PRODUCTION OF RED MUD COMPOSITE AS COAGULANT FOR HARVESTING *CHLORELLA VULGARIS*

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PRODUCTION OF RED MUD COMPOSITE AS COAGULANT FOR HARVESTING *CHLORELLA VULGARIS*

by

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NOR FATIHAH BINTI ABDULLAH

TABLE OF CONTENTS

LIST OF TABLES

LISTS OF FIGURES

LIST OF SYMBOLS

%	Percent
°C	Degree Celcius
±	Plus minus
<	Less than
>	More than
≈	Approximately

LISTS OF ABBREVIATIONS

h	hour
g	gram
L	liter
kg	kilogram
MPa	megapascal
L/ha	liter per hectare
gal/ac	gallon per acre
nm	nanometer
L/min	liter per minute
µm	micrometer
mm	millimetre
M	Molar
mL	millilitre
mg	milligram
mmHg	millimetre of mercury
ppm	parts per million
g/L	gram per litre
v/v	volume per volume

w/v	weight per volume
cm^{-1}	reciprocal centimetres
Al	Aluminium
Fe	Iron
$AlCl_3$	Aluminium chloride
$FeCl_3$	Iron (III) chloride
Fe_3O_4	Iron (III) oxide
Al_2O_3	Aluminium oxide
Fe_2O_3	Iron (III) oxide
SiO_2	Silicon dioxide
Na_2O	Sodium oxide
CaO	Calcium oxide
TiO_2	Titanium dioxide
MnO	Manganese (II) oxide
MgO	Magnesium oxide
K_2O	Potassium oxide
P_2O_5	Phosphorus pentoxide
$NaNO_3$	Sodium nitrate
K_2HPO_4	Dipotassium phosphate

$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
$CaCl_2.2H_2O$	Calcium chloride dihydrate
$EDTANa_2$	Ethylene Diamine Tetraacetic Acid Disodium
Na_2CO_3	Sodium carbonate
$MnCl_2.4H_2O$	Manganese (II) chloride tetrahydrate
H_3BO_3	Boric acid
$ZnSO_4.7H_2O$	Zinc sulphate heptahydrate
$Na_2MoO_4.2H_2O$	Sodium molybdate dihydrate
$CuSO_4.5H_2O$	Copper (II) sulphate pentahydrate
$Co(NO_3)_2.6H_2O$	Cobalt (II) nitrate hexahydrate
$TiCl_4$	Titanium tetrachloride
$SiCl_4$	Silicon tetrachloride
PCl_5	Phosphorus pentachloride
$MgCl_2$	Magnesium Chloride
NaCl	Sodium chloride
$MnCl_2$	Manganese (II) chloride
$CaCl_2$	Calcium chloride
KCl	Potassium chloride
$Al(OH)_3$	Aluminium hydroxide

$Fe(OH)_3$	Iron (III) hydroxide
$Ti(OH)_3$	Titanium (IV) hydroxide
$Si(OH)_2$	Silicon hydroxide
$P(OH)_5$	Phosphorus (V) hydroxide
$Mg(OH)_2$	Magnesium hydroxide
$Na(OH)$	Sodium hydroxide
$Mn(OH)_2$	Manganese dihydroxide
$Ca(OH)_2$	Calcium dihydroxide
KOH	Potassium hydroxide
H_2O	Water
CO_2	Carbon dioxide
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
KBr	Potassium bromide
UK	United Kingdom
USA	United State of America
US	United State
RM	Ringgit Malaysia
USD	US Dollar

PENGHASILAN KOMPOSIT LUMPUR MERAH SEBAGAI KOAGULAN UNTUK PENUAIAN *CHLORELLA VULGARIS*

ABSTRAK

Lumpur merah adalah sisa pepejal yang tidak boleh larut dan mempunyai kandungan alkali yang tinggi dimana dihasilkan semasa pengekstrakan alumina daripada bauksit melalui proses Bayer. Cara yang selalu digunakan oleh industry perlombongan untuk melupuskan lumpur merah adalah dengan membuangnya ke dalam tasik besar di kawasan yang tertutup. Tetapi, masalah yang paling besar mengenai kaedah ini adalah ia mewujudkan pencemaran yang disebabkan oleh kandungan alkali yang tinggi dan keberadaan logam berat dalam lumpur merah. Kajian ini akan tertumpu kepada penghasilan koagulan komposit daripada lumpur merah dengan menggunakan teknik melarut lesap asid yang terbahagi kepada melarut lesap asid, penghidrolisisan dan pempolimeran. Komposit ini akan digunakan sebagai bahan penggumpal dalam teknik penggumpalan-pengelompokan untuk menuai *Chlorella vulgaris*. Pengeluaran koagulan komposit daripada lumpur merah mempunyai potensi yang cerah disebabkan oleh kandungan aluminium oksida dan oksida ferum yang tinggi dalam lumpur merah. Matlamat kajian ini adalah mensintesiskan, mencirikan, pengubahsuaian, dan penggunaan koagulan komposit yang telah dihasilkan daripada lumpur merah untuk menuai *Chlorella vulgaris*. Lumpur merah sesuai dan mempunyai potensi dalam penuaian mikroalga tetapi ia mengambil masa lebih daripada 2 jam untuk berkumpal. Untuk mengoptimumkan kebolehlaksanaan, komposit lumpur merah telah diubahsuai dengan menambah garam klorida (Al, Fe) dimana keputusan menunjukkan penambahan $AlCl_3$ memberi hasil yang lebih baik berbanding $FeCl_3$. Kecekapan pengumpalan telah diselidik melalui pengaruh dos, pH kultur, pH sistem dan masa pengumpalan. Dibawah pengaruh dos Al komposit yang berbeza, 0.5 g/L Al komposit memdapatkan pemulihan biomass sebanyak 83.6 %. Peratus pemulihan biomass meningkat apabila pH kultur mencecah 10 dengan peratus pemulihan tertinggi sebanyak 87.6

%. Keputusan juga menunjukkan komposite yang diubahsuai boleh bergumpal sehingga 92 % dalam masa 25 minit dengan mengekalkan pH sistem dengan anggaran 6.8.

PRODUCTION OF RED MUD COMPOSITE AS COAGULANT FOR HARVESTING *CHLORELLA VULGARIS*

ABSTRACT

Red mud is a solid waste which is insoluble and has high alkaline content that is produced during alumina extraction from bauxite using Bayer process. The most common way used to dispose the red mud is by dumping it into large pond within the secured places. But the biggest concern regarding this method it does create pollutions due to high alkaline content and presence of heavy metals in red mud. This study will focus on the feasibility of developing composite coagulant from red mud by using acid leaching method which consists of acid leaching, hydrolyzation and polymerization. This composite was used as coagulant to harvest *Chlorella vulgaris* using coagulation-flocculation method. Production composite coagulant from red mud is promising because of its have high content of aluminium oxide and iron oxide. The aims of this study are to synthesize, characterize, modification and utilization of composite coagulant produced from red mud for *Chlorella vulgaris* harvest. Red mud composite was feasible and has potential in harvesting microalgae but it take more than 2 hour to coagulate. To optimize the feasibility, the red mud composite is modified by adding chloride salt (Al, Fe) which concludes the addition of $AlCl_3$ showed better results compared to $FeCl_3$. The coagulation efficiency was investigated through influence of dosage, pH of culture, pH of system and coagulation time. Under influence of different dosage of Al composite, 0.5g/L composite acquired 83.6% of biomass recovery. The percentage of recovery increased with increase of pH of the culture at 10 where the percentage of recovery at the highest of 87.6%. The results also showed that this modified composite can coagulate up till 92% within 25 minutes by maintaining pH of system at approximately 6.8.

CHAPTER 1 : INTRODUCTION

1.1 Research Background

Microalgae are usually found in freshwater or marine system depending on the species characteristics. Microalgae especially their biomass have been used widely and being applying for many purposes such as food supplement, cattle feed and bio fuel. Algal production require energy and cost intensive especially harvesting process as the existing method is less economical or sustainable (Abdul Razack *et al.*, 2015) and harvesting microalgae usually contributes for 20–30% of the total production cost (Blair *et al*, 2014). A lot of harvesting methods have been used and suggested such as centrifugation, flotation, filtration and others which have their advantages and disadvantages.

One of the harvesting method has been used is coagulation-flocculation method. Coagulation-flocculation is used to separate suspended solids portion from liquid portion. Coagulation is aggregation of suspended cell into large particles to enhance biomass recovery while flocculation is involving the coalescing of aggregates into large flocs that settle out of suspension (Kocamemi, 2012). This method usually has been applied in water treatment industries because the separation process is easier and energy saving. But, the commercial coagulants used in this methods presents in the market such as chitosan, iron-based coagulants and aluminium-based coagulants are expensive and not suitable for harvesting large quantities of biomass as it require high dosage in the process. In order to overcome this issue, there are many researcher have been trying to improve and develop coagulants by forming alternative coagulants from various resources such as polypyrrole/Fe_3O_4 magnetic nano-composite (Hena *et al.*, 2016), inorganic–organic composite coagulant consist of Polyferric Sulphate (PFS) and Polyacrylamide (PAA) (Moussas and Zouboulis, 2009), and polyaluminiumchloride (PAC) (Maholtra, 1994).

Nowadays aluminium is one of the valuable products and because of its high demand, they have been massively produced in many countries for example Australia, China, Jamaica, India and others. The alumina refinery industries produced waste known as bauxite residue or red mud. This waste being produced after undergoes a process involve in production of alumina which is Bayer process. Red mud can be seen as red dust like, have high alkalinity and contain various metal oxides which the variations is depend on the bauxite ore of their initial chemical composition. This red mud usually produced almost at 1 to 1 kg ratio to alumina and usually will be disposed into sealed or unsealed landfills. Due to abundant production, these landfills cannot accommodate this waste and lead to a lot of environmental problem. There are many research have been conducted in solving the problem by transforming red mud into valuable product or uses and one of the example is direct transformation of red mud into pig iron due to it high iron content (Balomenos *et al.*, 2011). Besides that, red mud also has been used in ceramic industry, cement industry, building material industry and agriculture industry to improve soil quality (Cabllk, 2007).

1.2 Problem Statement

As being mentioned before, red mud has been creating problems involving the environment although red mud is categorized as non hazardous materials. But due to its increasing and continuously production, the dump site was not enough to accommodate all the waste produced. This will change the condition of the soil due to its high alkalinity and also create water pollutant due to their high iron content. Although there is a lot of method to permanently disposed off or used of red mud (Cabllk, 2007) but still red mud has complex compound and cannot be completely dispose. Until this day, there is no approach involving using red mud as source of alternative coagulant or composite coagulant. Red mud has various metal oxide and mostly having high iron oxide and aluminium oxide. As the commercial coagulant are basically aluminium and iron based, the idea of using red mud for the coagulants formation is a good approach for the research.

1.3 Research Scope

The study focused on the red mud source from India. From this red mud, composite was synthesized using acid leaching methods for harvesting microalgae. The composite produced used as coagulant for coagulation-flocculation methods to see the potential efficiency in biomass recovery of microalgae (*Chlorella vulgaris*). This composite also undergoes modification to maximize the efficiency for harvesting microalgae. These modified composite were compared with the unmodified composite and commercial composite to see the differences on biomass recovery.

1.4 Research Objective

The purpose of this study is to investigate the potential of red mud in producing composite as coagulant for microalgae harvesting. This study was focusing on coagulation-flocculation method for harvesting microalgae.

Based on the above, this study has been conducted with the following objectives:

1. To synthesize, characterize, modification and utilization of a composite from red mud for harvesting microalgae.
2. To improve the efficiency of red mud composite by adding chloride salts of Al and Fe by quantify the optimum coagulants dosage, pH of culture, pH of system, flocculation time and concentration factor on microalgae to acquire maximum benefits.
3. To identify the effect of biomass and age of culture on efficiency of the composite.

CHAPTER 2 : LITERATURE REVIEW

2.1 Red Mud

2.1.1 Source of Red mud

Red mud can also be known as bauxite residue have constantly produced all over the world after extracting aluminium as targeting metal which have been used for making a lot of thing that can be utilized by humankind. There are a lot of country that have been produce large production of bauxite where general ratio of bauxite which yields alumina is 2:1 and because of high production, these country were having environmental issue because of the residue or red mud created. When the production of bauxite increases, the production of red mud also increases. Based on Table 2.1, Australia showed highest production of bauxite which keep increasing by year and at 2008, the production of bauxite reached 59 959 metric tonnes. This production may happen due to high demand and endless sources which provided by various countries for example Brazil, China, India and Jamaica where they produce more than 10 000 metric tonnes per year. But for a small country like Malaysia they produced quite low amount of bauxite due to their limited sources and generally their bauxite was sent to other country like China for further aluminium processing which was why the production became decrease by year.

Table 2.1 Bauxite world production of various country in thousand metric tonnes (Guidance, 2008)

Country	Bauxite production (thousand metric tonnes)			
	2001	2002	2004	2008
Australia	53 135	55 602	56 593	59 959
Brazil	13 260	17 363	19 700	19 800
China	12 000	13 000	15 000	18 000
India	9 647	10 414	11 285	11 957
Jamaica	13 120	13 444	13 296	14 118
Malaysia	40	6	2	2
Indonesia	1 283	1 263	1 331	1 400
Kazakhstan	4 377	4 737	4 706	4 800
Russia	4 500	5 500	6 000	6 400
Venezuela	5 191	5 446	5 842	5 900

Red mud is basically created from bauxite ore after undergoes two main standard industrial production steps to produce primary aluminium which are, production of high grade metallurgical alumina (Al_2O_3) from bauxite via Bayer Process and the electrolytic reduction of alumina to aluminium via Hall-Héroult process (Balomenos *et al.*, 2011). This residue is a side-product or waste that being produced after undergoes either both of the process. The amount and quality of red mud produced will depend on the raw material and variety of bauxite ore used.

2.1.2 Production of Red mud

The Bayer Process is basically a cyclic chemical process which consists of digestion stage, liquor clarification stage, precipitation stage, liquor surge stage and calcinations stage that was patented by Karl Joseph Bayer in 1888 (Balomenos *et al*., 2011). The first stage started with digestion stage where extraction of bauxite ore with sodium hydroxide solution, under high pressure (3.5 MPa) and elevated temperature (140 °C to 300 °C) to obtain alumina and then the cycle will go for precipitation stage which pregnant solution is cooled down to 60 °C and seeded with gibbsite in order to achieve the precipitation of trihydrate alumina where this stage is done after clarification in thickness of pregnant solution is complete (liquor clarification stage) (Balomenos *et al*., 2011). The cyclic process will complete after liquor surge stage where the spent caustic soda solution is regenerated through evaporation and calcinations stage where the trihydrate alumina is calcinated at 1100 °C to produce powdered, high grade metallurgical alumina (Balomenos *et al*., 2011).

This Bayer process is common process in production of aluminium with red mud as by-product. As the production of aluminium is increases, the amount of red mud also increases. After production is complete, red mud usually dump into nearby closed or sealed land area or sea lagoons depending upon industrial management. Some of them used depleted mine site for dumping and some of them reuse the red mud and recover some of the caustic soda value to avoid environmental issues (Evans and Speaker, 2015). There are two type of disposal tailings technology that usable where the first method is traditional slurry impoundments and the second is thickened tailings disposal impoundments (Gawu *et al*., 2012). The selection of the disposal technology depends on the risk of pollutant that might occur where the best option will be applied on.

2.1.3 Characteristic of Red mud

Red mud is having high alkalinity with general pH of approximately 10 to 12. The colour is basically brick red and slurry which has different states, particle sizes and water content or in a form of fine solids which depend on the type of disposal as can be seen in figure 2.2 or powdered on dry basis which can referred in figure 2.3.

Figure 2.2 Red muds in different points of disposal (Tóth *et al*., 2014)

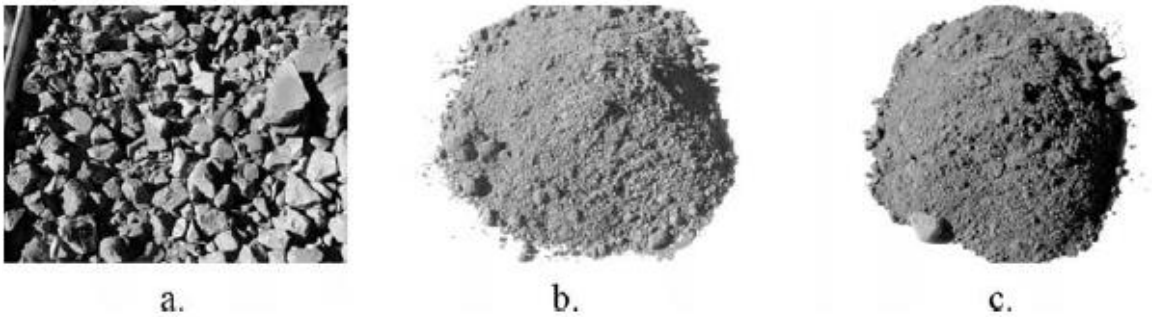

Figure 2.3 Visual appearance of autoclaved red mud after dried (Cabllk, 2007)

Red mud contains various metal oxides with high iron and aluminium oxide content. The variation of the metal oxide will depend on raw material of bauxite ore. Common metal oxide can be detect in the red mud are iron oxide, aluminium oxide, silica oxide, titanium oxide, calcium oxides and others which the common composition of red mud can be referred in Table 2.4. This table showed that iron oxide and aluminium oxide composition is two top elements that present in red mud. Due to their characteristics of non-soluble, high alkalinity and present of great amount of heavy metal elements, red mud have high potential causing pollutant in the environment.

Table 2.4 Typical composition of red mud (Deelwal *et al*., 2014)

Composition	Percentage (%)
Iron oxide	30-60
Aluminium oxide	10-20
Silicon dioxide	3-50
Sodium oxide	2-10
Calcium oxide	2-8
Titanium dioxide	Trace-25

2.1.4 Application of Red mud

Red mud is considered non-hazardous materials but due to its chemical composition which contain a lot of heavy metal that effect surrounding area which they have been disposed. Many pollutant issue involves red mud have been surface out and because of it continuation production of aluminium, these by-product also continuously created. In order to solve this problem, many research have been carried out and applied for example to made use of the red mud, creating better method for better and safer disposal, recoveries of a specific component in the red mud, use them as major component in manufacture a product or as a building material and convert red mud into useful compound (Evans and Speaker, 2015).

Red mud has made use in many industries and one of the industries is cement industry. In this industry, red mud either can takes role as crystallization in the production of cement clinker or involves in the production of cement by mixing the red mud, fly ash, lime and gypsum as raw material where fly ash can be used to absorb the water contained in the red mud and improve the reactive silica content of cement (Deelwal *et al*., 2014). It is reported that in India, approximately 2.5 million tonnes of red mud has been used in cement industries during 2006-2007 (Guidance, 2008). This application can improves the strength of the cement, resistance to sulphate attack and also reduces energy consumption in the production of cement (Deelwal *et al*., 2014).

In building material industry, red mud also have been used as raw material for the building manufacture, pavement blocks, road surfacing and brick production (Cabllk, 2007). They are using red mud as the raw material due to its extremely fine particles and their red colour characteristic which is suitable for building manufacture (Deelwal et al., 2014). In Zibo, Shandong a relevant department have been tested the sub grade stability and the strength of road using red mud as base material and concluded that the red mud base road meets the strength requirements of the highway (Deelwal et al., 2014). In China and Australia both of this country have been using red mud as raw material for brick production where China solely use 10% of red mud for this production while Australia also had made brick out of red mud to build homes in South-West of Western Australia which reported in February 2002 (Guidance, 2008)

2.2 Microalgae

Microalgae can be described as aquatic, oxygen-evolving photosynthetic autotrophs that are unicellular, colonial or are constructed of filaments or composed of simple tissue (Guiry, 2012). Algae essentially require light (energy), carbon source (CO_2 for autotrophic metabolism), growth medium (water) and nutrients (nitrogen and phosphorous) for reproduction (Blair *et al*., 2014) and because of that algae have become one of the new renewable resources in producing high value products where the sources are limited because of their photosynthetic characteristic which quite similar to higher plants. Due to their higher photosynthetic efficiency, high biomass productivity, high lipid productivity and fast growth rate compared to oilseed crops, they have become main option in making commercial product such as in food supplement, pharmaceutical product, antioxidant and bio-fuel or biodiesel, (Vaičiulytė *et al*., 2014). Algae also can achieve carbon fixation at a faster rate than most other plants and can be used for several products, such as bio-fuels, pharmaceuticals, as well as in agriculture (Cassidy, 2011). Under optimal growth condition or by creating stress environment for the microalgae, higher biomass or other desired content can be obtain. Microalgae also growing naturally in the environment whether it is growing in seawater, waste water or fresh water where it depends on the type of species. Even though microalgae will be used as the renewable sources, this approach will not affect the existence food chain compared to oilseed crops.

2.2.1 *Chlorella vulgaris*

Chlorella vulgaris basically characterizes as eukaryotic photosynthetic microalgae under phytoplankton and can be known as one of the fastest growing green microalgae due to their simple structure which is unicellular non-flagella where it can be found in fresh water. Usually the main reason *Chlorella vulgaris* was selected in research frequently because they have shown growing rapidly and easily, even in presences of elevated CO_2 levels (Lv., 2010). In the research, they mentioned that *Chlorella vulgaris* can grow at a fast rate (0.6 g/L day) and tolerate 10-15% carbon dioxide, can also grow in extreme environments, high temperatures of 30-35°C, and acidic environments such as a pH of 3 (Cassidy, 2011). *Chlorella vulgaris* was used to grow heterotrophically and autrophically to increase the biomass and while the carbon dioxide produced from the heterotrophic phase was used in the autotrophic phase (Ogbonna *et al*., 1997).

Chlorella vulgaris is rich in amino acids, complex carbohydrates, vitamins, minerals, fats (85% unsaturated fats), RNA (up to 10%), DNA (up to 3%), chlorophyll, an array of phytonutrients and carotenoids, enzymes (including pepsin for digestion), polysaccharides (Haresh, 2003). For secondary processes, *Chlorella vulgaris* has a high percent of proteins, minerals, vitamins (Cassidy, 2011) and also contain high fatty acid with high nutritional value (Nguyen *et al*., 2014). *Chlorella vulgaris* often choose by the researcher to work as a source for production of bio-fuels because of their capability in gather neutral lipid with high productivity. While under nutrient starvation phase, the lipid content in *Chlorella vulgaris* could be increased significantly, which are between 50% and 70% (Vaičiulytė *et al*., 2014) and lipids also would increase from 5.9 to 14.7% when the temperature decreased from 30°C to 25°C; at temperatures over 38°C oleic acid, a monounsaturated omega-9 fatty acid, production increased (Converti *et al*., 2009).

Chlorella vulgaris have been used in many sectors other than bio-fuel which depend on their abilities. This microalgae have been used as rotifer feed In Japan and Asian countries and also in waste water treatment (Oh *et al*., 2001). In Japan also, *Chlorella vulgaris* is used for making health supplement and almost 30% of their population was taking it and researchers have found that this microalgae can boost immune system, reducing the intensities of many chronic health problems, and reducing the side effects of medications (Haresh, 2003). Aside for being used as feed, *Chlorella vulgaris* also used to improve the nutritional value of food.

2.2.2 Application of microalgae

Microalgae contain variation of high value content which creating interest among industrial and research people in making them as sources of valuable product. Microalgae contain lipid where nowadays people used it in producing renewable biomass such as biodiesel or bio-fuel and they also contain proteins and carbohydrates. Algae especially marine algae was used generally long before as food, fertilizer, feed and on recent research which still on-going they were using algae for making cosmetics and pharmaceutical products (Enzing *et al*., 2014). Micro-algae are currently used both as dried whole algae and for the extraction of high- value food or feed supplements and colorants where the total production volume and their market size is quite small and one of the example is dietary supplement they have made out of *Spirulina* and *Chlorella* (Enzing *et al.*, 2014). But still there is always room for improvement where many research have been done to overcome the issue.

Recently in the mass markets microalgae have been popular for energy (bio-fuel) and green chemistry (Nguyen *et al.*, 2014) and they also showed to be the only source of renewable biodiesel that meet global demand for transport fuels which appear beat the promising sources such as bio-ethanol and oil crops. Using oil crops as the sources also require large land coverage which definitely involves higher expenses and this will raise issue in source of food competition. For example in one of the research some microalgae species containing lipid contents as high as 70% of the cell's biomass where this microalgae could potentially produce nearly 136,900 L/ha of biodiesel per year as compared to soybean which is capable of only 446 L/ha (47 gal/ac) per year (Chisti, 2008). In one of the previous research, they showed comparison of processes involving bio-fuel production from microalgae which is known as life cycle assessment (LCA) where overall flow of production can be referred in figure below as the conversion method for example can be done by using methanol or catalyst (Brentner *et al.*, 2011).

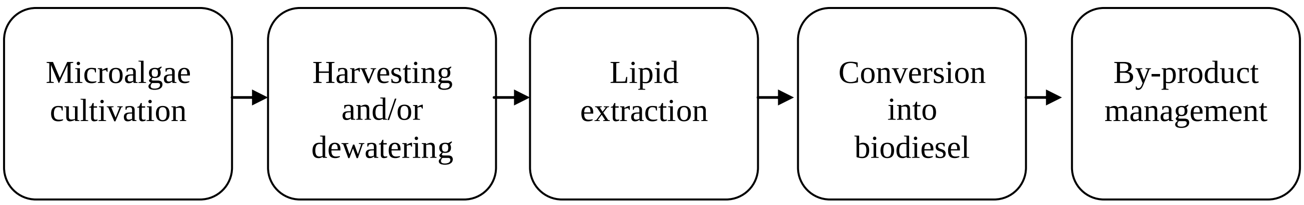

Figure 2.5 General processes involve in production of bio-fuel from microalgae (Brentner et al., 2011)

In other aspect, when the algae is used for carbon dioxide consumption, it can be used in a secondary process or product such as animal feed for example microalgae have mainly been farmed for juveniles and shellfish larvae feeding in aquaculture and now developing in the food and live-stock feed sectors (Nguyen *et al*., 2014).

2.2.3 Production of microalgae

Due to microalgae growing is faster and not complex where they need sunlight, CO_2 and water for optimal growth. Algae can be phototrophic or autotrophic where phototrophic algae can grow anywhere as long as there is enough sunlight where autotrophic algae can take CO_2 and exchange them into sugars through Calvin cycle. Because of these characteristics they have been chosen as preferred biomass feed stock but the production systems of microalgae usually different from open-air ponds to closed systems. Open-air ponds depend on the surrounding weather conditions, risk of contamination and with elevated consumption of water, they will have low construction costs and require easy maintenance while for closed systems required higher costs and higher production efficiency where the final results of all production systems require costly extraction expenses for making microalgae-derived products (Enzing *et al*., 2014). For example, algae used in biodiesel production is normally cultivated in open systems that are advantageous to other methods because due to their low cost and maintenance (Wilson, 2012).

2.3 Methods of Harvesting Microalgae

Harvesting or downstream process of microalgae is always a big challenge in the industry of producing based-product made of microalgae due to size of the cells especially for microalgae that having small size of cells where screening and sedimentation is not suitable for this type of species. In one of the article they mentioned that harvesting of microalgae usually contributes for 20–30% of the total production cost (Abdul Razack *et al*., 2015). Algal cultivation and harvesting/processing are also both energy and cost-intensive, especially for algal bio-fuel production, existing method not economical or sustainable (Blair *et al*., 2014). Because of these reasons, many researches have been done to improve and develop better harvesting methods. For example, one of the research suggest that to maintain a "clean" biomass product, high effluent quality, and low energy requirement, bio-flocculation of algae followed by gravity sedimentation is the ideal separation methodology (Lefebvre, 2012). Nevertheless, each of harvesting methods can be used single or combination of them but none of them can be view as the best and applied for all algae species. However, reasons for industrial to drawback in choosing harvesting methods are high energy cost, flocculant toxicity, or non-feasibility of scaling-up (Oh *et al*., 2001).

2.3.1 Gravity Sedimentation

Gravity sedimentation can be describes as natural settling which is self-generated after the algae cells being removed from cultivation system without addition of any chemical flocculants but this methods is only suitable for larger species. Based on Stokes law, only relatively large colonies or aggregates of cells or flocs with more than 100 nm in diameter will allow gravity sedimentation with reasonable time period in order to produce a biomass concentrate which typically around 1.5 to 4% (Reis Fernandes Montes, 2010).

Gravity sedimentation require usually approximately one hour for the recovery process (Hena *et al*., 2016). This method also usually suitable applied to primarily concentrate the culture of microalgae first before further to dewatering steps in order to reduce the energy requirement in harvesting methodology. Besides that, gravity sedimented sludge is generally more diluted which substantially influence the economics of product recovery further downstream (Martins *et al*., 2010). Gravity sedimentation also chosen often in wastewater treatment system to remove algae but the requirement of overflow rates in conventional clarifiers are too low (Divakaran and Pillai, 2002). But still, using only gravity sedimentation method for harvesting is possibly suitable for manufacturing low value product.

2.3.2 Magnetic Based Separation

Magnetic based separation is uncomplicated separation method for harvesting microalgae. This method usually used and applied to remove toxic algae from the fresh water. It can be described as bringing in contact the algal cells with the magnetic particles, and separating them from the liquid by an external magnetic force (Manousakis and Manariotis, 2016). Time required for magnetic separation to recover is usually approximately between 2-3 minutes with approximately 98 % of recovery efficiency (Hena *et al.*, 2015). Aside from useful in algae industry, magnetic separation is also proven to be useful for various industry such as wastewater treatment in steel factories and power plants, kaolin decolourization, enrichment of ores-mineral beneficiation, the removal of specific elements in the food industry, the removal of arsenic and metals in water treatment, and biochemical processes as the advantages are simple operation, low energy consumption and low cost (Wang *et al.*, 2015).

2.3.3 Flocculation

Flocculation involves the aggregation of two or more cells caused by cell-cell attraction or by chemical intermediates such as polymers and ions which can be categorized into chemical flocculation, auto-flocculation, bio-flocculation or microbial flocculation (Davis, 2011). Every each of microalgae cells having same charges on their cell surfaces which are negative charge and if they encounter in close distance there will be no attraction as they will react like magnet and started to repel each other. This characteristic makes it harder for the cells to attract naturally. Even if they were attraction occur it is not quite stabilized and it requires more times to aggregates. So in order to make the cells create attraction to each other and form Van der Waal forces, flocculation process is one of the ways to create the attraction and forces by using suitable flocculants. The addition of cationic metal ions as flocculants such as Ca2+ and Fe3+ can neutralize this charge, leading to the aggregation of cells which allows the treatment of large quantities of microalgae culture (J. Lee *et al*., 2013).

Flocculants can categorize into three groups that made up of inorganic flocculants (aluminium sulphate and polyaluminium choride), organic synthetic flocculants (polyacrylamide derivatives and polyethylene imine) and naturally occurring flocculants (chitosan, sodium alginate and bioflocculants) (Subramanian *et al*., 2007). Flocculation method is one of the harvesting methods that minify cost, energy usage and allows large quantities of culture to be treated. The flocculating reactions of an algal biomass are particularly sensitive to the pH, properties of the cellular surface, concentrations of the flocculants and divalent cations, ionic strength of the culture solution and other factors (Oh *et al*., 2001). Aside from that, when the ionic strength of water increases, the efficiency of flocculating agent also decreases (Garg *et al*., 2012).

Chemical flocculation uses salts or charged polymers to form flocks (Davis, 2011). One of the examples of chemical flocculants that commonly used is aluminium based (aluminium sulphate) and iron based. But aluminium sulphate cannot be applied over a wide pH range, moreover, floc size with alum when compared to ferric flocs is smaller resulting in ineffective sedimentation (J. Lee *et al*., 2013). Chemical flocculation also usually can be applied in wastewater treatment as microalgae can be harvested and pre-concentrated using flocculants based on aluminium or iron salts, or polyelectrolyte addition (Nguyen et al., 2014). Other flocculants like cations such as calcium and magnesium also have a positive effect on flocculation in high pH (Vandamme *et al*., 2012). But the most effective flocculants are cationic polymer (chitosan) and alkalis (NaOH).

Chemical flocculation is depend on pH for better effeciency, produces more sludge which is difficult to dehydrate, requires removal of excess flocculants from the medium before it can be reused and flocculants used are nondegradable, could cause adverse effects to humans and their intermediate by products of degradation are also harmful to the ecosystem (Abdul Razack *et al*., 2015).

Auto-flocculation involves the pH initiated precipitation of charged metallic ions dissolved in the growth media to cause flocculation (Davis, 2011). Auto-flocculate also can describe as using bacteria and extracellular polymeric substances to enhance flocculation activity of algae. In a research, they mentioned that auto-flocculation has been attributed to various causes including the ability of some microalgae strains to auto-flocculate at pH values that are weakly basic (< 8) or moderately basic (≈ 10−11), bridging between microalgae cells by extracellular organic matter excreted by the microalgae itself or by other microorganisms, and the precipitation of salts contained in the culture medium at high pH (≈ 8.5−10.5) reached by a slow, natural rise due to photosynthesis and the stripping of dissolved CO2 by air bubbling (Nguyen *et al*., 2014).

Bio-flocculation is a process in which flocculation is mediated by the presence of microorganisms and/or bio-flocculants (Subramanian *et al*., 2007). Bio-flocculation in detail can be refers to species that can experience a reduction in their cell wall charge commonly occurring during the cells stationary growth phase while microbial flocculation is an unconventional method that contaminates algae cultures with bacteria that secrete polymers causing flocculation (Davis, 2011). Due to their biodegradability, high efficiency, non-toxicity and eco-friendliness which made up of polysaccharides and protein materials generally produced from plants and micro- organisms, bio-flocculation agent is far better compared to chemical flocculants; however it requires many nutrients such as glucose, sucrose, and yeast extract, amino acids and sodium chloride for microbial growth and needs high amount of ethanol for isolation of exo-polysaccharide from microorganisms leading to extra operational cost (Abdul Razack *et al*., 2015).

2.3.4 Centrifugation

Centrifugation method basically suitable for harvesting biomass which is high-value product based on microalgae. Centrifugation process is the quickest and most effective process for the removal of particles from suspension without the aid of chemical additives but expensive due to use of energy inefficient electric motors and high capital cost, also existence of large shear forces during centrifugation that can lyse fragile cells and release algal oil into the dilute medium, thus, making oil recovery difficult if not economically impossible (Davis, 2011). As for high-value products, in order to recover high quality algae such as for food or aquaculture applications, it is often recommended to use continuously operating centrifuges that can process large volumes of biomass and it is more suitable to applied on microorganisms which can remain fully contained during recovery while the good side of this method is that the devices used during the process can be easily cleaned or sterilized which prevent contamination (Martins *et al*., 2010). Harvesting by centrifugation is generally characterized by high capture efficiency (>90%) under low flow rates and high energy consumption (Dassey and Theegala, 2013).

2.3.5 Flotation

Flotation can be describes as where air or gas is transformed into bubbles through a solid or liquid suspension, as a result solid particles get attached to gaseous molecules and are carried out and accumulated on the surface (Ndikubwimana *et al*., 2016). Due to the characteristic of the gas bubble, the efficiency of flotation process can be effective to capture smaller particles. In previous studies, they have been using flotation method to harvest fresh water microalgae and moreover there was also studied involve flotation method on marine algae but the efficiency is usually effected by salinity (Garg *et al*., 2012). There are many way to induce flotation where three main technique include dissolved air flotation (DAF), dispersed air flotation (DiAF), and electrolytic flotation (EF) while the rest technique that being used are jet flotation and dispersed ozone flotation.

DAF was more economical compared to the others, but, if the recovered algae were to be incorporated into animal feed, the use of flocculants such as alum could have undesirable effects on the growth rate of the animals however this problem could be overcome by the use of non-toxic flocculants (Sim *et al*., 1988). For dispersed air flotation which usually applied to upgrade coal and mineral at large scale (Garg *et al*., 2012), it was found to be less energy consuming while for jet flotation and dispersed ozone flotation are believed to be energy efficient microalgae flotation approaches (Ndikubwimana *et al*., 2016).

2.3.6 Filtration

Filtration is a method involving separation of solids from a suspension. Filtration usually performed as secondary dewatering process after increasing particle size using pre-treatment. However, membrane replacement is the major cost involved in filtration method and this also depends on the concentration of microalgae (J. Lee *et al*., 2013). This problem arises when large volumes of microalgae were being harvest. The filtration of large volumes of dilute microalgae cells by dead end or depth filtration can make many membrane concentration processes economically unattractive due to required capital cost, maintenance, and low throughput (Davis, 2011). Permeable membranes used for filtration and screening are also easily clogged by tiny microalgae and frequent scraping would significantly shorten the lifetime of these membranes, resulting in high operating costs (Garg *et al*., 2012). Although continuation of filtration process showing positive side in energy usage, economics and chemical-free operation but still the efficiency of the process was depended on the size and morphology of the algae.

2.3.7 Coagulation-flocculation

Function of coagulation-flocculation process is to aggregate the microalgae cells and increase the effective ‘‘particle’’ size, thus enhancing biomass recovery (Granados *et al*., 2012). The principle of this method involves both coagulation and flocculation process. In details, coagulation can be define as charges neutralization where the process is to neutralize the repulsive electrical charges surrounding particles which allowing them to attract each other to create flocks while flocculation is a process where it was occurred after coagulation process to facilitate the agglomeration of the coagulated particles to form larger floccules by bridging and thereby hasten gravitational settling (Borchate, 2014). For this process to occur, coagulants or flocculants agent is needed and later on forming cationic ions species in hydrated forms. The equation below showed chemical reaction between coagulants with OH^- to produce insoluble hydroxides of metals.

$$Al^{3+} + 3OH^- \rightarrow Al(OH)_3 \quad (1)$$

$$Fe^{3+} + 3OH^- \rightarrow Fe(OH)_3 \quad (2)$$

$$Ti^{4+} + 4OH^- \rightarrow Ti(OH)_4 \quad (3)$$

$$Si^{2+} + 2OH^- \rightarrow Si(OH)_2 \quad (4)$$

$$P^{5+} + 5OH^- \rightarrow P(OH)_5 \quad (5)$$

$$Mg^{2+} + 2OH^- \rightarrow Mg(OH)_2 \quad (6)$$

$$Na^+ + OH^- \rightarrow Na(OH) \quad (7)$$

$$Mn^{2+} + 2OH^- \rightarrow Mn(OH)_2 \quad (8)$$

$$Ca^{2+} + 2OH^- \rightarrow Ca(OH)_2 \quad (9)$$

$$K^+ + OH^- \rightarrow K(OH) \quad (10)$$

In this technique, material used for this process to occur can be divided into two which are inorganic and organic coagulants where the second category is organic flocculants. Among these materials, chitosan and alumn are common commercial of coagulation-flocculation reagent that can be found in the market while there are also various polymeric materials that have been developed for better improvement function of coagulation-flocculation process such as pre-polymerised coagulants for example polyaluminium chloride (PAC).

This method usually suitable and effective to apply in wastewater treatment on both physical and chemical treatment due to their time efficient and less costly compared to other technique. Coagulation-flocculation method usually use in the wastewater industry as colour removal, surfactant removal, and also uses for primary treatment before membrane application but the problem arise for using this method is production of sludge at end of the process.

2.4 Composite coagulants

Composite coagulants also can be known as composite flocculants or composite polymer can be describe as mixture of a few components or made of various parts or elements. Composite coagulant is a combination of other coagulants to improve and maximise the function of aggregation. This composite have been gaining interest among researcher due to their low cost production and improved performance than inorganic-based coagulants such as alumn-based and ferric-based coagulants.

Composite coagulants can be classified into three categories which are pure inorganic composite coagulants where it is made up of two or more of two types of inorganic coagulants for example poly-aluminum-ferric-silicate-chloride coagulants (Tolkou *et al.*, 2015), pure organic composite coagulants where it is a combination of two or more of two types of organic coagulants for example non-ionic polymer (polyacrylamide, PAA) and the third is a mixture of inorganic coagulant and organic polymer for example polyaluminium chloride (PAC) with addition of chitosan (Ng *et al*., 2012).

Among this three, inorganic-organic composite have received the most attention in treating wastewater due to the improvement of the bridging capacity and requirement of only one unit operation which is the flocculation system, compared to the conventional bi-operational system: coagulation-flocculation (K. E. Lee *et al*., 2012).

CHAPTER 3 : MATERIALS AND METHODOLOGY

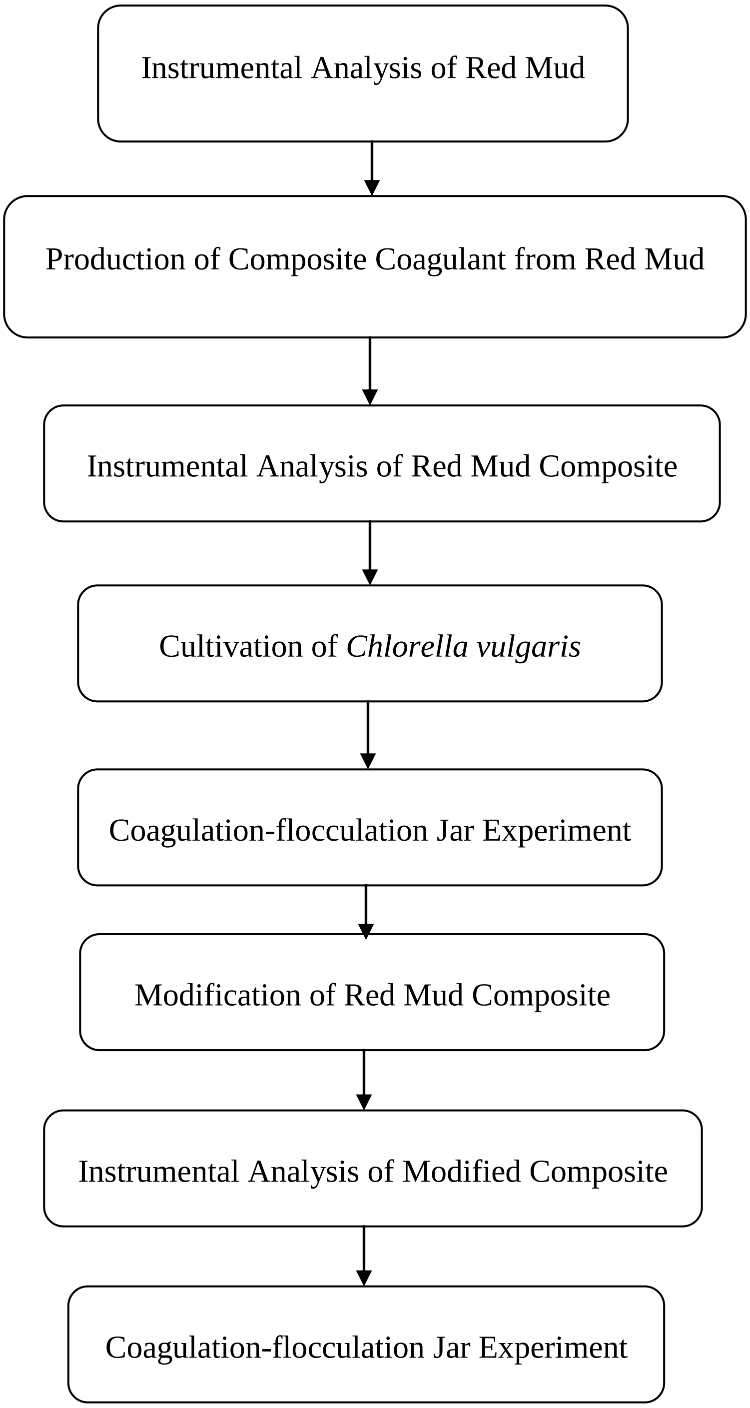

Figure 3.1 Flowchart methods for conducted experiment

3.1 Materials

Red mud sample was taken from Environment Group, Bauxite Division, JNARDDC, WADI India. The pure culture of *Chlorella vulgaris* which have been used in this research is obtained from Algaetech Laboratory, Technology Park, Malaysia. Chemicals used in this research were 37 % saturated hydrochloric acid (Sigma Aldrich, UK), 1% (w/v) sodium hydroxide (Qrec, New Zealand), aluminium chloride ($AlCl_3$), iron (III) chloride ($FeCl_3$), manganese (II) chloride ($MnCl_2$). All reagents used in this research were analytical grade reagents.

3.2 Experimental Methodologies

3.2.1 X-ray Fluorescence Spectrometry (XRF) Analysis of Red Mud

Red mud was analyzed for its major element content using X-Ray Spectrometry (XRF, PAN analytical Model Axias Max, Canada). The preparation of sample was started by using proper mechanical splitting technique; representative portion of sample was pulverized into approximately 20 µm grain size. The specimen for XRF major element analysis was made by igniting 0.5 g of sample and 5.0 g of spectruflux at 1050°C for 20 minutes, before casted into a glass disc, 32 mm in diameter. The specimen then analyzed for 10 major elements using fully automated Pan Analytical (Holland) AXIOS XRF spectrometer, with a standard elemental setup. Calibration technique was employed. The 10 element curves were constructed using 14 high quality international standard references materials, comparable in composition to the unknown sample.

The specimen for XRF major element analysis is in the form of pressed-powder pellet. The analysis was done by scanning the presence of elemental peaks from the lowest to the highest angle of incidents and the x-ray intensity at characteristic peak of a particular element was compared to the intensity of the same element in the series of standards, stored in the software name OMNIAN. Relative error of 5-10% was associated.