PREPARATION AND CHARACTERISATION OF HYBRID CHITOSAN FILMS FOR THE MITIGATION OF *Alexandrium minutum*

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by

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LIST OF SYMBOLS

$-NH_2$	Amine
°C	Degree Celcius
e^-	Electrons
h^+	Holes
-OH	Hydroxyl
•OH	Hydroxyl radicals
%	Percent
$O_2^{\bullet -}$	Superoxide anion

LIST OF ABBREVIATIONS

ATR-FTIR	Attenuated total reflection Fourier transform infrared
Cs	Chitosan
CB	Conduction band
EDX	Energy dispersive X-ray
eV	Electrovolt
Gly	Glycerol
GPTEOS	(3-glycidyloxypropyl)trimethoxysilane
HAB	Harmful algal bloom
Hrs	Hours
HCl	Hydrochloric acid
LbL	Layer-by-Layer
HNO_3	Nitric acid
PAC	Polyaluminium chloride
PZC	Point of zero charge
ROS	Reactive oxygen species
RH	Rice husk
RHA	Rice husk ash
SEM	Scanning electron microscopy
SiO_2	Silica
NaOH	Sodium hydroxide
SD	Standard deviation
SI	Swelling index
TGA	Thermogravimetric analysis
TiO_2	Titanium dioxide

UV-Vis	Ultraviolet-visible
VB	Valence band
WS	Wettability test
XRD	X-ray diffraction

LIST OF APPENDICES

PENYEDIAAN DAN PENCIRIAN FILEM HIBRID KITOSAN UNTUK MITIGASI *Alexandrium minutum*

ABSTRAK

Ledakan alga berbahaya telah menjadi isu yang semakin meningkat di seluruh dunia sejak beberapa dekad yang lalu, mendatangkan kemudaratan kepada ekosistem akuatik dan kualiti air minuman. Fenomena ini perlu dimitigasi dengan menggunakan agen mitigasi yang sesuai untuk mengurangkan kesan negatif. Oleh itu, penyelidikan ini menyiasat kebolehlaksanaan filem kitosan (CH), filem hibrid kitosan/silika (CH/SiO_2) dengan penambahan gliserol (CH/SiO_2/gly) dan (3-glycidyloxypropyl)trimethoxysilan (CH/SiO_2/GPTEOS), dan filem hibrid kitosan/TiO_2 yang disediakan melalui kaedah sintesis satu periuk untuk mitigasi sel alga toksik, *Alexandrium minutum*. Corak XRD bagi filem CH mengesahkan puncak yang berkaitan dengan satah kristalografi (002), (101) dan (220) kitosan masing-masing pada $2\theta = 10.1°$, $19.8°$ dan $22°$. Puncak XRD kelihatan lebih luas selepas pemautsilangan dengan SiO_2, gliserol dan GPTEOS, menunjukkan peningkatan dalam sifat amorfus polimer, manakala penambahan TiO_2 menyebabkan puncak kitosan menjadi lemah dan meluas selepas penggabungan. Analisis TGA filem menunjukkan penurunan berat filem selepas penggabungan SiO_2, gliserol, GPTEOS dan TiO_2. Tambahan pula, spektrum UV-vis bagi semua filme mendedahkan filem tersebut mempunyai jalur penyerapan di rantau UV. Kajian mitigasi *Alexandium minutum* dilakukan dengan menggunakan semua filem. Di antara semua filem, CH/0.2TiO_2 (1 mL) memberikan kecekapan penyingkiran yang lebih tinggi 76.1 ± 13.83 % pada 72 jam. Imej SEM dan mikroskopik digital

bagi filem yang digunakan mendedahkan kehadiran sel alga pada permukaan filem. Filem ini boleh dipisahkan dengan mudah daripada kultur alga tanpa pemendapan. Filem ini juga menunjukkan keterbiodegradasikan yang baik kerana semua filem telah terbiodegradasi sepenuhnya selepas 30 hari penanaman.

PREPARATION AND CHARACTERISATION OF HYBRID CHITOSAN FILMS FOR THE MITIGATION OF *Alexandrium minutum*

ABSTRACT

Harmful Algal Bloom (HAB) has been a growing issue worldwide for the past few decades, posing harm to aquatic ecosystems and drinking water quality. This phenomenon must be mitigated using a proper mitigation agent to reduce the negative impact. Herein, the research investigates the feasibility of a chitosan film (CH), hybrid chitosan/silica film (CH/SiO_2) a with the addition of glycerol (CH/SiO_2/gly) and (3-glycidyloxypropyl)trimethoxysilane (CH/SiO_2/GPTEOS), and hybrid chitosan/TiO_2 films were prepared via a one-pot synthesis method and characterised to mitigate toxic algae cells, *Alexandrium minutum*. The XRD pattern of the CH film confirms the peak related to the crystallographic planes (002), (101) and (220) of chitosan at $2\theta = 10.1°$, 19.8° and 22°, respectively. The XRD peaks appear broader after crosslinking with SiO_2, glycerol and GPTEOS, indicating an increase in the polymer's amorphous nature, while the addition of TiO_2 caused the peak of chitosan became weak and broaden after the incorporation. The films' TGA analysis shows that the film's weight loss reduces after the incorporation of SiO_2, glycerol, GPTEOS and TiO_2. In addition, the UV-vis spectra revealed that the films has an absorption innd at the UV region. All the films were subjected to the mitigation of *Alexandium minutum*. Among all the films, CH/0.2TiO_2 (1 mL) gave a higher removal efficiency 76.1± 13.83 % at 72 hrs. The SEM and digital microscopic images of the used films revealed the presence of algae cells on the surface of the films. The films can be easily separated from the algae culture

without sedimentation. The films also show good biodegradability as all the films were fully degraded upon 30 days of burial.

CHAPTER 1

INTRODUCTION

1.1 Baseline study

Harmful algal bloom (HAB), commonly called “red tides” in Malaysia, is a natural aquatic phenomenon that occurs worldwide. A single occasion of HAB can cause a massive loss to the aquaculture and tourism industries. Besides, HAB also reduces the water quality for the reservoir (Kim et al., 2021). Most algae are not harmful, but they cause serious problems such as causing discolouration of water, producing a smelly odour, or bad water taste. Some HAB species produce neurotoxins, such as domoic acid, saxitoxin, and gonyautoxin. Consumption of fish or shellfish infected with these neurotoxins by humans and animals can be lethal.

Besides, the algal bloom depletes the oxygen concentration available in the water through respiration. As a consequence, many organisms, particularly caged fish, will die. As the algae die at the end of the blooming season, the microbes will use the remaining oxygen to decompose the algae cells creating an oxygen-deprived zone known as hypoxic dead zones. In this zone, neither fish nor plants can survive.

Some algae tend to grow faster, denser, and persist longer due to nutrient enrichment, changes in climate, and habitat disturbance. Excess nutrients and minerals such as nitrogen and phosphorus in the water column, known as eutrophication, will induce extreme blooming. It has been suggested that climate change will lead to HAB species becoming more competitive than non-HAB species, becoming more toxic, and causing the occurrence of HAB to be on the rise (Kidwell, 2015).

Alexandrium minutum is one of the commonly reported toxic algae over several geographical areas and in a wide range of coastal hydrographic regimes (Usup et al., 2002). It is a single-celled dinoflagellate responsible for Paralytic Shellfish Poisoning (PSP) outbreaks in the Mediterranean Sea and Southeast Asia (Lim et al., 2004). The proliferation of this species worldwide was correlated to the nutrient-rich freshwater input and water salinity. *Alexandium minutum* can disperse since it can grow and generate a resting cyst under all conditions (Valbi et al., 2019). The red water discolouration (*Alexandium minutum*) was firstly observed in the Mediterranean sea at Alexandria Harbour, Egypt (Halim, 1960). Mussel farms along the Adriatic Sea's northwestern coast were contaminated by this species (Riccardi et al., 2009). In 2001, six people were hospitalised in Malaysia and one casualty due to a shellfish intoxication of *Alexandium minutum* (Lim et al., 2004).

The term "mitigation" is used to describe the actions taken to deal with an existing or ongoing bloom by taking necessary precautions to reduce the negative impact of the bloom. The devastating effects of HAB cause many researchers to find possible ways to overcome this occurrence. Several approaches are being explored, including chemical, biological and physical manipulations. The applications, effectiveness, and downside of each approach were described in Chapter 2.

Currently, studies are focused on using clay particles and polyaluminium chloride (PAC) as a successful mitigation agent which lowers the impact of HAB. However, the attachment of HAB cells and the toxins onto clay particles can be deemed weak. The possibility for the algae cells to detach from the clay and continue to reproduce is relatively high. The use of PAC as a flocculant to promote the attachment of HAB cells raised concern among researchers due to the known toxicity and promotes secondary contamination. In practice, the PAC and clays undergo sedimentation together to the bottom of the water column (Yu et al., 2017). In the long run, this practice may also affect the seawater balance.

The mitigation agents should have the following properties to mitigate the HAB: long-lasting effects, cost-effective, non-selective towards HAB species, safe towards aquatic organisms, and has buoyancy effect to avoid sedimentation. By considering all these factors, hybrid chitosan films are developed in this study via a simple one-pot solvent casting method to mitigate *Alexandium minutum* via a physical approach on the laboratory scale.

Chitosan (CS) can be proposed to replace PAC and clay particles due to its remarkable properties such as low toxicity, high biodegradability, and high film-forming ability (Kean & Thanou, 2010; Yang et al., 2014; Sumarni et al., 2016). However, the high alkalinity and ionic strength of seawater may prevent the unfolding of the polymer chain, thus weakening the netting and the bridging properties of the chitosan (Qun & Ajun, 2006; Bhalkaran & Wilson, 2016).

Hence, the matrix of chitosan needs to be modified to enhance the properties. Various functional groups can be introduced into chitosan due to numerous amines ($-NH_2$) and hydroxyl groups (-OH) on its framework. Silica (SiO_2) and titanium dioxide (TiO_2) are mostly introduced nanomaterials in the chitosan matrix to form crosslinks with chitosan molecules for better membrane stability, eventually improving their performances. Furthermore, chemical modification is also hypothesised to improve the adhesion of the algae cells and toxins. Previous studies on chitosan and modified chitosan composite applications using materials mentioned above were discussed in the literature review.

Several methods have been reported to synthesise a chitosan film or modified chitosan film. Layer-by-layer (LbL), nanoimprinting, and solvent casting methods are the common techniques used to obtain chitosan-based films. The LbL self-assembly takes place through electrostatic forces between molecules and particles with opposite charges. This process is repressed by the equilibrium of desorption and adsorption in cationic and anionic solutions as well as polyelectrolyte deposition, which includes basic steps of plunging, lifting, and drying in a repeated cycle (Bahrami et al., 2020). Meanwhile, nanoimprinting is a procedure that allows the imprinting of a design onto a thin film, typically in thermoplastic material. The film is plunged on a polymeric or silicon mould with the ideal design using controlled high temperature and pressure. The design is reproduced contrarily on the film upon removing the mould (De Masi et al., 2019). Solvent casting is the simplest technique that requires simple instrumentation with lower temperatures to produce the film. The film-forming solution will be dispersed on a designed mould and placed in the oven at the range of 50-60 ^{O}C. This helps the solvent evaporation take place, leaving a solid chitosan film that can be stripped off easily from the mould for further application.

1.2 Problem statements

Clays and modified clays that are currently being used tend to sediment together with algae cells and the toxins. The algae cells' sedimentation and toxins are dangerous to the benthic communities. The usage of PAC as a flocculant to promote the attachment of HAB cells can disrupt the seawater balance that eventually kills the other aquatic organisms.

One challenging aspect regarding the use of photosensitisers such as TiO_2 and SiO_2 in the seawater systems is that numerous amounts of salt ions present in the system will reduce the photocatalytic activity or inactivate the photocatalyst. In order to increase the production of reactive oxygen species (ROS), TiO_2 and SiO_2 need to be modified with net bridging properties material such as CS to enhance the performances.

1.3 Research objectives

This thesis reports the mitigation of *Alexandrium minutum* using hybrid chitosan films. Thus, the objectives of the research are:

1. To synthesize the hybrid chitosan films using a simple one-pot solvent casting method.
2. To evaluate the physicochemical properties of the hybrid chitosan films using different microscopic and spectroscopic analyses.
3. To investigate the films' ability to mitigate *Alexandium minutum* using the physical method.
4. To investigate the biodegradability of the films in the soil.

1.4 Scope of research

The focus of this research is to investigate the application of chitosan film, chitosan/SiO_2 film, chitosan/SiO_2/glycerol film, chitosan/SiO_2/(3-glycidyloxypropyl)trimethoxy silane and chitosan/TiO_2 films in the mitigation of *Alexandium minutum.* The synthesised films were characterised using various spectroscopic and microscopic analyses. Based on the collected data, possible mitigation mechanisms using the films were explored.

1.5 Outline of thesis

This thesis consists of six chapters as follows:

Chapter 1: General introduction on the HAB phenomena and the correlated mitigation agents. The problem statements and objectives of the research are stated as well.

Chapter 2: This chapter discusses a detailed literature review on HAB mitigation focusing on biological, chemical, and physical approaches. The application of chitosan and modified chitosan composite using SiO_2 and TiO_2 were discussed as well.

Chapter 3: The details on the materials used and the procedure for synthesising and characterising the films were mentioned.

Chapter 4: The results obtained from each characterisation and mitigation of *Alexandium minutum* using chitosan film and hybrid chitosan/silica film with the addition of glycerol and (3-glycidyloxypropyl)trimethoxysilane were discussed in this chapter.

Chapter 5: The results obtained from each characterisation and mitigation of *Alexandium minutum* using hybrid chitosan/TiO_2 films were discussed in this chapter. The possible mechanism that occurs during the mitigation was proposed.

Chapter 6: The overall conclusion and future recommendations were included in this chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 Harmful algal bloom (HAB) - Theory of Occurrence

Algal bloom takes place in both freshwater and marine environments. When the concentration of single-celled algae increases, the pigment from the different types of algae can turn the water bodies cloudy and foamy with patches of different colours such as blue-green, orange, yellowish-brown, and red (Zohdi & Abbaspour, 2019). The three major groups of microalgae that causes HAB are diatoms (e.g., *Pseudo-nitzchia, Skeletonema costatum*), dinoflagellates (e.g., *Alexandrium*, *Pyrodinium, Karenia* and *Prorocentrum*), and cyanobacteria (e.g., *Microcystis*, and *Nodularia*) (Lin et al., 2015; Ralston & Moore, 2019).

Research have suggested that algae cells grow faster, become denser and persist longer due to nutrient enrichment, changes in climate and habitat disturbance. An excess of nutrients and minerals such as nitrogen and phosphorus in the water column, known as eutrophication, will induce extreme blooming. The usage of high nitrogen urea fertilisers in agriculture, sewage spills, and improper wastewater disposal can increase nutrient levels in water (Sun et al., 2018; Paerl et al., 2018; Gilbert, 2020). Algal blooms are unpredictable since this phenomenon is also related to climate changes and habitat modifications. Global warming, rising sea level, stratification, ocean acidification, wind speed, and cyclone intensity are important factors that may trigger HAB (Anderson et al., 2015; Havens & Paerl, 2015; Wells et al., 2015; Pal et al., 2020). It has been suggested that climate change will lead to HAB species becoming more competitive than non-HAB species, becoming more toxic with higher biomass growth (Wells et al., 2019).

Intense blooming occurs when the temperature of surface waters lie in the range of 20-30 ºC, whereas algae growth rate slows down at a temperature less than 16 °C. Higher temperatures (> 35 ºC) can be fatal for particular algae species (Singh & Singh, 2015). The blooming accelerates when sodium chloride (NaCl) (salinity) content in the water is at 0-5% (Pal et al., 2020). Higher light intensity will increase the photosynthesis process of phytoplankton, increasing the growth rate (Zohdi & Abbaspour, 2019).

2.2 Chronology on the reported HAB cases

There have been numerous reported cases of HAB since a few decades ago. However, the severity has increased over the past few years. In 2015, the *Karlodinium australe* bloom in the East and West Johor Straits, Malaysia, caused the death of 500 to 600 tons of fish (Khew, 2015). In the same year, Sonoma County public health warned its people to avoid the Russian River when a dog died after swimming in the water that tested positive for blue-green algae toxin (Moore, 2015). In 2016, consumption of paralytic shellfish toxins (PST)-contaminated mussels caused poisoning at Qinhuangdao, China (Yu et al., 2018), and a massive bloom of *Creratium furca* had killed 47 tonnes of fish farmed and 300 kilograms of wild fish in northern Vietnam (Huang, 2016). The Singapore River was temporarily polluted as it turned emerald green and had a pungent smell due to phytoplankton's bloom in 2017 (Boh, 2017). The West Coast of Florida has experienced the blooming of the toxic alga *Karenia brevis,* which have brought millions of dead fish washed up on beaches and hundreds of manatees, turtles, and other marine animals in 2018 (Resnick, 2018). Two lakes in Northwest Ontario confirmed the blooming of blue-green algae, potentially posing a risk to human and animal health in 2020 (Rinne, 2020). Massive bloom of *Margelifidinium* sp. had killed tens of thousands of farmed fish in Penang and Perak, Malaysia, in May 2020 (Chern, 2020). The neurotoxin-producing dinoflagellate *Alexandrium catenellais* was detected at high concentrations and distribution in the Alaskan Arctic's bottom sediments and surface waters in 2021 (Anderson et al., 2021). Almost 476 algal bloom outbreaks were reported in 41 states in America in 2021. The highest cases were reported in Massachusetts (54 cases), followed by 47 cases identified in Florida (Schechinger, 2021).

The severity of this phenomenon exhorts many researchers to find the possible way to minimise HAB occurrences and measures for its control. Many approaches have been discovered to control and inhibit bloom formation. These approaches can be classified into biological, chemical and physical approaches.

2.3 Several approaches to inhibit HAB

2.3.1 Biological approach

The biological approach is considered the most promising approach due to its environmental friendliness and high algicidal potential (Backer et al., 2015; Harke et al., 2016). The biological method includes grazing the algae cells using aquatic organisms such as zooplankton, fish and bivalves, including microorganism-based methods (bacteria, vviruses and fungi). Using zooplankton to inhibit the HAB is considered a natural approach. The advantages of using zooplankton are low-cost removal, no secondary pollution and eco-friendly, but zooplankton cannot be applied in oxygen-poor conditions (Paerl et al., 2018). Filter-feeding fish and bivalves are always an option to remove HAB since some fish and bivalves can ingest and digest the toxin by themselves and balance the ecosystem. Tilapia , bighead carp, silver carp and mussels are used in many countries to control the cyanobacteria bloom.

The virus lytic cycle involves the reproduction of viruses using a host cell for manufacturing more viruses, followed by eventual release from the cell. Figure 2.1 shows the five stages of the virus lytic cycle. The first stage is the attachment of the phage onto the host cell, followed by the DNA penetration from the phage into the host cell. The third stage is biosynthesis, where the phage DNA replicates, and phage proteins are made. The fourth stage is the maturation of the new phage, and finally, the cell undergoes lysis, releasing the new phage. The advantage of this process is that the algae cells could be permanently killed (Ryu, 2017; Weynberg, 2018). However, the new phage will attack other marine organisms, producing more phages.

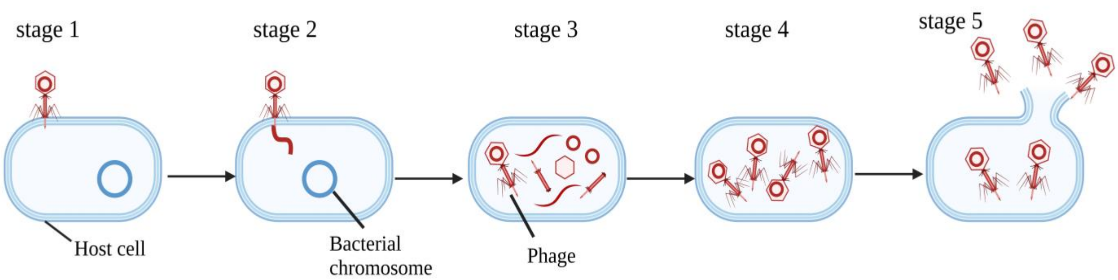

Figure 2.1: The general stages of the virus lytic cycle (The viral life lytic, accessed on 18th September 2021)

Fungal flocculants have been well studied for wastewater treatment and reducing microalgal biomass. Fungi act as natural flocculants by interacting with algal cells, leading to microbial aggregation (Sun et al., 2018). Formation and precipitation of fungus–alga pellets result in algae removal from water bodies through the pelletisation process. The pelletisation process is influenced by the cultivation parameters, such as pH values, salinity, and rheological behaviour (Espinosa-Ortiz et al., 2016). However, most microorganism-based experiments have been conducted in laboratories and on a small scale. The success rate appears relatively low in field management, even though many laboratory-scale reports were a success.

Seaweeds have also been identified as a potential mitigating agent. The presence of seaweed inhibits the HAB's growth through allelopathy effects and by competing for nutrients, such as nitrogen (Huo et al., 2011). The allelochemicals produced by the seaweeds will act as either growth stimulators or inhibitors. Several allelochemicals extracted from seaweed that showed an inhibitory effect on the algae cell growth are dithiolane, trithiane (Tang & Gobler, 2011), loliolide, N-phenethylacetamide, squamolone and 2-ethylidene-4-methylsuccinimide (Lu et al., 2011). These chemical compounds produced by the seaweeds did not harm other marine organisms. However, a large amount of seaweed is required to achieve the inhibitory effect. Even though field studies have been reported, most studies have focused on lab-based experiments. Table 2.1 summarises the different biological approaches for the mitigation of HAB.

Table 2.1: The latest reports on HAB mitigation via different biological approaches.

Method	Mitigation agent	Algae species	Result	Drawback	Ref.
	Notodiaptomus iheringe	*Microcystis aeruginosa Cryptomonas*	*N.iheringi* reduced the of Cryptomonas to negative value while increased the production of *Microcystis.*	Selective grazing on specific species.	(Leitão et al., 2018)
Grazing	Zooplankton	*Cyanothece* sp.	Grazing rate of 200-1000 μm zooplankton size class were higher in February (0.58 ± 0.093 SE d^{-1}), while significantly lower rates were observed in July (0.18 ± 0.045 SE d^{-1}).	Decreased in grazing impact due to increased salinity level.	(Du Plooy et al., 2019)
	Nile Tilapia	Cyanobacteria	Almost 60% of cyanobacteria was removed.	Increase in water transparency and ammonium concentration.	(Salazar Torres et al., 2015)
	Hyriopsis cumingii	*Microcystis aeruginosa*	High grazing impacts on *M.aeruginosa.*	Cannot perform in oxygen-poor zones.	(Görgényi et al., 2015)
	Paracoccus sp. Y42	*Prorocentrum donghaiense*	-5 % Y42 supernatant shows 90 % of algae cell removal in 72 h. -Stable in wide pH (3-12) and under different temperatures.	The inhibition of the HAB cell was species specific.	(Zhang et al., 2018)
Bacteria	Cultivable pelagic bacteria	*Pyrodinium bahamense*	Cell lysis and decline in total cell abundance were observed.	Formation of *Pyrodinium* pellicle cyst when exposed to bacterial cell.	(Dungca-Santos et al., 2019)
	Shewanella sp. IRI-160 cells immobilised within alginate beads	*Karlodinium veneficum Prorocentrum minutum*	The cell growth of *K.veneficum* and *P.minutm* increased by 1.44- and 1.62-fold, respectively, as compared to a control within a 6 day exposure	Increased the growth rate of the cell.	(Wang & Coyne, 2020)

Virus	Ma-LEP	*Microcystis aeruginosa*	Ma-LEP has reduced the bloom intensity of *M.aeruginosa* in 14 days	The inhibition took longer time while the production of Ma-LEP could be threat for other organisms.	(Jiang et al., 2019)
	HcRNAV	*Heterocapsa circularisquama*	Virus and sediment treatment was very effective in mitigating. *H. circularisquama* bloom under field conditions.	-Sedimentation of mitigation agent. -Might affect biodiversity	(Nakayama et al., 2020)
Fungi	Endophytic fungus	*Chlorella furca*	Shows algicidal properties against *Chlorella fusca* in 48 h.	- The fungus might affect the biodiversity.	(Hussain et al., 2014)
Seaweed	*Pyropia haitanensis* [Rhodophyta]	*Skeletonema costatum*	-Growth of *S. costatum* (96-99.47%) was inhibited in (0.625-10 g FW L^{-1}) fresh thalli at 21°C at the end of day 12. -The algae growth was also inhibited in culture filtrate, dry powder and water-soluble extract of *P. haitanensis* (mostly in higher concentration).	Studies using other species of microalgae which are toxic and involved in bloom scenarios need to be conducted as well.	(Patil et al. 2020)
	Ulva fasciata [Chlorophyta]	*Skeletonema costatum* *Nitzschia longissima* *Scrippsiella trochoidea* *Alexandrium minutum* *Heterosigma akashiwo*	-Almost 90 % inhibition rate of *S.costatum* was achieved using 2 g of macroalgal for six days. - Almost all the other species disappeared at the end of seventh day by using 2 g of macroalgae.	-Difficult to predict the interaction of allelopathic under natural conditions. -Appropriate method needed to develop a friendly mitigation agent.	(Shafay et al., 2019)

Ulva rigida [Chlorophyta]	*Ostreopsis cf. ovata* *Prorocentrum lima* *Coolia monotis* *Alexandium pacificum*	The growth of all HAB species were inhibited by *U. rigida* especially *A. pacificum.*	-Only certain species can be inhibited. -Allelopathic substances that effectively inhibit the macrophytes need to be explored.	(Gharbia et al., 2017)
Ulva lactuca [Chlorophyta]	*Chattonella marina* *Cochlodinium polykrikoides* *Karlodinium veneficum* *Karenia brevis* *Prorocentrum minimum* *Psuedo-Nitzschia multiseries*	The growth of all HAB species were lysed and strongly inhibited by fresh thalli of *U. lactuca.*	The effect on non-toxic HAB must be conducted.	(Tang & Gobler , 2011)

2.3.2 Chemical approaches

Herbicides, photosensitisers (hydrogen peroxide, TiO_2), metals (Al, Fe, Cu, Ca), and other chemicals are commonly used as agents to reduce the blooming of harmful algae under chemical-based approaches. Some examples of reported chemical approaches are listed in Table 2.2. Even though chemical approaches can effectively inhibit algae growth, it is less favourable than the biological approach. The chemical approach may pose secondary pollution, high cost and non-targeted toxicity to aquatic and human life (Yu et al., 2017; Sun et al., 2018).

Photosensitisers are chemicals that generate different reactive oxygen species (ROS) upon light irradiation. The ROS can destroy the algal cell and microorganisms by attacking the outer cell membrane and promoting intracellular substance leakage. In addition, ROS formation can damage ribosomes and DNA, leading to cell death (Sharma et al., 2012; Gali et al., 2016). Hydrogen peroxide is the best photosensitiser because it has a potent oxidising agent and represents a perfect decomposition property since it degrades itself into water and oxygen (Spoof et al., 2020). Unfortunately, the decomposition process is too fast to effectively mitigate the HAB cell growth. Hence, a large amount of hydrogen peroxide is required if it is to be applied in the field. Titanium dioxide is another example of photosensitisers that generate ROS effectively under UV irradiation. Modifying TiO_2 by doping with other elements such as iron, zinc, aluminium, copper, and sulfur has increased its efficiency in generating ROS. Figure 2.2 shows the general mechanism of the modified TiO_2 in the mitigation of HAB. Aluminium affects cyanobacteria by removing the phosphorus content and reducing cyanobacterial cell abundance in the water column (Şengül et al., 2016). Copper is mainly used due to its toxicity against algae as copper enters and disrupts cells' biochemical processes (Jaishankar et al., 2014).

Iron-based nanomaterial has also been considered because of its low environmental and human health impacts. Thus, these studies show that iron-based nanoparticles effectively cause the deactivation of HAB cyanobacteria during fully established bloom while reducing the nitrogen and phosphorus content in the water. However, the toxicity level when the TiO_2 and Fe-based materials are used varies depending on the organism evaluated and the parameters exposed (Skocaj et al., 2011). This may lead to inefficiency during the mitigation process.

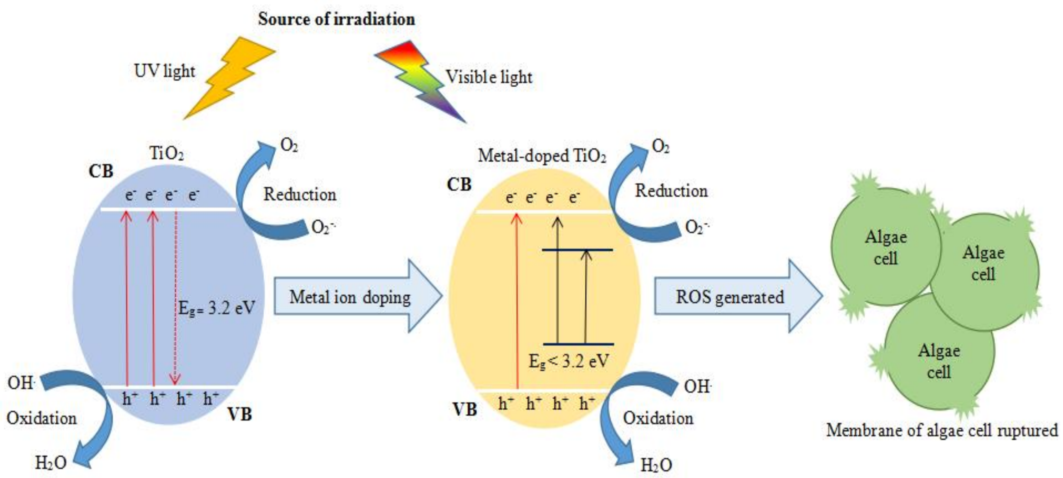

Figure 2.2: Mechanism of metal-doped TiO_2 in mitigation of HAB (He et al., 2020)

Under the chemical-physical approach, ballast (sand or clay particles) modified with organic and inorganic modifiers are added to improve flocculation and sedimentation properties. In addition, the presence of ballast can also increase the ROS generation (Yu et al., 2017; Gallardo-Rodríguez et al., 2018). Compared to sand, clays are widely used despite their low flocculation efficiency and are required in large amounts for field applications.

The organic modifier is mostly positively charged compounds with strong charge neutralisation and bridging effects that allow HAB mitigation. Synthetic and natural materials such as chitosan (Mucci et al., 2017) and starch (Shi et al., 2016) are commonly used organic modifiers. Poly aluminium chloride (PAC), aluminium chloride, and mixed-metal hydroxide are commonly used inorganic modifiers (Yu et al., 2017). Positively charged inorganic modifiers will change the negatively charged clay surface to positive and promote charge neutralisation between the clay and HAB cells. The charge neutralisation will enable more HAB cells to adhere to the clay surface for sedimentation and cell lysis. HAB cells' attachment onto clay particles is considered weakly bound. The possibility for the algae cells to detach from the clay and continue to reproduce is relatively high. Long-term sedimentation can affect the seawater balance. Figure 2.3 shows the effects of organic and inorganic modifiers on the algae cells flocculation and sedimentation.

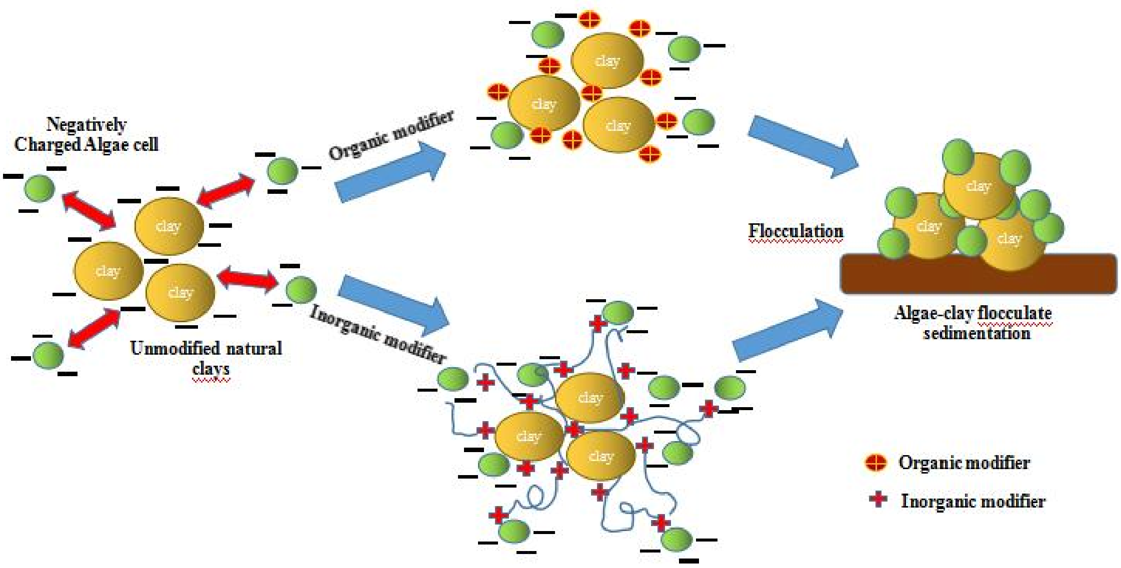

Figure 2.3: Schematic diagram on the interaction between clays and different modifiers in mitigation of HAB.

Table 2.2: Chemical approaches in controlling HAB.

Mitigation agent	Algae species	Result	Drawback	Ref.
0.6 Nb-N-TiO_2/C nanocomposite	*Microcystis aeruginosa*	Highest photocatalytic activity under visible light to degrade chlorophyll-a in algae cells in 8 h (92.7 %).	- High cost -Efficiency in the field application to be confirmed.	(Zhang et al., 2020)
Natural clay modified with PAC	*Karenia mikimotoi*	Increased the ROS production and removed 63 % of *K. mikimotoi* and inhibited the growth of residual algae cell within 48 h.	Sedimentation of the flocculant.	(Liu et al., 2018)
Hydrogen peroxide	*Microcystis aeruginosa*	Successfully mitigate all the *M.aeruginosa* cell within 2 h.	Increased the nutrient content in the water (nitrogen, phosphorus and carbon) and promote the growth of chlorophytes.	(Wang et al., 2019)
$CuSO_4$ and CuO NP	*Chlorella* sp.	$CuSO_4$ decreased the cellular contents of chl-a, chl-b, and carotenoids, ROS production as well as lipid peroxidation after exposure for 96 h.	CuO NP not suitable as it promotes toxicity.	(Wan et al., 2018)
Chitosan fiber	*Microcystis aeruginosa*	Almost 89% of cyanobacterial cells were eliminated in 24 h.	Colouration of fiber.	(Park et al., 2020)
Silica-quaternary ammonium "Fixed-Quat" nanofilm coated fiberglass mesh	-*Microcystis aeruginosa* - *Escherichia coli*	Successful control of *M. aeruginosa* with more than 99% inactivation and *E. coli* inactivation rate of 1.3×10^{-3} log reduction/cm min after 10 h of exposure.	High cost.	(Diaz et al., 2019)
Flumioxazin	*Prymnesium parvum*	Flumioxazin can inhibit *P.parvum* duringg winter season and not in the fall season.	Temperature dependant. Work best at a cooler temperature.	(Bloomer, 2017)
PAC modified kaolinite (0.4 g/ L)	*Prorocentrum donghaiense*	Approximately 60% removal of *P.donghaiense* within 3.5 h.	The cells deposits at the bottom of the container at the end of the experiment.	(Lu et al., 2016)

2.3.3 Physical approach

Physical approaches generally involve physically removing the algal cells from the water column, limiting the spatial extent of bloom by a physical barrier, resulting in killing the algal cells (Kidwell., 2015). The physical approach is conceivably more sustainable since it has minimal chemical inputs and can be carried out over multiple treatment cycles, unlike biological, chemical or chemical-physical approaches. Mitigation strategies that use physical approaches such as aeration, ultrasound, skimming, and membrane filtration have been demonstrated in the laboratory or at the mesocosm scale. The application of these devices is widely used in freshwater but limited in coastal systems as it gives little impact on a low-density of benthic HAB organisms and is often expensive when applied in a large volume of water. Table 2.3 shows the reported physical approaches in removing HAB.

Aeration is supplied by pumping water, pumping air or using surface agitators. Vertical aeration mixing prevents thermal stratification of the water column and warming surface waters that promotes algae growth. Horizontal aeration mixing impairs the algal buoyancy and inhibits the ability of the algae to move independently. Hence, removing the algae from the photic zone prevents photosynthesis from taking place (Gallardo-Rodríguez et al., 2018).

As the toxins from the algae bloom, especially microcystins in freshwater, increases, the protection of water supplies becomes more exacting (He et al., 2016). Therefore, there is a need for alternate water treatment technologies to remove toxic cyanobacterial blooms in drinking water. Figure 2.4 shows the basic steps involved during the physical removal of toxic algae in drinking water treatment.

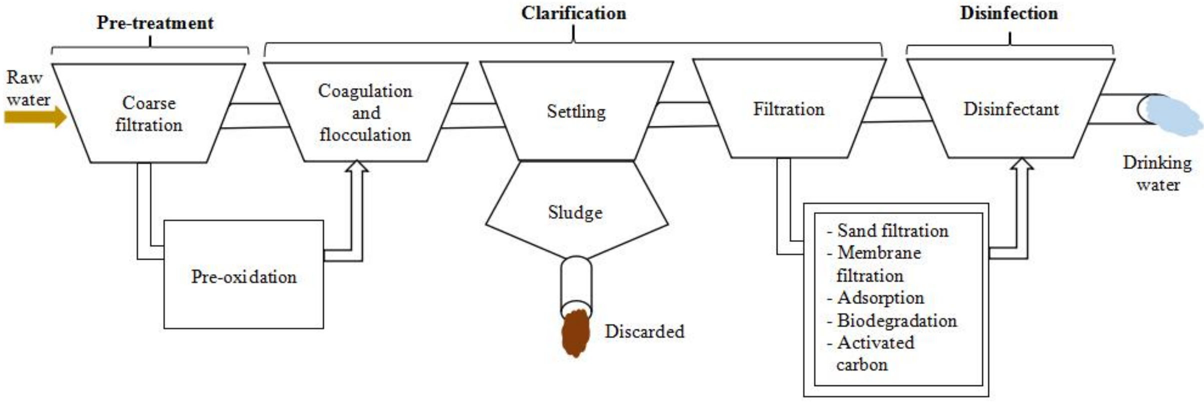

Figure 2.4: Conceptual diagram of the steps to remove algae cells in drinking water treatment (Merel et al., 2013).

Three basic steps are mainly implemented in the drinking water treatment process: pre-treatment, clarification, and disinfection. The first step involves coarse filtration, where the supplied raw water will undergo a pre-oxidation process followed by the clarification step to remove natural organic matter (NOM). There are specific techniques involved in this step. The first technique is based on the retention of contaminants (coagulation, flocculation, sand/membrane filtration and adsorption), and the second technique is based on the degradation of contaminants (biodegradation and advanced oxidation) (Merel et al., 2013). The coagulation-flocculation process involves mixing the organic or inorganic modifiers to neutralise the negatively charged cells. The combined flocculant and algal cell will be sedimented at the bottom, and cell lysis will occur. The sludge will be discarded. The skimming technique is often coupled with the implementation of some coagulant or flocculant. For example, oil-spill skimmers have been used to remove cyanobacteria from these surface scuAn ultrasoundound device emits ultrasonic waves at a particular frequency to destroy algae's cellular structure by rupturing internal gas vesicles used for buoyancy control. However, it is difficult to eliminate some cyanobacteria due to the positive buoyancy, low specific density, motility and y, and variable morphologies (Pham & Dang, 2018). Therefore, filtration will take place to remove the remaining algal cells. The direct filtration method could not potentially remove all the algal cells. Grützmacher et al., 2002 found that more than 90% of extracellular MC were removed during slow sand filtration, primarily due to the biodegradation on or inside the filter bed.

The final step is the disinfectant process. The main aim of this process is to remove organisms from the treated water before the effluent is released back into the water system. Disinfection prevents the spread of waterborne diseases by reducing microbes and bacterial numbers to a regulated level. One of the disinfect processes is the membrane filtration. The membrane filtration method analyses water quality using a special filter to trap the microorganisms that have not been trapped during coarse filtration. It is a very effective method for isolating microorganisms in the water sample. The membrane filtration efficiency depends on membrane pore size. Pores that are too large cannot retain all algae cells but pores too small reduce the permeate flux (Zhao et al., 2017). Membrane separation processes can recover microorganisms, resulting in stable yield and clean effluent water. Besides, membrane technology can remove viruses and protozoans from culture media while retaining the residual nutrients that allow for the reuse of the medium (Pavez et al., 2015).

Adsorption-based processes are a technology that has often been reported for achieving very low concentrations of o-phosphate, low footprint and minimal waste generation. One of the limitations of adsorption is its ability only to remove dissolved phosphate. Pre-treatment by advanced oxidation processes can also promote converting the organic forms of phosphorus into phosphate, which can then be targeted by adsorption (Mayer et al., 2013). Moreover, the flocculation and filtration method could also reduce the limitation of adsorption-based processes by targetting the particulate phosphorus (Langer et al., 2017).

Manufactured from wood, coal, peat, and coconut shell, activated carbon (AC) has high porosity and a large surface area. In both powdered activated carbon (PAC) and granular activated carbon (GAC) forms, activated carbon has been extensively used for decades to remove pollutants in drinking water and wastewater. Roegner et al., 2014 reported that almost 99 % of dissolved microcystins were effectively removed using activated carbon. Although effective, activated carbon filtration displays a limited lifetime for all contaminants, including microcystins (Pantelíc et al., 2013). The filtration needs to be changed frequently, varying between 2 months to 1 year, depending on the toxin type and the water quality. If it is not monitored, the removal efficiency will decrease.

Table 2.3: List of latest physical approaches in removing HAB.

Mitigation agent	Algae species	Result	Drawback	Ref.
0.1-µm membrane filter	*Chlorella pyrenoidosa*	0.1-µm membrane was suitable for harvesting *C.pyrenoidosa* compared to 0.03- and 0.05-µm membranes.	-0.1-µm membrane had the highest flux decline rate. -Less algae cell adsorption as the pore size increases.	(Zhao et al., 2017)
Ultrasonic technology	*Microcystis aeruginosa*	Removal rate of *Microcystin* reaches 99% after 15 min of ultrasound treatment (1200 W).	Removal depends on the species.	(Chen et al., 2019)
Water-lifting aerator (WLA)	*Microcystis aeruginosa*	Vertical mixing of WLA weakened the photosynthetic ability and reduced the biological activity of algae *in situ*.	This device can be used in a limited range.	(Zhang et al., 2020)
UV-radiation enhanced aluminum (Al)-based coagulation	-*Microcystis aeruginosa* -*Cyclotella* sp.	Approximately 93.5% of *M. aeruginosa* cells and 91.4% of *Cyclotella* sp. cells were removed after 240 s of UV irradiation with 0.4 mmol/L Al.	- Rapid sedimentation takes place which can lead in disturbance of the aquatic system.	(Dai et al., 2020)
Peptide HPA3NT3-A2	-*Microcystis aeruginosa* -*Haematococcus pluvialis* -*Chlorella vulgaris* -*Daphnia magna*	-Have an algicidal effect on *M. aeruginosa* which is 79.2 % in 48 h. -No algicidal effect on *H. pluvalis* and *C. vulgaris.* -Non-toxic to *D. magna.*	- Promotes sedimentation -Might affect other organisms.	(Han et al., 2019)

2.4 Chitosan

The history of chitin began in France at the beginning of the nineteenth century by chemist Henri Braconnot. Braconnot, in 1811 named 'chitin' the insoluble residue remaining after the extraction of fungi with water, alcohol, and dilute alkali fongine/fungine (Crini, 2019). Chitin is a major structural component of the exoskeleton of invertebrates and fungus' cell walls. Chitin is a glucosamine and N- acetylglucosamine-based linear copolymer. Protein and calcium salts are removed from chitin before deacetylated with concentrated NaOH to produce chitosan. The deacetylation levels can range from 70-95% (Marpu & Benton, 2018).

Chitosan (Figure 2.5), the derivative of chitin after deacetylation, is a linear amino polysaccharide with D-glucosamine and N-acetyl-D-glucosamine units. Chitosan has been applied in many fields, such as medicine, agriculture, food, textile, environment, and bioengineering, due to its excellent antimicrobial activity properties, non-toxicity, biocompatibility, biodegradability and chelating capability (Wang et al., 2018).

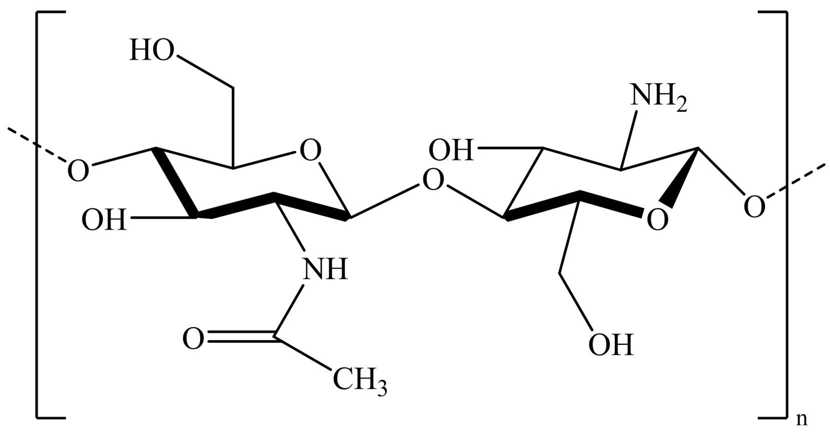

Figure 2.5: The chemical structure of chitosan