

PHYCOREMEDIATION OF ARTIFICIAL BATHROOM  
GREYWATER IN VILLAGE HOUSES USING MICROALGAE

*Botryococcus* sp.

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A thesis submitted in  
Fulfillment of the requirement for the award of the  
Degree of Doctor of Philosophy of Civil and Environmental Engineering



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MARCH, 2016

## DEDICATION

I dedicate this work to my beloved son Ahmed Rayyan and my darling wife Hauwa Atiku.



## ACKNOWLEDGEMENT

First of all most importantly, Allah, the all Mighty, who gave me the strength and courage to carry-on throughout my studies, making it all possible.

I wish to express my sincere appreciation and gratitude to the following persons and institution for their contributions to the successful completion of this study. My candid appreciation to my supervisor Dr. Radin Maya Saphira Radin Mohamed for her ceaseless encouragement, support, advice, tolerance. She is not just a supervisor but a dear sister and a times a mother, my gratitude please, only Allah can pay you back. My appreciation goes to my co-supervisors Prof. Ir. Dr. Amir Hashim Mohd. Kassim and Dr. Hazel Monica Matias-Peralta thank you. The staff of wastewater, analytical laboratory FKAAS, ceramic laboratory FKMP and UTHM with sponsorship under grant incentive scheme (GIPS), postgraduate scholarship and fundamental research grant scheme (FRGS) thanks a lot (Terima Kasih Banyu Banyu).

My sincere appreciation to my wife Hauwa Atiku with her resolute, patience, perseverance and tolerance. To my son Ahmed Rayyan am sorry, daddy is always not with you (crying on 14/12/20016 at 8 pm my daddy is not back home yet). Daddy will never let you go down dear, I love you.

My heartfelt gratitude goes to my dad Alhaji Ahmed Usman Wurochekke, mum Khadija Ahmed and siblings. My in-law Air commodore Atiku Umar (Rtd) and family, my Aunties especially Halima and Safiya Modibbo. My uncle Mahmud Usman, Isa Modibbo. Next is my brother Group Captain Shehu Bakari, Mr, Mohammed Giddado Modibbo and Huzaifa Ahmed, thank you for always praying, loving, providing and always being there for me. Lastly, a big thank you to my team in UTHM Suriani, Wahida, Hasila, Aznin, Azimah, other UTHM collique, Dr. Adel Algeethi and my grandma, friends, brothers, sisters, nieces and nephews, I love you all.

## ABSTRACT

The sources of water pollution in Malaysia are domestic sewage and industrial waste. Direct discharge of household bathroom greywater into drains cause eutrophication into the water bodies. Phycoremediation of bathroom greywater effluent to meet a certain level of discharge limit using microalgae *Botryococcus* sp. is suggested. The objectives of this study is to asses quality of nutrients in raw bathroom greywater, produce artificial bathroom greywater (ABGW) recipe with Response Surface Methodology, to study biokinetic absorption of microalgae through phycoremediation. To optimize *Botryococcus* sp. cell concentration, pH and the efficiency of laboratory scale treatment system with *Botryococcus* sp. was observed. The first objective results shown that NO<sub>3</sub>-N and PO<sub>4</sub>-P were 1.03-7.54 & 0.12-22.7 mg/L respectively and 63 L/c/day was discharged to drains as raw bathroom greywater. Secondly, ABGW recipe for soap, detergent, shampoo, shower gel, toothpaste were 0.13, 0.97, 0.88, 0.34, 0.37 mg/L respectively and pH= 6.55. The optimum concentration of *Botryococcus* sp. was 10<sup>6</sup> cells/mL and pH 7 for the third objective. Fourthly, the efficiency of *Botryococcus* sp. in removing NO<sub>3</sub>-N was 97% and PO<sub>4</sub>-P 87% in ABGW on the 30<sup>th</sup> day of phycoremediation, while biokinetic absorption rate using Michaelis-Menten coefficient were  $K = 0.46 \text{ mgNO}_3\text{-N mg/chl a/day}$  &  $K_m = 12.501 \text{ mg/L}$  ( $R^2 = 0.83$ ) and PO<sub>4</sub>-P coefficients were  $K = 8.53 \text{ mgPO}_4\text{-P mg/chl a/day}$  &  $K_m = 176.88 \text{ mg/L}$  ( $R^2 = 0.94$ ). Lastly, the efficiency of *Botryococcus* sp. in laboratory scale treatment system was 90.98% and 93.88% for NO<sub>3</sub>-N while 80.9% and 83% for PO<sub>4</sub>-P on the 13<sup>th</sup> day of phycoremediation in ABGW and raw bathroom greywater respectively. Statistically, algal days of culture, growth of algae, pH, temperature and light correlated well ( $p < 0.05$  &  $0.01$ ) influencing high nutrient removal in the system. Therefore, this proves that *Botryococcus* sp. has high potential to absorb NO<sub>3</sub>-N and PO<sub>4</sub>-P from household bathroom greywater. Hence, the system of this study represents an effective solution for remediation of bathroom greywater.

## ABSTRAK

Sumber-sumber pencemaran air di Malaysia adalah sisa kumbahan domestik dan sisa industri. Pelepasan langsung air sisa dari bilik air rumah kediaman ke dalam longkang menyebabkan berlakunya eutrophikasi ke dalam saluran air semulajadi. Penggunaan mikroalga *Botryococcus* sp. bagi pykoremediasi air sisa dari bilik mandi bagi memenuhi tahap tertentu had pelepasan adalah dicadangkan. Objektif kajian ini adalah untuk menilai kualiti nutrien dalam air sisa dari bilik mandi, menghasilkan resepi air sisa dari bilik mandi buatan (ABGW) dengan Kaedah *Response Surface*, untuk mengkaji penyerapan *biokinetic* mikroalga melalui pykoremediasi. Bagi mengoptimumkan kepekatan sel *Botryococcus* sp., pH dan kecekapan sistem rawatan skala makmal dengan *Botryococcus* sp. diperhatikan. Bagi objektif pertama, kajian menunjukkan bahawa NO<sub>3</sub>-N dan PO<sub>4</sub>-P bagi air sisa bilik mandi masing-masing mempunyai nilai 1.03-7.54 & 0.12-22.7 mg/L dan 63 L/c/ hari telah dilepaskan ke longkang. Kedua, resipi ABGW untuk sabun, bahan pencuci, syampu, gel mandian, ubat gigi masing-masing adalah 0.13, 0.97, 0.88, 0.34, 0.37 mg/L dan pH = 6.55. Kepekatan optimum *Botryococcus* sp. adalah 10<sup>6</sup> sel / mL dan pH 7 untuk objektif ketiga. Keempat, kecekapan *Botryococcus* sp. dalam menghapuskan NO<sub>3</sub>-N adalah 97% dan PO<sub>4</sub>-P 87% pada ABGW pada hari ke-30 pykoremediasi, manakala kadar penyerapan *biokinetic* menggunakan pekali Michaelis-Menten adalah  $K = 0.46$  mgNO<sub>3</sub>-N mg/chl a/hari &  $K_m = 12.501$  mg/L ( $R^2 = 0.83$ ) dan PO<sub>4</sub>-P pekali adalah  $K = 8.53$  mgPO<sub>4</sub>-P mg/chl a/hari &  $K_m = 176.88$  mg/L ( $R^2 = 0.94$ ). Akhir sekali, kecekapan *Botryococcus* sp. dalam sistem rawatan skala makmal bagi NO<sub>3</sub>-N adalah 90.98% dan 93.88% manakala 80.9% dan 83% untuk PO<sub>4</sub>-P pada hari ke-13 dalam pykoredemiasi ABGW dan air sisa dari bilik mandi yang belum diproses. Secara statistik, hari pembiakan alga, kadart pertumbuhan alga, pH, suhu dan cahaya mempunyai kaitan yang baik ( $p < 0.05$  &  $0.01$ ) mempengaruhi penyingkiran nutrien yang tinggi di dalam sistem. Oleh itu, ini membuktikan bahawa *Botryococcus* sp. mempunyai potensi yang tinggi untuk menyerap NO<sub>3</sub>-N dan PO<sub>4</sub>-P dari air sisa dari bilik air rumah kediaman. Oleh itu, sistem kajian ini merupakan penyelesaian yang berkesan untuk pemulihan air sisa mandian.

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## LIST OF ABBREVIATIONS

BOD	-	Biochemical Oxygen Demand
COD	-	Chemical Oxygen Demand
TSS	-	Total Suspended Solids
NH <sub>4</sub> <sup>+</sup>	-	Ammonium
NO <sub>3</sub> <sup>-</sup>	-	Nitrate
NO <sub>3</sub> -N	-	Nitrate-N
PO <sub>4</sub> -P	-	Phosphate-P
OP <sub>4</sub> <sup>3-</sup>	-	Orthophosphate
CO <sub>2</sub>	-	Carbondioxide
DHA	-	Docosahexaenoic Acid
GHG	-	Green House Gases
Mg	-	Magnesium
Ca	-	Calcium
TN	-	Total Nitrogen
N	-	Nitrogen
P	-	Phosphorus
TP	-	Total Phosphorus
DNA	-	Dioxoribonucleic Acid
RNA	-	Ribonucleic Acid
ABGW	-	Artificial Bathroom Greywater
ATP	-	Adesonine triphosphate
RSM	-	Response Surface Methodology
PDDA	-	Polyelectrolyte polydiallyldimethyl Ammonium Chloride
DAF	-	Dissolved Air Flotation
CTAB	-	Cationic N-cetyl-N-N-N trimethyl Ammonium
NaCl	-	Sodium Chloride
UTHM	-	Universiti Tun Hussein Onn Malaysia



- APHA - American public health association
- CCD - Central Composite Design
- SMBR - Submerged membrane bioreactor
- FSTPI - Fakulti Sains, Teknologi dan Pembangunan Insan



## LIST OF PUBLICATIONS

**Anwaruddin Ahmed Wurochekke**, Radin Maya Saphira Radin Mohamed, Amir Hashim Mohd. Kassim. Adel Algeethi, Hauwa Atiku, Hazel Monica Matias-Peralta. 2016. Household Greywater Treatment Methods using Natural Materials and their Hybrid System: A Review, *Journal of Water and Health*, 14(6), 914-928 (ISI, IF= 1.025, Q2).

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**Anwaruddin Ahmed Wurochekke**, Radin Maya Saphira Radin Mohamed, Hauwa Atiku, Suriani Sharuddin and Amir Hashim Mohd. Kassim. Improvement of Bathroom Greywater Quality after Phycoremediation with Microalgae *Botryococcus* sp. presented at the INTERNATIONAL CONFERENCE ON ENVIRONMENTAL FORENSICS (iENFORCE 2015), 19-20, August 2015.

Hauwa Atiku, Radin Maya Saphira Radin Mohamed and **Anwaruddin Ahmed Wurochekke**. Bathroom Greywater Bioremediation by Microalgae *Botryococcus* sp. (ICSESS 2016) presented at the 2<sup>nd</sup> INTERNATIONAL CONFERENCE ON SCIENCE, ENGINEERING AND THE SOCIAL SCIENCES, Universiti Teknologi Malaysia, Johor Bahru, Malaysia. Organized by UTM International and UTHM, 29 May-1 June 2016.

Al-Gheethi AA, Mohamed RMSR, **Wurochekke AA**, Nurulainee NR, Mas Rahayu J, Amir Hashim MK. Efficiency of *Moringa oleifera* seeds for Treatment of Laundry Wastewater presented at the INTERNATIONAL SYMPOSIUM ON CIVIL AND ENVIRONMENTAL ENGINEERING (ISCEE 2016), 25-26 October, 2016.

## CHAPTER 1

### INTRODUCTION

#### 1.1 Background

The speedy growth of the human population and their activities have caused serious water pollution and insufficient freshwater in many developing countries. It is a driving force for everybody to think of alternatives and sustainable solutions to manage this valuable resource (Qu *et al.*, 2013). In Jordan, water scarcity occurred due to the growing population over the previous decade. The availability per capita of water decreased to 198 m<sup>3</sup>/capita/year from the standard of 1000 m<sup>3</sup>/capita/year from 1996 to 2013 (Boufaroua *et al.*, 2013; Najib Al-Beiruti, 2005) which has affected the economic growth in Jordan. The high cost of usual sewers is one of the main restraints to expand wastewater services to small communities. As a result, domestic wastewater especially from greywater sources goes uncollected contributing phosphorus, nitrogen and other elements into water bodies. However, Jordanian water management has implemented some models to change how water is handled and appreciated due to the present water plan from the ministry of water and irrigation which is responsible for the management of operations, maintenance cost and supply, treating and distribution (Al-Beiruti, 2007). Hence, these would increase greywater reuse and reduce the amount of pollutants being discharged into water bodies.

In India, about 25 billion liters of untreated wastewater are discharged into the water bodies daily (Parjane & Sane, 2011). This untreated wastewater will lead to health problems and increase infectious diseases such as diarrhea, dysentery, skin and tissue

infections (Akpor *et al.*, 2011). Furthermore, in India, the International Water Management Institute (IWMI) forecast that by 2025 one person in three will face total water shortage. Similarly, rapid development and industrialization in Malaysia have affected water resources especially in the rural community. Besides, the decrease in quality of water in many rivers has mainly contributed to the water problem in Malaysia. The rivers of Kuantan and Belat were monitored by Kozaki *et al.*, (2016) and it was found that the pollution level of some ions were as follows: 4.44-14.9 for  $NH_4^+$ , 3.71-18.1 for  $NO_3^-$ , 154-6429 for  $Na^+$  and 33.8-1363 for  $Mg^{2+}$  respectively. These pollutants were influenced by human activities like the discharge of untreated water from human sewage and household waste, and also industrial point sources. Thus, they are becoming serious issues and need to be solved before worsening.

Greywater is another alternative source to substitute freshwater usage but it contains contaminants (Parjane & Sane, 2011). Greywater is a type of wastewater derived from the kitchen, bathroom (i.e. discharge from the hand basin, shower, and bath) and also laundry water. Bathroom contributes more than 50% of the total usable greywater volume in a typical household (Donner *et al.*, 2010; Coghlan & Higgs, 2003). Besides that, greywater which originates from bathrooms and showers make up over 30 per cent of household greywater flow (Edwin *et al.*, 2014). Water used for washing hands and showers generate about 50-60% of the total greywater and it can be considered as least polluted type of greywater compared to others. Common chemical pollutants include soap, shampoo, hair dye, toothpaste and cleaning products. It also has a number of faecal contaminations  $3.2 \times 10^5$  CFU/100mL through body wash (Saumya *et al.*, 2015).

However, greywater does not include the wastewater that is generated from toilet use which is normally considered as black water. However, greywater is generated in different quantities between household to household within one community and depends on different factors such as lifestyle and household activities (Al-Mashaqbeh *et al.*, 2012). Many communities in Malaysia, mainly villagers, dispose bathroom greywater directly into the nearest ditch. These inputs promote eutrophication in water bodies and naturally there will be a decline in water quality. At the same time, the removal of nutrients and organic compounds from greywater is an essential means to

avert eutrophication and water bloom. Therefore, bathroom greywater ought to obtain proper treatment prior to being discharged into water bodies.

Numerous systems operate for the removal of nutrients from greywater, although these are expensive and generate elevated thick soft mud (Ruiz-Marin *et al.*, 2010). Natural treatment systems via primary settling with cascaded water flow, aeration, agitation and filtration (Parjane & Sane, 2011; Pangarkar *et al.*, 2010) were used and are less expensive. Yet, there is lack of information when it concerns the removal of nutrients especially phycoremediation with microalgae *Botryococcus* sp. Greywater management treatment system is a system that allows direct utilization of the water. It can use the natural gravity by a hybrid treatment process with the use of natural materials and wetland system. It will facilitate in breaking down the organic compounds and recovery of nutrients (Parjane & Sane, 2011). Bathroom greywater should preferably be treated anaerobically because of lower treatment costs and the possibility of recovering energy (Leal *et al.*, 2010).

Therefore, in this study phycoremediation with microalgae *Botryococcus* sp. as a laboratory scale system will be adopted. Based on the aforementioned criteria, greywater treatment options for households will be advocated. Since uptake is the major means of removing nutrients by microalgae, the colony of microalgal growth squarely influences the nutrient removal rate. Greywater can contain nutrients such as total phosphorus (TP), total nitrogen (TN) from detergents (Maya *et al.*, 2013b; Park *et al.*, 2011b) and total organic carbon (TOC) (Beck *et al.*, 2013b; Li *et al.*, 2009) that benefit algal growth. Therefore, the usage of microalgae for treatment was proposed for a simple greywater treatment system especially for greywater from the bathroom source.

## 1.2 Problem Statement

Conventional discharge of greywater into drains gains the least attention in terms of environmental sanitation such as toilet waste and solid waste disposals. In individual village house areas in Malaysia, bathroom greywater is most often discharged untreated into storm water drains. These discharge can cause unpleasant odours, bread

mosquitos, flies, aesthetic of the environment is disturb and add nutrients (nitrogen and phosphorus) in the drain. Excess nutrients in bathroom greywater causes eutrophication. This discharge of untreated bathroom greywater in an uncontrolled manner to the main drain with excess nutrients flow in to the rivers. Rivers are the main source of water supply in Malaysia. Therefore, eutrophication of rivers with enrich nutrients rapidly grow algae. The decaying algae plant reduce dissolved oxygen, fish and other aquatic life die. In addition, the rapid increase of pollutants (nutrients and pathogens) will occur actively and result in an unhealthy environment for humans or animals with dangerous diseases. Bathroom greywater effluents have high concentrations of nitrogen and phosphorus content which are normally generated by activities such as bathing. It is anticipated that the utmost source of nitrogen is urine, as some people pass urine in the shower rooms or from body washing and bathing through the use of protein-rich shampoos and conditioners. Additionally, nutrient loads may come from washing babies, children after defecation, diaper changes or diaper washing. Phosphorus is mainly found in detergents. Therefore, bathroom greywater treatment is necessary before discharging the treated greywater to water bodies.

### **1.3 Aim of study**

The aim of this study is to evaluate the efficiency of phycoremediation in treating bathroom greywater from village households. This study intends to ascertain the potential for treated bathroom greywater to reduce pollutant loads of nearby water bodies.

### **1.4 Objective of study**

This study embarks on the following objectives:

1. To assess the nutrient, elements availability and the quality and quantity of bathroom greywater from selected case study houses.
2. To optimize bathroom products for the production of an artificial bathroom greywater (ABGW) recipe.

3. To optimize *Botryococcus* sp. cell concentration for best growth and pH of ABGW.
4. To determine ABGW nutrient removal efficiency and mechanism on biokinetic absorption rate.
5. To determine the efficiency of Laboratory Scale Greywater Treatment System for nutrient (nitrate-N and phosphate-P) removal and influence of environmental factors during phycoremediation of ABGW and raw bathroom greywater in a photobioreactor using *Botryococcus* sp.

### 1.5 Scope of Study

The scope of this study includes the isolation of a microalgae called *Botryococcus* sp. at the microbiology laboratory, Fakulti Sains, Teknologi dan Pembangunan Insan (FSTPi), Universiti Tun Hussein Onn Malaysia (UTHM). This microalgae was originally obtained from the Endau Rompin forest reserve in Johor, Malaysia.

Household activities were ascertained using interviews and a questionnaire in four households around Parit Raja. Bathroom greywater loading was measured during the composite sampling by comparing the production of bathroom greywater in the mornings and in the night. Water quality parameters were tested for pH, BOD<sub>5</sub>, COD, TSS, Turbidity, ammonium, nitrate-N, phosphate-P, Ca, Na and Mg to assess the quality of bathroom greywater collected. The artificial bathroom greywater recipe was produced using domestic products. Phycoremediation was done on bathroom greywater with *Botryococcus* sp. to determine its efficiency on nutrient removal (nitrate-N and phosphate-P) and chlorophyll-a content. The adsorption rate of *Botryococcus* sp. on nitrate-N and phosphate-P were determined with a biokinetic model of Michaelis-Menten equation  $\frac{1}{R_{xi}} = \frac{1}{K} + \frac{K_m}{K} \frac{1}{S_o}$  and environmental factors like light, temperature and pH on algal growth were observed during phycoremediation.

The Laboratory Scale Greywater Treatment System (photobioreactor) was built to represent the phycoremediation efficiency of ABGW and raw bathroom greywater for



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