SIMULTANEOUS PRODUCTION OF BIOFLOCCULATING AGENT AND TREATMENT OF CIBACRON YELLOW FN-2R BY *Sphingomonas paucimobilis* USING SEQUENCING BATCH REACTOR

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by

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LIST OF ABBREVIATIONS

AATCC	American Association of Textile Chemists and Colourist
ASM	Activated Sludge Model
BOD	Biochemical Oxygen Demand
CDS	Close Drainage Systems
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EPS	Extracellular Polymeric Substance
F/M	Food to microorganism ratio
HRT	Hydraulic Retention Time
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
OD	Optical Density
PGA	Polyglutamic Acid
SBR	Sequencing Batch Reactor
SMP	Soluble Microbial Products
SVI	Sludge Volume Index
SRT	Solid Retention Time
SS	Suspended Solids
TS	Total Solids

LIST OF SYMBOLS

k	first order substrate utilization reaction rate constant
K_d	decay coefficient
K_S	half saturation coefficient
k_{max}	maximum rate of substrate utilization per unit of weight of mass of substrate /mass of VSS time
R_2	correlation coefficient
R_s	rate of substrate utilization, mass/ volume-time.
X	concentration of biomass
Y	yield coefficient
μ_g	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})
μ_{net}	net specific growth rate
S	Substrate concentration
S_0	Initial Substrate Concentration Measured at the end of Fill Phase

PENGHASILAN AGEN BIO-PEMBUKUAN DAN RAWATAN UNTUK CIBACRON YELLOW FN-2R OLEH *Sphingomonas paucimobilis* SECARA SERENTAK MENGGUNAKAN REAKTOR KELOMPOK BERTURUTAN

ABSTRAK

Bakteria *Sphingomonas paucimobilis* didapati telah menghasilkan bahan bio-pembukuan dengan aktiviti pembukuan yang tinggi terhadap pemendapan kaolin dan pewarna larut air. Pelbagai jangkamasa pengkulturan telah diuji (2, 4, 6, 8, 10 jam) bagi menyiasat pertumbuhan *Sphingomonas paucimobilis* pada suhu pengkulturan (30°C, 35°C, 40°C, 45°C) ke atas penghasilan bio- pembukuan. Kajian ini mendapati kadar pembukuan bergantung kepada faktor masa dan suhu pengeraman. Penghasilan bio-pembukuan oleh bakteria *Sphingomonas paucimobilis* yang tertinggi dan yang terendah diperolehi pada suhu pengkulturan 35°C dan 40°C masing-masing. Dua ujikaji berasingan dengan kepekatan air sisa pewarna dan sukatan bakteria *Sphingomonas paucimobilis* yang berbeza telah dijalankan. Ujikaji pertama dijalankan untuk proses pengadaptasian *Sphingomonas paucimobilis* pada kepekatan pewarna berbeza iaitu 50, 100, 150, dan 200 mg/L di dalam empat reaktor R1, R2, R3 dan R4.

Ujikaji kedua merupakan lanjutan daripada ujikaji pertama menggunakan reaktor yang sama (R1, R2, R3 dan R4) tetapi pada kepekatan pewarna 500 , 1000, 1500, dan 2000 mg/L. Semua ujikaji dianalisa bagi mengenalpasti keperluan oksigen kimia (COD), larutan bercampur pepejal terampai (MLSS) dan penyingkiran warna pada setiap hari. Didapati tiada perubahan yang signifikan terhadap kecekapan penyingkiran COD dengan perubahan kepekatan pewarna. Kecekapan penyingkiran pewarna menurun apabila kepekatan pewarna meningkat. Perubahan peningkatan MLSS, EPS, dan SMP dalam masa satu bulan kajian telah mengesahkan kebolehan penghasilan bio-pembukuan dan penyingkiran warna oleh *Sphingomonas paucimobilis*. Masa tahanan pepejal (SRT) terbaik dicapai pada SRT 30 hari. Keputusan ujikaji menunjukkan pengurangan COD meningkat pada 5000 mg/L MLSS dengan nisbah F/M = 0.2. Model penyingkiran warna dengan kepekatan COD berbeza pada dua kepekatan MLSS (3000 dan 5000 mg/L) telah ditunjukkan melalui analisis regrasi. Kesan masa kitaran ke atas pengurangan COD dapat dilihat pada tiga kitaran SBR yang berbeza jangkamasa (12, 24 dan 48 jam). Kecekapan penyingkiran pewarna tertinggi telah diperolehi pada kepekatan pewarna larut air 500 mg/L. Keputusan ujikaji SBR berskala makmal telah digunakan dalam penentuan pekali bio-kinetik melalui persamaan Monod. Keputusan kajian kinetik menunjukkan $k_d = 0.182$ hari^{-1}, Y= 0.5 mg/L, k= 3.13 hari^{-1} dan $K_s = 24.07$ mg/L.

Di dalam proses SBR, nilai pekali Y yang tertinggi menujukkan peningkatan kepekatan MLVSS (biojisim) seterusnya merendahkan kepekatan COD dalam efluen terawat. Model kinetik untuk pembentukan EPS menggunakan Ni et al. (2009) dan juga modifikasi persamaan Monod telah diperolehi. Didapati EPS meningkat dengan pengurangan kepekatan COD. Kesesuaian pengaplikasian modifikasi persamaan Monod dalam penentuan pekali bio-kinetik pembentukan EPS telah memberi kesimpulan bahawa kepekatan EPS mengikuti model kinetik tertib-pertama dan mempunyai kesan positif terhadap proses bio-pembukuan.

SIMULTANEOUS PRODUCTION OF BIOFLOCCULATING AGENT AND TREATMENT OF CIBACRON YELLOW FN-2R BY *Sphingomonas paucimobilis* USING SEQUENCING BATCH REACTOR

ABSTRACT

Sphingomonas paucimobilis was found to produce a bioflocculant with high flocculating activity for kaolin suspension and water-soluble dyes. Various culture temperatures (2, 4, 6, 8, 10 hrs) in order to investigate *Sphingomonas paucimobilis* growth of different culture *temperature (*30°C, 35°C, 40°C, 45°C) *o*n the bioflocculant production. It was found that flocculating efficiency depends on incubation time and temperature. Highest and lowest production of bioflocculant by *Sphingomonas paucimobilis* were found at 35°C and 40°C incubation temperature respectively. Two separate experiments, with different range of the dye wastewater concentrations and dosages of *Sphingomonas paucimobilis* were conducted. First experiment was carried out for *Sphingomonas paucimobilis* acclimatization on the different dye concentration of 50, 100, 150, and 200 mg/L wastewater in four reactors (R1, R2, R3 and R4).

Second experiment was proceeding with the similar reactors with the dye concentration of 500, 1000, 1500 and 2000 mg/L. All experiments were analyzed for chemicals oxygen demand (COD) and mixed liquor suspended solids (MLSS) and colour removal every day. There was no significant influence on COD removal efficiency was observed by altering the dye concentration. The dye removal efficiency was decreased wherever the dye concentration increases. The increasing trend of MLSS, EPS and SMP within one month period of experiment confirmed the potential production of bioflocculating agent and dye removal by *Sphingomonas paucimobilis*. The best solid retention time (SRT) is achieved at 30 day SRT of SBR (24 cycled). Result show that COD reduction increase at 5000 mg/L MLSS with respect to F/M ratio = 0.2. Modelling for dye removal with different concentrations COD at two different MLSS concentrations (3000 and 5000 mg/L) were performed by regression analysis. Effect of cycle time on COD reduction was observed for three different SBR cycle (12, 24 and 48 h duration). The highest dye removal efficiency was obtained at the dye concentration of 500 mg/L. Laboratory scale SBR result were used for bio-kinetic coefficients determination via Monod equation. Results depicted that k_d = 0.1820 day^{-1}, Y = 0.5 mg/L, k = 3.13 day^{-1} and K_s = 24.07 mg/L.

High value of yield coefficient (Y) indicates the increase in MLSS concentration (biomass) in the SBR process resulted a lower COD concentration in the treated effluent. A kinetic model for EPS formation using Ni et al. (2009) via a modification of Monod equation. It was shown that EPS increase with the decrease in COD concentration. The suitability of modification in Monod equation for bio-kinetic coefficient determination of EPS production may infer that the EPS concentration follows first order kinetics and has a positive effect on the bio-flocculation process.

CHAPTER 1

INTRODUCTION

1.1 Bioflocculant and Wastewater Pollution

In wastewater treatment, bioflocculant have been used to treat dyes solution (Zhang et al., 2002; Deng et al., 2005). Bioflocculant is a kind of biodegradable macromolecular flocculant created by microorganisms. Because of their biodegradability, harmlessness and lack of secondary pollution, bioflocculants have gained much wider attention and research to date (Li et al., 1999; Patil et al, 2011). Flocculation is an essential process in the treatment of wastewater, tap water production and dredging or downstream processing techniques in a variety of industrial fields (Fujita et al., 2000).

Biodegradation using microorganisms is gaining importance as it is cost effective, environmentally friendly, and produces less sludge (Yang et al., 2003). Rapid developments of industrialization and human activities have led to increasing the discharge of waste and wastewater containing organic and inorganic pollutants such as dye wastewater to the environment. Dye wastewater pollution has become one of the most serious environmental problems today. Increasing pollutants have brought serious environmental pollution and threatened biolife (Bishop, 2002; Volesky, 1990; Wang, 2002; Wang and Chen, 2006). The primary purpose of wastewater treatment is to remove the suspended and soluble organic constituents measured as chemical oxygen demand (COD) or biochemical oxygen demand

(BOD) in the incoming liquid streams. Levine et al. (1985, 1991) have pointed out that the contaminants that must be removed from the wastewater are a complex mixture of particulate and soluble substances, both inorganic and organic, constituents that range in size from less than 0.001 µm to well over 100 µm, and a major fraction of the organic material in municipal wastewater is in the particulate form.

The first process results from the solubilization of biodegradable organic particulates (slowly biodegradable substrate) and the consumption of dissolved organic molecules (readily biodegradable substrate) originally present as well as resulting from the solubilization step. The second process, and ultimately the most important in the development of high-quality effluent, is the flocculation of microorganisms and other particulate and colloidal matter into a readily settleable biomass. Once the dissolved substrate depletes, biological flocculation is the mechanism bacteria uses to trap food particles for hydrolysis to occur (Logan and Hunt, 1988; Okutman et al., 2001; Morgenroth et al., 2002). Actually, biological flocculation is the first step in building the compact, readily settleable floc necessary to optimize suspended growth reactor settling characteristics (Grady et al., 1999). The third process is the final sedimentation of the flocculated particles. A well-flocculated sludge must leave a supernatant with very low concentrations of suspended solids (<15 mg/L) after 30 minutes of settling. If the biomass does not flocculate well, there will be some microorganisms, suspended and colloidal materials that end up in the final effluent, increasing the suspended solids total COD concentration.

The quality of the effluent discharged from the activated sludge process is significantly affected by the solid removal efficiency of the overall system. A significant fraction of the organic matter discharged from all activated sludge systems is in the particulate form (Pipes, 1979). In addition to being oxygen demanding, these organic particles contain significant concentrations of nutrients, nitrogen and phosphorous, the discharge of which is known to cause accelerated eutrophication in the receiving waters. Although flocculation seems to be important to constantly produce good effluents, its role in the overall particulate COD removal process is not well understood. Researchers agree that flocculation is the process responsible for producing settleable particles that can be separated easily by gravity in the final sedimentation tank.

However, most researchers have overlooked the effect of the kinetics of flocculation on the overall substrate removal process, and have concentrated their attention on the kinetics of hydrolysis and dissolved substrate removal when modeling activated sludge systems. The bioflocculation process is defined as the natural aggregation of colloidal particles and suspended solids by microbial activity. This process occurs because of biopolymer secretion by microorganisms present in the mixed liquor (Pavoni et al., 1972; Sheintuch, 1987; Urbain et al., 1993), and the subsequent aggregation of both organic and inorganic particles into microbial floc.

This process occurs naturally only under favorable conditions in the system. The extracellular polymers are considered important for the physico-chemical properties of activated sludge flocs and have been found to determine and define the floc structure (Eriksson and Hardin, 1984), the floc charge (Horan and Eccles, 1986), the flocculation process (Brown and Lester, 1980; Barber and Veenstra, 1986; and Eriksson and Aim, 1991), the sludge settling properties (Forster and Dallas-Newton ,1980; Goodwin and Forster, 1985; Urbain et al., 1993; and Stoll and Buffle, 1998), the sludge dewatering properties (Kang et al.,2002), and the floc chemical properties, affecting the probability that two particles will stick together when they collide (Stoll and Buffle, 1998).

Parker et al. (1970, 1971) and (Wahlberg, 1992) have been a few of the researchers that have studied the bioflocculation process as an effluent quality improvement mechanism. Parker et al. (1970, 1971) developed a theoretical relationship describing the kinetics of primary particles removal as a function of intensity and time of agitation during flocculation of activated sludge. Wahlberg (1992) extended and verified Parker's work and developed practical batch testing procedures for evaluation of flocculation kinetics.

Agreement between data collected in a series of continuous-flow flocculation experiments and batch flocculation studies were obtained. Increased primary particle removal after settling was observed for almost all combinations of time and intensity of agitation studied. Some studies focused on the "tail end" of the activated sludge process where the primary particles are not the influent particles, but bacteria eroded from floc in the turbulent aeration basin. However, they did not study flocculation as a process leading to the removal of influent organic particles from suspension and their subsequent use as food for the bacteria in the floc. This investigation study the initial contact of the influent particles with the biomass, where the primary particles are from the influent and most are not bacterial floc but particulate substrate for the process. The present study focuses on the incorporation of bioflocculation as removal for organic matter.

1.1.1 Benefits of Bioflocculant in the Environment

At present, flocculants are prevalent in a variety of industrial processes such as wastewater treatment, drinking water purification and downstream processes in fermentation processes (Shih et al., 2001). Although chemically synthetic flocculants are playing dominant roles associated with their effectiveness and low cost, they are hard degraded. Some of these synthetic flocculating substances are threats to public heath and increase environmental risks. For example, polyacrylamide, one of the most popular flocculants, includes acrylamide monomers which are verified as both neurotoxin and strong carcinogens to human beings (Dearfield and Abermathy, 1988). Recently, bioflocculants have attracted considerable attention as a promising substitute of the chemical flocculants (Jang et al., 2001) because of their biodegradability and safety for ecosystems (He et al., 2004).

Bioflocculants, secreted by algae, bacteria, fungi and yeast, are kinds of extracellular biopolymer including proteins, glycoproteins, polysaccharides, lipids and glycolipids (Salehizadeh and Shojaosadati, 2003). Many particular microorganisms and their bioflocculants had been announced recently.

(Jang et al., 2001) discovered the *gyrodinim impudicum* KG03, a bioflocculant produced by a marine dinoflagellate, which was characterized as an acidic heteropolysaccharide, with galactose and uronic acid as main and minor components, respectively (Yim et al., 2007). The bioflocculant secreted by Nannocystis sp. Nu-2 was found to be a glycoprotein which was effective in bleaching acid red and direct emerald blue (Zhang et al., 2002).

In addition, Rhodococcus erythropolis was able to generate a protein bioflocculant which lost the flocculating capability by enzymatic digestion (Takeda et al., 1991). Although these bioflocculants showed strong flocculating activities, it was noted that their flocculation abilities depended strongly on captions.

1.1.2 Aerobic Biological Treatment

The biological oxidation process can be defined as the biological process for the removal of readily biodegradable substrate by microorganisms whereas heterotrophic biomass is generated by growth on readily biodegradable substrate under either aerobic or anaerobic conditions (Metcalf and Eddy, 2003). The primary purpose of biological wastewater treatment has been to: remove organic constituents and compounds to prevent excessive DO depletion in receiving waters from municipal or industrial point discharges (Jimenez, 2002). Remove colloidal and suspended solids to avoid the accumulation of solids and the criterion of nuisance conditions in receiving waters.

1.2. Introduction to Reactive Dyes

Reactive dyes are an important class of textile dyes, and approximately 80% of them are based on the azo chromogen (Zollinger, 1991; Pereira and Alves ,2012).

The first reactive dye was introduced in 1956 by ICI, 100 years after the discovery of the first synthetic dye. Presently reactive dyes have a share of one-third of the money spent on dyes for cotton dyeing (Aspland, 1997). In 1988, the production of reactive dyes accounted for only 20% of the total amount of cellulosic dyes, and it increased to 38% in 1992. The annual production of reactive dyes is projected to increase to 178,000 tons in the year 2004 with a share of 50% of all cellulosic dyes. Therefore, the use of reactive dyes for cellulosic fibers has increased exponentially and is expected to increase even further as it brings about the issue of wastewater management from reactive dyeing operations.

In addition to the presence of dye, the use of high concentrations of salt (up to 100 g/L NaCl or Na_2S0_4) further complicates the management of wastewater from reactive dyeing operations, which is currently the single most pressing environmental problem in the textile dyeing industry. Reactive dyes are unique colorants which form covalent bonds between a carbon or phosphorus atom of the reactive group and an oxygen, nitrogen or sulfur atom belonging to hydroxyl, amino or mercapto group, respectively, of the substrate (Rys and Zollinger, 1989). Considering that the use of these dyes has been increasing rapidly and that they are not readily degraded in most conventional wastewater treatment systems, this class of dyes presents a very significant environmental problem (Lim, 2009). Wastewater from the textile industry are generally characterized by high chemical oxygen demand (COD), biochemical oxygen demand (BOD), suspended solids (SS), conductivity and high intense colours (Kim et al., 2002; Koch et al., 2002, Jain et al., 2003).

This is due to the textile dyes wastewater containing large amount of unexhausted dyestuffs (around 20-40% of initial dye load) together with high concentrations of dispersing agents (surfactants), bleaching agents, detergents trace and heavy metals, oil and grease, polyvinyl alcohol, starch, wax, urea, ammonia, caustic soda etc (Chaudhuri and Sur, 2000; Pala and Tokat, 2002; Kim et at., 2004; Golob et al., 2005). The reactive dyes-containing effluents from these industries have caused serious environment pollution because the presence of dyes in water is highly visible and affects their transparency and aesthetics even if the concentration of the dyes is low (Hao et al., 2000).

Cibacron yellow FN-2R is one of the main dyes that are used in textile industries in Malaysia. Therefore, industrial effluents containing dyes must be treated before it is discharged into the environment (Wong et al., 2003). The application of SBR to colour removal is rather a new approach compared to anaerobic–aerobic sequential treatment (Kapdan and Ozturk, 2005).

1.3 Sequencing Batch Reactor System (SBR) for Dye Wastewater Treatment through Biological Process

Sequencing batch reactor technology has been developed on the basic scientific assumption that periodic exposure of the microorganisms to defined process conditions is effectively achieved in a fed batch system in which exposure time, frequency of exposure and amplitude of the respective concentration can be set independently of any inflow condition (Wilderer et al., 2001). SBR technology differs in various ways from conventional technologies used in biological treatment of wastewater. The most obvious difference is that the reactor volume varies with time, whereas it remains constant in the traditional continuous flow system. From the process engineering point of view, the SBR system is distinguished by the enforcement of controlled short term unsteady state conditions leading in the long run to a stable steady state. With respect to composition and metabolic properties of the microbial population, they are growing in the reactor by controlling the distribution and physiological state of the microorganisms.

The success of the SBR technology depends upon the great potential provided by the possibilities of influencing the microbial system in the SBR and also upon the fact that SBRs are comparatively easy to prepare and are cost efficient. SBR processes are known to save more than 60% of expenses required for conventional activated sludge process in operating cost (Chang et al., 2000). SBR has been successfully applied for the treatment of domestic wastewater, medium and lower strength land fill leachates, simulated dye wastewaters and contaminated soils (Wei-Chi et al., 1986 and Mohan et al., 2002).

1.4 Problem Statement

The textile industry is one of the industry segments that make the largest contribution to Malaysia’s Gross Domestic Product (GDP). Nevertheless, they also discharge significant amounts of high level pollutants in their effluent (Sidhu and Hamid, 1993). Approximately 12% of the synthetic textile dyes used every year is lost into waste effluent during manufacturing and processing operations and 20% of them enter the environment from wastewater treatment plants, which cause considerable environmental pollution problems (Morais et al., 1999; Lall et al., 2003).

One of the major problems of textile wastewaters is coloured effluent, as colour is the first pollutant to be recognized in textile effluents because of its visibility (Chinwetkitvanich et al., 2000). The colour also will provide a strong negative impact on the aquatic environment if it is not dealt properly (Lin and Lin, 1993). The discharge of strongly coloured wastewater not only has an adverse aesthetic effect, but it also impedes light penetration and thus, causes the direct destruction of aquatic communities present in the ecosystems by affecting their photosynthetic activity. The growth of bacteria that biologically degrade impurities in the stream and start the food chain is also hindered by the strongly coloured effluent (AlDegs et al., 2000; Chaudhuri and Sur, 2000; Ozacar and Sengil, 2003; Papic et al., 2004).

Furthermore, dyes, which are bio-recalcitrant in wastewater, undergo chemical and biological changes, and consume dissolved oxygen (DO) from the stream (Can et al., 2003). Colour cannot be effectively removed by using simple chemical and physical treatment methods (Kuo, 1992; Morais et al., 1999; Armagan et al., 2003). Hence, colour removal from textile effluents has received a great attention and many environmental regulatory agencies impose the colour standards on receiving waters recently (Sadik and Shama, 2002).

Therefore, many of the textile dyes are carcinogenic, mutagenic and detrimental to our environment. The current environmental concern with azo dyes revolves around the potential carcinogenic health risk that azo dyes or their intermediate biodegradation products, such as aromatic amines which are formed as metabolites by reductive cleavage of the azo (-N=N-) bonds under anaerobic conditions present to humans (Arslan et al.,2000). Recently, new and more stringent regulations coupled with increased enforcement concerning wastewaters discharge are established. Legislation has controlled the effluent discharge by setting limits on the permissible biological and chemical properties of the effluent in the discharge standards (Papic et al., 2004).

In Malaysia, Industrial wastewater and sludge discharge is regulated under the Environment Quality Act. However, colour is still induced as a regulated parameter in Malaysia. According to United State Environmental Protection Agency (USEPA) Standards 1982, the discharge limit for colour is 100 (Pt Co). Colour, COD and BOD of most of the textile effluents in Malaysia have to be reduced drastically by up to 99% for colour, about 60-97% for COD and 90-97% for GOD, in order to meet the discharge standards (Rahman, 1993).

Recently, synthetic flocculants are widely used in industrial wastewater treatment plants due to their cost effectiveness and high flocculating activity. However, these kinds of flocculants can cause health and environmental problems due to their carcinogenic and neurotoxic monomers (Suh et al., 1997; Yokoi et al., 1997). Synthetic flocculants also produced more chemical sludge which cannot be directly discharged to municipal landfills but must be sent to an authorized company such as Kualiti Alam because chemical sludge is categorized as scheduled waste. Therefore, there is a need to develop technologies that are applicable similar to synthetic flocculants but safe.

The commonly used chemical treatment of wastewater by coagulation flocculation method by means of chemical coagulants produces large volume sludge. The use of bioflocculant for wastewater treatment largely reduces the amount of sludge due to its biodegradable nature. The interest in biotechnological methods for the production of bioflocculant lies in the possibility of using different microorganisms to synthesize extracellular substances with different compositions. Biofiocculants are generally nontoxic and benign to the environment. Bioflocculants have received considerable scientific and biotechnological attention in recent years because of their biodegradability, harmlessness and lack of secondary pollution due to their degradative intermediates (Lu et al., 2005). Bioflocculants also can minimize the production of chemical sludge during the treatment process. Besides that, biofiocculants also have the potential to improve the productivity and product quality in bioprocessing, wastewater treatment and many other industrial operations (Salehizadeh and Shojaosadati, 2001).

The major disadvantages of flocculation method are the production of sludge. The operating condition in a specific treatment plant depends on the composition of wastewater, quality requirements of the discharged water. A large amount of sludge created during the process becomes a pollutant itself and sludge disposal increase the treatment cost. However, the sludge amount could be minimized by treating the smaller volume of highly coloured dye bath directly after the dyeing process (Kuo, 1992; Fan et al., 2003; Papic et al., 2004; Golob et al., 2005). Furthermore, the sludge can be recycled and reused to enhance the treatment efficiency.

In the end, this study may aid to develop a biologically-based decolonization, SBR system to economically renovate and reuse spent reactive dye baths, which will result in the minimization of fresh water consumption, as well as reduction of wastewater volume and pollutant concentrations in textile discharges. Sequencing batch reactor (SBR) as a modified has been used for many industrial wastewaters such as fiber and dyes wastewater. Main advantages of this system are low build cost, high flexibility and low required space (Ganjesh et al., 2006). Disadvantages of this system are high excess sludge production and high SVI index (Bernet et al., 2000; Kargi and Uygur, 2002)

1.5 Research Objectives

a) To determine the flocculating activity by *Sphingomonas paucimobilis* in Cibacron yellow FN-2R wastewater.

b) To determine the percentage of removal of the dye and chemical oxygen demand (COD) in laboratory scale reactor inoculated with *Sphingomonas paucimobilis.*

c) To determine the effect of various solid retention time (SRT) of SBR (24 cycled) on the mixed liquor suspended solid (MLSS) concentration.

d) To determine the biokinetic coefficients (μ_{max}, $K_{s.}$ k_d, Y) on the bioflocculating agent production in reactive dye wastewater by *Sphingomonas paucimobilis*.

1.6 Thesis Outline

This thesis consists of five chapters. First chapter covers the research introduction and some related previous works, as well as the definitions of the terms used. Research objectives are clearly stated including the significance of this work pertaining to the present needs with regards to the biological treatment of water and dye wastewater.

Chapter two focuses on the literature review of the flocculation process, bioflocculant and its mechanisms, factors affecting the production of bioflocculant, reactive, and dyes biological treatment. Sequencing batch reactors (SBR) has been used for biological treatment and to determine of growth and degradation of kinetics organism.

Chapter three describes in detail the materials and methods that had been implemented during the research work. Chapter four depicts the research findings. The results are explained, discussed and supported with previous research work done by other researchers.

Finally, a conclusion detailing the findings of this research is available in chapter five (Conclusion and Recommendations) and suggestions for future research are listed to enlarge the scope as well as deepen the various areas of concern. The theoretical frame work of the study as follows:

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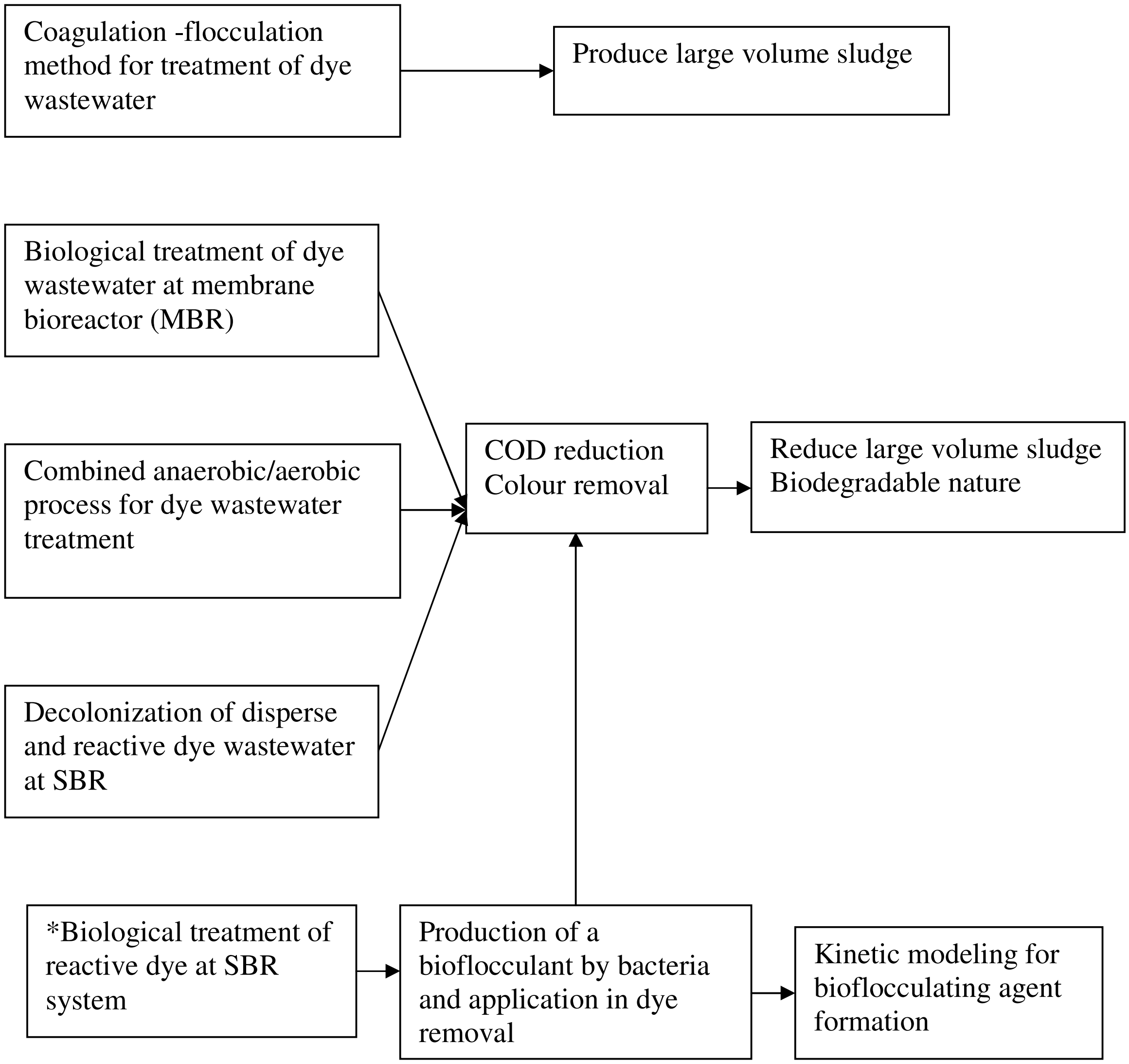

*Noted method used in this study

CHAPTER 2

LITERATURE REVIEW

2.1 Application of Flocculating Agent in Wastewater Treatment

At present, flocculants are prevalent in a variety of industrial processes such as wastewater treatment, drinking water purification and downstream processes in fermentation processes (Shih et al., 2001). Recent research has focused on the use of bio-coagulants and bio-flocculants (Roussy et al, 2005). The decolonization of wastewater is still a major environmental concern. Synthetic dyes used in textile industry, are difficult to be removed by conventional wastewater treatment systems based on adsorption and aerobic biodegradation (Lourenco et al., 2001). In recent years, in order to solve environmental problems the utilization of microbial flocculants has been anticipated due to biodegradability and harmlessness of their degradative intermediates (Salehizadeh et al., 2000).

The primary purpose of wastewater treatment is to remove the suspended and organic constituents measured as chemical oxygen demand (COD) or biochemical oxygen demand (BOD) in the incoming liquid streams (Levine et al, 1985, 1991). Sequencing batch reactor technology has been developed on the basic scientific assumption that periodic exposure of the microorganisms to defined process conditions is effectively achieved in a fed batch system in which exposure time, frequency of exposure and amplitude of the respective concentration can be set independently of any inflow condition (Wilderer et al., 2001).

In wastewater treatment microbial growth has been described by the Monod equation. Selection of the model will depend on the available data and on what is expected of the interaction between substrate and microorganisms (Reardon et al., 2000; Chang et al., 1993; Oh et al., 1994; Yerushalmi and Guiot ,1998).

2.1.1 Flocculation in Biological Wastewater Treatment

Treatment of rapid development of industrialization and human activities has lead to increase the discharge of waste and wastewater containing organic and inorganic pollutants (Patil et al, 2011). Bioflocculant is a kind of biodegradable macromolecular flocculants created by microorganisms. Because of their biodegradability, harmlessness and lack of secondary pollution bioflocculants have gained much wider attention and research to date (Li et al., 1999). Flocculation is an essential process in the treatment of wastewater, tap water production and dredging or downstream processing techniques in a variety of industrial fields (Fujita et al., 2000).

The essence of the flocculation process is the aggregation of suspended coagulated particles to form larger flocculate amenable to separation from the suspending medium by some subsequent physical process, generally sedimentation.

The flocculation process can be defined as the successful particle aggregation that occurs when hydraulic shear forces drive random particles toward each other in a flocculation basin. Several phases of flocculation growth occur during flocculation. Initially, particle growth is dominant, particles combine by coagulation or polymers, and their size increases rapidly (Spicer and Pratsinis, 1996). As flocculation continues, the flocs form large, porous open structures that are more susceptible to fragmentation by fluid shear (Tambo, 1991).

Finally, larger floc masses are formed and they can be easily separated from the liquid phase of water. While organic and inorganic particles can be aggregated by the addition of chemical coagulants, the ability of organisms to self-associate or bioflocculate is of considerable importance in the process design and operation of suspended growth biological reactors (Logan and Hunt, 1988). Coagulation and flocculation have their place among the conventional processes that are frequently cited for treating dye-containing effluents (Zhu et al., 2001; Georgiou et al., 2003 and Allegre et al., 2004). The main advantage of coagulation and flocculation is the decolorization of the waste stream by the removal of dye molecules from the dye bath effluents, and not by a partial decomposition of the dye, which can lead to even more harmful and toxic aromatic compounds (Golob et al., 2005).

2.1.2 Polymer Bridging Model

The most common way of particle flocculation in suspended growth biological processes is polymer bridging. This process occurs when a coagulant substance (i.e., extracellular polymers) forms threads or fibers that attach to several particles, capturing and binding them together. Deng at al .(2005) reported that the bridging mechanisms was found to play an important role in flocculating organic particles in wastewater .One important fact about bridging is that at a higher molecular weight, the molecules are longer and, consequently, more effective bridging between particles is produced as illustrated in Figure 2.1(Melia, 1970).

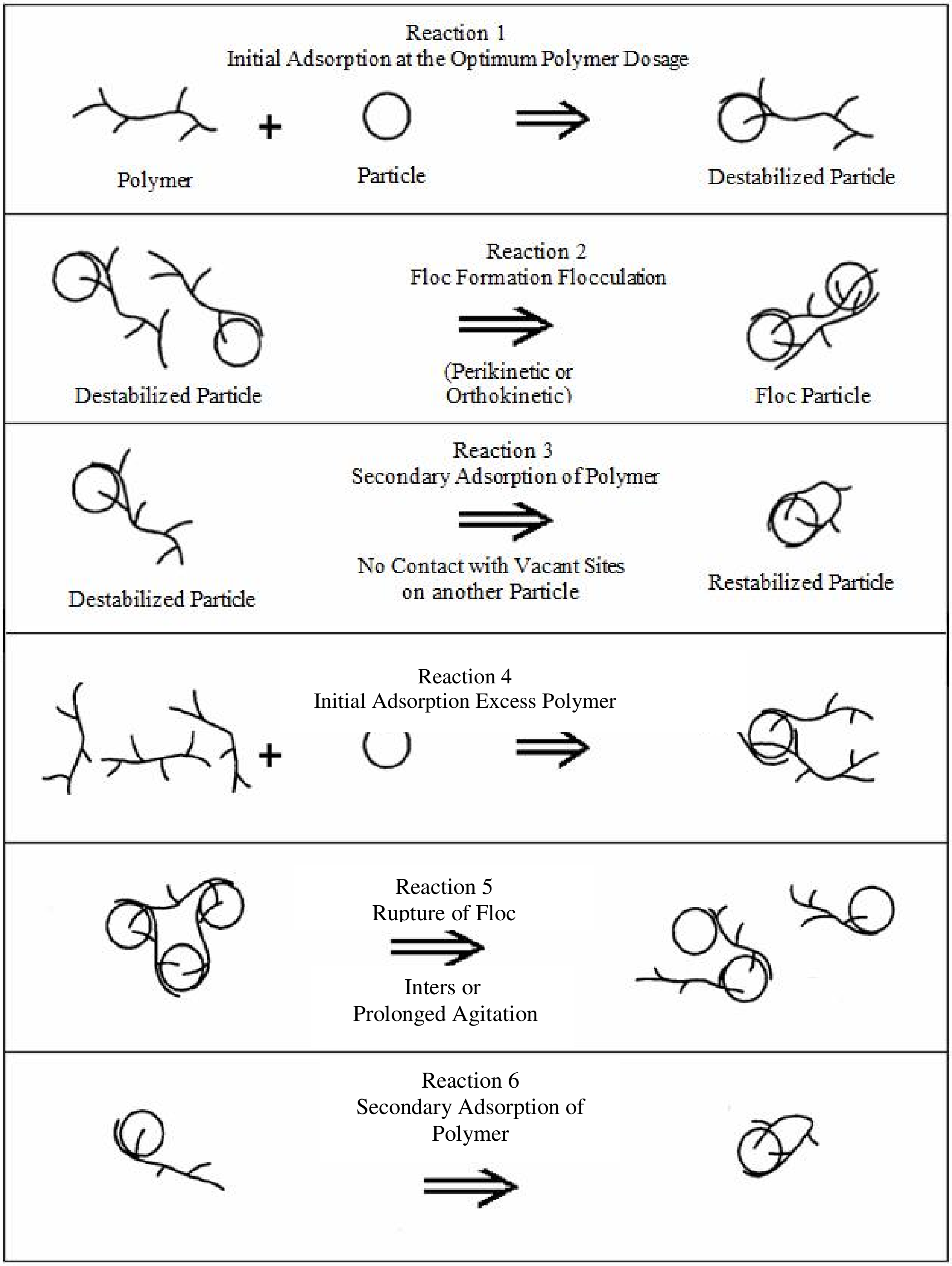

Figure 2.1 Polymer bridging model

Destabilization of the particles (Reaction 1) (Figure 2.1) occurs by adsorption of segments of long-chain polymers onto the particle surface. The un-adsorbed segment of a polymer extends from the surface of the destabilized particle out into the surrounding medium. These extensions adsorb to other particles in the suspension resulting in a floc (Reaction 2). The polymer molecules then form chemical bridges between particles. If another particle is not readily available for bridging to occur after destabilization, the free polymers segment may eventually fold back onto itself and adsorb to other sites on the original particle. If all the adsorption sites are occupied in this manner, the destabilized particle becomes re-stabilized (Reaction 3).

Theoretically, the optimum polymer concentration exists when there are just enough polymers to occupy, in the destabilization step, one half of the available adsorption sites, leaving the remaining sites available for bridging. Nevertheless, in the presence of excess polymers, all the sites can be occupied before any bridging occurs, therefore, preventing a successful aggregation of particles (Reaction 4).

Under turbulent conditions, floc particles can be broken into fragments (Reaction 5). A floc fragment can be incorporated into a floc by combining either with another floc fragment or with a destabilized particle that produced the fragment in the first place, causing the extended polymer segment to fold back onto itself and adsorb to unoccupied bridging sites on the original particles (Reaction 6).

The overall flocculation phenomenon, as described by the polymer-bridging model, is not easily confirmed experimentally because the reactions given in Figure 2.1 did not proceed in a simple sequence given; instead, they are thought to occur simultaneously (Gregory, 1987). Furthermore, in all turbulent environments, the degree and rate at which flocculation proceeds are governed by the relative magnitude of the competing reactions of aggregation and breakup (Argaman and Kaufman, 1968; Parker et al., 1970; Wahlberg, 1992; Wu and Ye, 2007). In spite of that, the polymer-bridging model accommodates most recorded observations of polymer-induced flocculation.

Most bridging flocculants therefore carry either a positive (cationic) or a negative charge. Wastewater which contain positive charge such as silica wastewater will show good flocculation process when anionic polymers is added to the wastewater (Hazana, 2009).The carboxyl groups (COO^-) of bioflocculants which are negatively charged could react with the positively charged suspended kaolin (hydrated aluminum silicate) particles (Wu and Ye ,2007).

Further support for the bioflocculation polymer-bridging model came from Pavoni et al. (1972) who similarly showed that extracellular polymers extracted from batch systems seeded with a municipal activated sludge could flocculate stable dispersions of kaolinite and alumina. Because the extracellular polymer-to-microorganism ratio in batch suspended growth systems increased during periods of pronounced bioflocculation, Pavoni et al. (1972) concluded that bioflocculation could be interpreted in terms of a surface coverage phenomenon, as predicted by the polymer-bridging model.

2.1.3 Biological Flocculation

The biological flocculation process was observed as early as 1947, when Steel (1947) stated that if air is bubbled through sewage, some coagulation of solids and greasy materials will occur, and BOD will be reduced to some extent.

According to Harif et al. (2012), several phases of floc growth occur during flocculation. Initially, particles (floc) growth is dominant, particles combine by extracellular polymers excreted by the cell activity and their size increases rapidly. As flocculation continues, the flocs form large, porous and open structures that are more susceptible to fragmentation by fluid shear. As a result, the final floc size distribution is the balance between particle growth and breakage (Rojas, 2004). The size of the biological floc ranges from 20 to 200 µm but Parker et al. (1971) showed a bimodal size distribution from 0.5 to 5 µm and 25 to 3000 µm.

Vatansever (2005) defined the process as the interaction of high-molecular-weight extracellular polymers, which have accumulated sufficiently at the microbial surface during endogenous growth. In addition, the bioflocculation kinetics suggests that during the initiation of the process, extracellular polysaccharides are responsible for bridging the distance between electrostatic cells to form a weak, elongated floc. Up to a certain level, further polysaccharide synthesis produces stronger flocs by binding cells more firmly (Eriksson and Hardin, 1984; Jackeline Luque ,2005).

Since bioflocculation is a microbial characteristic, there should be some advantage of growth within an aggregate (Logan and Hunt, 1988). There are a number of possibilities. First, there may be interactions between adjacent organisms such as commensalisms, mutualism, parasitism and genetic exchange. Second, growth within an aggregate may protect cells from predation. Third, microbial growth within aggregates or films may be selected by the hydraulics of the system. Finally, growth within an aggregate may increase nutrient uptake rate compared to freely dispersed cells.

According to Urbain et al. (1993), the overall floc structure is negatively charged and is the result of physico-chemical interactions between microorganisms (mainly bacteria), inorganic particles (silicates, calcium, phosphate, and multivalent cations), as shown in Figure 2.2. These researchers failed to consider organic particles as an integral part of the floc.

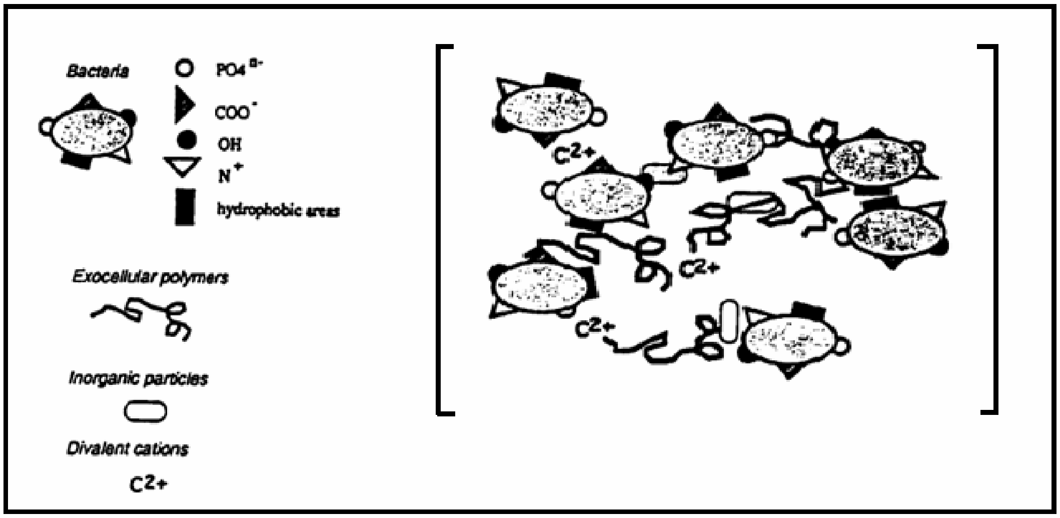

Figure 2.2 Schematic representation of activated sludge floc

Since the bacterial surface and EPS provide negative adsorption sites, the role of divalent cations in the floc stability must be emphasized. Divalent cations such as Ca^{2+} and Mg^{2+} are known to be involved in the chemical structure of bacterial flocculation (Sobeck and Higgins, 2002; Vatansever, 2005).

Bacterial agglutination is governed by the physiological state of the microorganism. Biological flocculation is not observed to occur until the microorganisms have entered into a restricted state of growth, known as the endogenous growth phase (Pavoni et al., 1972); which was later corroborated by Poliszuk (2004) who observed that biological flocculation is frequently observed at the end of logarithmic growth, when the soluble readily biodegradable substrate has been depleted.

For a pure culture of organisms to bioflocculate when the substrate is nearly depleted implies that cell associations confer some advantages over freely dispersed cells relative to increasing substrate uptake by aggregated cells (Logan and Hunt, 1988).

Pavoni et al. (1972) implied that the bioflocculation process inherently relies on two independent characteristics for the production of an acceptable effluent. The first is the complete assimilation of the suspended and colloidal organic material by the active biomass of microorganisms to a final end product of carbon dioxide, water, and inert material. This initial phase of the activated sludge process is commonly referred to as substrate utilization and deals primarily with active synthesis of the active microorganisms mass. The second phase, and ultimately the most significant, in the development of a high-quality effluent, is the flocculation of microorganisms and other colloidal or suspended components into a readily settleable mass so that a clear low end product may be obtained.