INVESTIGATION OF RECYCLABILITY OF *SARGASSUM BINDERI* FOR REMOVAL OF METHYL ORANGE

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A project report submitted in partial fulfilment of the requirements for the award of Bachelor of Engineering (Hons.) Chemical Engineering

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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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I certify that this project report entitled **"INVESTIGATION OF RECYCLABILITY OF *SARGASSUM BINDERI* FOR REMOVAL OF METHYL ORANGE"** was prepared by **WOO YEE PING** has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Degree (Hons.) Chemical Engineering at Universiti Tunku Abdul Rahman.

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Specially dedicated to

my beloved family and friends

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ABSTRACT

Dyes are contaminant that will cause damage to the environment as it will undergo for chemical changes, consume dissolved oxygen, and it may be toxic and carcinogenic to some aquatic living organism. There is an increasing water demand for industrial and public uses. Thus, needs of reclamation of the effluents and treatment of wastewater become highly desirous. There are several methods that can be applied to remove dye such as biological treatment (biodegradation using bacteria cells), coagulation or flocculation, chemical oxidation and photocatalytic processes, ozone treatment, membrane processes, and adsorption. By far, adsorption process is the best removal techniques as it is effective, economical, wide variety of adsorbent available, and capable to treat more concentrated dyes. Seaweed was chosen as it is a widely available biological resource, cheap, and presence of abundant dye removal functional group. In this experiment, *Sargassum binderi* has been choosen as biosorbent used to study the recyclability for removal of methyl orange in batch mode. Four different types of desorbing agent (HCl (0.1 M), CH_3COOH (10% v/v), distilled water, and NaOH (0.1 M)) was used to test the recyclability of biosorbent. The result is determined based on desorption efficiency. Based on the results, MO can be desorbed at all the three cycles by using HCl (0.1 M), CH_3COOH (10 % v/v), and distilled water as desorbing agent except for NaOH (0.1 M). Averagely, desorption process by using HCl (0.1 M), CH_3COOH (10 % v/v), and distilled water as desorbing agent for each cycle reached constant in 300 min. It was found that the percentage of desorption efficiency of the HCl (0.1 M) and CH_3COOH (10 % v/v) decreases after each cycle of desorption and regeneration process. However, desorption efficiency of distilled water did not decrease in all of the three cycles. Instead, it remains constant for all three cycles with a desorption efficiency of 45 %

and it was the desorbing agent that scores the highest in percentage of desorption efficiency. Therefore, distilled water is the best desorbing agent.

ACKNOWLEDGEMENTS

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LIST OF SYMBOLS / ABBREVIATIONS

C_0	Initial dye concentration (mg/L)
C_e	Equilibrium dye concentration (mg/L)
C_t	Concentration of dye in the desorption solution at time t
m	Mass of seaweed used in the adsorption process
q	Amount of dye adsorbed on seaweed (mg/g)
V	Volume of the solution (L)
W	Amount of adsorbent used (g)
DE	Desorption efficiency
MO	Methyl orange

CHAPTER 1

INTRODUCTION

1.1   Background

Dyes are a coloured element with an affinity to the substrate to which is being applied (Dye, 2011). Based on the third edition of Colour Index list which is edited by the Society of Dryers and Colourists and by the American Association of Textile Chemist and Colourists, there are more than 8000 colorants used on a large scale for fibres, plastics, printing inks, paints and liquids (Classifications of dyes, 2011). There are many structural varieties of dyes that can be classified into either the cationic type, non-ionic type or anionic type and the classification is as follow (Purkait, DasGupta, & De, 2005):

- Anionic   : direct, acid and reactive dyes
- Cationic   : basic dyes; and
- Non-ionic   : disperse dyes

Dyes are widely used in various industries such as dyestuffs, textiles, leather, papers and plastics (Chen & Chen, 2009) and it contributes to the coloured wastewater. The waste effluents of these industries normally are highly coloured. Thus, disposal of this coloured wastewater into receiving waters will cause damage to the environment as it will undergo for chemical changes, consume dissolved oxygen, and destroy aquatic creatures (Chen & Chen, 2009). The presence of dye in effluent even in a very low concentration of dyes which is less than 1 mg/L, it is still highly visible and is considered undesirable to the water user (Asouhidou, Triantafyllidis, Lazaridis, Matis, Kim & Pinnavia, 2009). Besides, these dye effluents reduces light penetration

and affecting the photosynthetic activity in aquatic life (Asouhidou et al., 2009). Some of the dye effluent may also be toxic and carcinogenic to some aquatic living organism as it contains metals and chlorides (Asouhidou et al., 2009). Furthermore, increasing water demand for industrial and public uses, the needs of reclamation of the effluents and treatment of wastewater become highly desirous (Mittal, Mittal, Malviya, & Gupta, 2009). Thus, it is necessary to develop an effective and efficient method to remove the colour from wastewater before being discharged to natural water stream.

There are several methods that can be applied to remove dye such as biological treatment (biodegradation using bacteria cells), coagulation or flocculation, chemical oxidation and photocatalytic processes, ozone treatment, membrane processes, and adsorption (Wu, Liu, Khim, & Suen, 2007). However, there are some difficulties in treating the coloured wastewater as they are recalcitrant organic molecules, resistance to aerobic digestion, and is stable to light, heat, and oxidising agents (Sun & Yang, 2003). Due to the presence of large degree of aromatic rings in the dye molecules, the treatment of coloured wastewater via a biological process is difficulty and ineffective as dyes have low biodegradability (Chen & Chen, 2009). There are few limitations that taken into account when choosing the right techniques to treat coloured wastewater such as excess amount of chemical usage, expensive plant requirements and operational costs, lack of effective colour reduction and sensitivity to a variable wastewater input (Hasan, 2011). Among all wastewater treatment techniques, the one which has been recognized to be an effective and economical wise method to remove colour dye from industrial effluents is by using adsorption process (Chen & Chen, 2009). The main advantages of adsorption treatment for the control of water pollution are less investment in terms of initial development cost, simple design, easy operations, free from generation toxic substances, and easy and safe recovery of the adsorbent as well as adsorbate materials (Mittal et al., 2009).

There is a large variety of adsorbent materials have been tested to absorb the dye molecules and then remove the colour from wastewater included natural or synthetic adsorbents, such as zeolites, activated clays, activated carbons, activated slag, chitosan beads, cellulosic resins, polymer resins, modified rice husk, cross-

linked starch, palm kernel fiber, red mud, bottom ash, ion exchangers and granulated ferric hydroxide (Wu et al., 2007). The efficiency and cost of these absorbents vary from one material to another too but among these large varieties of adsorbent, activated carbon still is one of the most widely used adsorbent for colour removal due to its excellent adsorption capacity for organic pollutants (Chen & Chen, 2009). Yet, the carbon loss as wasted sludge and the difficulty to completely regenerate the exhausted activated carbon is rather significant and causes other problems (Chern & Wu, 2001). Besides, it is also very costly for developing countries like Malaysia (Acenioglu, 2004). Hence, cheaper and effective new alternatives adsorbents inclusive of biosorbents, natural resources, and waste materials from industry and agricultural are being studied.

Among all the alternatives mentioned, biosorbents is one of the most favourable choices in recent research in environmental or bioresource technology for the application of removing toxic pollutants or to recover valuable properties from wastewater. The advantages of using biosorbents is to remove toxic pollutants in high efficiency, produce minimum chemical or biological sludge, ability to regenerate biosorbents, and the possibility of metal recovery following adsorption (Volesky, 2001; Tang, 2011). Removal of colour dye using biosorbents is a non-conventional technology that uses biomaterials such as bacteria, fungi, algae, industrial wastes, agricultural wastes and polysaccharide materials are known to bind pollutants. Biosorption mainly takes place on the biomass surface and the binding site at the surface will activating and thus increasing the effective approach of enhancing the biosorption capacity (Mao, Sung, Vijayaraghavan, & Yeoung, 2009). In the present project, marine algae which are popularly known as seaweed was used as biosorbent to remove dye and it was regenerated by testing with various types of regenerant. The regeneration process involves restoring the capacity of saturated biosorbent by desorbing adsorbed contaminants like colour on the surface of the biosorbent (Activated Carbon, 2011). The purpose of regeneration is to minimise the process cost and opening the possibility of recovering colour dye extracted from wastewater (Volesky, 2001). Then, the regenerated biosorbent was used for another cycle of application. Seaweed was chosen as it is a fairly cheap, widely available biological resource and most importantly is that it contains alginate gel in their cells

walls which offer a convenient basis for the production of biosorbent particles that are suitable for sorption process (Vieira, 2003).

1.2   Aims and Objectives

The purpose of current study is to use *Sargassum binderi* as biosorbent to remove methyl orange (MO) from aqueous solution and to desorb MO with different desorbing agent. Following that, the regeneration of biosorbent was conducted. The objectives of the study are:

1. To investigate the possibility of using *Sargassum binderi* in removal of methyl orange solution.
2. To study desorption efficiency of a suitable regenerant in regenerating and recycling of the *Sargassum binderi* for subsequent adsorption process.

CHAPTER 2

LITERATURE REVIEW

2.1 Contaminants in Water

Water is essential to life and it is one of the nature's most important gifts to mankind. However, human activities and industrialization has caused water contamination to the water source and it has become an important issue to Malaysia. Thus, several ways and methods have been developed to prevent water contamination in order to protect all living organisms and to remove contaminants in the water. The following sections mainly discuss on the major categories of contaminants in water due to a lengthy list of contaminants.

2.1.1 Pathogens

Pathogens are bacteria that cause diseases and even death to human, animals and even plants. In years ago, waterborne diseases caused by pathogens has accounted for millions of deaths. Until today, in those undeveloped countries, an estimated of 25,000 people will die daily from waterborne disease (Excel Water Technologies Inc., 2007). Pathogens are able to grow and multiply in a host and the resulting growth in a host causes infections (Masters & Ela, 2008). There are several pathways where pathogens can invade a host, which can be through the use of sewage sludge, agricultural practices, animal waste and others. Contaminated water is one of the pathways that responsible for the spread of pathogens and causes diseases too. The

survival of pathogens is influenced by the factors like sunlight intensity, adsorption to particles, the presence of aquatic microorganism that may be used by the pathogen as food source or as a host for pathogens and also temperature (Masters & Ela, 2008). The pathogens survival extended when the water temperature is low. Thus, pathogens are the first and important consideration in making water acceptable for consumption.

2.1.2 Oxygen-demanding Wastes

Oxygen demanding waste is any substances that can be decomposed by oxygen-requiring bacteria for growth (Lenntech, 2011). When large populations of decomposing bacteria are converting these wastes, it poses threats to the aquatic life as it depletes oxygen levels in water and reduces the amount of dissolved oxygen (DO). As DO level drops, it can suffocate and kill aquatic life. Besides, it can also cause undesirable taste, odours, and colour that is unpleasant for human consumption or recreational use (Stormwater pollution: oxygen-demanding waste, 2008). Most of the oxygen-demanding wastes are biodegradable organic substances derived from municipal wastewater, industrial effluents and various sources from urban environments. Apart from that, oxidation of certain inorganic compound and natural organic matter like leaves, grass clippings and animal waste that reach streams are decomposed by bacteria and leads to oxygen demand (Masters & Ela, 2008).

2.1.3 Nutrients

Nutrients are made off water-soluble nitrates and phosphates (Lenntech, 2011). Nutrients are vital to the growth of all livings. However, the presence of nutrients in lakes and slow moving water will affect the water quality. The enrichment of nutrients in water is known as 'eutrophication' and it will lead to excessive growth of aquatic plants. As high population of aquatic plants death and left on decay, it removes oxygen from water and it depletes dissolved oxygen (DO), causing the

survival of aquatic life to be problematic (Masters & Ela, 2008). Moreover, these decomposing aquatic plants will result in colour, turbidity, odours, and objectionable taste to water which are hardly to be removed and reduces its acceptability as a domestic water source (Masters & Ela, 2008). If enrich nutrients and low DO water is being consumed by young children, it may cause death (Excel Water Technologies Inc., 2007).The source of nutrients pollutants in water is usually due to agricultural operations (Masters & Ela, 2008).

2.1.4 Salts

Salts like sodium, calcium, magnesium, chloride sulphate and bicarbonate or a variety of dissolved solids are naturally accumulate by water when it passes through soils and rocks on its way to sea and this process of dissolving salt into water is known as salinity (Masters & Ela, 2008). Salinity is measured by the concentration of total dissolved solids (TDS) and it is an important indicator to determine the usefulness of water for different type of applications (Masters & Ela, 2008). For example, water with high salinity is not suitable for irrigation as some crops have low tolerant to salt. Therefore, good water management is important to maintain crop yields.

The source of increasing salinity inclusive of industrial discharged which is high in content of salt, urban runoff where salts are used to keep ice from forming on the road during winter time (Masters & Ela, 2008). Salinity usually happens on the irrigated agriculture, especially in arid areas because when the salinity increases, the irrigation water percolates through the soil and return to river. High salinity of water occurs mainly by water evaporation. As soon as water evaporates, the salts are left behind, and since there is less remaining fresh water to dilute them, their concentration increases. Still, salt accumulations can be controlled by flushing away the salts with additional amount of irrigation water and construction of adequate drainage.

2.1.5 Heavy Metals

Heavy metals are toxic and most of this toxic metal can be present in industrial, municipal and urban runoff which can be harmful to humans and aquatic life. Increased urbanization and industrialization are part of the main cause for an increased level of trace metals, especially heavy metals in our waterways. There are over 50 elements that can be classified as heavy metals and 17 out of it are considered to be both very toxic and relatively accessible (APEC, 2011). Toxicity levels are depending on the type of metal, the metal's biological role and the type of organisms that are exposed to it (APEC, 2011). Some of the metals like zinc, chromium and iron are essential nutrients in our diets if their presence in human's body is in small amount (Masters & Ela, 2008). However, they can cause a range of adverse impact on the body if in high doses. The heavy metals linked most often to human poisoning are lead, mercury, arsenic and cadmium (APEC, 2011).

Heavy metals are dangerous as they tend to bioaccumulate in biological organism over time. In addition, the heavy metals taken up and stored is faster than they are broken down (Kilic, 2011). Presence of high doses of heavy metals in human body might cause negative effects such as chronic damage to nervous system, creation of mutations, lung cancer, anemia, kidney and liver damage (Kilic, 2011). Thus, it is crucial to revivify metal pollution to avoid further environmental and human impacts. There are various techniques available to remove heavy metals from polluted water, which include chemical oxidation, reverse osmosis, ion exchange, electrochemical applications, and adsorption (Masters & Ela, 2008). On the other hand, application of biosorption by using seaweeds, microorganism, activated sludge, fermentation waste, and other specially propagated biosobents is also applied to remove heavy metals from soultuion (Kilic, 2011).

2.1.6 Pesticides

Pesticides are composite that includes all chemicals that are used intended for preventing, destroying, repelling or mitigating any pest (Pesticides, 2011). Pesticides

that commonly used in agricultural can be delineated as herbicides (weeds), insecticides (insects), fungicides (fungi), nematocides (nematodes), and rodenticides (vertebrate poisons) (Department Natural Resources Management and Environment, 1996). The application of pesticides widely in agricultural is to control the pest that would otherwise diminish the quantity and quality of food product. Unfortunately the benefits of pesticides have also become drawbacks to the long-term survival of major ecosystem and also potential toxicity to humans and other animals (Department Natural Resources Management and Environment, 1996). Some of the pesticides remain in the environment for a long time before broken down into other substances (Masters & Ela, 2008). Pesticides are soluble in hydrocarbon solvents and thus it can easily accumulate in fatty tissue (Masters & Ela, 2008). Animals may be poisoned by pesticide residues remain on food shortly after spraying and the poison of pesticides will store in the animals' fatty tissue (Masters & Ela, 2008). Once it was consumed by human, the poison of pesticides will adsorb by human body too. Hence, at the top of the food chain, body concentrations of these pesticides are the highest and toxicity is most recognised (Masters & Ela, 2008). Pesticides exposure can cause a variety of adverse health effects. These effects can range from simple irritation of the skin and eyes to more severe effects such as affecting the nervous system, mimicking hormones causing reproductive problems and also causing cancer. Therefore, usage of pesticides should be decreased.

2.1.7   Volatile Organic Chemicals

Volatile organic compounds (VOCs) consist of a group of chemicals that contain organic carbon which are readily evaporated (Colorado Department of Public Health and Environment, 2000). This includes chlorinated solvents such as carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethylene, cis-1,2-dichloroethylene, methylene chloride, tetrachloroethylene, vinyl chloride, and trichloroethylene; and fuel components like benzene, toluene, xylenes, and methyl tert-butyl ether (New Jersey Department of Health and Senior Services, 1997). These chemical components are widely found in household products like degreasing fluids, inks, wood furniture cleaner, water pipes, plastic wrap, paints, glues, and heating oil

(Colorado Department of Public Health and Environment, 2000). VOCs are usually found in underground water instead of drinking water that source from a lake, reservoir, or stream as VOCs tend to evaporate into the air (New Jersey Department of Health and Senior Services, 1997). VOCs can sip easily onto the ground if it is improperly discarded where these chemicals can travel downward through the soil and ended up in the groundwater (Masters & Ela, 2008). The most common treatment method to remove VOCs from water is to aerate the water and allow the VOCs to vaporize and dispersed into the atmosphere (Masters & Ela, 2008).

2.1.8   Dyes

Dye is a coloured compound that has an attraction to the substrate which is being applied (Dye, 2011). Substrate is the material such as textile fibres, polymers, leather, oils, and foodstuff where colour can impart on to by using one of the various processes like dyeing, printing, surface coating, and so on (Rangnekar & Singh, 1980). Natural organic and inorganic colorants have been used since prehistoric times (Maschmeyer, 2004). Application of dyes and colour manipulation in products may add value to the products commercially.

During ancient time, dyes are extracted from minerals, plants, and animals. Around 1856, scientist has discovered ways to produce synthetic dye which is cheaper to produce, brighter, more colours, and easy to apply to fabric (Brit, 2008). Synthetic dyes had brightened up the new world with colours but not without a down side. Dyes are complex compounds with a big complicated structure. The properties of dyes and the chemicals used to produce dyes are highly toxic, carcinogenic, or even explosive (Brit, 2008). Most of the dye effluent emitted by the industry are not treated before being discharged into water sources like river, even if it is treated, it is still contains traces of dye. Therefore, when these dye effluents is being discharged into water sources, it pollutes the water source and affecting the aquatic life, human health and ecological system (Molen, 2008).

Pigment which appeared to be similar to dyes which is a material that changes colour due to adsorption of some wavelengths of lights (Pigment, 2011). It is also one of the major types of colorants in the colouring industry. Dye is a water-soluble organic compounds which shows affinity towards the substrate which is being applied by being absorbed into the substrate and destroying the crystal structure of the substances. In contrast with a dye, pigment is an insoluble compound which has no affinity for the substrate (Dye, 2011). Normally, pigments retain their crystalline form during application without being destroyed.

Generally, synthetic dyes are made of aromatic organic compounds. The structure of synthetic dyes consists of aryl rings that has delocalised electron systems. These structures named as chromogen (electron acceptor) are responsible for the absorption of electromagnetic radiation that has varying wavelengths based on the energy of the electron clouds (KOLORJET Chemicals, 2011), while auxochrome (electron donor) is a part that regulates the solubility and dyeing properties. With the presence of chromophore ("chromo" means colour and "phore" means bearer) in the dyes causes energy changes in the delocalised electron cloud of the dye (Molen, 2008). This alteration invariably results in the compound absorbing radiation within the visible range of colours and not outside it (KOLORJET Chemicals, 2011).

Chromophore group is a color giver and is represented by the following radicals, which form a basis for the chemical classification of dyes when coupled with the chromogen: azo (-N=N-); carbonyl (=C=O); carbon (=C=C=); carbon-nitrogen (>C=NH or -CH=N-); nitroso (-NO or N-OH); nitro ($-NO_2$ or =NO-OH); and sulfur (>C=S, and other carbon-sulfur groups). The chromogen–chromophore structure is not adequate to impart solubility and cause adherence of dye to fiber. Thus, auxochrome ("auxo" means augment) or bonding affinity groups like amine, hydroxyl, carboxyl, and sulfonic radicals or their derivatives are salt-forming which is needed to aid the solubility and adherence of dye in acidic and basic medium (MIGA, 2006).

There are several types of dyes which can be classified as acetate rayon dyes, acid dyes, azoic dyes, basic dyes, direct dyes, mordant or chrome dyes, lake or pigment dyes, sulfur or sulfide dyes and vat dyes. These dyes may be classified

according to a dual system managed by the Society of Dryers and Colourists (United Kingdom) and the American Association of Textile Chemists and Colourists (AATC). Both of this society and association is in cooperation and has published the Colour Index (C.I.) which provides a detailed classification of commercial dyes and pigments by generic name and chemical constitution. Besides, this sourcebook also provides useful information on technical performance, physical properties, and application areas (MIGA, 2006). The classification of dyes by usage is summarized in Table 2.1.

Table 2.1: Usage Classification of Dyes

Class	Applications	Method of application	Chemical Type
Acid	Nylon, wool, silk, paper ink, and leather	Usually from neutral to acidic dye-baths	Azo (including premetallized), anthraquinone, azine, triphenylmethane, xanthene, nitro, and nitroso
Basic	Leather, wool, silk paper, modified nylon, polyacrylonitrile, polyester, and inks	Applied from acidic dye-baths	Cyanine, azo, azine, hemicyanine, diazahemicyanine, triarylmethane, xanthen, acridine, oxazine, and anthraquinone
Direct	Cotton, paper, rayon, leather, and nylon	Applied from neutral or slightly alkaline baths containing additional electrolyte	Azo, phthalocyanine, stilbene, nitro, and benzodifuranone
Disperse	Polyester, polyamide, acetate, acrylic, and plastics	Fine aqueous dispersions often applied by high temperature, pressure or lower temperature carrier methods; dye may be padded on cloth and baked on or thermo fixed	Azo, anthraquinone, styryl, nitro, and benzodifuranone

Table 2.1: (Continued)

Class	Typical Applied	Method of application	Chemical Type
Reactive	Cotton, wool, silk, and nylon	Reactive site on dye reacts with functional group on fiber to bind dye covalently under influence of heat and alkaline condition	Azo, anthraquinone, phthalocyanine, formazan, oxazine, and basic
Solvent	Plastics, gasoline, varnishes, lacquers, stains inks, fats, oils, and waxes	Dissolution in the appropriate solvent or medium	Phthalocyanine, azo, anthraquinone, and triphenylmethane
Sulfur	Cotton and rayon	Dissolved in water (with the addition of sodium sulfide for the insoluble types); exhausted with glauber's salts	Indeterminate structures
Vat	Cotton, rayon, and wool	Water-soluble dyes solubilization by reducing with sodium hydrogen sulfite, then exhausted on fiber and reoxidized	Anthraquinone (including polycyclic quinones) and indigoids

(Sources: Shenai, 1977; Rangnekar & Singh, 1980; Hunger, 2003)

2.2   Basic Dye

Dyes can be defined as coloured, aromatic organic compounds that can be ionised. Hence, dyes are able to interact with oppositely charged tissue constituents (KOLORJET Chemicals, 2011). Basic dye is a stain that is cationic (which is positively charged) and so it will react with material that is negatively charged (Jagson Colorchem, 2011).

Basic dyes are the class of dyes which is most commonly synthetic. Their primary nature is to act as bases and basic dyes are actually aniline dyes (Dyes-

pigments standardcon, 2011). Initially, basic dye's colour base is not water soluble but it can be solute in water with the base being converted into a salt (Jagson Colorchem, 2011). Basic dyes are not generally light fast even it has great tinctorial strength and brightness. Thus, it is largely restricted from using in the dyeing of archival materials and also to those materials not requiring this characteristic (Jagson Colorchem, 2011). At chemical level, basic dyes are cationic or positively charged as it possesses functional groups like $-NR^{3+}$ or $=NR^{2+}$. Since basic dye is cationic, therefore it can react well with the material that is anionic or negatively charged. Some commonly used examples of basic dyes includes of methylene blue, crystal violet, basic fuchsin, and safranin (Dyes-pigments standardcon, 2011).

2.3   Acid Dye

Dyes are being grouped according to the lines of being coloured and also the aromatic compounds contain in the structure that can be ionise. Acid dyes contain mainly negative charge and it is used to bind with positively charged tissue components (Acid Dyes, 2005).

Acid dyes are very complex in structure but the groups that have ionising capability are known as the auxochromes (Acid Dyes, 2005). Auxochromes is a salt-forming group which consist of hydroxyl, carboxyl or sulphonic groups that attach to non-ionising compound yet it can retain its ability to ionise and it will produce dye when it is introduced into chromogen (Auxochrome, 2011). Therefore, auxochromes is the coloured part of the molecule which consequently has an overall negative charge. Besides, these auxochromes group are the one that makes the acid dye to be soluble in water (Hanu, 2010). There are few examples of acid dyes which are acid yellow 24, acid red 66, acid red 87, and methyl orange (Acid Dyes, 2005).

2.3.1   Methyl Orange (MO)

The dye used in this experiment is Acid Orange 52 which is commonly known as methyl orange. Methyl orange is often being used as an indicator in titrations for acid due to its clear and distinct colour change when pH changes (Wiki, 2011). Table 2.2 illustrates the properties of methyl orange used in the present study.

Table 2.2: Properties of Methyl Orange

Property	Data
CAS Number	547-58-0
C.I. Number	13025
C.I. Name	Acid Orange 52
Class	Azo
Ionisation	Anionic
Solubility Aqueous	Soluble in hot water
Solubility Alcohol	Insoluble
Absorption Maximum	464 nm
Colour	Orange
Molecular Formula	$C_{14}H_{14}N_3NaO_3S$
Molecular Weight	327.33 g/mol
Chemical Name	4-[4-(Dimethylamino)phenylazo]benzenesulfonic acid sodium salt
Chemical Structure	
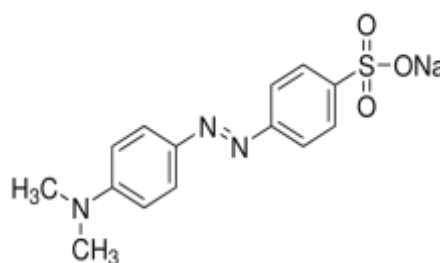
(Sources: Methyl Orange, 2010; Sigma-Aldrich, 2011)

2.4 *Sargassum binderi*

Algae are small aquatic plants or plant like organisms that has simple bodies but grows in large proportions (Essortment, 2011). It is simple chlorophyll and other pigments containing organisms that trap light from the sun. This light energy was converted into food molecules by a process called photosynthesis. Algae are simple organisms that composed of one cell, or grouped together in colonies to form large multicellular organisms. This aquatic plant does not have true roots, stems, or leaves but they have tissues that organize into structure that serves particular functions. The cell walls of algae generally made of cellulose and also contain pectin which causes the algae to have slimy feel that are suitable for sorption process application (UXL Encyclopedia of Science, 2002).

Algae can be divided into a variety of types like Cyanophyta (blue-green algae), Chlorophyts (green algae), Rhodophyta (red algae) and Phaeophyta (brown algae). There are several characteristics used to classify algae which inclusive of the nature of the chlorophyll, the cell wall chemistry, flagellation and even phytopigments (UXL Encyclopedia of Science, 2002). *Sargassum* is a type of brown algae which is in the class of Phaeophyceae (Sargassum, 2011). This brown algae obtains it characteristic colour from the presence of large amounts of the carotenoid fucoxanthin which contained in *Sargassum* chloroplasts and also with the presence of various pheophycean tannins (Guiry, 2011). The colours of the algae types are due to their particular mixtures of photosynthetic pigments, which typically include a combination of one or more of the green-coloured chlorophylls as their primary pigments (UXL Encyclopedia of Science, 2002). Generally, the cell walls of Phaeophyta (brown algae) consist of two different layers which is the innermost layer and the outer layer. The innermost layer is a microfibrillar skeleton that imparts rigidity to the walls of Phaeophyta whereas the outer layer is an amorphous embedding matrix. The embedding matrix of Phaeophyta algal is mainly alginic acid or alginate (the salt of alginic acid) with a less amount of sulphated polysaccharide (Davis, Volesky, & Mucci, 2003).

Majority of the acidic functional group in the brown algae are carboxylic groups (Davis et al., 2003). The carboxylic group in Phaeophyta plays an important

role in adsorption process by a reduction in calcium and lead uptake by dry *Sargassum* biomass with partial or complete esterification of the carboxylic sites (Fourest & Volesky, 1996). The analysis results of Fourier-transformed infrared (FTIR) shows that cadmium biosorption to *Sargassum* arises from bridging or bidentate complex formation with the carboxylate groups of the alginate (Fourest & Volesky, 1996). The second most acidic functional group found in Phaeophyta is the sulfonic acid of fucoidan which plays a secondary role except when metal binding occurred at low pH. Besides, hydroxyl group which presents in all polysaccharides tend to become negatively charged at pH more than 10. Thus, the hydroxyl group does not favour in metal binding during low pH.

The division of Phaeophyta can be subdivided into orders, which will be classified into families, follow by familiar genus and also species. For biosorption process, there are only two orders which are important, the Laminarials and Fucales. *Sargassum binderi* shown in Figure 2.1 is under the orders of Fucales with the family named Sargassaceae (Davis et al., 2003). *Sargassum* is commonly found in Malaysian waters and only the subgenus *Sargassum* is found in Malaysia. It is usually grown on a reef flats substrate rocks extend out to sea where the water movement is relatively strong and constant. The stem of *Sargassum binderi* is short above the holdfast. It has a flattened rod of 1 to 5 mm wide which is smooth in surface in about 60 cm long. The terete primary branches have orderly secondary branches. Mature thalli have fewer and smaller oblanceolate leaves and the leaves is 5 cm long and 1 cm wide (IPTEKnet, 2002). *Sargassum* species in Malaysia is gaining more attention because of its economic value as low cost biosorbent with high regeneration rate (Tan, Wong, Ong, & Hii, 2009). Apart from that, *Sargassum binderi* consists of high alginic acid content which attributes to the binding capability towards dye compound in solution (Davis et al., 2003).

Figure 2.1: *Sargassum binderi*

2.5 Conventional Wastewater Treatment Methods

Dye pollutants from various industries are one of the important sources of environmental contaminations. Discharge of dye effluents imparts colour to receiving streams and affect its aesthetic value. There are more than 10,000 different commercial dyes and pigments and over 7×10^5 tonnes of dye stuff produced annually (Mital et al., 2009). The complex chemical structure and synthetic origin of these dyes causes it to be hard to decolourised and decomposed biologically (Ahmad, Harris, Syafiie, & Ooi, 2002). Dye wastewater is extremely variable in composition and thus, it causes difficulties in selecting of the wastewater treatment methodologies.

Conventional ways of wastewater treatment consist of physical, chemical, biological, and acoustical, radiation and electrical processes. The major existing and emerging process for dye removal with its advantages and drawbacks is shown in Table 2.3. Most of the conventional methods of treating dye wastewater are not being applied as a result of high cost and disposal problems. Besides, it is difficult to treat complex dye-bearing effluents by using conventional ways due to its diverse and often changing composition (Kannan & Rajamohan, 2008). Hence, desired water quality in most economical way can be obtained by applying a combination of different processes.

Table 2.3: Principle Exiting and Emerging Processes for Dye Removal

	Technology	Advantages	Disadvantages
Conventional treatment process	Coagulation Flocculation	Simple, economically feasible	High sludge production, handling and disposal problems
	Biodegradation	Economically attractive, publicly acceptable treatment	Slow process, necessary to create an optimal favorable environment, maintenance and nutrition requirements
	Adsorption on activated carbons	The most effective adsorbent, great capacity, produce a high-quality treated effluent	Ineffective against disperse and vat dyes, the regeneration is expensive and results in loss of the adsorbent, non-destructive process
Establish recovery	Membrane separations	Removes all dye types, produce a high-quality treated effluent	High pressure, expensive, incapable of treating large volumes
	Ion-exchange	No loss of sorbent on regeneration, effective	Economic constraints, not effective for disperse dyes
	Oxidation	Rapid and efficient process	High energy cost, chemicals required
Emerging removal processes	Advanced oxidation process	No sludge production, little or no consumption of chemicals, efficiency for recalcitrant dyes	Economically unfeasible, formation of by-products, technical constraints
	Selective bioadsorbents	Economically attractive, regeneration is not necessary, high	Requires chemical modification, non-destructive process

	selectivity	
Biomass	Low operating cost, good efficiency and selectivity, no toxic effect on microorganisms	Slow process, performance depends on some external factors (pH, salts)

(Source: Ramachandra, Ahalya, & Kanamadi, 2011)

2.5.1   Biological Treatments

Biological treatment is a common and widely used technique in dye wastewater treatment. It is often the most economical alternatives compare with other processes like chemical and physical process. Biological treatment applies biodegradation methods like fungal decolourisation, microbial degradation, and adsorption by (living or dead) microbial biomass. Besides, bioremediation systems are also commonly applied to the treatment of industrial effluents as many microorganisms such as bacteria, yeasts, algae, and fungi are able to accumulate and degrade different pollutants (Fu & Viraraghavan, 2001; McMullan et al., 2001). This method has advantages like being relatively inexpensive and low operating cost. Nevertheless, this method has restriction too such as technical constraints. According to Bhattacharyya & Sharma (2003), biological treatments needs a large land area and is limited by sensitivity toward diurnal variation as well as toxicity of some chemicals, and less flexibility in design and operation. Moreover, with current conventional biodegradation processes, biological treatment is unable to obtain satisfactory colour elimination (Robinson, McMullan, Marchant, & Nigam, 2001). Even though many organic molecules are degraded but there are still other recalcitrant which is not degraded because of their complex structure and synthetic organic origin or due to their xenobiotic nature, azo dyes are not totally degraded (Ravi, Bose, Kumar, & Siddaramaiah, 2006).

Biological treatment process can be aerobic, anaerobic or combined aerobic-anaerobic (Gupta & Suhas, 2009). The characteristic of aerobic treatment process

involves providing a suitable oxygen rich environment for microorganism to reduce the organic portion of the waste by producing enzyme which is able to break down the organic compounds into carbon dioxide and water. The most common microorganism groups that are used to study for dye aerobic wastewater treatment are bacteria and fungi. In general, the wastewater from textile contains several types of dyes and *Aeromonas hydrophila*, an azo-degrading bacterium have been found to have the ability to decolourizing a wide range of dyes and also the capability of degrading textile dyes (Chen, Wu, Liou, & Hwang, 2003). However, under aerobic condition, azo dyes are not readily metabolised and the intermediate formed during degradation disrupt the metabolic pathways and causes the dyes not fully mineralised.

In fungal decolourization of dye effluent, these fungi can be classified into two kinds according to their live state: living cell to biodegrade and biosorb dyes, and dead cells (fungal biomass) to adsorb dyes. For living cells, the main mechanism is biodegradation since it can produce lignin modifying enzymes like laccase, manganese peroxidise, and lignin peroxidise to mineralize synthetic to decolourization or oxidation of dyes (Miao, 2011).There are several factors that are affecting the decolourisation and degradation process. For instance, the concentration of pollutants, dyestuff concentration, initial pH, and temperature of effluent are those factors that will affect microorganism perform in optimum condition. Actually not all dyes are suitable to be treated aerobically as some of them are recalcitrant to biological breakdown and are non-transformable under aerobic condition (Ravi et al., 2006).

For anaerobic process, it takes place without the presence of oxygen. The anaerobic reduction of wide variety of synthetic dyes has been well established. Azo dyes cannot be fully degraded by using conventional aerobic system. Degradation of azo dyes using microbial sludge by anaerobic reduction is found to be more effective and economically compare to aerobic systems which requires expensive aeration and also having problems with bulking sludge (Delee, O'Neil, Hawkes, & Pinheiro, 1998; Wallace, 2001). Studies have shown that anaerobic bacteria are capable to reductively cleave the azo linkages in reactive dyes which effectively alter the chromogen of synthetic dyes and also destroy the observed colour of the dye. Besides, anaerobic process can remove BOD levels and retained heavy metals

through sulphate reduction efficiently without foaming problems (Delee et al., 1998). Yet, many aromatic groups are not susceptible to anaerobic reduction. Anyhow, there is also evidence shows that some azo dye metabolites may be fully stabilized in an anaerobic environments (Miao, 2011).

In order to obtain a better remediation of coloured compound and textile effluents, a combination of aerobic and anaerobic treatment is being suggested. Aerobic-anaerobic process is suitable for a variety of dyes and it can achieve a complete mineralisation due to synergistic action of different organisms which cannot be done by using either aerobic or anaerobic system only. With this combination system, the reduction of the azo bond can be completed under the reducing conditions of anaerobic bioreactors while the process of decolourisation of aromatic amines may be mineralised under aerobic condition. Usually it is recommended that this aerobic-anaerobic process is initially done by anaerobic decolourisation first then only follow by aerobic post-treatment (Brown & Laboureur, 1983; Stolz, 2001). Although this combination process has a better remediation of colour but it is still be affected by the concentration of dyes, initial pH and temperature of the effluent. Overall, biological treatment has low biodegradability of dyes, less flexibility in design and operation as it needs a large land area, and it took a long time for decolourisation-fermentation process which making it impossible to remove dyes on a continuous basis.

2.5.1.1 Bioaccumulation

Bioaccumulation is the gradual build up over time of a chemical in a living organism. Accumulation of chemical in a living organism is caused by the uptake of chemical is faster than it can be used, or because the chemical cannot be metabolized by the organism (Hoyle, 2012). Bioaccumulation is the sum of two processes which are bioconcentration and biomagnification. Bioconcentration is the direct uptake of a substance by a living organism from the medium like through skin, gills, or lungs, whereas biomagnification is resulted from dietary uptake (Bioaccumulation, 2012).

A good example of bioaccumulation is mercury contamination. Once the mercury enters to the water source, the mercury was taken up by bacteria and phytoplankton and it was eaten by the fish and the mercury started to accumulate. The fish then was consumed by humans and animals where large concentrations of mercury were building up in human and animal tissue through food chain. This process is known as biomagnifications (Hoyle, 2012). Human exposure to mercury occurs through consumption of contaminated marine or aquatic foods. The mercury that exposed by human through food consumption would affect the central nervous system and brain as mercury is able to cross the blood brain barriers (Gbaruko & Friday, 2007).

Chemical pollutants can be bioaccumulated from many sources. Pesticide is one of the common contaminant that bioaccumulates in organisms as rain can wash away freshly sprayed pesticides into creeks. Then the pesticides will make their way into rivers, estuaries, and the ocean. Other major source of toxic contaminants is the presence of compounds from industrial smokestacks and automobile emissions that will return to the ground during rainfall. Once these toxic pollutant run into the water or soil, it can easily enter to the food chain. The bioaccumulation and biomagnification of toxic contaminants also can put human health at risk. When humans eat organisms that are relatively high in the food web, the higher doses of harmful chemicals human can get (Hoyle, 2012).

Efforts are being made to lessen the bioaccumulation of toxic compounds where legislation banning certain compounds to be disposed in water as an attempt to reduce the level of toxic compounds presence in the environment. Research of using genetically engineered microorganism to remove toxic material such as mercury from food source has been done to avoid bioaccumulation of toxic compound to be worsened.

2.5.1.2   Biostimulation

Biostimulation is the most widely used bioremediation procedure where the existing microorganism is being stimulated by addition of nutrients. The input of large quantities of carbon source from nutrients resulted in rapid depletion of the contamination (Morgan & Watkinson, 1989). Biostimulation can be enhanced by bioaugmentation. The main difference between biostimulation and bioaugmentation is that in bioaugmentation, only specifically selected pre-grown microorganisms used to degrade contaminants (Bioaugmentation and Biostimulation 2011). The purpose of enhancing the microbial population present at a site is to improves the process of contaminant clean up. With the presence of sufficient quantities of microorganisms in the soil, complete biodegradation can be achieved faster where it additionally reduces clean up costs and time (Bioaugmentation and Biostimulation, 2011).

2.5.1.3   Biodegradation

Biodegradation is the decay or breakdown of organic matter that happens when microorganisms use an organic substance as a source of carbon and energy (Erickson & Davis, 2012). The microorganisms that are responsible for biodegradation are bacteria and fungi. The purpose of biodegradation is to minimize pollution problems to the environment (Biodegradation, 2009). Moreover, biodegradation processes are also important in wastes recycling process where all the elements in the organic matter can be reused (Erickson & Davis, 2012).

Biodegradation is a microbial process that would occur when all of the nutrients and physical conditions involved are suitable for growth. Suitable temperature (10°C to 35°C), presence of water and also nutrients such as carbon, nitrogen, oxygen, phosphorus, sulfur, calcium, magnesium, and several metals is essential for microorganisms to grow and reproduce. Biodegradation process can take place under both aerobic conditions and anaerobic conditions. Aerobic condition happens where oxygen is the electron acceptor while anaerobic conditions where

nitrate, sulfate, or another compound is the electron acceptor (Erickson & Davis, 2012).

2.5.2   Chemical Treatments

There is a number of chemical treatment methods used for wastewater treatment which includes coagulation or flocculation, chemical precipitation, ion exchange process, chemical oxidation and solvent extraction. Treatment of dye wastewater using chemical techniques are very costly even though chemical treatment methods are able to mineralisation those non-degradable compounds in a smaller reactor volume. Moreover, this chemical treatment has increase the production of sludge which causes disposal problem (Akpor & Muchie, 2010). Besides, excessive chemical usage in treating wastewater might also cause pollution problem too (Tang, 2011).

In chemical treatment of dye wastewater, coagulations or flocculation are employed to remove colour dye (Tang, 2011). Coagulants like aluminium sulphate, poly aluminium chloride, ferrous sulphate, and sodium aluminate which provides positive electric charges will reduce the negative charge colloids and causes the particles collide to form larger particles (flocks). Flocculants are also positively charged groups such as amino, imino or quatery amino that enhanced the agglomeration or aggregation of the coagulated particles to form larger floccules to ease gravitational settling or filtration (Sivaramakrishnan, 2011). Coagulation and flocculation process is economically feasible and it also give a satisfactory result in removal of disperse, sulphur, and vat dyes (Gupta & Suhas, 2009). The main drawback of this process is that the sludge formation is very high which leads to high disposal cost and making the treatment non-user friendly (Sivaramakrishnan, 2011). In addition, this process is also ineffective for highly soluble dyes like azo dye, reactive dye, acid dye and basic dye (Raghavacharya, 1997; Hai, Yamamoto, & Fukushi, 2007).

Oxidation is one of the most commonly used methods of decolourisation by chemical means due to its simplicity of application which only requires low quantities and short reaction times (Robinson et al., 2001; Gupta & Suhas, 2009). Powerful oxidizing methods such as chlorine, ozone, Fenton's reagent (peroxide and ferrous sulphate), UV assisted oxidation using peroxide and ozone, and other oxidizing techniques or combination are used for treating effluents contain modern dyes as dyes are resistant to mild oxidation condition (Robinson et al., 2001).Oxidation process are capable of completely oxidizing a dye by reducing the complex molecules to carbon dioxide and water. There are two important factors in oxidation process which is pH and catalysts (Gupta & Suhas, 2009).

Electrochemical method is a relative new technique that was developed in the mid 1990s (Pelegrini, Peralta-Zamora, Andrade, Reyes, & Durán, 1999). This process take place in decolourisation of dye effluents by electro oxidation with non-soluble anodes, like iron, conducting polymer and boron doped diamond electrode or by electro-coagulation using consumable materials (Gupta & Suhas, 2009). Electrochemical techniques has significant advantages for use as an effective way for dye removal as there is little or no consumption of chemicals and no sludge build up (Pelegrini et al., 1999). Besides, it is able to decolourise both soluble and non-soluble dyes with reduction of chemical oxygen demand (COD) (Gupta & Suhas, 2009). The difficulties of using this technique is that relatively high flow rates will cause a direct decrease in dye removal, and the cost of electricity used is comparable to be price of chemicals (Pelegrini et al., 1999).

2.5.3   Physical Treatments

Physical treatments are widely used in wastewater treatment and this process usually treats suspended rather than dissolving the pollutants. The physical methods that are being used in wastewater treatment include membrane-filtration process and adsorption techniques. The technologies for membrane-filtration are microfiltration, ultrafiltration, nanofiltration, and reverse osmosis (Hasan, 2011). Membrane-filtration is applying the principal of a liquid passed through a physical barrier where

particle size which is larger is retained on one side of the barrier while the remaining liquid is allowed to pass through. Reverse osmosis is employed to remove charged ions by applying a pressure that exceeds the osmotic pressure of a solution across a semi permeable membrane. This technique is normally used to reject non-ionised weak acids and bases, and also to reject smaller organic molecules (Gupta & Suhas, 2009).

In the dye industry, this membrane-filtration process can be applied for material recovery from waste for the treatment of effluent prior to final disposal to reduce production cost (PCI membrane, 2008). Among all four technologies of membrane-filtration, microfiltration is rarely to be used for wastewater treatment process due to its large pore size. Ultrafiltration and nanofiltration techniques which have smaller pores are widely used in removal of all classes of dyes (Marmagne & Coste, 1996; Tang, 2011). However, dye molecules often clogged on the membrane pores which causes it seldom to be used in textile effluent treatment (Gupta & Suhas, 2009). Furthermore, membrane has limited lifetime before fouling occurs and the cost of periodic replacement is also very costly (Hasan, 2011).

In between of all methods mentioned, adsorption is still one of the most popular methods for the removal of pollutants from wastewater. Adsorption is a process by which atoms, molecules or ions are retained on the surfaces of solids by chemical or physical bonding (Adsorption, 2011). This process can be classified into physical sorption and chemisorptions (Gupta & Suhas, 2009). Adsorption occurs because of the attraction between the solid surface and the adsorbed molecules are physical in nature while the latter's attraction forces are due to chemical bonding (Gupta & Suhas, 2009). This technique gives the best results in treatment of various dye contaminated waters and the sorbent used for this technique is relatively inexpensive and does not require an additional pre-treatment step before applying this techniques (Hasan, 2011).

Adsorption techniques have successfully proven its ability on lowering dye concentration of the industrial effluents by using adsorbents such as activated carbon, peat, chitin, clay, and others (Jaafar & Sharifah, 2006). Adsorption techniques is best used for water re-use in terms of initial cost, flexibility and simplicity of design, ease

of operation and insensitivity to toxic pollutants. Besides, it does not result in the formation of harmful substance (Hasan, 2011). According to Kumar et al. (1998), there are few factors that would influenced the results of decolourisation such as dye or sorbent interaction, sorbent surface area, particle size, temperature, pH, and contact time. Generally, a good adsorbent would have a porous structure which results in high surface area exposed and increases the efficiency of adsorption. The time taken for adsorption equilibrium to be established has to be as small as possible for a good adsorbent as the lesser the time it takes, the faster the adsorbent able to remove dye wastes. In addition, the quantity of adsorbate that an adsorbent can accumulate is also one of the most important characteristics of adsorbent (Tang, 2011).

In this experiment, biosorbent (*Sargassum binderi*) was employed as the adsorbent for sorption process, and this process is named as biosorption process. Biosorption is a physiochemical process that occurs naturally in certain types of inactive and dead microbial biomass to bind and concentrate pollutants from even very dilute aqueous solution. This property exhibits in non-growing or non-living microbial biomass act like a chemical substance or as an ion exchanger of biological origin. This special property is available particularly at the cell wall structure of certain algae, fungi, and bacteria which enable them to adsorb a variety of pollutants from wastewater (Volesky, 2010). Biosorption indicates a number of metabolisms like physical adsorption, chemical adsorption, electrostatic interaction, ion exchange, complexation, chelation, and microprecipitation takes place in the cell wall rather than oxidation through anaerobic or aerobic metabolism (biodegradation) (Aksu, 2005). The major benefits of biosorption compare to others conventional treatment methods includes low cost, high efficiency, minimisation of chemical and biological sludge, no additional nutrient requirement, ability to regenerant biosorbent, and also the possibility of material recovery (Volesky & Holan, 1995). Biosorption process involves a solid phase biosorbent and a liquid phase solvent which containing a soluble species to be adsorbed like sorbate, metal ions or dyes. Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms. These processes will only stops when equilibrium is reached between the amount of solid-bound sorbate species and its portion remaining in the solution (Ahalya, Kanamadi, & Ramachandra, 2003).

The choices of binding mechanism is based on the type of biomass, chemical nature of the pollutants, and the environmental conditions like the pH, temperature, and ionic strength of effluents. The most commonly used biosorbents are bacteria, fungi, algae, industrial wastes, agricultural wastes, and others polysaccharide materials (Mao et al., 2009). Another inexpensive source of biomass is seaweeds where it is available in large quantities in oceans. Actually, living biomass can be used to remove the pollutants too but it is not advisable to use living biomass due to difficulty in maintaining a viable biomass during adsorption as it requires continuous supply of nutrients and avoidance of organic toxicity to the microorganism (Aksu, 2005). Whereas for non-living biomass, it is not affected by toxic wastes and also does not require continuous supply of nutrients. It can also be regenerated and reuse for many cycles too. Besides, non-living biomass can be kept and stored for long periods before being used as biosorbents without deterioration. Non-living biomass are able to accumulate an amount of pollutants which is as much as living biomass or more. Furthermore, it is easier to prepare and regenerate the non-living biomass than the living ones (Aksu, 2005).

There are few factors that are affecting the biosorption efficiency of the biomass during industrial application of the biosorption like pH, temperature, initial dye concentration, salts, heavy metals ions, other dyes, surfactants, shaking rate, and particle size on dye biosorption. The most important parameters that affect the biosorption efficiency will be the pH of biosorptive process. The pH of biosorption process will affects the solution chemistry of the pollutants, the activity of the functional groups in the biomass, and the competition of metallic ions (Ahalya et al., 2003). Biosorption capacity can be influenced by the relatively high temperature wastewater effluents which contain salts, heavy metals, surfactants and other dye species. Additionally, larger particle size of biosorbent could enhance the absorption process by exposing a larger surface area for biosorption. Studies also indicate that adequate stirring rate in a batch biosorption process can overcome the external mass transfer resistance (Tang, 2011).

2.6   Desorption and Regeneration

Desorption is a phenomena whereby a substance is being released from or through a surface. This process is the opposite of sorption process where it is the process of removing a sorbed substance by the reverse process of adsorption and absorption. Desorption process will only occurs when the concentration of substance in the bulk phase is lowered, and some of the sorbed substance changes to the bulk state (Desorption, 2011). Desorption is utmost importance for inexpensive biomass regeneration as it may decrease the operating cost and also the dependency of the process on a continuous new biosorbent supply (Vijayaraghavan, Sung, Mao, & Yeoung-Sang, 2008). Hence, a proper selection of elution is required in order to obtain a successful desorption process. The criteria of a good elution inclusive of the capability of desorbing bound material quickly and completely without deteriorating the biomass. Besides, it should not decrease the material sorption capacity of biomass during the successive cycles of material sorption (Kordialik-Bogacka, Smoli´nska, Cedzy´nska, & Ambroziak, 2009). The selection of suitable elutions is also strongly depends on the type and mechanism of the biosorption (Vijayaraghavan et al., 2008). There are a few common used desorbing agent for desorption experiment such as alcohol, HCl, and NaOH.

In the present project, dye-loaded *Sargassum binderi* was desorbed by using four different types of dye-desorbing agent, which are 0.1 M HCl, 10% v/v of CH_3COOH, distilled water, and 0.1 M NaOH. Selection of suitable eluent for dye recovery is made by understanding the affinity of desorbing agent with the dye material (Mital et al., 2009). According to Lodeiro et al. (2006), the appropriate desorbing agent for the biosorbent must have lower affinity towards the dye solution to avoid difficulty on removing the dye during regeneration process.

Desorption and regeneration process is conducted to study the ability of the biosorbent to be recycled and reused. After desorption process has been done, desorption efficiency is calculated by using Eq. 2.1 to obtain the amount of adsorb dye on the biosorbent which is being remove in terms of percentage.

$$DE = \frac{C_t V}{qm} \times 100\% = \frac{released\ dye\ (mg)}{initially\ sorbed\ dye\ (mg)} \qquad (2.1)$$

where,

C_t = concentration of dye in the desorption solution at time t

V = volume of desorption solution (L)

q = amount of dye adsorbed on seaweed before desorption process (mg/g)

m = mass of seaweed used in the adsorption process (g)

CHAPTER 3

METHODOLOGY

3.1   Sorbent Preparation

3.1.1   Preparation of *Sargassum binderi*

Sargassum binderi was collected of the coast of Cape Rachado, Port Dickson, and these fresh seaweeds was packed and stored in $-4\,℃$. *Sargassum binderi* was washed with tap water to remove salt, some epiphytes and other species of the seaweed. After that, these seaweeds were rinsed with distilled water until the water became clean and clear. Then, the seaweeds were air dried for 3 to 5 days.

3.1.2   Chemical Modification

3.1.2.1   Formaldehyde and Acid Treatment

The cleaned and dried seaweed was subjected to chemical modifications with formaldehyde (0.2% v/v) to replace the natural mix of ionic species with protons. Seaweed (1.5 g) was placed together with 150 mL of 0.2% formaldehyde solution and shaked in an incubator shaker at 150 rpm and 30℃ for 24 hours. The mixture was subjected to undergo the cross-linking reaction. The modified seaweed was separated from the solutions and washed by distilled water followed by air dried. Then the dry formaldehyde-treated seaweed was further treated with 1 M of

hydrochloric acid (HCl) for protonation purposes. Formaldehyde-treated seaweed (20 g) was placed into 300 mL 1 M HCl and agitated in an incubator shaker at 130 rpm and 30°C for 3 hr. After that, the formaldehyde and acid treated seaweed was filtered out from the solutions and rinsed through with distilled water. The formaldehyde and acid treated seaweed was dried in oven overnight at a temperature of 60°C. The dried mass was sealed well and stored with silica gel at room temperature.

3.1.2.2   Acid Treatment Only

Without any formaldehyde treatment, the dried seaweed (20 g) was placed into 300 mL HCl (1M) and agitated in an incubator shaker at 130 rpm and 30°C for 3 hr. After that, the acid treated seaweed was filtered out from the solutions and rinsed through with distilled water. The acid treated seaweed was dried in oven overnight at a temperature of 60°C. The dried mass was sealed well and stored with silica gel in room temperature.

3.2   Dye Solution Preparation

The dye used in this study is Methyl Orange (MO). The stock solutions was prepared by dissolving accurately weighed dye powder in distilled water at a concentration of 1 g/L and left overnight to make the dye powder fully dissolved. The bottle was covered with aluminium foil in order to prevent decolourisation caused by light and the bottle was stored in dark environment in room temperature.

Dye solutions with different concentration were obtained by dissolving the stock solutions. The concentration of dye solution was determined by a UV-Vis spectrophotometer (Varian Cary 100) operating in the visible range on absorbance mode. Absorbance values were recorded at the corresponding maximum absorbance wavelength (λ_{max}). The λ_{max} of MO is 464 nm. Dye solution was calibrated initially for concentration in terms of absorbance units. The calibration curve of MO solutions

was plotted respectively from dye solution prepared in the concentration range from 10-500 mg/L. The calibration curve for MO is shown in Figure 3.1.
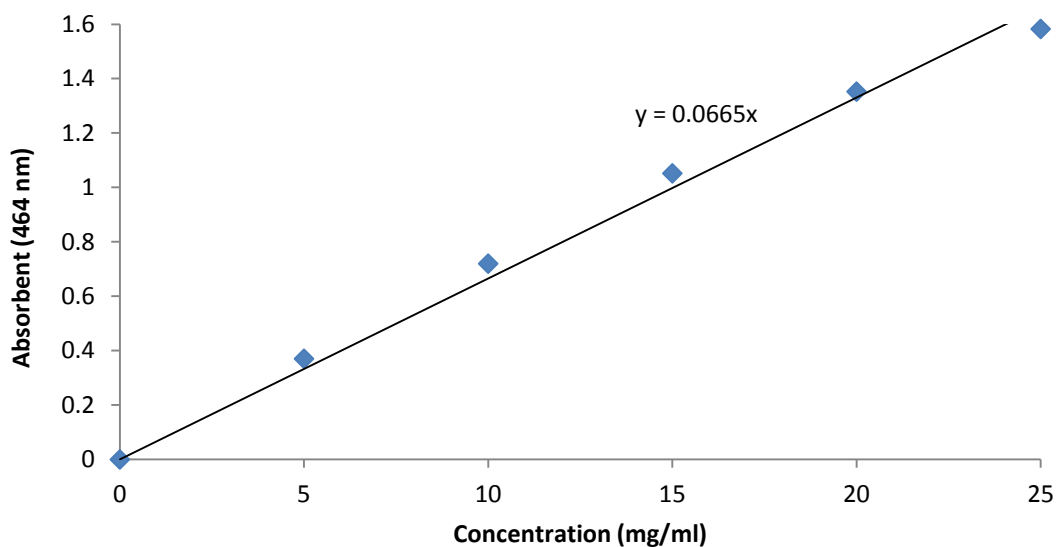
Figure 3.1: Calibration Curve of Methyl Orange

3.3　　Preliminary Test (Batch Adsorption)

Batch adsorption of MO was conducted to investigate the efficiency of biosorption process. In this experiment, the efficiency of seaweed which was treated with different types of treatments was investigated. The experiment was conducted by using 250 mL conical flasks that contain 100 mL of dye solution and 1 g of dried seaweed. The batch experiment was performed in an incubator shaker, with shaking speed 150 rpm at 30 °C. The samples were taken out to measure the dye removal at predetermined time intervals. The concentration of dyes solution was determined by using single-beam UV-Vis spectrometer at maximum absorbance wavelength.

The amount of dye adsorbed on seaweed, also known as sorption capacity, $q(mg/g)$, was obtained as according to Eq. 3.1:

$$q = \frac{(C_0 - C_e)V}{W} \qquad (3.1)$$

where,

C_0 = Initial liquid phase concentration (mg/L)

C_e = Equilibrium liquid phase concentration (mg/L)

V = Volume of the solution (L)

W = Amount of adsorbent used (g)

To express the percent of dye removal, the following equation was used:

$$\% \, removal = \frac{C_0 - C_e}{C_0} \times 100\% \qquad (3.2)$$

where,

C_0 = Initial liquid phase concentration (mg/L)

C_e = Equilibrium liquid phase concentration (mg/L)

3.4   Desorption of Dye

After adsorption process, the dye-loaded seaweed was filtered and placed into oven for drying overnight at 50°C. Then, the dye-loaded seaweed was placed into a flask containing the desorbing agent to undergo for desorption process. The flask is then agitated at 150 rpm and 30°C. Sample was taken to determine concentration after predetermined time intervals. These desorption and regeneration experiments were repeated for 3 cycles by using the same batch of seaweed. In this study, there was four type of desorbing agent used, i.e., 0.1M hydrochloric acid, 10 % v/v acetic acid, 0.1M sodium hydroxide and distilled water.

3.4.1 Adsorption Process

The dye loaded seaweed was prepared by putting in 1 g of formaldehyde treated *Sargassum binderi* into a 250 mL conical flask with 100 ppm of dye solution. First, sample was taken before the seaweed was added into the conical flask. The conical flask was agitated with 150 rpm at 30°C and left overnight. The final sample was then taken to calculate for adsorbed dye on seaweed using Eq. 3.1. The seaweed was then rinsed for few times to remove any unbound dye and left in oven to dry overnight at 50°C.

3.4.2 Desorption Process

Subsequently, 150 mL of desorbing agent was then added into the conical flask containing the 1 g dried dye loaded seaweed. First sample was taken before the desorbing agent come into contact with the seaweed. The conical flask was then agitated with 150 rpm at 30°C. Sample was taken at time interval 10 min, 20 min, 40 min, 60 min, 80 min, 100 min, 120 min, 150 min, 180 min, 240 min, 300 min, and 360 min. The sample was tested for pH using pH meter and concentration of dye solution using single beam UV–Vis spectrometer at maximum absorbance wavelength. After the final sample was taken, the seaweed was rinsed with distilled water a 3-5 times to remove any remaining desorbing agent and let dry in an oven at 50°C overnight. After dried, the seaweed undergoes adsorption process and the cycle continues.

3.4.3 Desorption Efficiency

Desorption efficiency was used to calculate in terms of percentage the ability of absorbed dye to be removed on seaweed. This expression can be calculated using Eq. 3.3:

$$DE = \frac{C_t V}{qm} \times 100\% = \frac{released\ dye\ (mg)}{initially\ sorbed\ dye\ (mg)} \quad\quad (3.3)$$

where,

C_t = concentration of dye in the desorption solution at time t (mg/L)

V = volume of desorption solution (L)

q = amount of dye adsorbed on seaweed before desorption process (mg/g)

m = mass of seaweed used in the adsorption process (g)

3.5   Chemical Characterization of Adsorbent

The surface acidic and basic function groups were determined by Boehm titration method. To measure the surface acidity, 0.2 g of adsorbent were contacted with 50 mL of 0.05 N NaOH and shaken for 2 days. The mixture was filtered and then back-titrated with 0.05 N HCl. To measure the surface basicity, 0.2 g of adsorbents were contacted with 50 mL of 0.05 N HCl and shaken for 2 days. The mixture was filtered and back-titrated with 0.05 N NaOH. Both were shaken at 150 rpm and 30°C.

3.6 Experiment Design

Figure 3.1 shows the general plan of the experimental design of the present study.

| **Stage 1** |
| Preparation of biosorbent (*S. binderi*) and basic dye solution (Methyl Orange) |

| **Stage 2** |
| Maximum wavelength detetmination and calibration curve preparation of Methyl Orange |

| **Stage 3** |
| Preliminary Test (Batch Adsorption) |

| **Stage 4** |
| Desorption using different desorbing agent and regeneration |
| (a) Desorption using HCl (0.1 M) |
| (b) Desorption using CH_3COOH (10%) |
| (c) Desorption using NaOH (0.1 M) |
| (d) Desorption using distilled water |

| **Stage 5** |
| Chemical characterization of adsrobent |

| **Stage 6** |
| Result analysis and discussion |
| (a) Desorption efficiency |

Figure 3.2: Proposed Experiment Works

CHAPTER 4

RESULTS AND DISCUSSION

## 4.1   Effect of Different Pretreatment

A preliminary experiment was conducted to determine the efficiency of methyl orange (MO) biosorption process by using *Sargassum binderi* for determination of maximum adsorption capacity of *Sargassum binderi*.

In this experiment, biosorbents were chemically treated by using two different approaches. One set of the *Sargassum binderi* was treated by formaldehyde followed by 1 M HCl while the other is being treated only with 1 M HCl. Based on Figure 4.1, the percentage removal of dye for *Sargassum binderi* treated by formaldehyde and 1 M HCl is almost 80 % uptake which is comparable with *Sargassum binderi* pretreated only with 1 M HCl, (almost 70 % of uptake).

Sargassum binderi without any pretreatment shows no affinity towards MO, while the acid-treated seaweed showed some significant result on dye removal (Figure 4.1). According to Chaisena & Rangsriwatananon (2005), pretreatment process are able to modify and increase the porosity of the biosorbent's surface which causes interacition in between of some new functional groups with dye. Under acidic condition, there will be electrostatic interaction among the seaweed and the dye particles where the nitrogen-containing functional groups in the seaweed will be protonated (O'Mahony, Guibal, & Tobin, 2002). Thus, the seaweed will have a net positive charge (hydrogen ion) that was introduced from the HCl during modification

process and it is likely to be the binding sites for negatively charged dye molecule (Vijayaraghavan & Yun, 2008).

Although the percentage removal of dye by using non-formaldehyde and 1 M HCl treated seaweed is higher by around 6% in compare with the seaweed treated by both formaldehyde and 1 M HCl (Figure 4.1), the formaldehyde and 1 M HCl treated seaweed was chosen to conduct the following desorption and regeneration experiment. This is due to that acid-treated seaweed does not remove all phenolic compounds which causes discolouration of seaweed. On the other hand, pre-treatment with formaldehyde can further reduce discolouration because phenolic compounds and formaldehyde react to avoid discolouration of seaweed (McHugh, 1987).
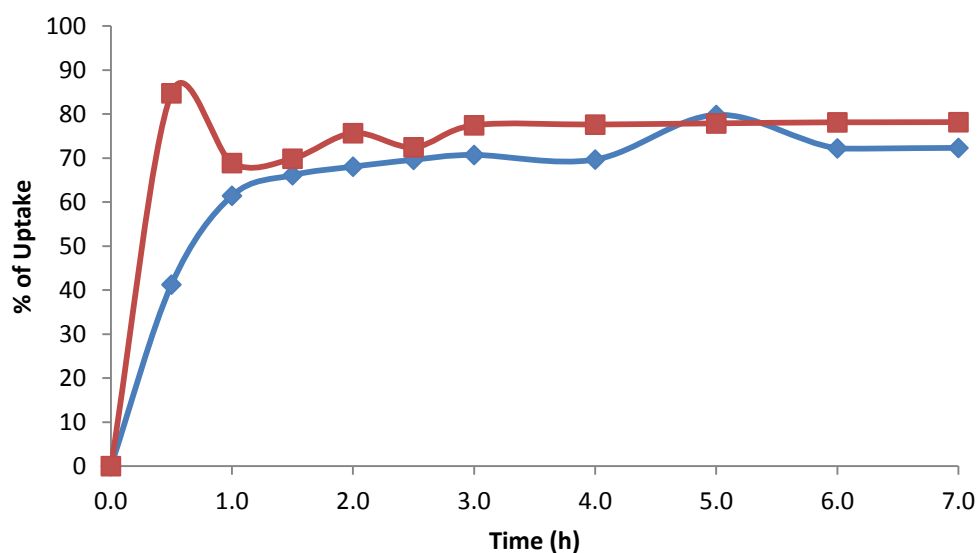
Figure 4.1: Removal MO of *Sargassum binderi* Treated with Both Formaldehyde and 1 M HCl (♦) and *Sargassum binderi* Treated with 1 M HCl Only (■).

4.2 Desorption and Regeneration

Regeneration and reuse of a biosorbent is important for industrial applications in order to reduce the process costs, the continuity dependency of the process on biosorbent and also the possibility to recover dye molecules. The purpose of desorption and regeneration of biosorbent is to determine the desirable of the biosorbent material to desorb the sorbed dye and regenerate for another cycle. Under this experiment, efficiency of *Sargassum binderi* to be regenerated and reused was studied under different types of chemical desorbing agents which are HCl (0.1 M), CH_3COOH (10%), NaOH (0.1 M) and distill water.

Prior to each experiment, *Sargassum binderi* that had undergone for biosorption was washed repeatedly with distill water to remove the unbound dye particles. Before each desorption study, the amount of MO absorbed to 1 g of *Sargassum binderi* was measured too. For the desorption study, 1 g of *Sargassum binderi* with adsorbed dye was mixed with 150 ml of the desorbing medium (HCl (0.1 M), CH_3COOH (10%), NaOH (0.1 M) and distill water) and it was placed into the incubator shaker to shake for 6 hrs. Samples were taken at a time interval of 10 minutes for the first two samples, then follow by a time interval of 20 minutes for the following five samples, and a time interval of 30 minutes for the next two samples. For the last four samples, it was taken at every 1 hour.

HCl were found to be a very powerful desorbing agent, and it may have damaging effects for the algae as it was demonstrated by the high TOC (total organic carbon) and biomass weight loss values. However, the selection of the best desorbent agents also depends on the previous state of the algae. For instance, if a protonated biomass is used, desorption with acid could be more advantageous, provided that the release of metal and regeneration of the alga can be achieved just in one step, diminishing the overall process cost (Lodeiro et al., 2006). Hence, CH_3COOH which is a weaker acid compare to HCl might worked as a powerful desorbing agent too. NaOH is a strong base chemical solution that has been widely used as desorbing agent in desorption process. Mital et al. (2009) reported that, NaOH as desorbing solution exhibit good attraction towards the dye under considearation is acidic.

After desorption process, regeneration step is applied to prevent biomass deterioration or loss of biosorption capacity. The selection of regenerating agent is widely depends on the type of biosorbent used and also the materials of being adsorbed as it determines the type of ion interaction with the biosorbent material (Mata, Blázquez, Ballester, González, & Mũnoz, 2010). For cationic biosorbents like *Sargassum binderi*, it could be regenerated by a simple acidic wash that will quickly releases the deposited metal and making way for its very high concentration in the desorbing solution (Volesky, 2007). In some cases, simple wash with distilled water will do to regenerate the biosorbent (Mata et al., 2010). The purpose of regeneration is to have a better overall process economy and also to recovered the materials like metals from the metal concentrated solution after elution by using electrochemical or other conventional techniques (Volesky, 2007; Mata et al., 2010).

4.2.1 Desorption and regeneration using HCl (0.1 M)

The desorbing agent used in the experiment is 0.1 M HCl and comparison have been made based on desorption in between of cycle 1 to cycle 2, and in between of cycle 2 to cycle 3. By comparing the desorption efficiency of Cycle 1 and cycle 2 in Table 4.1 and desorption efficiency of Cycle 2 and Cycle 3 (Table 4.2), it can be observed that the % difference of desorption efficiency is getting bigger when the desorption process proceed except for the data after 100 min which caused by reaching equilibrium. This indicates that the amount of dye being desorbed out from the biosorbent (*Sargassum binderi*) is getting lesser within each cycle of desorption and regeneration and it has reached constant at time 100 min (Figure 4.2).

According to Lodeiro, Herrero, & Sastre de Vicente (2006), the maximum dye uptake during the first cycle adsorption has resulted in a considerable reduction after first desorption cycle afterwards. The values of desorption efficiency obtained after the first cycle desorption are practically constant or in a lower variations. The reason of reduction in dye capacity uptake in the second cycle is due to the effect of acid desorption step where alteration occurred in some part of the seaweed in its

structure and chemical composition, with the consequent loss or blockage of binding sites even though the seaweed was previously acid-treated (Lodeiro et al., 2006).

Table 4.1: % Difference of Desorption Efficiency of HCl (0.1 M) in Cycle 1 and Cycle 2

Time (min)	Desorption Efficiency, DE (%)		Difference (%)
	HCl (0.1 M)Cycle 1	HCl (0.1 M) Cycle 2	
0	0.00	0.00	0.00
10	11.63	10.06	1.57
20	14.77	13.60	1.16
40	21.16	17.97	3.18
60	22.53	20.48	2.05
80	24.06	22.01	2.05
100	25.42	22.69	2.73
120	26.29	23.63	2.66
150	26.89	24.13	2.76
180	27.31	24.87	2.44
240	28.02	25.61	2.41
300	28.32	25.85	2.47
360	28.50	25.85	2.66

Table 4.2: % Difference of Desorption Efficiency of HCl (0.1 M) in Cycle 2 and Cycle 3

Time (min)	Desorption Efficiency, DE (%)		Difference (%)
	HCl (0.1 M) Cycle 2	HCl (0.1 M) Cycle 3	
0	0.00	0.02	0.02
10	10.06	7.78	2.29
20	13.60	10.20	3.41
40	17.97	13.37	4.61
60	20.48	15.21	5.27
80	22.01	16.20	5.81
100	22.69	17.11	5.57
120	23.63	17.57	6.06
150	24.13	18.27	5.86
180	24.87	18.89	5.99
240	25.61	19.55	6.06
300	25.85	19.81	6.04
360	25.85	20.00	5.84
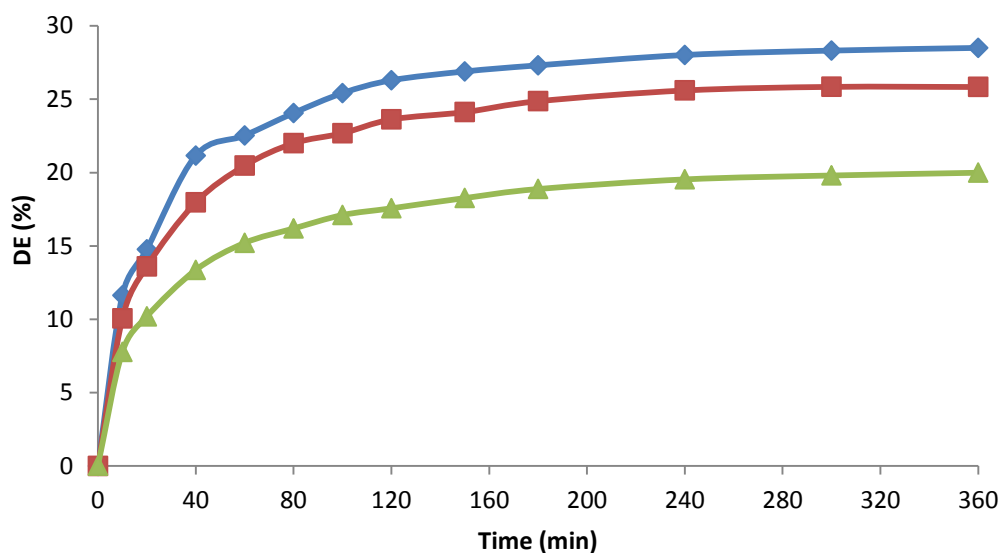
Figure 4.2: Desorption Efficiency (%) by Using 0.1 M HCl as Desorbing Agent. Symbols: (♦), 1st cycle; (■), 2nd cycle; (▲), 3rd cycle

4.2.2 Desorption and regeneration using CH_3COOH (10% v/v)

Under this part, an investigation of desorption and regeneration was carried on by using a weaker acid, which is acetic acid (CH_3COOH). CH_3COOH (10 % v/v, 150 mL) was used to desorp and regenerate the seaweed that had been loaded with dye after adsorption. From Table 4.3 and Table 4.4, the percentage difference of desorption efficiency from both cycle 1 with cycle 2 and cycle 2 with cycle 3 is getting bigger as the time of desorption process goes by. According to Table 4.3, the time needed for desorption of seaweed reach equilibrium was at 150 min while the time taken by the cycle 2 with cycle 3 to reach equilibrium was 240 min.

Figure 4.3 exhibits that the amount of MO desorbed from the biosorbent (*Sargassum binderi*) is getting lesser within each cycle of desorption and regeneration and it reached constant at 240 min. The main cause of the reduction in amount of dye after every cycle of desorption and regeneration is same as the cause of reduction in dye desorption by 0.1 M HCl, which is due to acid desorption step

that caused losses and blockage of binding sites of the seaweed after desorped and regenerated for few cycles (Lodeiro et al., 2006).

Table 4.3: % Difference of Desorption Efficiency of CH_3COOH (10 % v/v) in Cycle 1 and Cycle 2

Time (min)	Desorption Efficiency, DE (%)		Difference (%)
	CH_3COOH (10 %) Cycle 1	CH_3COOH (10%) Cycle 2	
0	0.00	0.00	0.00
10	16.77	16.15	0.62
20	21.63	22.08	0.45
40	29.77	29.01	0.76
60	33.78	32.30	1.49
80	36.42	34.31	2.11
100	37.92	35.46	2.47
120	39.34	36.83	2.51
150	40.62	37.57	3.04
180	41.34	38.45	2.89
240	42.28	39.18	3.10
300	42.84	39.63	3.21
360	43.19	40.01	3.18

Table 4.4: % Difference of Desorption Efficiency of CH_3COOH (10 % v/v) in Cycle 2 and Cycle 3

Time (min)	Desorption Efficiency, DE (%)		Difference (%)
	CH_3COOH (10%) Cycle 2	CH_3COOH (10%) Cycle 3	
0	0.00	0.00	0.00
10	16.15	14.55	1.60
20	22.08	19.12	2.95
40	29.01	25.07	3.93
60	32.30	28.02	4.27
80	34.31	30.05	4.27
100	35.46	30.88	4.58
120	36.83	31.61	5.21
150	37.57	32.27	5.30
180	38.45	33.03	5.42
240	39.18	33.74	5.44
300	39.63	34.35	5.28
360	40.01	34.65	5.36
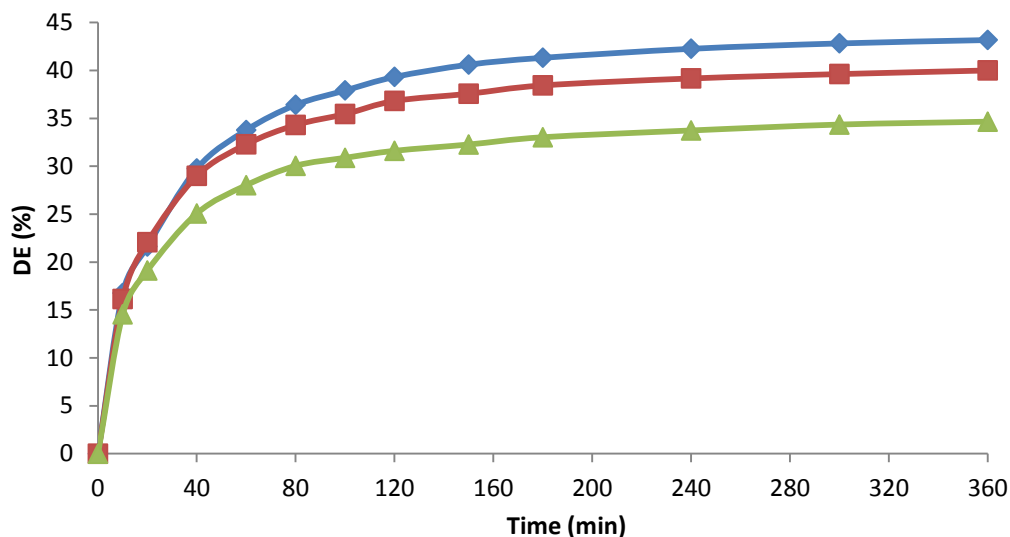
Figure 4.3: Desorption Efficiency (%)by Using (10 % v/v) CH$_3$COOH as Desorbing Agent. Symbols: (♦), 1st cycle; (■), 2nd cycle; (▲), 3rd cycle

4.2.3 Desorption and regeneration using distilled water

Distilled water is a desorbing agent that is neutral in terms of pH (pH 7) where there is no amount of free hydrogen ion and hydroxyl ion in the water (Water chemistry, 2011). An experiment of desorption and regeneration of *Sargassum binderi* which had adsorbed MO is carried on with distilled water and the results was shown in Table 4.5, Table 4.6, and Figure 4.4. Table 4.5 illustrates the percentage difference of desorption efficiency of distilled water for cycle 1 and cycle 2 while Table 4.6 display the percentage difference of desorption efficiency of distilled water for cycle 2 and cycle 3. Figure 4.4 shows the % of desorption efficiency (DE) for each cycle within the time frame of 360 min.

The results show that there are only small variations in % of DE at each cycle. The reason of small variation in % DE for each cycle is because distilled water which has its impurities removed through distillation process (left only H+ and OH- and neutral in terms of pH) did not have damaging effects to the seaweed that could result in decreased of dye uptake capacity in the next-cycle unlike acidic desorbing agent which alters the binding sites of seaweed (Davids, Volesky, & Vieira, 2000;

Disitlled Water, 2012). Thus, there is no significant reduction in % desorption efficiency as the seaweed was recycled and regenerated for another 2 cycles.

Table 4.5: % Difference of Desorption Efficiency of Distilled Water in Cycle 1 and Cycle 2

Time (min)	Desorption Efficiency, DE (%)		Difference (%)
	Distilled Water Cycle 1	Distilled Water Cycle 2	
0	0.00	0.00	0.00
10	10.64	12.12	1.48
20	16.28	20.13	3.84
40	23.63	27.52	3.89
60	29.04	33.22	4.18
80	33.29	36.36	3.07
100	35.94	38.25	2.32
120	37.82	40.09	2.27
150	39.64	41.68	2.04
180	41.19	42.99	1.80
240	43.19	44.06	0.86
300	44.13	44.79	0.66
360	45.16	45.22	0.05

Table 4.6: % Difference of Desorption Efficiency of Distilled Water in Cycle 2 and Cycle 3

| Time (min)	Desorption Efficiency, DE (%)		Difference (%)
	Distilled Water Cycle 2	Distilled Water Cycle 3	
0	0.00	0.00	0.00
10	12.12	17.49	5.37
20	20.13	22.22	2.10
40	27.52	28.60	1.08
60	33.22	32.53	0.69
80	36.36	35.43	0.93
100	38.25	37.46	0.80
120	40.09	38.98	1.12
150	41.68	40.61	1.06
180	42.99	41.88	1.12
240	44.06	43.37	0.69
300	44.79	44.42	0.37
360	45.22	44.98	0.24
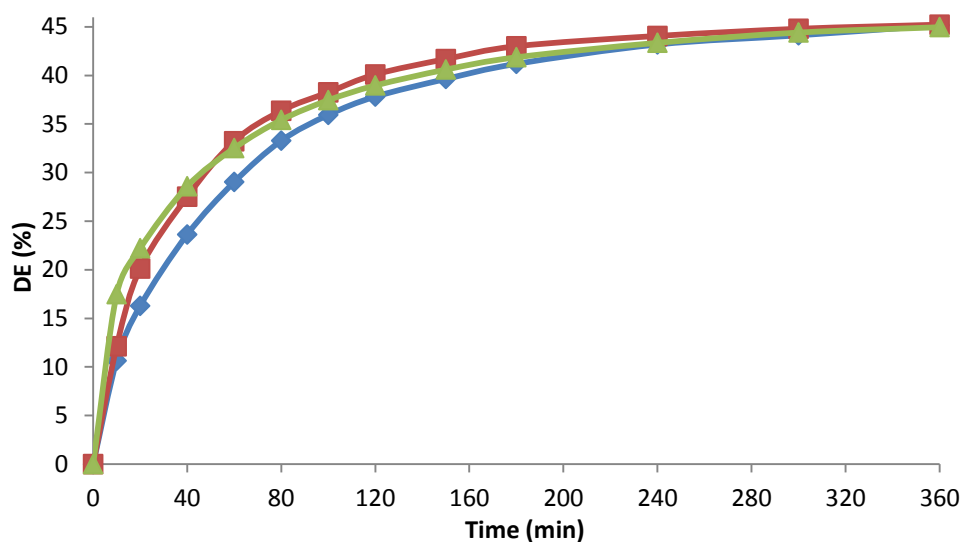
Figure 4.4: Desorption Efficiency (%) by Using Distilled Water as Desorbing Agent. Symbols: (♦), 1st cycle; (■), 2nd cycle; (▲), 3rd cycle.

4.2.4 Desorption and regeneration using NaOH (0.1 M)

Sodium hydroxide (NaOH) is the only basic desorbing agent that was used in this experiment and sodium hydroxide is known as a strong base chemical agent (Sodium hydroxide, 2012). Figure 4.5 presents % of desorption efficiency for cycle 1 only by using NaOH as desorbing agent.
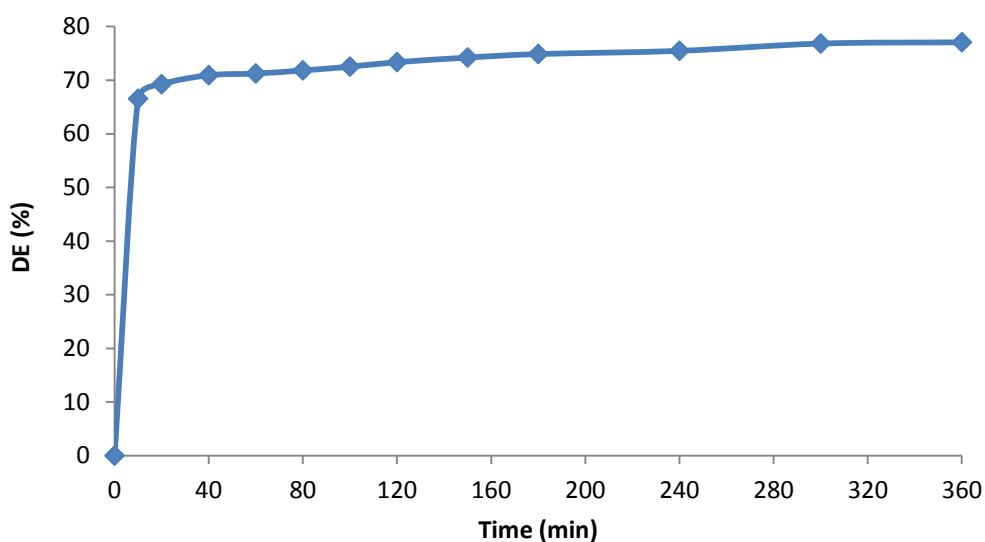
Figure 4.5: Desorption Efficiency (%) by Using NaOH (0.1 M) as Desorbing Agent for Cycle 1. Symbols: (♦), 1st cycle.

In this experiment, there is only one cycle of desorption process can be done. This is due to the seaweed (*Sargassum binderi*) started to degrade and dissolve wwhen inside the NaOH solution, as shown in Figure 4.6. According to McHugh (1987), alkaline extraction is a process used to convert alginate to a soluble form so that it can be removed from the rest of the seaweed. In this part of experiment, alkaline extraction process occurred the dried and acid pre-treatment seaweed is prone to breakdown and degrade when it is in contact with alkaline solution (McHugh, 1987). Although the percentage of desorption efficiency shown in Figure 4.5 for the first cycle is quite high but the result might be not accurate as some of the colour of the seaweed was leached out to the solution due to degradation of seaweed.

Figure 4.6: Degradation of *Sargassum binderi* in NaOH (0.1 M)
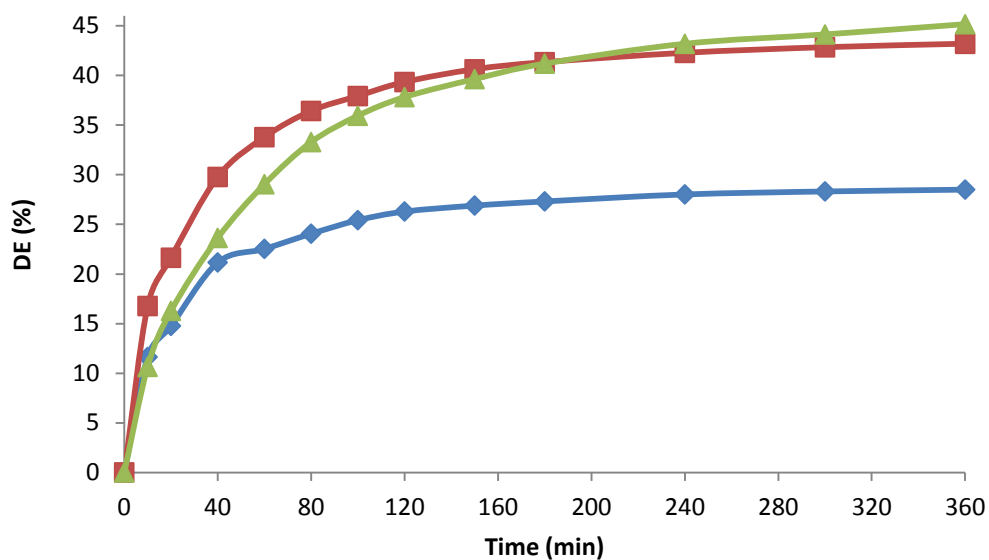
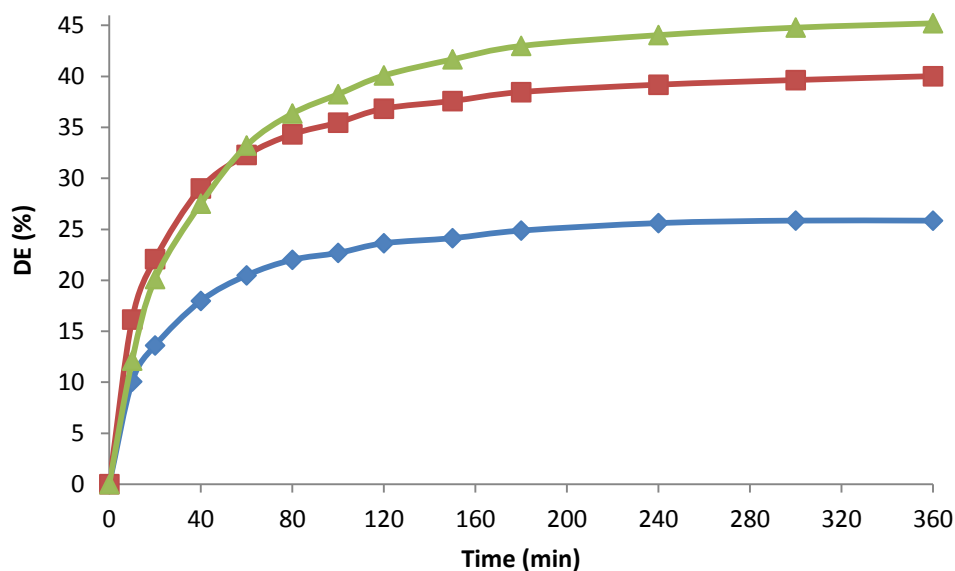**4.2.5   Comparison Between Different Desorbing Agent Based on Each Cycle**

Results of three different type of desorbing agent, which are 0.1 M HCl, 10 % v/v of CH₃COOH, and distilled water was used to compare the percentage of desorption efficiency of each cycle. The purpose is to identify the most efficient desorbing agent for *Sargassum binderi* to desorp MO and ability to regenerate and reuse for the following cycles.

Figure 4.7, Figure 4.8, and Figure 4.9 indicates desorption efficiency of different desorbing agent for cycle 1, cycle 2, and cycle 3, respectively. Based on Figure 4.7, it can be observed that no obvious variation in between the % of DE by CH₃COOH (10 % v/v) with distilled water, 45 % of DE. However, during desorption and regeneration at cycle 2, the percentage of desorption efficiency for CH₃COOH (10 % v/v) has dropped to 40 % while distilled water is still remaining at 45 % of DE. As for the third cycle of desorption and regeneration process, a significant difference of % DE of distilled water and CH₃COOH (10 % v/v) can be observed for Figure 4.9. Desorption efficiency of distilled water remained at 45 % while the desorption efficiency of CH₃COOH (10 % v/v) has dropped to 35 %.

Experiment using 0.1 M HCl as desorbing agent is also facing the same phenomena as CH₃COOH (10 % v/v) where desorption efficiency of 0.1 M HCl decreased roughly by 5 % as each desorption and regeneration cycles goes by. This shows that there is possibility of loss or blockage of binding site of biosorbent, for both 0.1 M HCl and CH₃COOH (10 % v/v) (Lodeiro et al., 2006). Nevertheless, biosorbent of distilled water does not affected as its solution does not contain freely moved hydrogen ion and hydroxyl ion in the solution which will alter the structure and chemical composition of the biosorbent. Hence, it can be concluded that distilled water is the best desorbing agent among all due to its efficiency in desorbing MO as it does not alter the structure nor chemical composition of the biosorbent.

Figure 4.7: Comparison Desorption Efficiency of Different Desorbing Agent for Cycle 1. Symbols: (♦), HCl (0.1 M); (■), CH₃COOH; (▲), Distilled water.


Figure 4.8: Graph of Comparison Desorption Efficiency Versus Time (min) of Different Desorbing Agent for Cycle 2. Symbols: (♦), HCl (0.1 M); (■), CH₃COOH; (▲), Distilled water.
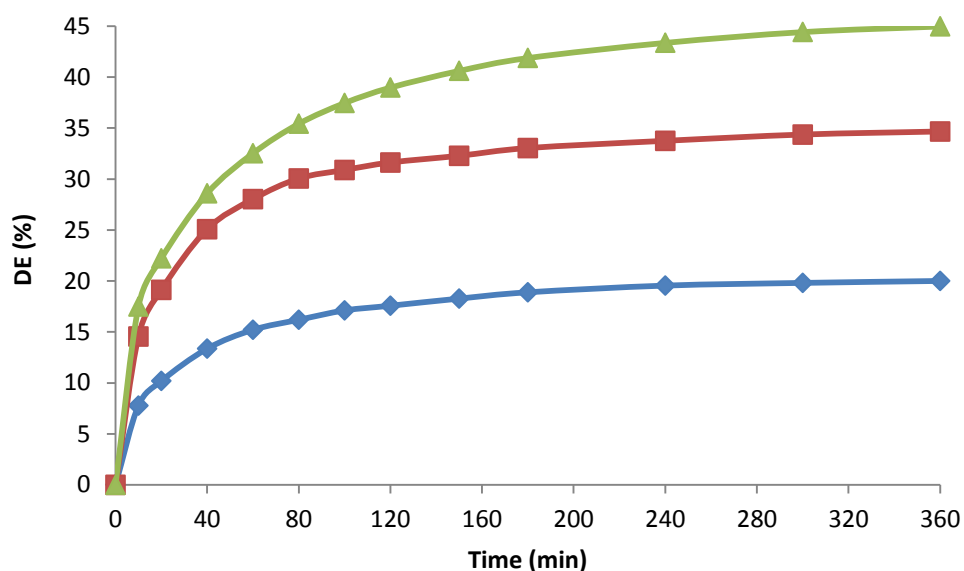
Figure 4.9: Comparison Desorption Efficiency of Different Desorbing Agent for Cycle 3. Symbols: (◆), HCl (0.1 M); (■), CH$_3$COOH; (▲), Distilled water.
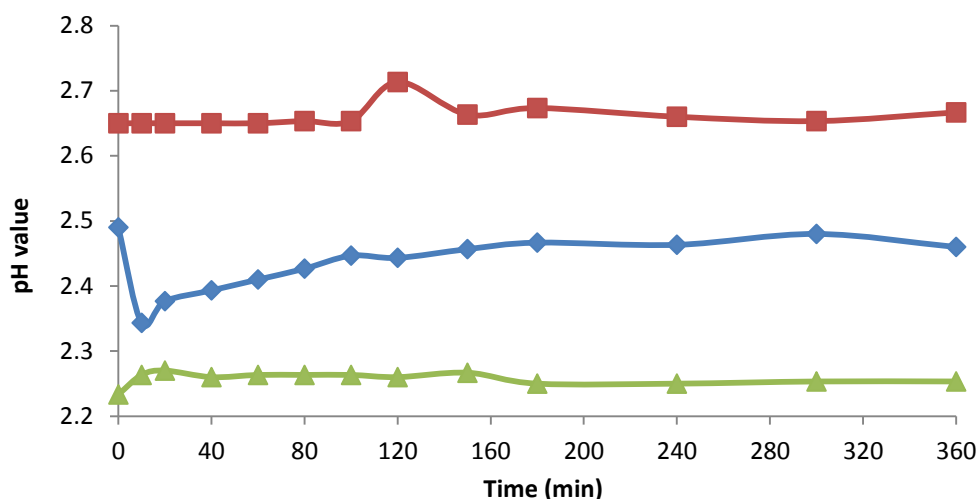
4.2.6   Change of pH During Desorption

Figure 4.10 presents the pH changes where desorption of MO from *Sargassum binderi* by using 0.1 M HCl as desorbing agent took place. Based on Figure 4.10, the pH values for each cycle remain almost constant for all the time. However, the pH value for cycle 2 and cycle 3 is much lower in compare to the pH value for cycle 1. This is attributed to human error while preparing the 0.1 M HCl desorbing agent from concentrated HCl solution. Besides, there is a sudden drop and increase of pH value for cycle 1 at time of 80 min which is due to inaccuracy of the pH meter.
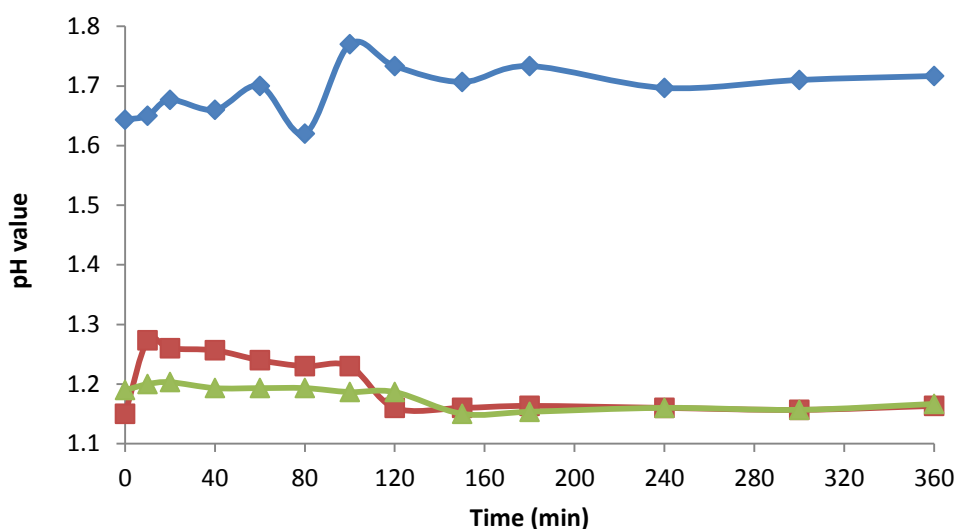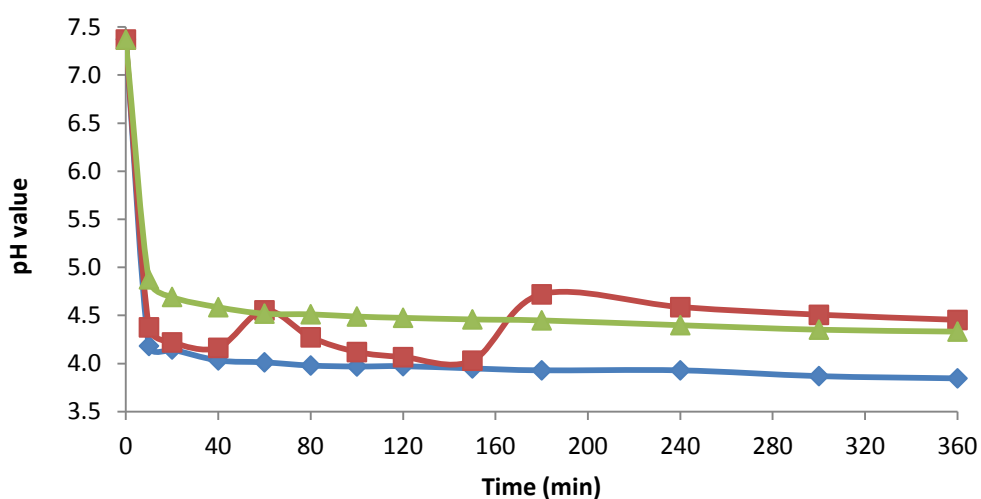
Figure 4.10: Changes of pH During Desorption for HCl (0.1 M). Symbols: (♦), 1st cycle; (■), 2nd cycle; (▲), 3rd cycle.

For CH$_3$COOH, the pH value of cycle 1 has started to drop at the point of 0 min to 10 min from pH 2.49 to pH 2.34 (Figure 4.11). The reason of insignificant changes of pH at the first point of cycle 1 might be due to the acid pre-treatment seaweed which has not been washed completely to pH 7 and there is a possibility that the residue of acid leached to the desorbing solution. The pH value for cycle 2 and cycle 3 does not shows any significant changes in pH value along the time interval of desorption process. As observed from Figure 4.11, the overall pH value for cycle 2 is higher than pH value for cycle 3 which might be due to inconsistency in preparation of the 10 % v/v CH$_3$COOH solution for each cycle.

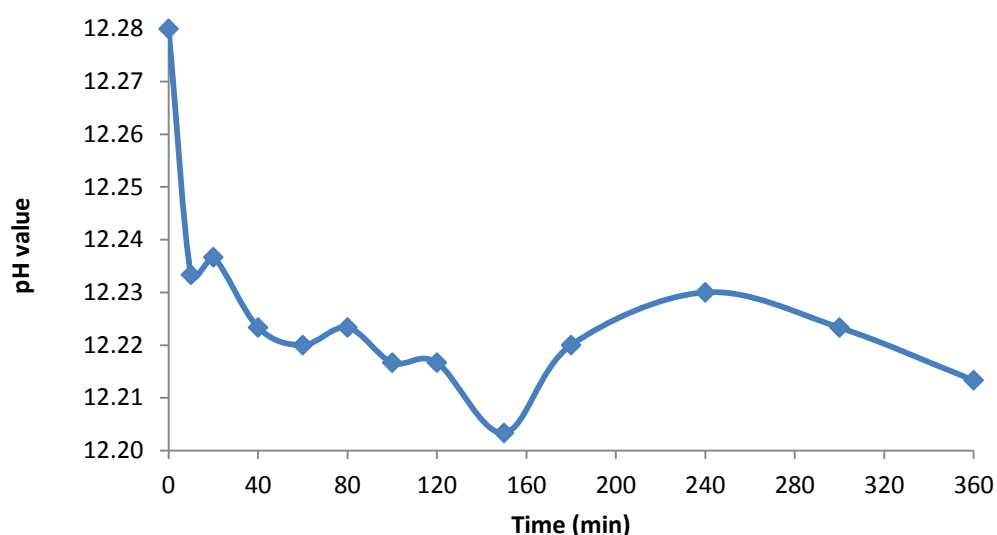**Figure 4.11:** Changes of pH During Desorption for CH_3COOH (10 % v/v). Symbols: (♦), 1st cycle; (■), 2nd cycle; (▲), 3rd cycle.

From Figure 4.12, the pH value of cycle 1 has started to drop at the point of 0 min to 10 min to CH_3COOH had occurred for all three cycles of pH value for distilled water. Acid pre-treatment seaweed which is not being washed truly to pH 7 is the main cause of causing the huge pH drop at the starting point. After the significant changes in pH value at the started point from 0 min to 10 min, there is only some small pH fluctuation for cycle 2 while the pH for cycle 1 and cycle 3 remain almost constant along the time interval of desorption process.

Figure 4.12: Changes of pH Over Time During Desorption for Distilled Water. Symbols: (♦), 1st cycle; (■), 2nd cycle; (▲), 3rd cycle.

By using 0.1 M NaOH as desorbing agent (Figure 4.13), there is only the first cycle of pH value available as the seaweed has degraded by the desorbing agent. According to Figure 4.13, the pH value has an insignificant dropped of pH value at the starting point. Later then, the pH value keeps on fluctuating along the time interval of desorption process. The inconsistent pH value along the time of desorption process for 0.1 M NaOH is due to the alkaline extraction phenomena.

Figure 4.13: Changes of pH Over Time During Desorption for NaOH (0.1 M). Symbols: (♦), 1st cycle

4.2.7   Chemical Characterization of Adsorbent

According to Ip, Barford, & McKay (2009), it is important to identify the chemical characteristics of the adsorbent (*Sargassum binderi*) as the chemical characteristics of adsorbent affects the attraction or repulsive interactions between the adsorbent surface and the adsorbate, i.e., MO used in the present study. Thus, the chemical characterization test is used to determine the net charge of the adsorbent surface in the solution (Ip, Barford, & McKay, 2009). Based on Table 4.7, the acid-treated *Sargassum binderi* is acidic due to the extensive presence of carboxylic groups at the binding site of the seaweed.

Table 4.7: Acidity and Basicity of Acid-treated *Sargassum binderi*

Adsorbent	Total acidic groups (mmol/g)	Total basic groups (mmol/g)
Acid treated *S.binderi*	5.825	3.200

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1   Conclusions

The objective of this experiment is to investigate the recyclability of acid-treated *Sargassum binderi* for removal of methyl orange by using four different types of desorbing agent, which are HCl (0.1 M), CH_3COOH (10% v/v), distilled water, and NaOH (0.1 M). Based on the results, MO can be desorbed from acid-treated *Sargassum binderi* at all the three cycles by using HCl (0.1 M), CH_3COOH (10 % v/v), and distilled water as desorbing agent except for NaOH (0.1 M) where alkaline extraction occurred after first cycle of desorption.

In average, desorption process by using HCl (0.1 M), CH_3COOH (10 % v/v), and distilled water as desorbing agent for each cycle reached constant after 300 min of desorption process. It was found that the percentage of desorption efficiency by using HCl (0.1 M) and CH_3COOH (10 % v/v) decreased after each cycle of desorption and regeneration process. However, no obvious desorption efficiency could be observed by using distilled water in all of the three cycles. Instead, it remains constant for all three cycles with a desorption efficiency of 45 % and it was the desorbing agent that scores the highest in percentage of desorption efficiency. Besides, a chemical characterization test for adsorbent and acid-treated *Sargassum binderi* was tested to be acidic.

According to the present study and analysis of results, it is suggested that the best desorbing agent for acid-treated *Sargassum binderi* is distilled water as the

biosorbent can be reused and recycle for three times or more without losing its structure and also binding sites. Distilled water is considered as very high purity water where most of the impurities are being removed. Thus, it will be easier for dye recovery process in future.

5.2   Recommendation and Future Research

Regarding to the improvement on this part of thesis, the following future research and recommendations of application in industrial field are suggested:

(a) Desorption of acid-treated *Sargassum binderi* with other desorbing agent should be investigated in future to make desorption process more ecologically and economically feasible.

(b) To further study on desorption process by using different concentration of a desorbing agents to identify the optimum concentration of desorbing agent.

(c) More desorption and regeneration cycles should be carried in order to identify the maximum limit of recyclability of acid-treated *Sargassum binderi*.

(d) To investigate the effects of others operating parameters such as effect of temperature, agitation speed, and dye concentration.

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