IN-SITU TURBIDITY MEASUREMENT OF MODEL WASTEWATER USING CATIONIZED BIOFLOCCULANT INDUCED FROM ASPERGILLUS FLAVUS

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DEDICATION

This thesis is dedicated to my late father, who granted me knowledge from my childhood and thought me that efforts and struggles could reach you to your goals. To my beloved mother, who taught me to rely on Allah (SWT) and always be hopeful to Him in any critical moment, to spread love and serve for humanity. To my respected supervisor who taught me that facing challenges can teach you the best lessons in your life. To my dear siblings (Ziaurrahman, Sadiqullah and Fayaz Muhammad) for their support and assistance. To my spouse, and children (Zainab, Abdullah, Ibrahim and Fatima Zahra) for their love and patience.

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ABSTRACT

The aim of this study was to develop a cost-effective alternative technique for turbidity measurement. Nowadays, different instruments have been used for turbidity measurement such as turbidimeter and spectrophotometer. However, each technique encountered with limitations. Conventional techniques for turbidity measurements usually are expensive, unsafe data collection, the delay between samples collection and posting results. Furthermore, conventional techniques for estimating flocculation efficiency through spectrophotometer can reduce the accuracy of bioflocculant efficiency. In-contrast, in-situ turbidity measurement techniques that mainly use in-situ sensors, could be a possible solution to the mentioned problems because it measures turbidity directly and continuously. To find an appropriate solution and shrink the expenses of bioflocculation, a relatively inexpensive and novel millilitre scale-based bioflocculator was successfully fabricated for in situ turbidity measurements of cationized bioflocculant. Bioflocculator was fabricated with different control systems such as rotational speed controller, temperature controller and microcontroller platform. Moreover, bioflocculator was equipped with different sensors such as turbidity sensor, hall-effect sensor and temperature sensor to ensure the automation and high throughput of bioflocculator. Also, the data retrieve system was developed to avoid manually data collection and save time. The entire process of wastewater treatment such as mixing and online measurements of turbidity was performed within the bioflocculator. The flocculating activity of bioflocculant produced by *A. flavus* S-44 was compared using conventional techniques and bioflocculator. The average flocculating efficiency of 94 % was recorded using conventional techniques, however, it was reduced to 89.87 % by on-line measurements using bioflocculator. The possible reason for low flocculating efficiency using bioflocculator could be due to the real measurements which may give the actual bioflocculant efficiency. Using conventional techniques, the samples collection delay shows the flocculating efficiency higher than the real rate. The whole bioflocculation process was performed within 11 min in bioflocculator. However, using conventional techinques, the whole bioflocculation process was conducted within 17 min because of the manual sampling. Besides that, bioflocculator is cost-effective, high throughput, automated, and control system for real-time turbidity measurements of cationized bioflocculant. Meanwhile, the results can be posted online via PLX-DAQ. The data obtained from the online measurements were able to easily analysed. The data retrieved via PLX-DAQ can also be plotted to make graphs, tables and figures. However, using conventional techniques for turbidity measurements, data need to be manually collected and analysed, which cause time-consuming.

ABSTRAK

Penyelidikan ini dijalankan bertujuan untuk membangunkan satu teknik alternatif yang boleh mengjimatkan kos untuk mengukur tahap kekeruhan. Pada masa kini, pelbagai alat telah digunakan untuk mengukur tahap kekeruhan seperti turbidimeter dan spektrofotometer. Walau bagaimanapun, setiap teknik tersebut mempunyai batasnya yang tersendiri. Teknik konvensional yang digunakan untuk mengukur kekeruhan kebiasaannya mahal, pengambilan data yang tidak selamat, pengambilan sampel dan penghantaran keputusan yang lambat. Selain itu, teknik konvensional yang digunakan untuk menilai kecekapan flokulasi melalui spektrofotometer juga boleh mengurangkan ketepatan dalam menganggar kecekapan bioflokulan tersebut. Sebagai perbandingan, teknik pengukuran kekeruhan in-situ yang terutamanya menggunakan pengesan in-situ, mungkin boleh dijadikan sebagai satu daripada penyelesaian kepada masalah ini kerana teknik ini boleh mengukur kekeruhan secara langsung dan berterusan. Untuk mencari satu penyelesaian yang sesuai dan mengurangkan perbelanjaan bioflokulan, satu bioflokulator berskala mililiter yang baru dan tidak mahal telah berjaya direka untuk mengukur kekeruhan in-situ untuk bioflokulan berkation. Bioflokulator yang telah direka menggunakan sistem kawalan yang berbeza seperti kawalan kelajuan yang berpusing, kawalan suhu, dan pelantar mikropengawal. Di samping itu, bioflokulator ini juga dilengkapkan dengan pengesan yang berbeza seperti pengesan kekeruhan, pengesan kesan-dinding, dan pengesan suhu untuk memastikan mesin berjalan secara automatik dan hasil bioflokulator yang tinggi. Sistem pengambilan semula data juga telah dibina untuk mengelakkan pengambilan data secara manual dan ini sekaligus dapat mengjimatkan masa. Keseluruhan proses rawatan air kumbahan seperti campuran dan pengukuran kekeruhan dalam bioflokulator telah dilaksanakan secara atas talian. Aktiviti memflokul of bioflokulan yang dihasilkan oleh *A.flavus* S-44 telah dibandingkan penggunaanya antara teknik konvensional dan bioflokulator. Purata kecekapan memflokul telah direkodkan sebanyak 94 % apabila menggunakan teknik konvensional, namun, purata tersebut meturun kepada 89.87 % apabila pengukuran oleh bioflokulator secara atas talian digunakan. Kecekapan memflokul yang rendah yang ditunjukkan oleh bioflokulaot mungkin disebabkan oleh pengukuran sebenar yang memberi kecekapan bioflokulan yang nyata. Dengan menggunakan teknik konvensional, pengumpulan sampel yang lambat menunjukkan kecekapan memflokul lebih tinggi berbanding kadar sebenar. Keseluruhan proses bioflokulasi telah dilakukan selama 11 minit apabila menggunakan bioflokulator. Walau bagaimanapun, dengan menggunakan teknik konvensional, keseluruhan proses bioflokulasi telah mengambil masa selama 17 minit disebabkan sampel yang diambik secara manual. Selain itu, penggunaan bioflokulator juga boleh menjimatkan kos, penghasilan yang banyak, automatik dan sistem kawalan untuk pengukuran kekeruhan bioflokulan berkation secara serta-merta. Pada masa yang sama, keputusan yang diperolehi itu boleh dihantar secara atas talian melalui PLX-DAQ. Data pengukuran yang didapatkan secara atas talian sangat mudah untuk dianalisis. Pengambilan semula data melalui PLX-DAQ kemudiannya boleh diplot untuk membuat graf, jadual dan lukisan gambar. Manakala, penggunaan tenik konvensional untuk pengukuran kekeruhan, data hendaklah diambil secara manual dan dianalisis, yang dimana akan mengambil masa yang lama.

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LIST OF ABBREVIATIONS

2D	-	Two Dimensional
3D	-	Three Dimensional
5V	-	Five Volts
A0	-	Analog pin 0
AMI	-	Advanced Metering Infrastructure
CFD	-	Computational Fluid Dynamics
CFU	-	Colony Forming Unit
COD	-	Chemical Oxygen Demand
D1	-	Digital pin 1
DADMAC	-	poly (diallyl dimethyle ammonium chloride)
DC	-	Direct Current
EEPROM	-	Electrically Erasable Programmable Read-Only Memory
EPA	-	Environmental Protection Agency
EPS	-	Extracellular Polymeric Substances
FOPID	-	fractional order PID
GLI2	-	Great Lakes Instrument Method 2
GND	-	Ground
GPG	-	Gel Permeation Chromatography
HCl	-	Hydrochloric Acid
I2C	-	Inter-Integrated Circuit
ICSP	-	In-Circuit Serial Programmer
IDE	-	Integrated Development Environment
INN	-	Inverse Neural Network
IR	-	Infrared
LCD	-	Liquid Crystal Display
NGIC	-	Nonlinear Guided Intelligent Controller
NTU	-	Nephelometric Turbidity Unit
OD	-	Optical Density
OH	-	Hydroxyl
PAA	-	Polyacrylamide

PAC	-	Polyaluminium Chloride
PBS	-	Phosphate-Buffered Saline
PDA	-	Potato Dextrose Agar
pH	-	Power of Hydrogen
PID	-	Proportional Integral Derivative
PLA	-	Polylactic Acid
PMMA	-	Polymethyl methacrylate
PVC	-	Polyvinyl Chloride
PWM	-	Pulse Width Modulation
RPM	-	Revolutions Per Minute
SD	-	Secure Digital
SRAM	-	Static Random-Access Memory
STL	-	STereoLithography
TSS	-	Total Suspended Solid
UNICEF	-	United Nations International Children's Emergency Fund
USB	-	Universal Serial Bus
USEPA	-	United States Environmental Protection Agency
USGS	-	United States Geological Survey
UV	-	Ultraviolet
VCC	-	Voltage Common Collector
WHO	-	World Health Organisation

LIST OF SYMBOLS

°C	-	Degree Celsius
%	-	Percent
<	-	Less than
>	-	Greater than
Al	-	Aluminium
C	-	Carbon
Ca	-	Calcium
Cd	-	Cadmium
Cn	-	Copernicium
Co	-	Cobalt
Cr	-	Chromium
Fe	-	Iron
g	-	gram
H	-	Hydrogen
h	-	Hour
K	-	Potassium
L	-	Litre
M	-	Molar
m^3	-	Cubic metre
mg	-	milligram
Mg	-	Magnesium
mL	-	Millilitre
µL	-	Microlitre
mm	-	millimetre
Mn	-	Manganese
N	-	Nitrogen
Na	-	Sodium
Ni	-	Nickel
nm	-	Nanometre
O	-	Oxygen

⌀	-	Diameter
Pa	-	Pascal
Pb	-	Lead
Pt	-	Platinum
S	-	Sulfur
W	-	watt
Zn	-	Zinc

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Water is essential for industrial, agriculture, domestic, and environmental purposes. However, impurities in the water is one of the challenging environmental issues. The release of agricultural, domestic wastes and toxic industrial sewage are the significant sources of water pollution (Okaiyeto *et al.,* 2016). According to WHO/UNICEF (2000), in developing countries, 70-80 % of all diseases are connected with the utilization of contaminated water, particularly women and children are defenceless against the waterborne disease as cited by Bhatnagar and Sillanpää, (2010). Pollutants from wastewater are toxic to aquatic life and cause an increase in waterborne diseases. Thus, the demand for clean and safe water for domestic use, municipal and industrial purposes is imperative (Yang *et al.,* 2012).

Wastewater from industries and agriculture need to be treated to make them usable. Through the process of flocculation, the contaminants from the sewage can be removed (Shih *et al.*, 2001; Polizzi *et al.*, 2002; Tripathy and De, 2006). Flocculants are generally polymers widely used for wastewater treatment, including inorganic flocculants, organic synthetic flocculants, and naturally occurring flocculants. Most of the people used synthetic chemical flocculants due to their low cost and effective flocculating performance. However, the use of chemical flocculants caused some environmental and health problems.

In contrast, naturally occurring flocculants such as bioflocculants, are non-toxic, biodegradable (Li *et al.,* 2015), safe and eco-friendly biopolymers, and have high efficiency for wastewater treatment (Zaki *et al.,* 2014). Bioflocculants are secreted by some microorganisms such as bacteria, fungi, algae, and yeast (Li *et al.,* 2015). Most researchers are recently concentrated on bioflocculants due to harmlessness, biodegradability, and lack of secondary pollutants (Gong *et al.*, 2008).

There are a variety of instruments and techniques used to measure bioflocculant efficiency. However, the accuracy of measurements is still questionable (to the author's knowledge). The most common drawback is inaccuracy in turbidity measurement, mainly related to techniques and instrument design. Thus, to address this problem, there is a need to develop a new scientific method to increase bioflocculant accuracy measurements.

1.2 Problem Statement

Conventional techniques of estimating the flocculation efficiency of bioflocculants involve measurement of the optical density of the treated wastewater mostly with the spectrophotometer (Azni *et al.*, 2013). However, using these conventional techniques reduces its accuracy (Anderson, 2016). Furthermore, conventional techniques usually are expensive, unsafe data collection, the delay between samples collection due to manually sampling and posting results. In-contrast, in-situ turbidity measurement techniques that mainly use in-situ sensors, could be a possible solution to the mentioned problems because it measures turbidity directly and continuously. Continuous monitoring of in-situ turbidity leads to develop a better estimation of water quality and it is relatively accurate and sensitive method. It also provides a direct view of in-situ conditions (Chai *et al.*, 2017; Azman *et al.*, 2017; Mulyana and Hakim, 2018).

The present study attempts to evaluate the effectiveness of the cationized bioflocculant activity through a series of experimentation involving the treatment of a Kaolin clay suspension using millilitre range prototype bioreactor integrated with turbidity sensor for on-line measurements of bioflocculant activity. Finally, it is essential to develop a new scientific technique to measure the turbidity directly in a bioreactor integrated with the turbidity sensor to accurately reflect in-situ conditions.

1.3 Objectives

Followings are the objectives proposed for this study: -

1. To develop a millilitre range prototype bioflocculator for in situ turbidity measurement.

2. To determine cationized bioflocculants efficiency using the conventional method by measuring optical density manually using a spectrophotometer.

3. To evaluate cationized bioflocculants efficiency using millilitre range prototype bioflocculator integrated with the turbidity sensor.

4. To compare the cationized bioflocculant efficiency using model wastewater between the conventional method and prototype bioflocculator.

1.4 Scope of the Study

This study is focusing on developing a new technique to measure turbidity in-situ of treated wastewater using bioflocculants. Bioflocculant was induced from *Aspergillus flavus* and cationized with calcium ion, which has been widely reported as an excellent cation for bioflocculant stimulation (Tripathy and De, 2006). The cationized bioflocculant is tested using model wastewater, Kaolin clay solution via conventional technique, and prototype bioflocculator. A comparison of bioflocculant efficiency is evaluated between these two techniques.

1.5 Significance of the Study

This study addresses the significance of in-situ cationized bioflocculants explicitly used for wastewater treatment. The in-situ turbidity measurement of cationized bioflocculant used millilitre range prototype bioreactor integrated with a turbidity sensor system for flocculating efficiency measurement. The accuracy of in-situ turbidity measurement is improved and saved operating time. In-situ turbidity measurement helps researchers to collect data safely, reduce or diminish the time delay between samples collection, and post the results immediately after measurements.

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