate was performed by using the light microscope (100X, Olympus, BX53F-CCD, Serial No. 1A589796, Japan). Some part of the mycelia was taken from the centre and edge of the growing colony and then placed onto glass slide that contained one drop of distilled water and covered by a cover slip and observed under the microscope. The microstructure of the fungal conidiophore was observed using SEM. Approximate 0.2 × 0.2 cm of the fungal colony grown on PDA medium for 2 d at 28°C was transferred into an aluminium petri dish and subjected to drying using liquid nitrogen, coated with gold powder and then observed using SEM. The observation was carried out at School of Biological Sciences, Universiti Sains Malaysia (USM), Penang Malaysia.

2.2.2. Molecular identification of isolated fungus

The molecular identification of fungal isolates was performed at Centre of Chemical Biology (CCB-USM). The identification was performed by polymerase chain reaction on the thermal cycler "Veriti 96 Well Thermal Cycler" (Applied Biosystems, USA). The amplification was targeted at 28LrDNA regions using a set of primers namely F63 and LR3 to amplify the 600 bp amplicon of D1/D2 region; forward primer (F63) 5′-GCATATCAATAAGCGGAGGAAAAG-3′ and reverse primer (LR3) 5′-GGTCCGTGTTTCAAGACGG-3′ [37]. The amplicon was further sequenced and analysed using NCBI BLAST analysis. The sequences were then deposited in the GeneBank with accession no. MK517570 (https://www.ncbi.nlm.nih.gov/nuccore/MK517570). Nine sequences that give 100% homology to the genus *Schizophyllum* were used to construct the phylogenetic tree including *Schizophyllaceae* sp. ZLY-2010b (HM595606.1), *Schizophyllum commune* MH874930.1, *Schizophyllum commune* MH877686.1, *Schizophyllum* sp. isolate M29 MH121059.1, *Schizophyllum radiatum* LT217575.1, *Schizophyllum fasciatum* LT217593.1, *Schizophyllum amplum* AF141873.1, and *Auriculariopsis ampla* AY293169.1 (Fig. 1). *Aspergillus aculeatus* (MK392046.1) strain was also included in the analysis for interrelation between homologous strains of genus *Schizophyllum*. The sequences alignment was analysed using ClustalX2.1 and the phylogenetic tree was constructed using PAUP4 software and analysed based on the neighbour-joining UPGMA algorithm.

2.3. Determination of HABs in freshwater samples

The freshwater samples (20 L) with HABs were collected from a domestic water tank that was used for concrete soak located near to the wood factory at Universiti Tun Hussein Onn Malaysia (UTHM). The samples were collected in a plastic bottle (20 L) and subjected to the analysis within 24 h. The presence of HABs in the freshwater sample was determined using the KDHE procedure as described by the Kansas Department of Health and Environment, USA [38]. The method was performed based on the formation of upper layer on the surface of firewater after keeping the sample in a cold refrigerator overnight [38]. The HABs used in this present work is similar to *Microcystis aeruginosa* based on the cell morphology as determined by SEM.

The quality of the freshwater samples was determined in terms of biochemical oxygen demand (BOD), dissolved oxygen (DO), ammoniacal nitrogen (NH_3–N), pH and total suspension solids (TSS) by using the procedure described by APHA [34]. The concentrations of Chl-a content, in the raw freshwater samples were determined by the methods described by Jeffrey and Humphrey [39]. The Chl-a was extracted from the water samples by mixing 1 mL of the water

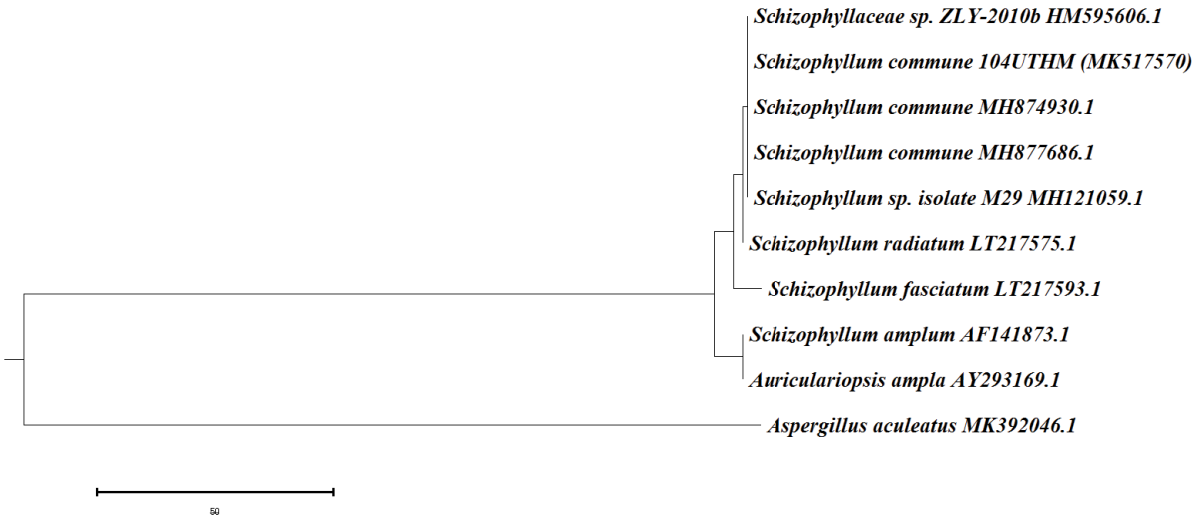


Fig. 1. Phylogenetic tree showing the relationship between *Schizophyllum commune* 104LD and the related selected sequences based on 28S LrDNA sequence comparisons.

sample with 9 mL of acetone (90%). The absorbance of the mixture was determined using spectrophotometer (HACH DR 6000, USA) at ƛ = 664 nm, ƛ = 647 nm and ƛ = 630 nm. The Chl-a concentrations were calculated using Eq. (1).

$$\mathrm{Chl(a)}\left(\frac{\mu g}{l}\right) = 11.85E_{664} - 1.54E_{647} - 0.08E_{630} \quad (1)$$

where E_{664} = value of absorbance at wavelength 664 nm; E_{647} = value of absorbance at wavelength 647 nm; E_{630} = value of absorbance at wavelength 630 nm.

The water quality in the collected samples was determined according to WQI formula (Eq. (2)) as described by Environmental Quality Report (EQR) [40].

$$\mathrm{WQI} = (0.22\times\mathrm{SiDO}) + (0.19\times\mathrm{SiBOD}) + (0.16\times\mathrm{SiCOD}) + (0.15\times\mathrm{SiAN}) + (0.16\times\mathrm{SiSS}) + (0.12\times\mathrm{SipH}) \quad (2)$$

where SiDO = Sublndex DO (% saturtlon); SiBOD = Sublndex BOD; SiCOD = Sublndex COD; SiAN = Sublndex NH_3–N; SiSS = Sublndex SS; SipH = Sublndex pH; 0 ≤ WQI ≤ 100.

2.4. Optimization set up for bio-flocculants process using S. commune 104UTHM

The experimental set-up of the current work consists of *S. commune* 104UTHM inoculum preparation, algicidal activity experiments, as well as pseudo-first and second order kinetic model for inactivation process using RSM. The factorial complete randomized design (CRD) (4 × 1 × 1) in duplicates was used to study the optimal factors affecting the inactivation process with four independent factors, four (1 dependent variable and one (1) control as suggested by RSM. *S. commune* 104UTHM was sub-cultured in PD broth, one loopful of *S. commune* 104UTHM was inoculated into 1 L of sterilized PD medium and incubated at 28°C for 3 d. The best operating parameters for achieving highest inactivation of HABs in the freshwater samples were optimized using RSM. Four independent factors were selected and used for designing the experimental runs using Design Expert 6.0.10, central composite design (CCD) (Stat-Ease, Inc., Minneapolis, USA) software that suggested 30 experimental runs. There are several independent factors including, inoculum size (mL [10^6 CFU mL^{-1}]) of *S. commune* 104UTHM (x_1), pH of water sample (x_2), mixing rate (x_3), and hydraulic retention time, HRT (x_4). The maximum (+1), intermediate (0), and minimum (–1) values of each independent variable are illustrated in Table 1. In contrast, the dependent variables include the removal percentage of chlorophyll (Chl-a) (y_1) that was used to assess the inactivation rate of *M. aeruginosa*.

2.5. Bioflocculant processes

30 experimental runs were conducted in order to assess the efficiency of algicidal activity of *S. commune* 104UTHM against *M. aeruginosa* in freshwater. Each freshwater sample (1 L) was placed in jar test and inoculated with different inoculum size of *S. commune* 104UTHM (10–100 mL [10^6 CFU mL^{-1}]), pH was adjusted using NaOH (0.1 M) and HCl (0.1 N) to be within range (pH 4–9) based on the RSM designed in this study. The freshwater with *S. commune* 104UTHM was mixed at 50–180 rpm for different HRT (30–180 min). The samples were subjected for mixing at 30 rpm for 30 min and left to settle for 6 h and then the supernatant was used to estimate the inactivation efficiency based on the reduction in the Chl-a contents. The inactivation percentage was determined according to Eq. (3) [41]:

$$\text{Removal ratio of Chl a}(\%) = \left(1 - \frac{\mathrm{Chl(a)}_2}{\mathrm{Chl(a)}_1}\right)\times 100 \quad (3)$$

where Chl $(a)_1$: Chlorophyll-a concentration of control sample; Chl $(a)_{2:}$ Chlorophyll-a concentration of treated sample

2.6. Determination of HABs cell morphology using SEM

In order to understand the effect of algicidal *S. commune* 104UTHM on *M. aeruginosa* cells which have spherical shape, therefore, any changes of the shape are used as indication of inactivation or deformation of *M. aeruginosa* cells [42]. *M. aeruginosa* cells morphology was observed before and after the inactivation process using SEM. The *M. aeruginosa* cells were collected from the treated and untreated water sample using centrifugation at 4,020 × g for 20 min. The pellets of *M. aeruginosa* cells were observed under the SEM (Zeiss Supra 50 VP, Germany).

2.7. Statistical analysis

The experiments were performed in triplicates to ensure the accuracy of the results. Design-Expert software was used to analyse the data and investigate the first order response surface equations of the model. The significance of the independent variables on the dependent variables were analysed using ANOVA ($p < 0.05$). The R^2 was used for checking the fit of the linear model. The interactions between the independent factors and their role in the inactivation of HABs were presented using a three-dimensional graphical representation of the system behaviour, called RSM.

3. Results and discussion

3.1. Algicidal fungal strain

The colony morphology of *S. commune* 104UTHM on different selective media is presented in Fig. 2. *S. commune* 104UTHM showed diverse morphological shape and form dependent on the culture medium used for culturing. The form texture, colour, margin, growth pattern, elevation are useful for primary identification of the fungus strain.

S. commune 104UTHM exhibited a rapid growth with purely white and dense woolly texture, odour absent, clamps present and yellow reverse. The growth initiates near the inoculum and spreads throughout the surface of the medium (Fig. 2). Similar findings were observed for this fungal strain by Amee et al. [43], who isolated the *S. commune* from the fungus natural habitat. The colony size depends on the culture media and recorded as 76.76 ± 2.3 mm on PDA, 80 ± 12.1 mm on V8, 73 ± 9.1 on MEA, 50 ± 4.9 mm on CYA, 55 ± 1.7 mm on CZ, 50 ± 3.5 mm on SDA (4% sucrose) and 60 ± 3.7 mm

Table 1
Coded and un-coded levels of the independent variables used in the current study

Factor	Symbol	Level		
		Low (–1)	Middle (0)	High (+1)
Inoculum size (mL)	x_1	10	55	100
pH	x_2	4	7	9
Mixing rate (rpm)	x_3	50	110	180
Hydraulic retention time, HRT (min)	x_4	30	100	180

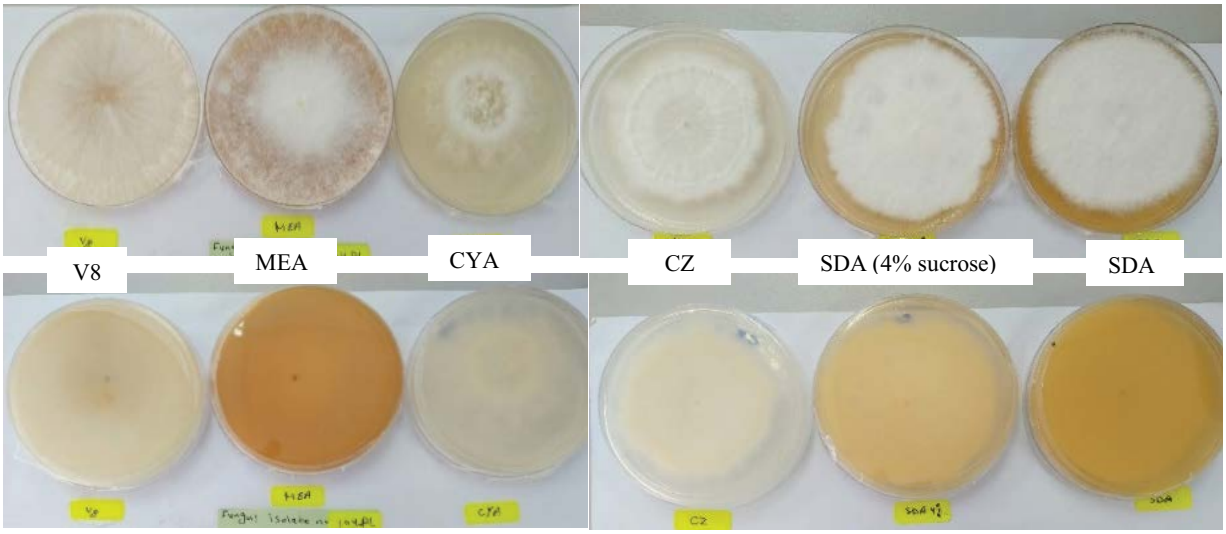


Fig. 2. Culture of *S. commune* 104UTHM isolated from peat land sample on different culture media. The plates were incubated at 28°C for 7 d.

on SDA where PDA, MEA and SDA belong to the different culture media. These findings indicated that MEA, PDA and V8 were the best growth medium for *S. commune* 104UTHM. It has been reported that PDA medium supported the growth for wide range of fungi [44]. However, Choi et al. [45] claimed that V8A is necessary for isolating the fungus with complex requirements for their growth and sporulation. In the present study, both V8A and PDA exhibited similar growth pattern for *S. commune* 104UTHM which indicate that the selection of isolation medium is depending on the fungal species and their requirements for the growth.

The scanning electron micrographs of *S. commune* 104UTHM showed a hyaline, smooth, septate and branched hyphae of 2 widths that included a wider hyphae and a narrower width hyphae, the SEM images also showed the clamp connections and spicules (Fig. 3). Similar observation was also recognized in previous work of this fungal strain [46,47]. *S. commune* 104UTHM has monokaryotic or dikaryotic (binucleate condition) stages in life cycle. In addition, the microscopic morphology of *S. commune* 104UTHM shows binucleate in the hyphae (Appendix A) as well as spicules and clamp connections on hyphae. Won et al. [46] show that the dikaryotic phase of *S. commune* isolated from the environment is a distinctive feature. However, the fungi isolated from human specimens fail to exhibit the dikaryotic phase. Therefore, *S. commune* 104UTHM from the clinical sample is more difficult to be identified by morphological characteristics only and should be identified by molecular gene sequencing. The *S. commune* 104UTHM isolated from peat land showed the presence of dikaryotic phase and easily identified by using light microscope at 40X. The microstructure was recognized by SEM which has been used as a technique for the identification of fungi since 1950s [48,49]. SEM provides critical details for the fungal morphology and microstructure [50]. However, the culture and morphological identification were insufficient for accurate identification of the fungi. The molecular analysis based on 28S LrDNA sequences was deciphered as *S. commune* 104UTHM which belongs to the family of Basidiomycetes [51]. In the present work, the gene sequencing of D1/D2 regions of the 28S ribosomal DNA was very helpful for the accurate identification. According to Balajee et al. [52], D1/D2 regions sequencing has been recognized as the gold standard for fungal identification in addition to the morphological analysis.

S. commune 104UTHM has been isolated from different location in Malaysia [53]. The application of this fungus for inactivation of HABs has never been reported. Due to the non-sporulation attribute, this fungus has high applicability in inactivating HABs without adverse effect on the environment. In addition, the growth of mycelium enhanced the efficiency of the system against *M. aeruginosa*. The assessment of the effects of metabolites on the environment should be

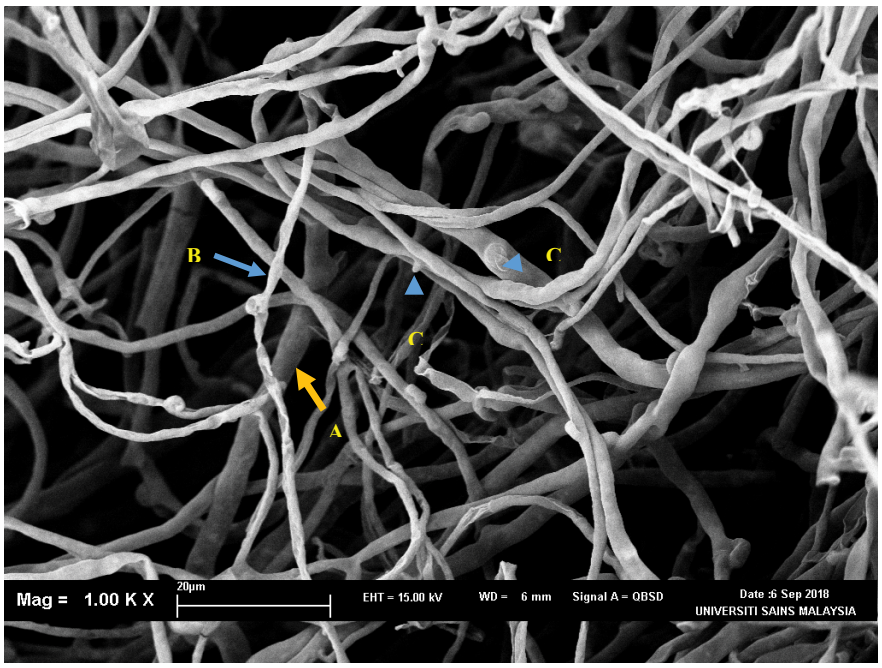


Fig. 3. Scanning electron micrographs of *S. commune* 104UTHM show, hyaline, smooth, septate and branched hyphae of 2 widths; (A) a wider hyphae; (B) a narrower width hyphae; (c) the clamp connections and spicules (1380 X); (B) spores ellipsoidal/pyriform to fusiform/smooth to spinulose (1,000 X).

investigated as indicated by Liao et al. [33]. Although the *S. commune* 104UTHM does not produce spore, the effects assessment of metabolites requires further investigation.

3.1.1. Characteristics of raw freshwater samples

The freshwater sample used in the current work contained Chl-a in the average of 920 µg L^{-1}, BOD_5 (44.79 mg L^{-1}), NH_4-N (4.75 mg L^{-1}), pH 6.81, COD (2.48 mg L^{-1}), TSS (125 mg L^{-1}) and DO (9.56 mg L^{-1}) (data not shown). The high concentrations of Chl-a in the freshwater sample indicate the presence of high concentrations of HABs. Moreover, the results of KDHE test revealed the presence of the upper layer on the surface of the water sample in the test tube which indicates that the HABs cell concentrations exceeded 10,000,000 cells mL^{-1} (Appendix B). These findings are inconsistent with the Hollister and Kreakie [54], who stated that Chl-a is associated with various microcystin health advisory concentrations which is one of the HABs. Other studies have also mentioned that the concentration of Chl-a more than 550 µg/L is an indication for the presence of HABs [20,55–57]. Therefore, in the current work, the freshwater samples with 920 µg L^{-1} of Chl-a were considered as samples containing HABs. Based on WQI formula, the water sample has 31.93 which is less than the water quality index of Malaysia and classified under class IV. According to EQR [40], the water with WQI less than 60 is considered as highly polluted.

3.2. Optimizing of bio-flocculation of M. aeruginosa in the freshwater using S. commune 104UTHM

The bio-flocculation of *M. aeruginosa* in freshwater using *S. commune* 104UTHM as a function of the Inoculum size (x_1) (10–100 mL), pH (x_2) (4–9), mixing rate (x_3) (50–180 rpm) and HRT (x_4) (30–180 min) is illustrated in Table 2. *S. commune* 104UTHM as a live strain was used as indicated by Jia et al. [25] who mentioned that the live fungal strain exhibited more efficiency in inactivating HABs than the culture filtrate. *S. commune* 104UTHM exhibited the maximum activity against *M. aeruginosa* with 100 mL of the fungal inoculum size, at pH 4, 50 rpm and within 30 min of the HRT, where 55.32% vs. 49.79% of observed and predicted reductions of Chl-a (y) was recorded. In comparison with the control experiment, which was conducted without fungal inoculation, the reduction of Chl-a was 5.43%.

Palletisation is a process that takes place during the flocculation process by interacting of *M. aeruginosa* with the fungus mycelium leading to forming fungus–alga pellets [23]. The optimization of the palletisation using *S. commune* 104UTHM has not been reported before. However, Grimm et al. [58] revealed that the palletisation of HABs using fungi is depending on the pH. This might be related to the effect of pH on the charge of the functional group on the surface of fungal cell wall and thus effect on the interaction with the HABs cells. Zhou et al. [29] indicated that acidic conditions which is optimal for the fungal growth might be non-existent for treating HABs in rivers and reservoirs. Therefore, the pH should be adjusted to values close to those of water bodies. In the present study, *S. commune* 104UTHM was isolated from acidic soil (peat soil has pH 4), while pH of the freshwater was 6.81. However, the maximum reduction of Chl-a was reported at pH 4. More reduction (86.79%) in Chl-a was observed at pH 1.5 (out of the investigated range for pH, 4–9). One explanation for these findings might be due to the nature of *S. commune* 104UTHM which was more active for catching the *M. aeruginosa* cells at acidic conditions.

Table 2
Central composite design arrangement and responses for algicidal activity of *S. commune* 104UTHM against *M. aeruginosa* in freshwater

Run	x_1	x_2	x_3	x_4	y_1 (R%)	
					Observed	Predicted
1	–1.000	0.000	0.500	–0.176	5.43	2.99
2	0.379	–0.500	–0.500	–0.765	55.32	53.87
3	–0.862	0.500	–0.500	–0.765	6.50	2.69
4	–0.241	0.000	0.500	–0.176	32.60	32.92
5	–0.241	–1.000	0.500	–0.176	86.79	70.49
6	–0.241	0.000	–1.000	–0.176	27.17	23.27
7	–0.241	0.000	0.500	1.000	33.36	28.17
8	–0.241	1.000	0.500	–0.176	32.06	43.28
9	–0.241	0.000	0.500	–0.176	33.80	32.92
10	–0.862	0.500	–0.500	0.412	5.43	6.13
11	–0.862	–0.500	0.850	0.412	15.21	22.35
12	–0.862	–0.500	–0.500	0.412	16.30	17.40
13	–0.241	0.000	0.500	–0.176	33.47	32.92
14	–0.241	0.000	0.500	–0.176	33.58	32.92
15	–0.862	0.500	0.850	–0.765	6.52	2.14
16	–0.241	0.000	0.500	–1.000	27.70	27.92
17	–0.241	0.000	0.500	–0.176	28.26	32.92
18	–0.862	–0.500	–0.500	–0.765	5.76	11.19
19	0.379	0.500	0.850	–0.765	54.34	48.62
20	0.379	0.500	–0.500	0.412	55.43	52.95
21	–0.862	0.500	0.850	0.412	5.21	2.68
22	–0.862	–0.500	0.850	–0.765	15.21	19.04
23	0.379	–0.500	0.850	0.412	55.86	62.96
24	1.000	0.000	0.500	–0.176	72.60	68.43
25	0.379	0.500	0.850	0.412	50.00	48.30
26	0.379	0.500	–0.500	–0.765	47.82	50.38
27	0.379	–0.500	0.850	–0.765	52.17	60.53
28	0.379	–0.500	–0.500	0.412	53.04	59.21
29	–0.241	0.000	1.000	–0.176	33.69	29.96
30	–0.241	0.000	0.500	–0.176	31.84	32.92

x_1 (Inoculum size, mL); x_2 (pH); x_3 (mixing rate, rpm); x_4 (HRT, min); y_1 (Chl-a).

The analysis for the effect of investigated independent factors on the inactivation of *M. aeruginosa* was performed by using one-way ANOVA. The results revealed that these factors contributed by 87.62% in reducing Chl-a (Table 3). These findings indicated that the investigated factors occurred significant ($p < 0.01$) models for reduction of Chl-a. However, the lack-of-fit for these models was also significant indicating that these factors have more than quadratic effects and more or less range for each factors should be conducted. Nonetheless, the range selected in the current study was performed based on the previous studies that investigated each factor separately, while their effects might be different due to their interactions.

The results of the linear and quadratic model for the independent factor as well as interaction between these factors and their effect on the bio-flocculation of *M. aeruginosa* using *S. commune* 104UTHM are illustrated in Table 3. It was noted that the increases of pH (x_2) from 4 to 9 are correlated with a significant decrease in Chl-a reduction ($p < 0.021$) from 55.32% at pH 4 to 6.5% at pH 9. In contrast, the bio-flocculation of *M. aeruginosa* has a positive and significant correlation with the dosage factor (x_1) ($p < 0.001$). However, the mixing rate (x_3) and HRT factors (x_4) have no significant effect on the reduction of Chl-a. The positive correlation between the inoculum size and the formation of pellet flocs has been reported in the literature. Gang et al. [59] has revealed that the pellets size flocculated was increased from 2.25 to 3.52 mm (the settling velocity increased from 3.28 to 7.37 mm/s) by increasing the polyacrylamide from 0.59 to 1.18 mg/L. In the present study, the pellets size was not determined but the reduction of Chl-a has increased from 6.5% with 10 mL of the fungal inoculum size to 55.32% with 100 mL of the inoculum size.

These findings could indicate that the palletisation process takes place as a result of bio-flocculation using

Table 3
Analyses of the variance (ANOVA) of the response surface quadratic model for algicidal activity of *S. commune* 104UTHM against HABs in freshwater

Source	Sum of squares	df	Mean Square	*F* value	*P* value Probe > *F*	
Model	12,299.09	14	878.5065	15.66639	<0.0001	significant
X_1	5,274.508	1	5,274.508	94.06022	<0.0001	
X_2	368.3573	1	368.3573	6.568911	0.021633	
X_3	56.46553	1	56.46553	1.006949	0.331549	
X_4	3.755901	1	3.755901	0.066979	0.799305	
X_1X_2	25.05003	1	25.05003	0.446717	0.514053	
X_1X_3	1.512511	1	1.512511	0.026973	0.87174	
X_1X_4	0.7569	1	0.7569	0.013498	0.909051	
X_2X_3	76.18522	1	76.18522	1.35861	0.261979	
X_2X_4	7.645225	1	7.645225	0.136337	0.71711	
X_3X_4	8.873227	1	8.873227	0.158236	0.696389	
X_1^2	74.06783	1	74.06783	1.320851	0.268436	
X_2^2	1,022.771	1	1,022.771	18.23907	0.00067	
X_3^2	64.77352	1	64.77352	1.155105	0.299468	
X_4^2	56.18688	1	56.18688	1.00198	0.332707	
Residual	841.1379	15	56.07586			
Lack of Fit	819.2679	10	81.92679	18.73033	0.002373	significant
Pure Error	21.87008	5	4.374017			
Cor Total	13,140.23	29				

S. commune 104UTHM. Therefore, the investigated factors have similar effects on the bio-flocculation process in comparison with the previous studies [60,61]. However, the algicidal activity against *M. aeruginosa* occurs during the settling time which was conducted for 6 h, leading to the destruction of HABs cells.

The quadratic effect for the investigated factors is presented in Table 3, x_2 has a significant positive quadratic effect on the reduction of Chl-a ($p < 0.01$), while no significant effect for other independent factors on the reduction of Chl-a. The linear and quadratic effects for the independent factor are presented in Eq. (4).

$$y_1 = +41.40 + 32.97x_1 - 9.93x_2 + 2.85x_3 + 0.89x_4 + 4.03x_1x_2 - 0.72x_1x_3 - 0.60x_1x_4 - 6.23x_2x_3 - 2.35x_2x_4 - 1.83x_3x_4 - 5.42x_1^2 + 23.97x_2^2 - 6.17x_3^2 - 5.05x_2^2 \quad (4)$$

The interactions between the independents factor are illustrated in Table 3 and depicted in Fig. 4. It was noted that x_1 interacted positively with x_2 for reduction of Chl-a, x_1 exhibited more efficiency reduction of Chl-a compared with x_2, x_3 and x_4 (Figs. 4a, b and c), while no interactions were recorded between x_3 and x_4 (Fig. 4d), x_2 and x_3 and between x_2 and x_4.

Based on the abovementioned results, It can be indicated that the inactivation of *M. aeruginosa* taken place due to the flocculation process with *S. commune* 104UTHM leading to form the flocs during the mixing time as well as due to the algicidal substances from the fungus that led to destroy the *M. aeruginosa* cells and release the cytoplasm contents into the surrendered medium, the process taken place during the HRT of 30–180 min and settling time of 240 min, because the algicidal activity needs more time in comparison with the flocculation process. The fungal species have exhibited high potential as flocculent. Aljuboori et al. [62] have reported that the purified bio-flocculant from *Aspergillus flavus* contained 69.7% of sugar which lead to the agglomeration the pollutants. Besides, the bioflocculant contained hydroxyl, amide, carboxyl and methoxyl groups which played important role in the flocculation process. Espinosa-Ortiz et al. [23] indicated that fungal species have high potential in the wastewater treatment because the fungal pellets are well settling aggregates formed by self-immobilization. Moreover, Jia et al. [25] indicated that the fungal strains rapidly degrade microcystins within 12–72 h. However, in this study the degradation of HABs occurred within a short time (3 h for the HRT and 6 h for the settling time) indicating that the bioflocculation with fungus accelerates HABs inactivation by algicidal activity.

The best operating parameters for reducing Chl-a was optimized using Design Expert software, the results revealed that the maximum reduction (54.63%) in Chl-a might be achieved with 100 mL of the fungus inoculum size, pH 6, with 125 rpm of the mixing rate and within 135 min of HRT with 72% of the coefficient (R^2). These parameters were confirmed in the laboratory and the recorded reduction was 50.34%. In comparison with previous studies, Jia et al. [25] indicated that *Trichaptum abietinum* removed all the test algal cells (*Microcystis aeruginosa*, *Microcystis flosaquae*, *Oocystis borgei*) within 48 h. The removal in the present study was less than that reported by Jia et al. [25], however,

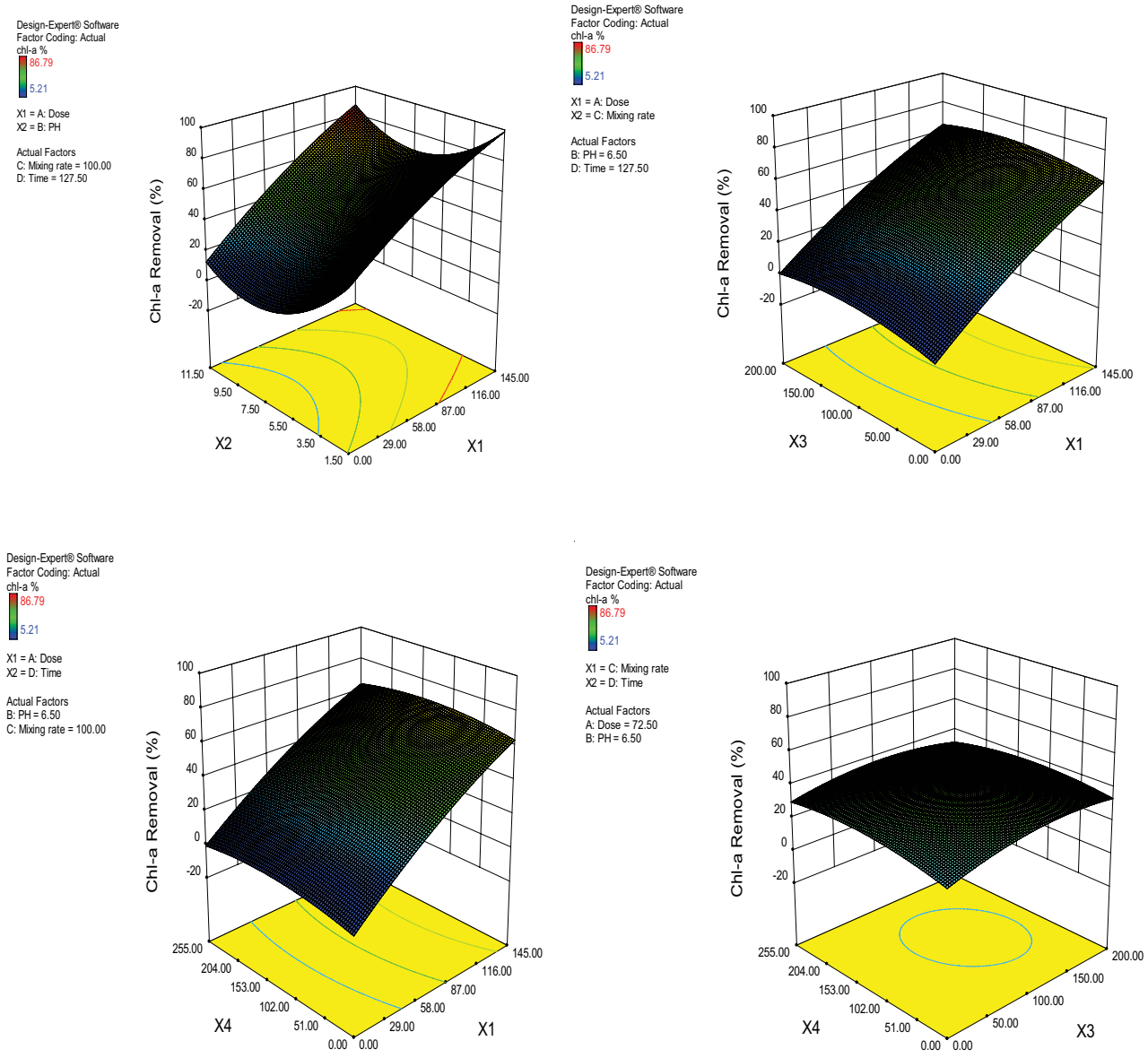


Fig. 4. Three-dimensional response surface plot for Chl-a reduction in freshwater by *S. commune* 104UTHM as a response of interaction between independent factors. x_1 (Inoculum size, mL); x_2 (pH); x_3 (mixing rate, rpm); x_4 (HRT, min).

it has to be mentioned that the team investigated the inactivation process in a culture medium and for long period of HRT (48 h) compared with this study which was conducted in a real sample and for short time (max HRT was 3 h and settling time was 6 h). The salinity of the water with high COD concentrations might inhibit the activity of algicidal substances produced by *S. commune* 104UTHM, where the inactivation of HABs was a result of the production of diffusible extracellular substances as stated by Redhead and Wright [26]. Du et al. [63] reported that *Trametes versicolor* degrades *Microcystis aeruginosa* by 96% within 60 h and explained the degradation process as a function for the extracellular enzymes activity. However, the maximum reduction of Chl-a by *S. commune* 104UTHM was 50% and might be enhanced by optimizing the algicidal process with different factors such as temperature with different ranges. Nonetheless, *S. commune* 104UTHM has more applicability for the inactivation of HABs in the environment compared with *T. abietinum* and *T. versicolor* due to the absence of spore production and toxins.

3.3. Degradation mechanism of *M. aeruginosa* cells

The results of SEM imaging showed that the fresh *M. aeruginosa* in the raw water sample (before the treatment) have spherical shape with smooth surface, where there was

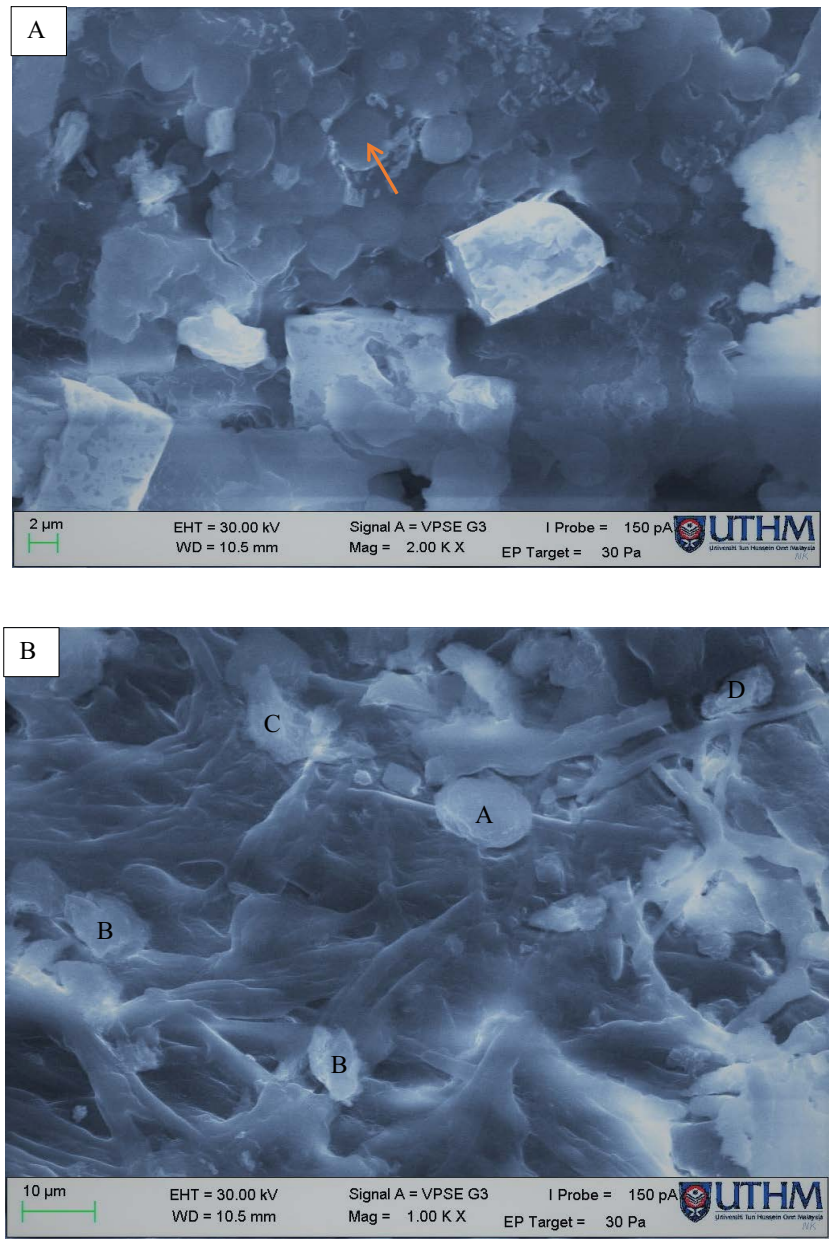


Fig. 5. SEM image shows destruction of *M. aeruginosa* cells in freshwater using *S. commune* 104UTHM, (a) before the inactivation (control); (b) after the inactivation. The label in Fig. 5b show the stages of the degradation process of the *M. aeruginosa* cells adsorbed into the *S. commune* mycelium, (A) *M. aeruginosa* cells adsorbed on *S. commune 104LD* without damage for the cell morphology; (B–D) show the damaged cells.

no influence on the algal cells under the normal environmental conduction (Fig. 5a). In contrast, the results in Fig. 4b revealed the SEM analysis for *M. aeruginosa* in the water sample after the bio-flocculation process. The results show that *M. aeruginosa* cell adsorbed to the mycelial networks of *S. commune* 104UTHM, without clear destruction of damage. In comparison, many of the *M. aeruginosa* have subject for algicidal activity and lost the spherical shape of the cells with clear damage in the cell wall. Jia et al. [25] suggested that inactivation of HABs was due to the direct preying capability of the fungus mycelium as well as suppressive effect on HABs by the substances secreted by the living fungi. Other studies have shown that the algal cells are lysed only by a fibrous glycocalyx or directly invaded after physical contact [64]. The study of Jia et al. [25] claimed that the inactivation of HABs took place in three stages included physical contacted of HABs with the fungal mycelia under a shaking condition. HABs were surrounded by the mucous membrane secreted by the fungal mycelia which lead to degrade the HABs cells. On the other hand, a similar mechanism of the algicidal bacteria lies by inhibiting the host antioxidase activities of algae. Many authors in the literature have claimed that the algicidal bacteria can promote the activity of antioxidase after a few hours of the inoculation into algae

culture [21,65–67]. The current study revealed two stages including the adsorption of *M. aeruginosa* to *S. commune* 104UTHM mycelia (Fig. 5b) which occur during the flocculation process and settling time and the degradation of *M. aeruginosa* cells was observed.

4. Conclusion

The algicidal activity of *M. aeruginosa* was optimized using *S. commune* 104UTHM through bio-flocculation process. The optimal inactivation rate was determined based on the reduction of Chl-a was recorded with 100 mL of the fungus inoculum size, pH 6, with 125 rpm of the mixing rate and within 135 min of HRT with 72% of the coefficient (R^2). The SEM analysis confirmed that the damage of *M. aeruginosa* cells were due to the physical contacted of the cells with the fungal mycelia during the flocculation process and secrete of algicidal substances by fungal mycelia which lead to degradation of HABs cells. It can be concluded that *S. commune* 104UTHM has high potential in inactivating *M. aeruginosa* through bio-flocculation. However, more independent factors with different ranges should be considered for further investigation.

Acknowledgements

The authors wish to thank The Ministry of Education Malaysia under Fundamental Research Grant Scheme (FRGS) Vot No. K101 (Enzymatic Degradation of Xenobiotics Organic Compounds in greywater), Universiti Tun Hussein Onn Malaysia (UTHM) under grant Tier 1 Vot No. H207 and Micropollutant Research Centre, Faculty of Civil and Environmental Engineering, UTHM for supporting this research.

References

[1] S. Jeong, K. Ishida, Y. Ito, S. Okada, M. Murakami, Bacillamide, a novel algicide from the marine bacterium, *Bacillus* sp. SY-1, against the harmful dinoflagellate, *Cochlodinium polykrikoides*, Tetrahedron Lett., 44 (2003) 8005–8007.

[2] K. Davidson, R. Gowen, P. Tett, E. Bresnan, P. Harrison, A. McKinney, S. Milligan, D. Mills, J. Silke, A. Crooks, Harmful algal blooms: how strong is the evidence that nutrient ratios and forms influence their occurrence?, Estuar. Coast. Shelf Sci., 115 (2012) 399–413.

[3] L. Villacorte, S. Tabatabai, N. Dhakal, G. Amy, J. Schippers, M. Kennedy, Algal blooms: an emerging threat to seawater reverse osmosis desalination, Desal. Wat. Treat., 55 (2015) 2601–2611.

[4] L. Grattan, S. Holobaugh, J. Morris Jr, Harmful algal blooms and public health, Harmful Algae, 57 (2016) 2–8.

[5] A. Magdaleno, M. Saenz, A. Juárez, J. Moretton, Effects of six antibiotics and their binary mixtures on growth of *Pseudokirchneriella subcapitata*, Ecotoxicol. Environ. Safety, 113 (2015) 72–78.

[6] J. Hao, B. Lian, R. Zhou, H. Liu, L. Shi, C. Liu, Microbial flocculant combined ferric trichloride facilitates floating aggregation of Microcystis aeruginosa for efficient removal, Desal. Wat. Treat., 57 (2016) 20483–20493.

[7] T. Nagai, K. Taya, I. Yoda, Comparative toxicity of 20 herbicides to 5 periphytic algae and the relationship with mode of action, Environ. Toxicol. Chem., 35 (2016) 368–375.

[8] J. Pohl, I. Saltsman, A. Mahammed, Z. Gross, B. Röder, Inhibition of green algae growth by corrole-based photosensitizers, J. Appl. Microbiol., 118 (2015) 305–312.

[9] M. Kuroiwa, T. Masuda, T. Omura, A. Wongrueng, K. Oguma, H. Sakai, M. Murakami, S. Takizawa, Membrane fouling in seawater desalination processes caused by harmful dinoflagellate Cochlodinium polykrikoides, Desal. Wat. Treat., 51 (2013) 950–957.

[10] M. Darwish, H. Abdulrahim, A. Hassan, B. Shomar, Reverse osmosis desalination system and algal blooms Part I: harmful algal blooms (HABs) species and toxicity, Desal. Wat. Treat., 57 (2016) 25859–25880.

[11] J. Park, J. Church, Y. Son, K. Kim, W. Lee, Recent advances in ultrasonic treatment: challenges and field applications for controlling harmful algal blooms (HABs), Ultrason. Sonochem., 38 (2017) 326–334.

[12] Y. Tao, X. Mao, J. Hu, H. Mok, L. Wang, D. Au, J. Zhu, X. Zhang, Mechanisms of photosynthetic inactivation on growth suppression of *Microcystis aeruginosa* under UV-C stress, Chemosphere, 93 (2013) 637–644.

[13] B. Ghernaout, D. Ghernaout, A. Saiba, Algae and cyanotoxins removal by coagulation/flocculation: a review, Desal. Wat. Treat., 20 (2010) 133–143.

[14] Y. Sun, H. Zheng, Z. Xiong, Y. Wang, X. Tang, W. Chen, Y. Ding, Algae removal from raw water by flocculation and the fractal characteristics of flocs, Desal. Wat. Treat., 56 (2015) 894–904.

[15] M. Marzbali, A. Mir, M. Pazoki, R. Pourjamshidian, M. Tabeshnia, Removal of direct yellow 12 from aqueous solution by adsorption onto spirulina algae as a high-efficiency adsorbent, J. Environ. Chem. Eng., 5 (2017) 1946–1956.

[16] C. Liao, X. Liu, High-cell-density cultivation and algicidal activity assays of a novel algicidal bacterium to control algal bloom caused by water eutrophication, Water Air Soil Pollut., 225 (2014) 2120.

[17] G. Weiss, D. Kovalerchick, J. Lieman-Hurwitz, O. Murik, R. De Philippis, S. Carmeli, A. Sukenik, A. Kaplan, Increased algicidal activity of *Aeromonas veronii* in response to Microcystis aeruginosa: interspecies crosstalk and secondary metabolites synergism, Environ. Microbiol., 21 (2019) 1140–1150.

[18] X. Guo, X. Liu, L. Wu, J. Pan, H. Yang, The algicidal activity of Aeromonas sp. strain GLY-2107 against bloom-forming Microcystis aeruginosa is regulated by N-acyl homoserine lactone-mediated quorum sensing, Environ. Microbiol., 18 (2016) 3867–3883.

[19] L. Backer, D. McGillicuddy Jr, Harmful algal blooms: at the interface between coastal oceanography and human health, Oceanography (Washington, DC), 19 (2006) 94.

[20] R. Sun, P. Sun, J. Zhang, S. Esquivel-Elizondo, Y. Wu, Microorganisms-based methods for harmful algal blooms control: a review, Bioresour. Technol., 248 (2018) 12–20.

[21] C. Liao, X. Liu, R. Liu, L. Shan, Two novel algicidal isolates kill *Chlorella pyrenoidosa* by inhibiting their host antioxidase activities, Appl. Biochem. Biotechnol., 177 (2015) 567–576.

[22] Y. Yu, Zeng, J. Li, C. Yang, X. Zhang, F. Luo, X. Dai, An algicidal Streptomyces amritsarensis strain against Microcystis aeruginosa strongly inhibits microcystin synthesis simultaneously, Sci. Total Environ., 650 (2019) 34–43.

[23] E. Espinosa-Ortiz, E. Rene, K. Pakshirajan, E. Hullebusch, P. Lens, Fungal pelleted reactors in wastewater treatment: applications and perspectives, Chem. Eng. J., 283 (2016) 553–571.

[23] E. Soliman, E. Ali, G. Masoud, A. Mohamed, S. El-Moneim, Antifungal activities of methanolic extracts of some marine algae (ulvaceae and dictyotaceae) of Benghazi Coasts, Libya, Egypt. J. Exp. Biol. (Bot.), 9 (2013) 75–79.

[24] G. Han, X. Feng, Y. Jia, C. Wang, X. He, O. Zhou, X. Tian, Isolation and evaluation of terrestrial fungi with algicidal ability from Zijin Mountain, Nanjing, China, J. Microbiol., 49 (2011) 562.

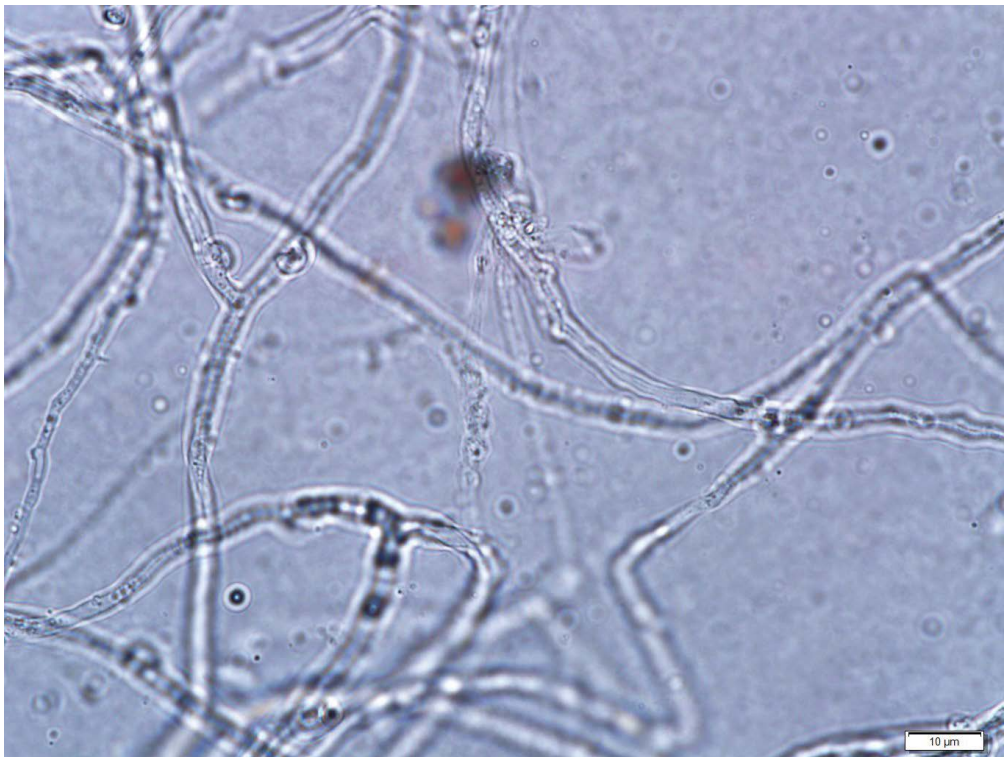
[25] Y. Jia, J. Du, H. Fang, G. Zhao, X. Tian, Inhibition of freshwater algal species by co-culture with two fungi, Mat. Sci. Eng. C, 33 (2013) 2451–2454.

[26] K. Redhead, S. Wright, Isolation and properties of fungi that lyse blue-green algae, Appl. Environ. Microbiol., 35 (1978) 962–969.

[27] P. Stamets, Comprehensive Assessment of Mycofiltration Biotechnology to Remove Pathogens from Urban Storm Water, Final Report submitted to US Environmental Protection Agency, 2010.

[28] R. Paterson, N. Lima, Climate change affecting oil palm agronomy, and oil palm cultivation increasing climate change, require amelioration, Ecol. Evolut., 2018.
[29] W. Zhou, Y. Cheng, Y. Li, Y. Wan, Y. Liu, X. Lin, R. Ruan, Novel fungal pelletization-assisted technology for algae harvesting and wastewater treatment, Appl. Biochem. Biotechnol., 167 (2012) 214–228.
[30] H. Bhamare, H. Jadhav, R. Sayyed, Statistical optimization for enhanced production of extracellular laccase from *Aspergillus* sp. HB_RZ4 isolated from bark scrapping, Environ. Sus., 1 (2018) 159–166.
[31] M. Kumar, G. Singh, S. Arya, J. Bhatti, P. Sharma, Artificial Neuro-Fuzzy Inference System (ANFIS) based validation of laccase production using RSM model, Biocatal. Agr. Biotechnol., 14 (2018) 235–240.
[32] L. Copete-Pertuz, F. Alandete-Novoa, J. Plácido, G. Correa-Londoño, A. Mora-Martínez, Enhancement of ligninolytic enzymes production and decolourising activity in *Leptosphaerulina* sp. by co–cultivation with *Trichoderma viride* and *Aspergillus terreus*, Sci. Total Environ., 646 (2019) 1536–1545.
[33] C. Liao, X. Liu, L. Shan, Optimization of liquid media and biosafety assessment for algae-lysing bacterium NP23, Canadian J. Microbiol., 60 (2014) 593–597.
[34] APHA, Standard Methods for the Examination of Water and Waste Water. 22nd Edition, American Public Health Association, American Water Works Association, Water Environment Federation, 2012.
[35] E. Noman, A. Al-Gheethi, N. Rahman, H. Nagao, M. Kadir, Assessment of relevant fungal species in clinical solid wastes, Environ. Sci. Pollut. Res., 23 (2016) 19806–19824.
[36] I. Promputtha, R. Jeewon, S. Lumyong, E. McKenzie, K. Hyde, Ribosomal DNA fingerprinting in the identification of non sporulating endophytes from *Magnolia liliifera* (Magnoliaceae), Fungal Diversity (2005).
[37] J. Fell, T. Boekhout, A. Fonseca, G. Scorzetti, A. Statzell-Tallman, Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis, Int. J. Sys. Evolut. Microbiol., 50 (2000) 1351–1371.
[38] KDHE (2015) Harmful Algae Blooms: A tool kit for health departments. kansas department of health and environment bureau of epidemiology and public health informatics, 1000 SW Jackson Street, Suite 330. Topeka, KS. 66612–1365. www.kdheks.gov/algae-illness/index.htm.
[39] S. Jeffrey, G. Humphrey, New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton, Biochemie und physiologie der pflanzen, 167 (1975) 191–194.
[40] Environmental Quality Report (EQR), 2006, Retrieved from https://www.doe.gov.my/portalv1/en/lainlain/laporan-kualiti-alam-sekeliling-eqr-2013/316956.
[41] X. Jiang, C. Ren, C. Hu, Z. Zhao, Isolation and algicidal characterization of Bowmanella denitrificans S088 against *Chlorella vulgaris*, World J. Microbiol. Biotechnol., 30 (2014) 621–629.
[42] E. Eckersley, B. Berger, An engineered polysaccharide lyase to combat harmful algal blooms, Biochem. Eng. J., 132 (2018) 225–232.
[43] P. Amee, P. Nagadesi, A. Susy, A. Arya, Morphology, anatomy and cultural characters of two wood decaying fungi *Schizophyllum commune* and *Flavadon flavus*, Mycol. Plant Pathol., 39 (2009) 27.
[44] A. Saha, P. Mandal, S. Dasgupta, D. Saha, Influence of culture media and environmental factors on mycelial growth and sporulation of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl, J. Environ. Biol., 29 (2008) 407.
[45] Y. Choi, K. Hyde, W. Ho, Single spore isolation of fungi, Fungal Diver., 3 (1999) 29–38.
[46] E. Won, J. Shin, S. Lim, M. Shin, S. Suh, D. Ryang, Molecular identification of *Schizophyllum commune* as a cause of allergic fungal sinusitis, Ann. Lab. Med., 32 (2012) 375–379.
[47] A. Chowdhary, H. Randhawa, S. Gaur, K. Agarwal, S. Kathuria, P. Roy, C. Klaassen, J. Meis, *Schizophyllum commune* as an emerging fungal pathogen: a review and report of two cases, Mycoses, 56 (2013) 1–10.
[48] H. Clarke, D. Griffiths, Ascospores of some common species of Eurotium (*Aspergillus glaucus*) as shown by scanning electron microscopy, Tran. British Mycol. Soc., 55 (1970) 117-IN13.
[49] W. Eduard, P. Sandven, B. Johansen, R. Bruun, Identification and quantification of mould spores by scanning electron microscopy (SEM): analysis of filter samples collected in Norwegian saw mills, In Inhaled Particles, VI (1988) 447–445.
[50] E. Noman, A. Al-Gheethi, N. Ab Rahman, N. Hideyuki, T. Talip, R. Mohamed, M. Ab Kadir, Phenotypic identification of *Penicillium* spp. isolated from clinical wastes based on microstructure characteristics, Malay. J. Microbiol., 14 (2018) 88–95.
[51] W. Buzina, D. Lang-Loidolt, H. Braun, K. Freudenschuss, H. Stammberger, Development of molecular methods for identification of *Schizophyllum commune* from clinical samples, Clinic. Microbial., 39 (2011) 2391–2396.
[52] S. Balajee, L. Sigler, M. Brandt, DNA and the classical way: identification of medically important molds in the 21st century. Medical Mycol., 45 (2007) 475–490.
[53] S. Lee, Checklist of Fungi of Malaysia, Forest Research Institute Malaysia, 2012.
[54] J. Hollister, B. Kreakie, Associations between chlorophyll a and various microcystin health advisory concentrations, F1000 Research, 5 (2016) 151, doi: 10.12688/f1000research.7955.2.
[55] M. Mu, Z. Fan, H. Pei, X. Yuan, S. Liu, X. Wang, Isolation and algae-lysing characteristics of the algicidal bacterium B5, J. Environ. Sci., 19 (2007) 1336–1340.
[56] C. Park, J. Lee, S. Kim, Y. Park, Selective algicidal action of peptides against harmful algal bloom species, PLoS One, 6 (2011) e26733.
[57] H. Zhang, H. Wang, W. Zheng, Z. Yao, Y. Peng, S. Zhang, Z. Hu, Z. Tao, T. Zheng, Toxic effects of prodigiosin secreted by *Hahella* sp. KA22 on Harmful Alga Phaeocystis globosa, Fron. Microbiol., 8 (2017) 999.
[58] V. Grimm, U. Berger, F. Bastiansen, S. Eliassen, V. Ginot, J. Giske, J. Goss-Custard, T. Grand, S. Heinz, G. Huse, A. Huth, A standard protocol for describing individual-based and agent-based models, Ecol. Model, 198 (2006) 115–126.
[59] Z. Gang, H. Ting-Lin, T. Chi, L. Zhan-Peng, H. Wen-Jie, H. Hong-Da, L. Chen, Settling behaviour of pellet flocs in pelleting flocculation process: analysis through operational conditions. Water Sci. Technol., 62 (2010) 1346–1352.
[60] H. Farajnezhad, P. Gharbani, Coagulation treatment of wastewater in petroleum industry using poly aluminum chloride and ferric chloride, IJRRAS, 13 (2012) 306–310.
[61] S. Vishali, R. Karthikeyan, A comparative study of *Strychnos potatorum* and chemical coagulants in the treatment of paint and industrial effluents: an alternate solution, Sep. Sci. Technol., 49 (2014) 2510–2517.
[62] A. Aljuboori, A. Idris, N. Abdullah, R. Mohamad, Production and characterization of a bioflocculant produced by *Aspergillus flavus*, Bioresour. Technol., 127 (2013) 489–493.
[63] J. Du, G. Pu, C. Shao, S. Cheng, J. Cai, L. Zhou, Y. Jia, X. Tian, Potential of extracellular enzymes from *Trametes versicolor* F21a in *Microcystis* spp. degradation, Mat. Sci. Eng. C, 48 (2015) 138–144.
[64] Y. Li, W. Hongyi, M. Komatsu, K. Ishibashi, L. Jinsan, T. Ito, T. Yoshikawa, H. Maeda, Isolation and characterization of bacterial isolates algicidal against a harmful bloom-forming cyanobacterium *Microcystis aeruginosa*, Biocontrol Sci., 17 (2012) 107–114.
[65] C. Liao, X. Liu, R. Liu, L. Shan, Characterization and effects of two algicidal isolates on antioxidase activities of *Chlorella pyrenoidosa*, Environ. Progr. Sustain. Energy, 34 (2015) 1647–1651.
[66] S. Shi, D. Tang, Y. Liu, Effects of an algicidal bacterium *Pseudomonas mendocina* on the growth and antioxidant system of *Aphanizomenon flosaquae*, Current Microbiol., 59 (2019) 107–112.
[67] J. Luo, Y. Wang, S. Tang, J. Liang, W. Lin, L. Luo, Isolation and identification of algicidal compound from *Streptomyces* and algicidal mechanism to *Microcystis aeruginosa*, PloS One, 8 (2013) e76444.

Appendix A



Appendix B

Appendix B Shows upper layer of *M. aeruginosa* on freshwater surface after 24 h at 4°C.