PRODUCTION OF BIOPOLYMER BY *BACILLUS SUBTILIS* ISOLATED FROM PALM OIL MILL EFFLUENT (POME) AND ITS APPLICATION FOR COLOR REMOVAL

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PRODUCTION OF BIOPOLYMER BY *BACILLUS SUBTILIS* ISOLATED FROM PALM OIL MILL EFFLUENT (POME) AND ITS APPLICATION FOR COLOR REMOVAL

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LIST OF SYMBOLS

%	Percent
±	Plus-minus
µL	Microliter
µm	Micrometer
°C	Degree Celcius
Al^{3+}	Aluminum ion
Al(OH)	Aluminum hydroxide
$Al(OH)_6$	alumina
C	Carbon
Ca^{2+}	Calcium ion
$CaCl_2$	Calcium chloride
CFU/mL	Colony forming unit per milliliter
CH_4	Methane
$C_5H_9NO_4$	Glutamic acid
cm	centimeter
cm^{-1}	Per centimeter
CO_2	Carbon dioxide
Fe_3O_4	Ferrosoferric oxide

g	Gram
g/L	Gram per liter
H	Hydrogen
H^+	Hydrogen ion
h	Hour
H°	Hydrogen radical
H_2O	Hydrogen dioxide
H_2O_2	Hydrogen Peroxide
HCl	Hydrochloric acid
K^+	Potassium ion
KBr	Potassium bromide
Kg	Kilogram
L	Liter
M_1	Initial Mass
M_2	Final Mass
M	Molar
Mg^{2+}	Magnesium ion
Mg/L	Milligram per liter
$MgCl_2$	Magnesium chloride
mL	Milliliter

mM	Millimolar
mV	Millivolts
Mn^{2+}	Manganese ion
N	Nitrogen
NaOH	Sodium Hydroxide
ng	Nanogram
nm	Nanometer
O	Oxygen
OH°	Hydroxyl radical
r/min	Revolutions per minute
R_2NH	Secondary amine
rpm	Revolutions per minute
S	Sulfur
SO_4^-	Sulfate ion
SiO_4	Silicon oxygen
TiO_2	Titanium dioxide
U/µL	Unit per microliter
V_1	Initial Volume.
V_2	Final Volume
-C=C-	Ethenyl

-C-H	Hydrocarbon
-C-N	Imino
-C=O	Carbonyl
-COO-	Carboxylate
-COOH	Carboxylic
-N-H	Amines
-N=N-	Azo

LIST OF ABBREVIATIONS

AGS	Aerobic granular sludge
AN	Ammoniacal nitrogen
AOMBP	Anaerobic-oxic membrane bioreactor
AOP	Advanced oxidation process
APTES	Aminopropyltriethoxysilane
B.subtilis	*Bacillus subtilis*
BOD	Biochemical oxygen demand
CaHP	Calcium hybridized polyglutamate
CAS	Chemical abstracts service
CFU	Colony forming unit
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
dNTP	Deonucleoside triphosphate
DO	Dissolved oxygen
E.cloacae	*Enterobacter cloacae*
Fig	Figure
FT-IR	Fourier transforms infrared spectroscopy
HMDS	Hexamethyldisilazane

HRT	Hydraulic retention time
MCA	Mesoporous carbon aerogel
MMT	Montmorillonite
NF	Nanofiltration
NTU	Nephelometric turbidity units
OD	Optical densities
P.pabuli	*Paenibacillus pabuli*
PACl-PAMlPCl	Polyaluminum chloride - poly(3-acrylamido-isopropanol chloride)
PAM	Polyacrylamide
PCR	Polymerase Chain Reaction
POME	Palm oil mill effluent
RO	Reverse osmosis
RR120	Reactive red 120
SBR	Sequential batch reactor
SEM	Scanning electron microscopy
Sp	Species
sPPSU	Sulfonated polyphenylenesulfone
TN	Total nitrogen
TS	Total solids

UV-Vis	Ultraviolet-visible spectrophotometer
VS	Volatile solids

PENGHASILAN BIOPOLIMER DARIPADA *BACILLUS SUBTILIS* YANG DIASINGKAN DARIPADA EFLUEN KILANG MINYAK SAWIT DAN PENGGUNAANNYA UNTUK PENYAHWARNAAN

ABSTRAK

Dalam kajian ini, proses pengumpulan bakteria telah diasingkan daripada kolam anaerobik efluen kilang minyak sawit daripada MALPOM Industri, Pulau Pinang. Menurut hasil dapatan penjujukan daripada perisian *Blast* Pusat Kebangsaan daripada Maklumat Bioteknologi menyatakan *B.subtilis*, *P.pabuli* dan *E.cloacae* merupakan bakteria yang berfungsi untuk proses pengasingan. Optimum kecekapan pemberbukuan dalam rawatan ampaian kaolin tanah liat bagi *B.subtilis* mencapai 71.26 % dalam 48 jam, manakala *P.pabula* dalam 96 jam memperolehi sebanyak 15.46 % dan 29.95% dalam 72 jam didapati pada kadar 29.95 % adalah *E.cloacae.* Maka itu, bakteria *B.subtilis* mampu menghasilkan biopolimer daripada proses pemberbukuan. Dengan itu, kajian analisis telah dilakukan ke atas *B.subtilis* terhadap kadar pertumbuhan optimum dan hasil pengeluaran biopolimer. Dalam tempoh 48 jam merupakan kadar optimum pertumbuhan *B.subtilis* di mana ia berkolerasi dengan penghasilan biopolimer. Didapati bahawa kecekapan pemberbukuan menunujukkan kecekapan yang tertinggi iaitu 71.26% dalam tempoh 48 jam. Oleh yang demikian, kecekapan pemberbukuan untuk merawat pepejal terampai dan pewarna air dengan menggunakan biopolimer yang dihasailkan oleh *B.subtilis* pada 48 jam dapat ditentukan. Biopolimer mampu merawat 71.64 % daripada pepejal terampai pada dos optimum 42 mg/L, manakala 98.64 % dapat menyinkirkan penyahwarnaan reaktif merah 120 pada dos optimum 38 mg/L. Kekeruhan air sisa dari 13.70 pada 14 mg/L menurun kepada 3.78 NTU pada dos optimum biopolimer (38 mg/L). Caj peneutralan dan pemberbukuan adalah mekanisma yang berlaku apabila biopolimer meneutralkaan

caj zarah dan gumpal seluruh zarah koloid untuk penurunan. Di samping itu, kajian turut melibatkan pencampuran biopolimer dengan kation untuk meningkatkan kecekapan pemberbukuan. Penambahan ion logam akan meningkatkan kecekapan pemberbukuan akibat daripada pengikatan biopolimer. Kajian perbandingan empat jenis ion logam (K^{+}, Ca^{2+}, Mg^{2+}, dan Al^{3+}) dengan campuran Biopolimer dijalankan dan didapati bahawa kecekapan pemberbukuan sebanyak 44.34 % daripada K^{+}, 70.10 % kecekapan pemberbukuan daripada Ca^{2+}, Mg^{2+} didapati 61.45 % kecekapan pemberbukuan, dan 53.66 % kecekapan pemberbukuan untuk Al^{3+}. Ini adalah kerana penambahan ion logam dapat menghasilkan ikatan dengan biopolimer dapat meningkatkan caj positif permukaan biopolimer. Selain daripada itu, kajian perbandingan kecekapan pemberbukuan antara alsium hybridized biopolimer (CaHP) dan polyacrylamide turut dilakukan, dengan memperolehi penyingkiran reaktif merah 120 untuk CaHP adalah 98.64% pada 38 mg/L, manakala PAM adalah 68.29 % pada 30 mg/L selepas dirawat oleh alum. Kajian ini telah memberikan inspirasi bahawa CaHP mempunyai keupayaan untuk meningkatkan lagi penyahwarnaan berbanding dengan PAM yang tidak menunujukkan peningkatan yang ketara penyahwarnaan selepas pengentalan proses yang menggunakan alum (62.59 %). CaHP mempunyai keupayaan untuk bertindak sebagai caj peneutralan dengan ikatan pencampuran kationik (Ca^{2+}) pada biopolimer untuk menambahkan pengumpalan zarah pewarna, di samping meningkatkan kemampuan penyingkiran warna. Perbezaan PAM dengan CaHP adalah disebabkan PAM berfungsi untuk membentukan saiz terampai menjadikan lebih besar dan ia tidak melibatkan prosese peneutralan. Secara keseluruhannya, biopolimer yang dihasilkan oleh *B.subtilis* mampu merawat pepejal terampai dan pewarna air sisa melalui caj peneutralan dan pemberbukuan. Biopolimer bercampuran dengan ion logam dapat membantu untuk meningkatkan kecekapan proses pemberbukuan.

PRODUCTION OF BIOPOLYMER BY *BACILLUS SUBTILIS* ISOLATED FROM PALM OIL MILL EFFLUENT (POME) AND ITS APPLICATION FOR COLOR REMOVAL

ABSTRACT

In this study, bacteria were isolated from Palm Oil Mill Effluent (POME) collected from anaerobic pond of MALPOM Industry, Penang. According to the sequencing results using Blast software of National Centre for Biotechnology Information, the bacteria isolated are *B.subtilis*, *P.pabuli* and *E.cloacae*. The optimum flocculation efficiency in treating kaolin clay suspension for: *B.subtilis* at 48 hours with 71.26 % flocculation efficiency; *P.pabuli* at 96 hours with 15.46 % flocculation efficiency; *E.cloacae* at 72 hours with 29.95 % flocculation efficiency. Therefore, *B.subtilis* is the only isolated bacteria that able to produce biopolymer for flocculation. Subsequently, *B.subtilis* was further analyzed for its optimal growth rate and biopolymer production. The optimum growth rate for *B.subtilis* is at 48 hours which is correlated to the highest amount of biopolymer yielded. Additionally, the flocculation efficiency showed the highest flocculation efficiency (71.26 %) at 48 hours. Hence, flocculation efficiency in treating suspended solid and dye wastewater by applying biopolymer produced by *B.subtilis* at 48 hours was determined. The biopolymer was able to treat 71.64 % of 5000 mg/L kaolin suspension at the optimal dosage of 42 mg/L whereas 98.64 % removal for reactive red 120 at the optimal dosage of 38 mg/L. The turbidity of the color solution decrease from 13.70 at 14 mg/L to 3.78 NTU at optimum dosage of biopolymer (38 mg/L). The mechanism involves are charge neutralization and sweep flocculation whereby the biopolymer neutralized the particles charges and agglomerate entire colloidal particles for settling. Besides that, the hybridization of biopolymer with cation in enhancing the flocculation efficiency

was distinguished. Addition of metal ions to hybridize with biopolymer greatly affects the flocculation efficiency. Biopolymer hybridized with four different types of metal ions (K^+, Ca^{2+}, Mg^{2+}, and Al^{3+}) respectively are compared. The results show 44.34 % flocculation efficiency for K^+, 70.10 % flocculation efficiency for Ca^{2+}, 61.45 % flocculation efficiency for Mg^{2+}, and 53.66 % flocculation efficiency for Al^{3+}. This is because addition of metal ions to hybridize with biopolymer could help to increase the positive charge of the biopolymer surface. Lastly, the flocculation efficiency between calcium hybridized biopolymer (CaHP) and polyacrylamide was compared. Reactive red 120 color removal for CaHP is 98.64 % with 38 mg/L whereas PAM is 68.29 % with 30 mg/L after treated by alum. The novelty of this study is CaHP has the capability to further enhance the color removal compared to PAM which has no significant increment of color removal after coagulation by alum (62.59 %). CaHP has the capability to act as charge neutralizer since the cationic compound (Ca^{2+}) bonded at the biopolymer can further agglomerate dye particles, resulted to higher color removal. In contrast to PAM, there is no further neutralization happened but agglomeration to form larger flocs size for settling. In overall, biopolymer produced by *B.subtilis* is able to treat suspended solid and dye wastewater through charge neutralization and sweep flocculation mechanisms. Additionally, biopolymer hybridized with metal ions can help to enhance the flocculation efficiency.

CHAPTER ONE

INTRODUCTION

Currently, large scale manufacturing industries are considered as the main contributors for the country development and economic growth such as automobile, food and beverages, electronic, textile, leather and pharmaceutical industries. Indirectly, generation of wastewater which contain suspended solid, remaining dye substances, organic compounds and surfactants is unavoidable. Ong et al. (2014) reported a kg of textile products could generate 2 to 180 L of textile wastewater. Introduction of inappropriate treatment method or capability of existing treatment plant to cope with the high capacity of wastewater to be treated daily potentially caused the contaminants to flow into water stream. The release of incompletely treated wastewater with contaminants into water stream could cause environmental and health issue. These contaminants would create issue due to their toxic, non-biodegradable and mutagenic nature. For instance, benzene could affect human being's nervous and vascular systems (Li et al., 2014a). The presence of low concentration of dyes (10 to 50 mg/L) in watercourse will cause esthetic problems of the aquatic ecosystem (Sheydaei et al., 2014). Therefore, an appropriate wastewater treatment methodology should be applied in order to treat the wastewater effluent effectively before discharged to water stream.

1.1 Suspended Solid

Suspended solids are one of the pollutants in the water quality which can cause depletion of dissolved oxygen (DO) in the water effluent. The main sources that cause suspended solids in the watercourse are construction, mining, agriculture and dicing industries. Although suspended solids are a small solid particles or colloid that mobilized in water, it required high treatment cost to remove the suspended solids in the water system (Bilotta and Brazier, 2008). Hence, it is imperative to know the concentration of suspended solid in the watercourses in order to maintain the desired characteristics of the effluent. Suspended solids can be measured by turbidity which is referring to the haziness of a fluid caused by a large number of individual particles (Cartensen et al., 1996; Choi and Park, 2002).

1.2 Dye

Textile industries are the largest industry that used dye to impart colors on materials or fibres. Dyes are arising by two combined molecules which are chromophores and auxochromes. Chromophores are responsible for its color; whereas electrons withdrawing or donating substituents that responsible to intensify the color of chromophores are known as auxochromes (Christie, 2001). Owing to chromophores do not have the affinity to unite with tissue or fibre, it is color but not a dyes. To unite with tissue or fibre, chromophores needed to integrate with auxochromes group to make up a dye that potentially imparts color on fibres. Auxochromes are amino, carboxylic, sulphonated and hybroxyl group whereas chromosphores are ethenyl, carbonyl, imino, thio-carbonyl, azo, nitroso and nitro (Verma et al., 2012).

1.3 Wastewater treatment method

For decades, variety of wastewater treatment methods have been developed to treat wastewater effluent. The treatment methods can be classified into biological, chemical, physical and physicochemical processes (Ong et al., 2014). Among those processes, there is no significant single process and technology that can be applied universally for remediate variability composition of water and wastewater (Aquino et al., 2014). In wastewater containing suspended solid, physical and physicochemical processes are the most common treatment method used such as coagulation-flocculation and filtration. This is because suspended solid is highly affected by the turbidity level, therefore physical and physicochemical treatment are the effective treatment methods in controlling water quality (Zemmoui et al., 2012). In wastewater treatment, biological process such as aerobic, anaerobic and sequential anaerobic-aerobic systems is used for wastewater treatment process that contains high organic load and Chemical Oxygen Demand (COD) (Ong et al., 2014). On the other hand, chemical process is applicable for wastewater that contains high heavy metals and dye. Chemical treatment is efficient in treating wastewater containing heavy metals such as zinc, copper, nickel, mercury, cadmium and lead (Gaikwad and Gupta, 2008). This is because chemical treatment is very efficient in precipitating of heavy metals by forming metal hydroxides. However, it required high chemicals cost and proper discharge of chemical sludge. This caused a large economic burden for the industries. Coagulation-flocculation process is the most commonly utilized treatment method in industry for dye wastewater treatment (Tan et al., 2000). Freitas et al. (2015) used low amount of okra mucilage to remove 93 % of textile wastewater.

1.4 Problem Statement

In this regard, industrial wastewaters produced from different kinds of industries contains fine suspended solids, dye substances, organic compounds and surfactants. Presence of these substances in waterbodies will cause severe environmental and health problem. High suspended solids will have hindered the light penetration into water stream, thus causing the depletes dissolved oxygen (DO) in the water. Moreover, dyes are considered as stable, stubborn, colorant, and potentially carcinogenic and toxic pollutants, their discharge will pollute the river water. Therefore, the demand for adequate treatment to floc the suspended solids as well decontamination of dyes effluents from the textile and dyes industries has become imperative.

Wastewater treatment such as adsorption, coagulation-flocculation, advance oxidation process (AOP), photocatalytic process, membrane filtration and sequencing batch reactor has been reported in treating the industrial wastewater (Gobi et al., 2011; Ong et al., 2012a; Ong et al., 2012b; Yeap et al., 2014; Ong et al., 2014). Among those methods, coagulation-flocculation is preferred as the alternative means and high efficiency for suspended and dissolved solids, colloids contained wastewater treatment (Tan et al., 2000). The most commonly used coagulant in industry is aluminum-based coagulant. However, usage of aluminum-based coagulants alone has limited efficiency in color removal of dyes wastewater (Jangkorn et al., 2011). Therefore, enhancement of aluminum-based coagulant towards high removal efficiency of wastewater is essential to ensure the dye wastewater is well-treated before being discharged to water stream. Flocculants such as chemical flocculant, biopolymer and synthetic organic flocculant have developed great attention of researches to effectively floc the suspended solid or dye particles before discharged into water stream. The challenging of flocculation is to floc the small particles and colloids which carries surface charges, the function of flocculant is to make make these colloids heavier mass for efficient settlement. In this research, flocculation efficiency of between chemical flocculant and biopolymer are compared. Furthermore, additional of auxiliary agent such as cation to improve the flocculation efficient was investigated.

Therefore, in this study, bacteria from POME is used to identify presence of biopolymer agent to treat suspended solid and dye industrial wastewater. Subsequently, cation is introduced to enhance the flocculation efficiency. Lastly, flocculation efficiency between biopolymer and chemical flocculant is compared.

1.5 Research Objectives

The objectives for the present research project are:

1. To identify bacteria from POME for biopolymer production.
2. To determine the flocculation efficiency of biopolymer in treating suspended solid and dye wastewater.
3. To compare the efficiency of cation in the flocculation efficiency.
4. To compare the flocculation efficiency between biopolymer and Polyacrylamide (PAM).

1.6 Scope of research

In the present work, POME sample that was collected from an anaerobic wastewater pond was screened and isolated. Preliminary study on each biopolymer produced by isolate will be analyzed and the highest flocculating efficiency biopolymer will analyze to find out the optimum in term of polymer dosage, favorable metal ions and metal ions' dosage. This was intended to enhance the production of biopolymer as well as the behavior of the biopolymer. Removal of kaolin clay suspension and reactive red 120 was determined using biopolymer in flocculation process. The flocculating efficiency of biopolymer is compared with organic synthetic polymer in term of color removal, turbidity removal and zeta potential. FT-IR analysis and UV-Vis spectra were used to identify the products in the treatment process and to establish pathway of coagulation and flocculation.

1.7 Organization of the Thesis

The thesis is organized into several chapters:

Chapter 1: This chapter is briefly discussing about type of wastewater treatment to treat wastewater effluent, type of effluent such as suspended solids, dyes and biopolymer. It is also cover the problems confronted with the steps of technology developments. Hence, this research is carried out to overcome the stated problem. The scope and objective of this research are also stated.

Chapter 2: Literature review in this chapter focuses on a review of present practices for the treatment of industrial wastewater with their advantages and limitations. Coagulation-flocculation mechanisms, factors of flocculation process, type of polymer are discussed. Special topics related to POME such as characteristic of POME, type of bacteria in POME and factor affect bacteria growth are also discussed.

Chapter 3: This chapter describes material and method that has been used and implemented during the analysis. The methodology of isolation, identification, flocculating activity determination, purification, and application of biopolymer in flocculation are explained in detail in this chapter.

Chapter 4: Results and discussion in this chapter illustrates and discusses the results of the research studies conducted and detailed evaluation concerning to the result analysis.

Chapter 5: Final conclusive is based on the results obtained towards the objective of this research. Recommendation section suggests ideas for further studied in the related field.

CHAPTER TWO

LITERATURE REVIEW

2.1 Treatment of wastewater

Industries produced wastewater contain fine suspended solid, dissolved solids, metals, inorganic and organic matter, colors and other impurities. The treatment of these wastewaters becomes challenging due to the presence of surface charge on the fine particles (Bratby 2006). Wastewaters from textile industries are considered as one of the major sources of pollution because of their synthesis and chemical stability (Balapure et al., 2014). Dyes are toxic to aquatic biota and may affect the photosynthesis activity of aquatic plant by reducing the light penetration into water stream, subsequently limit the oxygen production for aquatic life (Tauber et al., 2008; Hao et al., 2000). The breakdown products of dyes could be toxic and carcinogenic such as azo bond and aromatic amines since it can accumulate in food chain and affect human health as well as ecosystem (Levin et al. 2012; Li et al. 2012). Therefore, direct discharge of these dyes without proper treatment can cause adverse impact to environment and human health. Currently, many treatment methods have been applied to decolorize dyes before discharged into water bodies such as physical chemical and biological treatment processes.

2.1.1 Physical Treatment

Physical treatment system or known as membrane process such as filtration, adsorption and reverse osmosis is commonly applied in treating industrial wastewater. Filtration is a pressure-driven separation process in a liquid phase by using semipermeable membrane. Filtration process has the ability to treat soluble and ionic dyes due to its size exclusion and convection effect (Schäfer et al, 2002). However, the disadvantages of filtration is membrane fouling and high energy consumption when treating when used for the treatment of industrial effluent (Van der Bruggen et al., 2008). The membrane pore is easily constricted or blocked due to the cake formation (Zuriaga-Agusti et al., 2014). Adsorption is an easy operating method to treat dye by using adsorbent material through the fundamental study of surface modification, easy separation, large surface area and adsorption capacity. Nowadays, nano-adsorbents have been intensively investigated by researchers (Shirmadi et al., 2012). Besides that, reverse osmosis is a widely applied physical treatment technology for reuse of textile and dyeing wastewater due to its affordability and reliability (Liu et al., 2011). Reverse osmosis is a diffusion-controlled process which performed with solution–diffusion mechanism and the dissolved solutes can be separated from water through a semipermeable membrane that passes water in preference to the solute. RO membrane is described as hydrophilic, therefore ease of the diffusion of water to be readily diffuse into and out of the membrane polymer structure (Lee et al., 2011). The limitation of reverse osmosis is membrane fouling caused by the interaction between membrane surface and foulants due to surface hydrophilicity, charge and roughness (Li and Wang, 2010).

2.1.2 Chemical Treatment

Chemical treatment for industrial wastewater includes coagulation-flocculation, advanced oxidation process (AOP), ozonation, Fenton reagents, sonolysis. Sonolysis or ultrasonic irradiation is a technique that using phenomenon of acoustic cavitation to accelerate the chemical process resulting in the formation of strong and active radicals such as OH°, H° and H_2O_2 (Adewuyi, 2005; Reddy et al., 2010). However, sonolysis degradation rate for organic pollutants is low due to low efficiency of reactive radical formation (Zhai et al., 2013). Advanced oxidation process (AOP) is a process where oxygen based radical are generated in-situ for industrial wastewater treatment. Oxygen based radicals that generated such as $\cdot OH$, $HO_2\cdot$ and $O_2^{-}\cdot$. AOP able to decompose dye structure into low molecular weight compounds like carbon dioxide, carboxylic acid, small inorganic compounds (Hisaindee et al., 2013). TiO_2 is broadly used as a photocatalyst for degrading a wide range of organic pollutants because of its nontoxicity, photochemical stability, and low cost (Thompson and Yates, 2006). In ozonation process, ozone is the commonly used chemical oxidation which is a powerful oxidizing agent used for treatment of industrial wastewater. Ozone can decompose to oxygen by splitting the radical such as hydroxyl radical, super oxide (Wijannarong et al., 2013). However, the ozone production cost is high and the transfer rate of ozone is poor (Konsowa et al., 2010; Tehrani-Bagha et al., 2010). Chemical coagulation-flocculation process is the application of coagulant to destabilize molecules, subsequently flocculant is used to bridge the destabilized particles into larger agglomerates for higher settling rate. Coagulation-flocculation is the common treatment method utilized in industry due to its low capital cost (Golob et al., 2005). However, the major limitation of this process is the generation of sludge (Hai et al., 2007). Among all the method stated, coagulation-flocculation is one of the most popular and common method used for the removal of suspended and dissolved solids, colloids and organic matter in the solid-liquid separation process as well as treatment of industrial dye wastewater (Renault et al., 2009a; Lee et al., 2012).

2.1.3 Biological Treatment

Biological treatment that has been commonly used by industries are aerobic, anaerobic processes and sequential batch reactor (SBR). Aerobic and aerobic processes involve bacterial degradation of dyes which is initiated by an enzymatic step with the aid of an azoreductase and an electron donor (Sarayu and Sandhya 2010). Aerobic took place with the presence of oxygen whereas anaerobic process without presence of oxygen and the dye degradation rate is depending on the microbial communities and their synergistic metabolic activities (Jain et al., 2012). Sequential batch reactor (SBR) is a modified activated sludge process used to treat dye wastewaters (Sathian et al., 2014). Biological treatment is an environmental friendly treatment process since less secondary pollutant is produced. However, this treatment process required nutrient supplement as well as the degradation process is slow and ineffective (Padhi and Gokhale, 2014).

Table 2.1 presents the physical, chemical and biological treatment that has been applied to treat various types of wastewater and their efficiency in treating wastewater.

Table 2.1 Treatment of wastewater

Treatment	Method	Concluding remarks	References
Physical	Filtration	Polyvinylidene difluoride ultrafiltration membranes was able to treat 99 % of wastewater containing indigo dye and 80 % of COD reduction.	Buscio et al., 2015
		Positively charged hollow fiber nanofiltration membranes was able to remove Brilliant green, Victoria blue B and Crystal violet with 99.8, 99.8 and 99.2% removal, respectively through submerged filtration under the pressure of 0.7 bar.	Zheng et al., 2013
		A thin-film composite nanofiltration (NF) hollow fiber membrane formed by interfacial polymerization on a polysulfone/polyethersulfone was able to remove 99.99 % of Reactive brilliant blue X-BR.	Wei et al., 2013
		Positively charged nanofiltration (NF) membranes have been fabricated using sulfonated polyphenylenesulfone (sPPSU) was able to remove 99.98% to Safranin O dye.	Zhong et al., 2012

	Adsorption	Fe_3O_4 magnetic nanoparticles modified with L-arginine (Fe_3O_4@L-arginine) was able to remove 96.34 % of 50 mg/L Reactive Blue 19 at pH 3 with adsorbent dose of 0.74 g/L.	Dalvand et al., 2016
		HDTMA-modified Spirulina sp. Was able to adsorb 75% and 88% for crystal violet and Safranin dye, respectively.	Guler et al., 2016
		Leather shaving waste was able to remove 87.37 % of tannery-dye-containing effluent at pH 2.3.	Gomos et al., 2016
		Aminopropyltriethoxysilane (APTES) modified MIONPs were prepared by chemical precipitation method was able to decolorize sunset yellow at pH 3.1.	Rajabi et al., 2015
	Reverse osmosis	Reactive dye was able to remove by reverse osmosis.	Srisukphun et al., 2009
		More than 90 5 of Reactive Black 5 was remove through anaerobic-oxic membrane bioreactor (AOMBR) / reverse osmosis processes.	You et al., 2008

Chemical	Sonolysis	99.5 % of rhodamine B was removed by synthesis of Ni@graphene nanocomposite microspheres (NGs) in hydrazine hydrate solution.	Zhao et al., 2015
		sonocatalytic performance of the synthesized TiO_2/Montmorillonite K10 (TiO2/MMT) nanocomposite was was able to remove Basic Blue 3 (BB3) at pH 7.	Khataee et al., 2015
		Er^{3+}:$YAlO_3$/TiO_2–SnO_2 was able to degrade Acid Red B by using under ultrasonic irradiation at 550 ˚C for 60 minutes.	Zhai et al., 2013
	Advanced Oxidation Process (AOP)	H_2O_2 assisted photochemical oxidation was able to remove 50 % of phthalocyanine dye, Remazol Turquoise Blue in 10 mins.	Kalsoom et al., 2012
		Bi-based photocatalyst Bi_4TaO_8I was able to remove 60 % of methyl orange after 3 cycles of 28 hours visible-light irradiation.	Fan et al., 2012

	98.9 % of crystal violet (triphenylmethane) dye was removed under UV light irradiation using a Pt modified TiO_2 photocatalyst.	Fan et al., 2011
Ozonation	Mesoporous carbon aerogel (MCA) supported copper oxide as a catalyst in ozonation process was able to remove 80 % of reactive black 5 and 46 % of COD reduction.	Hu et al., 2016
	Generation of active radicals on the MoO_3 catalyst surface was able to enhance the degradation of an azo dye orange II.	Manivel et al., 2015
	90 % of reactive dyes solutions of Nova cron super black G and Terasil red ww 3BS was decolorized by using ozone as an oxidant with 6 hours reaction time.	Wijannarong et al., 2013
Coagulation-Flocculation	A synthesized polyaluminum chloride–poly(3-acrylamido-isopropanol chloride) (PACl–PAMIPCl) was able to remove Reactive Cibacron Blue F3GA and Disperse Terasil Yellow W-4G with 95 % and 96 %, respectively.	Yeap et al., 2014

	Green refined laterite soil is a bi-functionalized coagulant and flocculant, it was able to remove acid orange 7 with 98.43% color removal at pH 2.	Lau et al., 2014
	Okra mucilage was able to remove 97.24% of turbidity, 85.69% of COD and 93.57% of color at pH 6.0.	Freitas et al., 2015
	Staphylococcus cohnii ssp. Isolated from Palm Oil Mill Effluent (POME) was able to remove kaolin suspension up to 88.9% with Al^{3+} as cation.	Wong et al., 2012
	Bacillus mojavensis strain 32A from salt production pond was able to treat clay suspensions with 96.11% flocculation efficiency.	Elkady et al., 2011
	Chitosan is an aminopolysaccharide which was able to remove anionic dye, acid blue 92 up to 99% color removal.	Szygula et al., 2009
	Kaolin clay suspension was treated by bioflocculant TJF1 from a mixed activated sludge, with flocculating efficiency reached up to 93.13%.	Xia et al., 2008

		Bioflocculant-producing bacterium isolated from soil and identified as *Enterobacter aerogenes* was able to treat 78% of trona suspension.	Lu et al., 2005
Biological	Aerobic	90 % of Acid Scarlet 3R could be decolorized by using salt-tolerant yeast *Scheffersomyces spartinae* TLHS-SF1 within 16 hours.	Tan et al., 2015
		Bacterium *Bacillus* sp. YZU1 was able to remove 95 % of Reactive Black 5 in 120 hours at static conditions with pH 7.0 and 40 °C.	Wang et al., 2013
		Yeast strain *C. tropicalis* TL-F1 was able to decolorize 97.2% Acid Brilliant Scarlet GR within 10 hours at pH 4-6 and 45^{o}C.	Tan et al., 2013
		More than 90% of Acid Red B (100 mg/L) was decolorized within 10 h in the Martin Broth at 30 °C and 150 r/min by using *Pichia* sp. TCL which isolated from sea mud.	Qu et al., 2012

	Anaerobic	Anaerobic fluidized bed bioreactor was able to decolorize more than 90 % of dyeing effluent and more than 80 % of COD.	Deng et al., 2016
		A modified internal circulation (MIC) anaerobic reactor was able to decolorize up to 90 % of dyeing wastewater.	Wang et al., 2015
		Dyella ginsengisoli LA-4 was able to remove 89 % of Acid Red GR with inoculation amount 6.49%, pH 7.06 and temperature 29 °C.	Zhao et al., 2010
	Sequential batch reactor (SBR)	Aerobic granular sludge (AGS) sequencing batch reactor (SBR) system operated with 6-h anaerobic-aerobic cycles was able to treat 90 % of Acid Red 14.	Franca et al., 2015
		Non-tubular sequential batch reactor (SBR) is able to treat 85 % of Acid Red 14 and 80 % COD reduction.	Mata et al., 2015
		Azo dye Acid Orange 7 was decolorized by Macrocomposite based sequencing batch biofilm reactor within 3 hours and more than 80 % of COD was removed.	Lim et al., 2014

2.2 Coagulation-Flocculation Mechanism

Coagulation and flocculation processes occur by destabilizing the repulsive forces between dissolved and particulate contaminants when coagulant is introduced into the system and followed by flocculant substitution to promote formation of floc. The main mechanism of coagulation and flocculation processes are electrostatic patch, bridge formation and charge neutralization (Bolto and Gregory, 2007).

2.2.1 Electrostatic patch

Electrostatic patch mechanism as shown in Fig. 2.1 applied when an oppositely charge electrolyte was used and caused strong attraction. This mechanism is more relevant to high charge density polyelectrolytes with low molecular weight adsorb on negative surface. Blanco et al, (2002) stated there is not physically possible to have overall neutrality when a highly charged cationic polymer adsorbs on a weak charged negative surface. Hence, the cationic patches or islands will form between regions of uncoated, negatively charged particle surface.

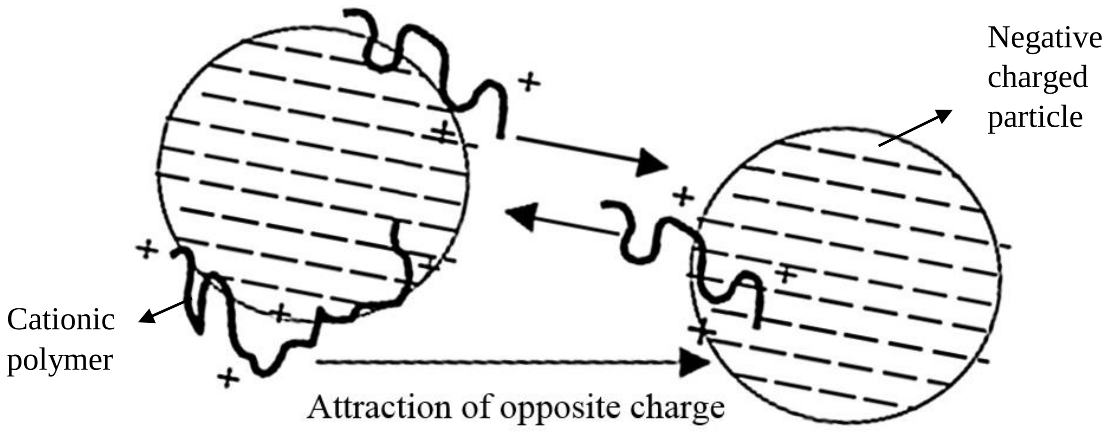

Fig. 2.1 Schematic view of charge neutralization flocculation by patch mechanism (Source: Sharma et al., 2006)

2.2.2 Bridging

Polymer bridging in Fig. 2.2 occurred dominated in the system with long chain polymers and high molecular weight (Caskey and Primus, 1986). Bridging mechanism took place effectively with longer length of the polymer chains that attach and interact with particle from one particle surface to another. Shorter chains polymers are less effective than the longer chains polymers (Razali et al., 2011). This is due to shorter chains polymers have insufficient length to extend or stretch some way into solution far beyond the electrical double layer (Caskey and Primus, 1986). On the other hand, there should be sufficient unoccupied particle surface in the system for the attachment of long chains polymers where else the particles are said to be restabilized (Sher et al., 2013).

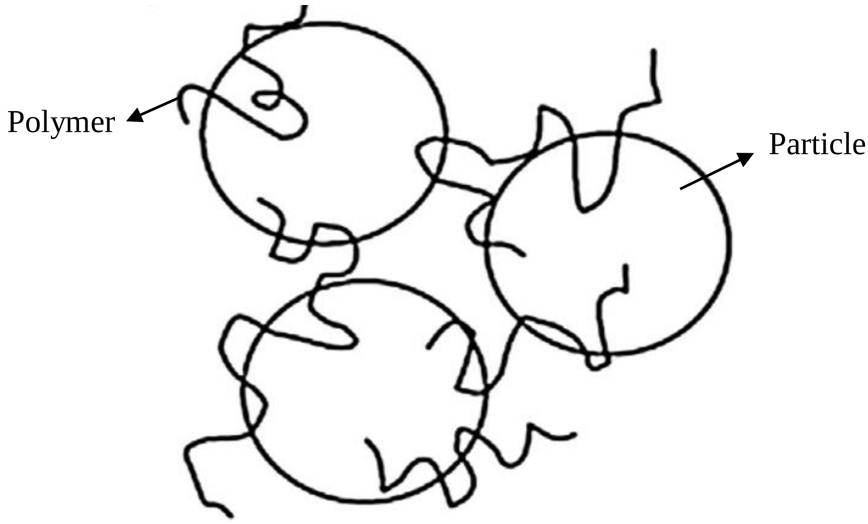

Fig. 2.2 Schematic view of polymer bridging mechanism (Source: Sharma et al., 2006)

2.2.3 Charge neutralization

Charge neutralization mechanism in Fig. 2.3 occurred with the colloids stability in the system was demolished by adding inorganic flocculants and cationic polymers. The surface charged of the particles has been reduced with the reduction of zeta potential. The decrease of electrical repulsion force between colloidal particles is encouraging the formation of van der Waals force of attraction. Hence, the colloidal and fine suspended materials are aggregate to form microfloc. Researchers found that the most effective flocculation takes place at the optimal polymers dosages required to neutralize the particle charge. The zeta potential in the case will close to zero which known as isoelectric point. At isoelectric point, the colloidal particles are tending to agglomerate and become destabilized (Kleimann et al., 2005).

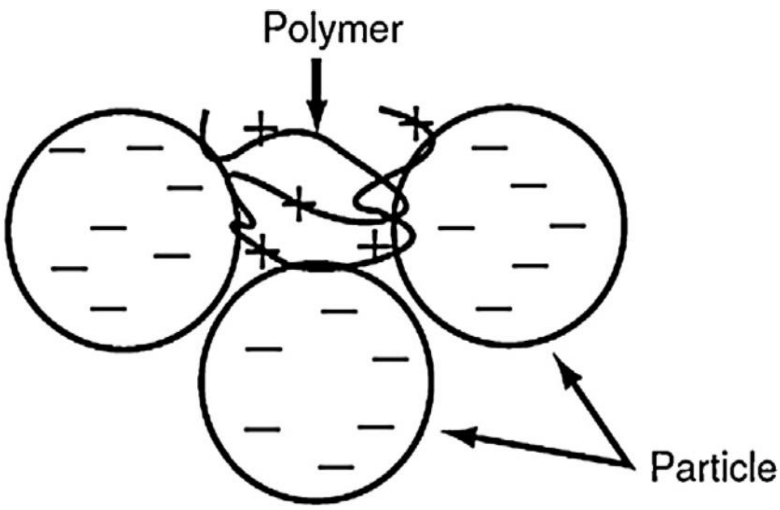

Fig. 2.3 Schematic view of flocculation charge neutralization mechanism (Source: Bohuslav Dobias, 2005)

2.3 Factors affecting flocculation process

2.3.1 Types of flocculant

Selection of flocculant is important to ensure high achievements of removal in wastewater treatment. Chemical-typed flocculants are the most prevalently utilized in industrial wastewater treatment. Different types of flocculant are efficient in treating specific types of wastewater. With the aid of coagulant (polyaluminium chloride), Liang et al. (2014) found that the flocculant (poly(diallyl dimethyl ammonium chloride)) was able to remove approximately 90 % of dye at the optimal dosage and pH. Yeap et al. (2014) synthesized polyaluminum chloride–poly(3-acrylamido-isopropanol chloride) gives removal of color by 96 % and COD by 93 % at the optimum dosage and pH. polyaluminum chloride–poly(3-acrylamido-isopropanol chloride) is a hybrid polymer from inorganic polyaluminium chloride and organic poly(3-acrylamido-isopropanol chloride).

2.3.2 Dosage of flocculant

Dosage of flocculant is one of the factors affecting flocculation performance. Inadequate addition of flocculant induces inefficiency of flocculation performance. This is because small amount of flocculant present in the system is inadequate to destabilize the colloidal particles due to low collision frequency and rate between flocculant and the particles. However, overdose of flocculant may cause restabilization of the colloids into the system, leading to inefficiency of flocculation and high volume of sludge produced (Lee et al., 2011). As a result, it is important to determine the optimum dosage of flocculant needed to achieve optimum treatment efficiency.

2.3.3 Agitation condition

Agitation condition such as mixing speed (rpm) is one of the significant factors for the ideal performance of coagulation-flocculation. Agitation promotes the dispersion of coagulant and flocculant molecules and enhances collision of dye particles and coagulant-flocculant. Therefore, high agitation rate is vital to ensure sufficient kinetic energy is provided to reduce the repulsion forces between colloids prior to charge neutralization. Besides, slow agitation rate should be high enough to facilitate homogenization of the coagulant-flocculant and the wastewater colloids, but it should be low enough not to break the aggregation of flocs (Zahrim et al., 2011).

2.4 Types of polymer

Types of polymers are categorized into two types: direct flocculation and coagulation-flocculation polymer. The difference between these two types of polymers is that direct flocculation polymer has the ability to perform the function of neutralization and bridge the aggregated destabilized particles together to form flocs (Chong, 2012), whereas the coagulation-flocculation polymer required coagulant to perform destabilization of particles in the system, and subsequently the flocculant will aggregate the particle to become floc.

Direct flocculation polymer is able to neutralize the particles in the solution, thus form a bridge between the destabilized particles for the formation of (Chong, 2012). Direct flocculation does not require addition of coagulant and pH adjustment. This is because it is workable in all range of pH value from pH 1 to 14. It only needed sole cationic or anionic polymers to clarify wastewater. However, the polymer should contain medium charge density with higher molecular weight. Fig. 2.4 represents direct flocculation process

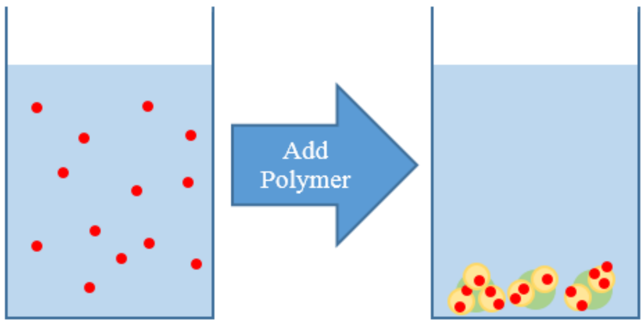

Fig. 2.4 Direct flocculation process

On the other hand, coagulation-flocculation polymer is a long chains non-ionic or anionic structure which will be added after coagulation process (Chong, 2012). In the coagulation process, coagulant will be added into the system, subsequently the coagulant will hydrolyse rapidly in wastewater to form cationic species coagulant. The cationic species coagulant will undergo charge neutralization mechanism through reaction with negatively charged colloidal particles causing reduction of surface charges and micro-flocs formation (Suopajärvi et al., 2013). Coagulation process is performed in a rapid mixing whereas flocculation is performed in a slow mixing. In flocculation process, anionic/non-ionic polymeric polymer will have introduced into the system for the bridging of slow-settling micro-flocs and agglomerate the flocs to form larger and denser flocs by facilitating their removal in sedimentation, filtration or flotation as shown in Fig. 2.5 (Lee et al., 2012).