Science and Technology

RMUTT Journal

Vol.7 No.1 (2017) : 43-57
www.sci.rmutt.ac.th/stj
Online ISSN 2229-1547

REMOVAL OF HARMFUL BLUE-GREEN ALGAE *Microcystis* spp. USING AGRICULTURAL AND AQUACULTURAL WASTE

Sirikae Pongswat[1*], Natthaphon Chaosuan [1] and Watcharapol Kapphimai [2]

[1]Division of Biology, Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Thanyaburi, Pathumthani.

[2]Regional Medical Sciences Center 9 Nakhon Ratchasima, Nong Bua Sala, Mueang, Nakhon Ratchasima.

*E-mail: sirikhae_p@rmutt.ac.th

Abstract

Eutrophication is an indicator of the decline of water quality and aquatic ecosystem changes. This problem occurs in the main water resources in Thailand and is getting more severe, particularly in fresh water. This research focused on using agricultural residues and aquacultural waste - rice straw and shrimp shells to eliminate dominant species of toxic algae *Microcystis* spp. in eutrophication. In the laboratory scale experiment, 5 groups of the two-substance mixture, including one controlled group were used. The ratios of the straw extracts and chitosan from shrimp shells mixture were 3:1, 2:1, 1:1, 1:2, and 1:3. The experiment result indicated that the mixture was effective in inhibiting the growth of toxic algae *Microcystis* spp. The different efficiency among the five groups was statistically significant at the level of .05. The best achievement was from the ratio of 1:2 group that the cell numbers (42.42×10^5 cells/mL) were reduced to 3.98×10^5 cells/mL and a reduction of chlorophyll a content from 1667.13 µg/L to 511.94 µg/L. This optimum mixture was then used in the field experiments, resulting in the inhibition of cell number and chlorophyll a at 76.97 and 46.51%, respectively. In addition, the amount of ammonia nitrogen (NH_3-N), nitrate nitrogen (NO_3-N) and orthophosphate (PO_4-P) were reduced by 85.82, 51.67 and 36.36%, respectively.

Keywords: Agriculture waste, *Microcystis* spp., Chitosan from shrimp shells, Extracted rice straw.

Received: May 31, 2017
Revised: June 20, 2017
Accepted: June 27, 2017

1. Introduction

The rapid growth of toxic algae and blue-green algae in a eutrophication the new sources of fresh water is a common problem all over the world and escalates the violent skirmishes during the past decade [28]. Algal blooms, which are increasingly becoming a global concern, represent a significant threat to human health, tumor promoter of liver cancer and water ecosystems [36]. Algal blooms will reduce the water quality, alter the food webs through effects on zooplankton and fish species, and cause highly toxic pollutions [3]. It is caused by the water with nutrients and suitable conditions for the growth of toxic algae, and blue-green algae [14]. The issue was found in both the pool river basins and fresh water lakes. Toxic algae and, blue-green algae that grow quickly in water with high levels of nutrients were *Anabaena* spp., *Microcystis* spp., *Aphanizomenon* spp., *Planktothrix* spp., *Gloeotrichia* spp., *Cylindrospermopsis* spp., and *Lyngbya* spp. [26]. Especially a toxic blue-green algae, *Microcystis* spp. predominantly found in the eutrophication. *Microcystis* spp. can increase to very high densities, often forming a surface scum [6]. It can create toxins called microcystins a chemical cyclic heptapeptides [22]. Microcystins were toxic to the liver when toxins into the human body, causing diseases–hepatotoxic and classified as tumor-promoting compounds, which would accelerate liver cancer [8]. It also affects other living cells which are therefore multifaceted and include disruption of the cytoskeleton, DNA damage and apoptosis related to mitochondrial damage. Microcystins have also been demonstrated to be toxic to reptiles, amphibians, and aquatic species, as well as invertebrates and plant species [23], [11].

For this reason, the whole world is aware of the problems caused by the rapid growth of *Microcystis* spp. Scientists are trying to solve these problems by getting rid of toxic algae in various ways, including physical, chemical and biological fields. The physical and chemical fields are costly and bring about the impact on the environment. However, this research will focus on using agricultural and aquacultural residues to eliminate the toxic algae *Microcystis* spp. for the reason of cost savings and environmental friendly by rice materials from agricultural waste. There are various types of compounds from the extracts that can inhibit the growth of toxic algae, *Microcystis* spp. such as oak extracts [19]. Many compounds can be isolated from rice hulls [3]. Shrimp shells are the waste from aquaculture in the production of frozen shrimp [20]. Pure chitosan can be extracted from shrimp shells [7]. Chitosan has taken algal blooms, as well as clusters of sediments that eventually sink to the bottom of the lake [16], [24], [30]. The oldest traditional coagulants to eliminate the toxic algae *Microcystis* spp. whether the ferric chloride ($FeCl_3$) aluminum chloride ($AlCl_3$) or polyacrylamide brought about algae in the form of lumpy sludge and the use of such substances may result in increasing doses of heavy metals in the water that has been treated and may affect the health of humans and animals that live in freshwater ecosystems [4].

However, chitosan is not heavy metal elements and it is a substance that is biologically degradable naturally and friendly on the treatment waters which are contaminated with toxic algae *Microcystis* spp.

The extract substance from rice straw and chitosan from shrimp shells has an active performance in the elimination of toxic algae; this research, therefore, used the mixture of the two in the removal of toxic algae *Microcystis* spp. This can also serve the cost saving purpose because the chitosan is commercially sold at a relatively high price.

2. Materials and Experiment

2.1 Exploration of the area with the growth of algae, *Microcystis* spp.

Water samples were taken from the Sammakorn Village reservoir, Soi Ramkhamhaeng 110, Ramkhamhaeng Road, Saphan Sung, Bangkok, Thailand. Here the growth of *Microcystis* spp. was found to be continuing throughout the year.

2.2 Preparation of rice straw extracts and chitosan

Chitosan and chitosan from shrimp shells: 100 kg of raw shrimp shells enclosures of the selected shrimps were immersed in a 4% NaOH volume of 270 L at 25°C for 24 hrs. and were again immersed in 4% HCl volume of 270 L at 25°C for 18 hrs. and dried at a temperature of 60 °C. 3 kg dry chitin from the earlier drying process was then immersed in 50% NaOH volume 30 L at 25°C for 48 hrs. Again chitosan was immersed in 50% NaOH the volume of 12 L at 60°C for 48 hrs and dried at a temperature of 60 °C by step to adapt neutral pH [2]. The solution was prepared by dissolving 100 mg of chitosan into 1% concentration of HCl solution. The solution was stirred with a glass rod until it became homogeneous and then adjusted to one liter by distilled water [33].

Extract straw, rice RD 31 (*Oryza sativa*) was cut into pieces of approximately 2 cm. Then, straw pieces were dried at room temperature for 1 week. 20 g of straw were mixed with reverse osmosis (RO water) volume of 400 mL were incubated at 25°C for 5 days. Solution was filtered through filter GF/C to remove the straw. The concentration of straw extract was adjusted to 50 g / L [27].

2.3 Laboratory Experiment Design

Concentrations of extracts from agricultural and aquacultural residues (Table 1) were sprayed on the surface of the samples in 32 cm. x 20 cm. glass jars with 5.82×10^5 cells/mL and last volume of 6 L of water. The glass jars were placed under a cool white fluorescent light of 2,500-3,000 lx. on 12 hr. light/day and 12 hrs. dark/day for 44 days and analysis of the sample was carried out every 3 days.

Table 1 Ratio of extracted from agricultural and aquacultural residues.

Extract	Concentration/Ratio
Straw extract	2 4 6 8 10%
Chitosan from shrimp shells	2 4 6 8 10%
The mixture of extract of rice straw on chitosan from shrimp shells	3:1 2:1 1:1 1:2 1:3

Table 2 Methods for physico-chemical analysis [5].

Physico-chemical Variable	Methods of analysis [37]
Dissolved oxygen; DO (mg/L)	Azide modification method
Biochemical oxygen demand; BOD (mg/L)	5 Days incubation and azide modification method
Ammonia nitrogen; NH_3-N (mg/L)	Nesslerization method
Nitrate nitrogen; NO_3-N (mg/L)	Cadmium reduction method
Orthophosphate; PO_4-P(mg/L)	Ascorbic acid method

2.4 Experimental Design in Experimental Ponds

The best ratio of rice straw extract and chitosan from shrimp shells: 200 mL of straw extract mixed with 400 mL of chitosan from shrimp shells, the result from the experiment in the laboratory, was used with $42.42x10^5$cells/mL of *Microcystis* spp. from natural sources in a final volume concentration of 500 L in 1 diameter of a pond. The experiment was repeated 3 times. For the control group, straw extract and chitosan from shrimp shells were not added.

2.5 Water quality analysis

Microcystis spp. were isolated into single cells by ultrasonic and counted by haemacytometer. Chlorophyll a were analyzed by the method according to Nusch, 1975 modified by Yuwadee [32] for 44 days. Samples were analyzed every three days. Seven parameters of physical and chemical water quality were studied. (Table 2) The samples were analyzed every three days for 44 days.

2.6 Statistical analysis

Each series of experiments between rice straw extracts and chitosan from shrimp shells were

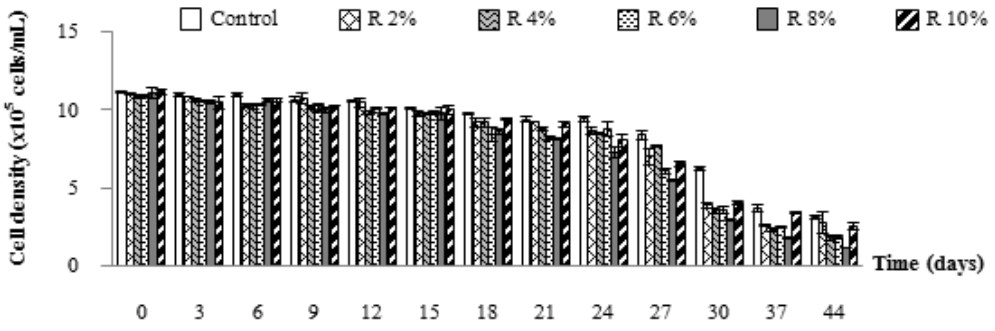

Figure 1 The effects of an extract of rice straw in ratio of the number of *Microcystis* spp. cells. R= rice straw extracts.

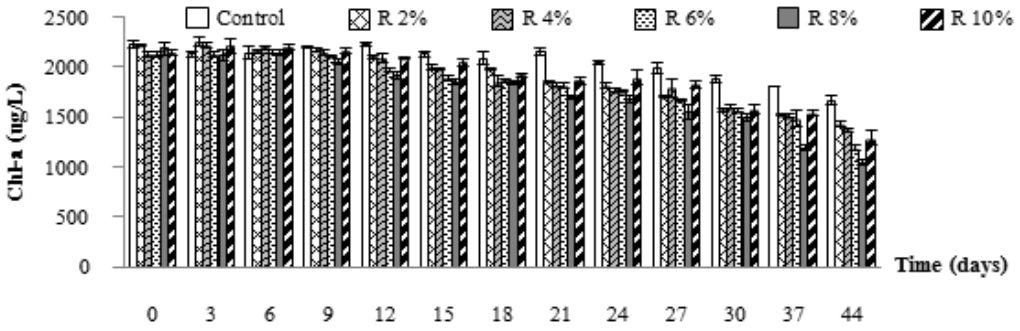

Figure 2 The effects of an extract of rice straw in ratio of chlorophyll a. R= rice straw extracts.

compared using One Way Analysis of Variance (SPSS version 15.0) to analyze the performance of different concentration rice straw extracts and chitosan from shrimp shells in inhibiting the growth of *Microcystis* spp.

3. Results and Discussion

3.1 Elimination of *Microcystis* spp. by rice straw extracts

Among the concentration of 2, 4, 6, 8 and 10% of rice straw extract, 8% straw extract was highly effective in inhibiting the growth of *Microcystis* spp. The number of cells and the amount of chlorophyll a were reduced during the treatment. Both value decreased continuously until the end of the trial on the last day of the experiment. The cell numbers were decreased to 64.21% and the amount of chlorophyll a decreased to 37.39% (Figure 1-2).

3.2 Elimination of *Microcystis* spp. by Chitosan from shrimp shells

The 10% chitosan from shrimp shells resulted in the highest performance at 79.69% inhibition and chlorophyll a decreased to 47.85% compared to the control group in 44 days. (Figure 3-4) Chitosan effective removal of phytoplankton and biological sludge generated by phytoplankton [20]. It can destroy the cell walls of the algae can also be precipitated cell algae floating on the surface at 10 g/l [31].

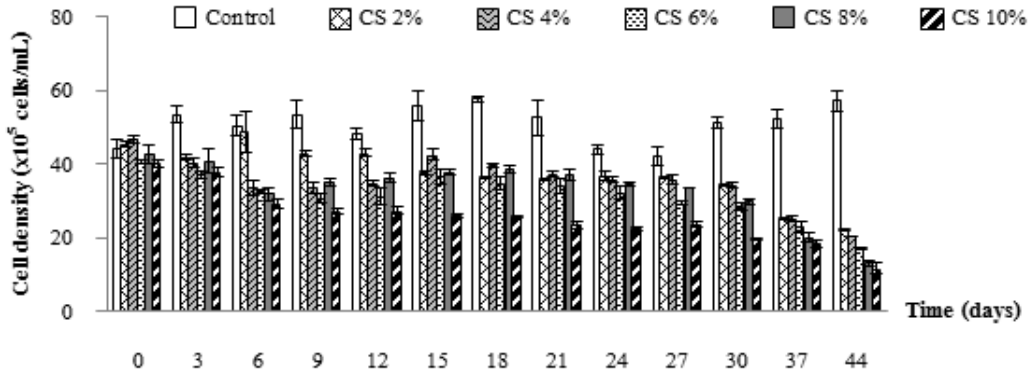

Figure 3 The effect of chitosan from shrimp shells in ratio of the number of cells *Microcystis* spp. CS=chitosan from shrimp shells.

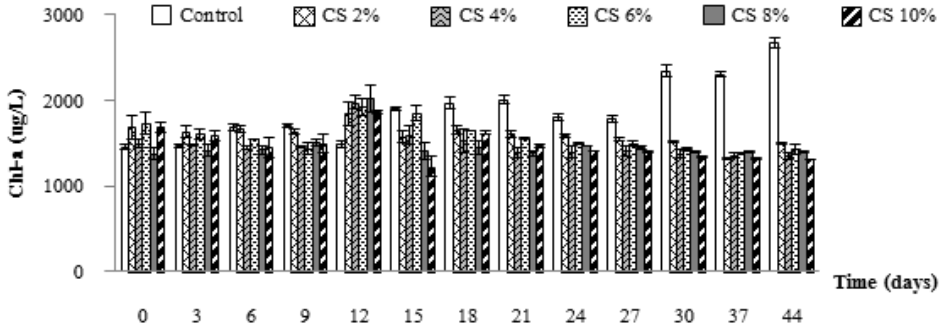

Figure 4 The effects of an extract of chitosan from shrimp shells in ratio of chlorophyll a. CS=chitosan from shrimp shells.

3.3 Elimination of *Microcystis* spp. by rice straw extract and chitosan from shrimp shells

In addition, treatment with the extract of rice straw mixed with chitosan from shrimp shells in the ratios of 3:1, 2:1, 1:1, 1:2 and 1:3 in each treatment resulted in significant decrease of cell algae *Microcystis* spp. continuing from 3 to 44 days of the trial. Straw extracts and chitosan from shrimp shells in the 1:2 ratio formula is most effective in the removal of cell algae *Microcystis* spp. by 92.35%. (Figure 5) Concerning the effective inhibition of chlorophyll a, straw and extract chitosan from shrimp shells in the ratio of 1:2 can inhibit the highest amount of chlorophyll a at 77.76% compared with the control. (Figure 6) When analyzing the variance in individual sets, it was found that the difference among them is statistically significant at the .05 level and the mixture of rice straw extract on chitosan from shrimp shells in the ratio of 1: 2 demonstrated the highest performance. In other words, the mixture was more efficient than the straw extract or chitosan from shrimp shells alone.

3.4 Studies to inhibit the growth of algae *Microcystis* spp. in experimental pond

Studies to eliminate algae *Microcystis* spp. in laboratory, extracts straw chitosan from shrimp shells and the mixture of extract of rice straw on chitosan from shrimp shells showed that the ratio (rice straw extracts: chitosan from shrimp shells, R: CS = 1:2), is the best formula to inhibit the growth

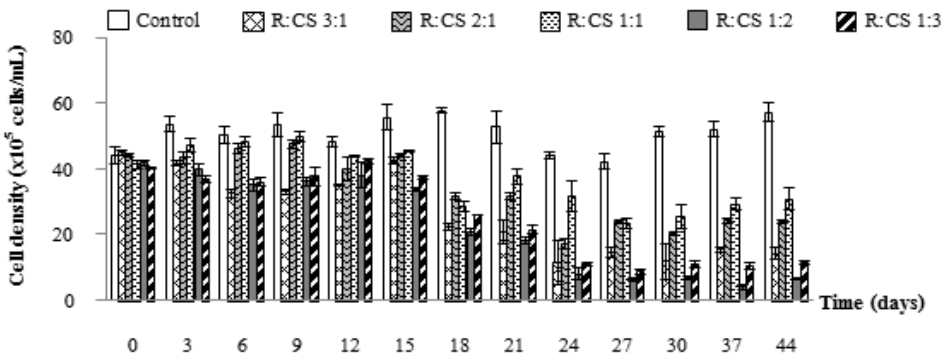

Figure 5 The effects of an extract made from two types of agriculture to the number of *Microcystis* spp. cells. R=rice straw.

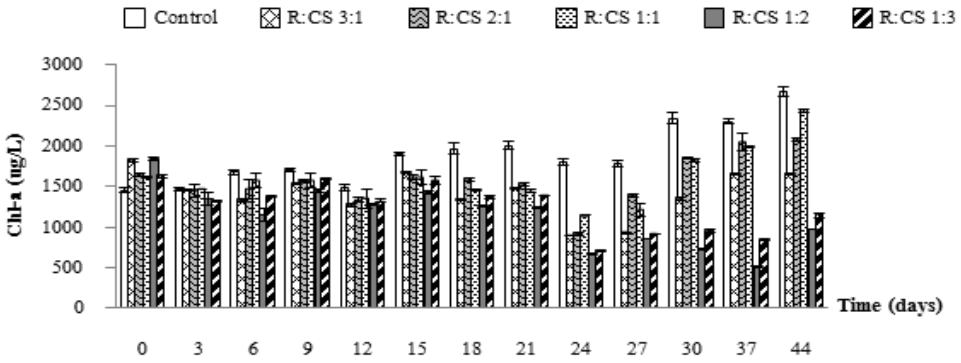

Figure 6 The effects of an extract made from two types of agriculture to chrophyll a. R= rice straw extracts, CS=chitosan.

of toxic algae *Microcystis* spp. This result has been field-tested. The decrease of cell number and chlorophyll a over the period was that the cell number decreased 76.97% and chlorophyll a decreased 46.51% over the 44-day experiment compared with the control. (Figure 7-8) This research studied how to get rid of harmful blue-green algae *Microcystis* spp. bloom by using extracts from two kinds of waste–agriculture and aquaticultural residues i.e. rice straw and shrimp shells. By counting cells and measuring the amount of chlorophyll a, as a way to measure the inhibition of the growth of toxic algae *Microcystis* spp., it was found that the mixture of both could inhibit the growth of *Microcystis* spp. and the mixture of 1:2 ratio was highly effective in inhibiting *Microcystis* spp. in accord with Wen *et al*. [27] the treatment with the extract of rice straw. Concentration of 10 g/L is the most effective in inhibiting the growth of *Microcystis* spp., followed by extracts of straw at concentrations of 8, 6, 4 and 2 g/ L, respectively. Park *et al*. [17] showed that rice hull extract could inhibit *Microcystis* spp. higher than green algae. The above extracts decreased the cell density of *Microcystis* spp. by more than 95%, and green algae by less than 24%. Xiao *et al*. [29] reported that barley straw extract (2 g/L to 8 g/L) could reduce

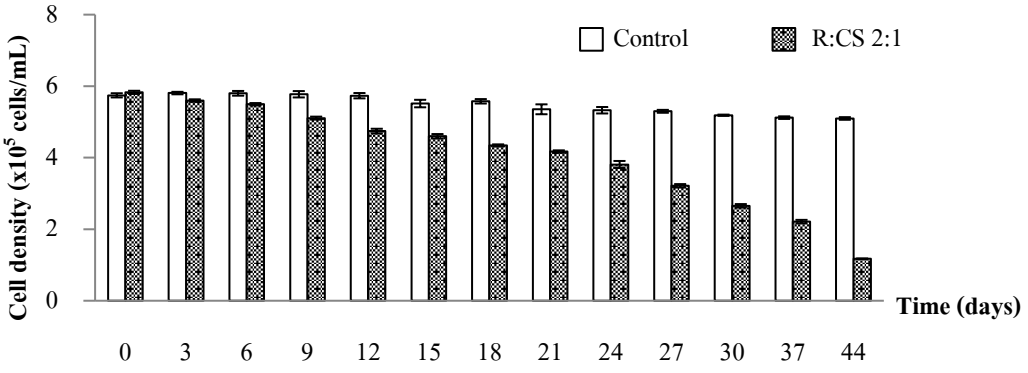

Figure 7 Variation of cell number *Microcystis* spp. during the therapy. R= rice straw extracts, CS=chitosan from shrimp shells.

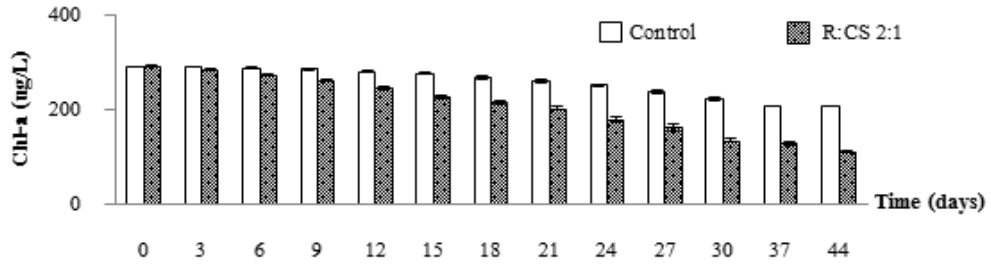

Figure 8 Variation of chlorophyll a during the therapy. R= rice straw extracts, CS=chitosan from shrimp shells.

the amount of chlorophyll a post-treatment. Iredale *et al*. [9] reported that fresh barley straw at a concentration of 5 g could inhibit the growth of *Microcystis aeruginosa* SD053 and *Microcystis aeruginosa* PCC7806 in condition 20°C and 27°C. Several studies have indicated that rice hulls contain a variety of bioactive compounds. Park *et al*. [17] studied extracts of rice hull on the growth of *Microcystis aeruginosa*. It was found that β-sitosterol-β-D-glucoside and dicyclohexanylorizane powerfully inhibited the growth of colonial *Microcystis aeruginosa* cells. Ahmad *et al*. [1] discovered oleioyl-β-D-arabinoside from Rice straw. This substance is the most effective to inhibiting *Microcystis aeruginosa* UTEX 2388 at a concentration of 100 ppm. Park *et al*. [18] studied three types of phenolic compounds: p-coumaric, salicylic and benzoic acid, and the mixture of these compounds showed inhibitory effect the growth of *Microcystis aeruginosa*. Mechanism of inhibiting algal cells- the extract from rice straw, was transported into the cytosol in cell. The enzyme in PS II reaction center was inhibited and destroyed, and the photosynthesis of cells was also inhibited [27]. Effect of chitosan treatment caused toxic algae *Microcystis* spp. to precipitate by protons of the amino groups of chitosan and induced a positive charge to bind to the negatively charged algal cells [31]. The surface of the cell covered with a thick layer of chitosan and connected by long-chain polymers of chitosan with *Microcystis* spp. became chunk of sediment. *Microcystis* spp. immersed under the water and could not be photosynthesized [21]. Several studies found that chitosan, a substance that makes the toxic algae *Microcystis* spp. precipitated. Dong *et al.* [4] reported that chitosan concentration of 7.31 mg/L and optimized

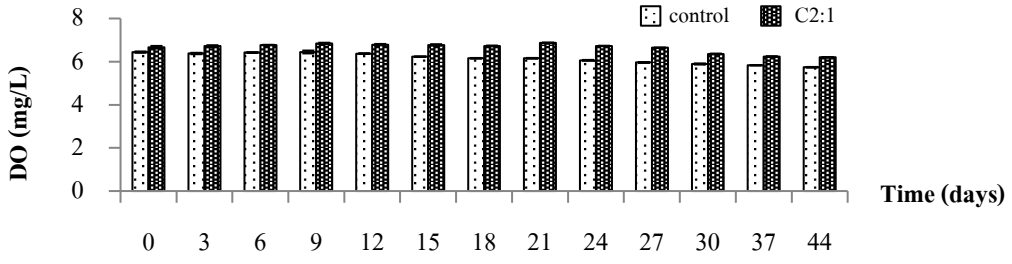

Figure 9 The change of dissolved oxygen (DO) value during therapy. R=rice straw extracts, CS=chitosan from shrimp shells.

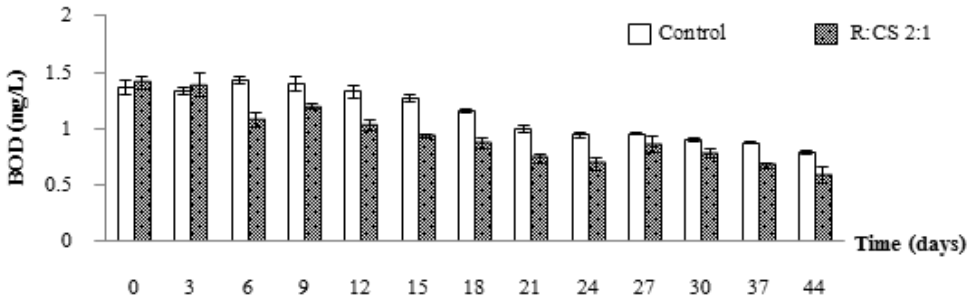

Figure 10 The change of biochemical oxygen demand (BOD) value during therapy. R=rice straw extracts, CS=chitosan from shrimp shells.

mechanical conditions can get rid of *Microcysis aeruginosa* cell. Wang *et al.* [25] found that chitosan-montmorillonitenano composite with chitosan and montmorillonite. A combination effectively eliminates the toxic algae *Microcystis aeruginosa* passenger makes such toxic algae settling. Li and Pan [10] found that *Moringa oleifera* coagulant form seeds-chitosan modified sand contain with chitosan, sand and *Moringa oleifera* coagulant *Microcystis aeruginosa* together with sediment substances in the bottom. Pan *et al*. [15] studied modified local soil induced ecological restoration (MLS-IER) technology which could flocculates the algal blooms and was sinked them to the bottom of the lake. In addition, chitosan also absorbed nutrients and suspended solids in the water.

3.5 Physical and chemical water quality

The study of some parameters of water quality by using an extract from rice straw and chitosan from shrimp shells with the ratio of 1:2 had shown the effect of dissolved oxygen in the water along with the duration of the experiment. From treatment with straw extracts and chitosan from shrimp shells (R: CS = 1: 2) it was found that the DO decreased from 6.67 mg/L to 6.20 mg/L within 44 days of the experiment. (Figure 9) This trend, as well as the BOD values decreased from 1.41 mg/L to 0.58 mg/L, which was less than 44 internal control 0.21 mg/L. (Figure 10) when the photosynthesis enzyme in cell was destroyed by straw extract, cells components were incapacitated and decomposed naturally. As a result, none of the oxygen produced by photosynthesis process tends to lower the dissolved oxygen during the trial. The low DO concentration was due to waste discharges high in organic matter and nutrient near by the water and due to increase of microbial activity occurring during the degradation of the organic matter [34]. However, the dissolved oxygen in the ground water quality standard criteria Category 4 requires that the dissolved oxygen should not be less than 6 mg/L [13]. Ma *et al*. [12] reported dissolved oxygen between 5-6 mg/L suitable for the growth of the fish. According to Xu *et al*. [30], after the treatment, dissolved oxygen of water is higher and can be used for aquaculture. The BOD has lowered from 1.41 mg/L to 0.58 mg/L. This may be due to higher rate

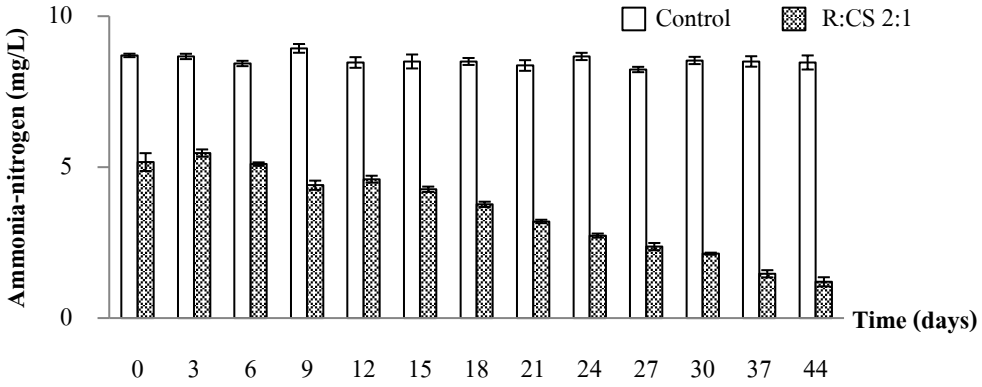

Figure 11 The change of ammonia nitrogen (NH_3-N) value during therapy. R=rice straw extracts, CS=chitosan from shrimp shells.

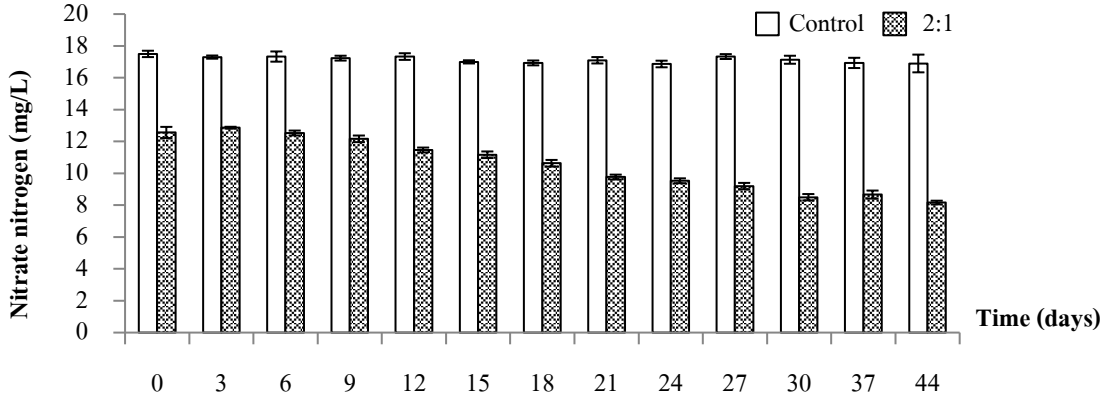

Figure 12 The change of nitrate nitrogen (NO_3-N) value during therapy. R=rice straw extracts, CS=chitosan from shrimp shells.

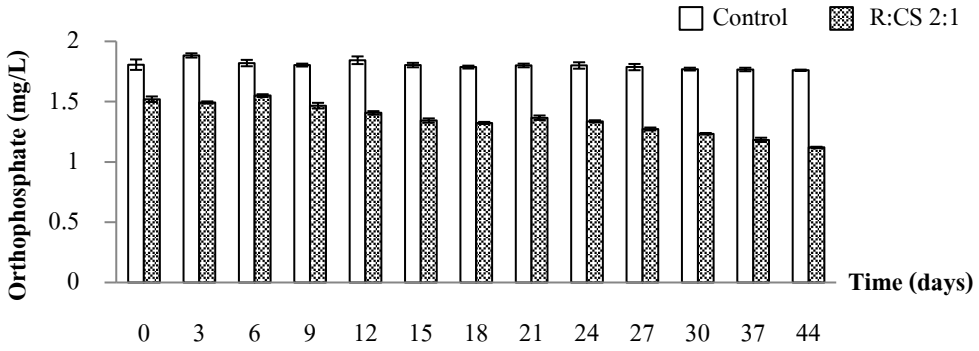

Figure 13 The change of orthophosphate (PO_4-P) value during therapy. R=rice straw extracts, CS=chitosan from shrimp shells.

of organic matter decomposition at higher temperature and less water current [35].

From (Figure11-13), the results showed the quantities of nutrients such as ammonia nitrogen, nitrate nitrogen and orthophosphate. After a trial period of 44 days, the amount of ammonia nitrogen decreased from 5.46 mg/L to 1.2 mg/L, representing 85.82% according to the amount of nitrate nitrogen decreased from 12.56 mg per liter to 8.16 milligrams on liter, representing 51.67% and orthophosphate was reduced by 1.52 mg/L to 1.12 mg/L as a percentage of 36.36%. This indicated that ammonia nitrogen, nitrate nitrogen and orthophosphate volume were decreased at 85.82, 51.30 and 36.36%, respectively when compared with the control According to Lochmatter *et al*. [38] studied nutrient removal in water sources by biological methods. Aerobic granular sludge reduced nitrogen content by 73-77% and phosphorus reduction by 98% within 60-150 days. Nitrate ammonium and nitrite may be removed from water by aquatic plants, algae and bacteria which imbibe it as a source of nitrogen [39]. Moreover, concentrations of dissolved oxygen reduce to minimum values, facultative anaerobic bacteria such as *Pseudomonas, Micrococcus, Bacillus, Chromobacter* can apply nitrate as a final acceptor of electrons for resulting in the ultimate formation of nitrogen [40]. In this experiment, the nutrient content in the sample water is high and can't be eliminated within 44 days.

4. Conclusions

From the present study, we found that chitosan from shrimp shells and rice straw extracts were able to remove *Microcystis* spp. in water. The best ratio between chitosan from shrimp shells and rice straw extracts was 2:1 (w/w). This ratio could reduce the cell number of *Microcystis* spp. and chlorophyll a at 46.51% and 76.97%, respectively. Furthermore, chitosan from shrimp shells and rice straw extracts demonstrated its nutrients adsorption ability. The amount of ammonia nitrogen (NH_3-N), nitrate nitrogen (NO_3-N) and orthophosphate (PO_4-P) being absorbed were 85.82%, 51.67% and 36.36%, respectively.

5. Acknowledgements

The authors would like to thank Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Thailand, financial in fiscal year 2014.

6. References

[1] Ahmad, A., S.H. Kim, M. Ali, I. Park, J.S. Kim, E.H. Kim, J.J. Lim, S.K. Kim, and I.M. Chung. New Chemical Constituents from *Oryza sativa* Straw and Their Algicidal Activities against Blue-Green Algae. J. Agric. Food Chem. 2013.61: 8039-8048.

[2] Aye, K.N., C.H. Ng, and W.F. Stevens. Pilot scale production of chitin and chitosan from shrimp shells. Adv. Chitin Sci. 2002.5:12-14.

[3] Chung, I.M., M.A li, A. Ahmad, S.C. Chun, J.T. Kim, S. Sultana, J.S. Kim, S.K. Min, and B.R. Seo. Steroidal constituents of rice (*Oryza sativa*) hulls with algicidal and herbicidal activity against blue-green algae and duckweed. Phytochem. 2007.18: 133–145.

[4] Dong, C., W. Chen, and C. Liu. Flocculation of algal cells by amphoteric chitosan-based flocculant. Bioresour. Technol. 2014.170 : 239–247.

[5] Eaton, A.D., L.S. Clesceri, E.W. Rice, A.E.Greenberg, and M.A.H. Franson. *Standard methods for the examination of water and wastewater: centennial edition.* American Public Health Association (APHA), Washington D.C. 2005.

[6] Gray, N.F. *Water Pollution*. In N.F. Gray (ed) Water Technology. Butterworth-Heinemann, Oxford. 2010: 117-148.

[7] Hossain, M. S., and A. Iqbal. Production and characterization of chitosan from shrimp waste. J. Bangladesh Agril. Univ. 2014.12: 153–160.

[8] Humpage, A.R., and I.R. Falconer. Microcystin-LR and liver tumor promotion: Effects on cytokinesis, ploidy, and apoptosis in cultured hepatocytes. Environ. Toxicol. Water Quality. 1999.14: 61–75.

[9] Iredale, R.S., A.T. McDonald, and D.G. Adams. A series of experiments aimed at clarifying the mode of action of barley straw in cyanobacterial growth control. Water Res. 2012.46: 6095-6103.

[10] Li, L., and G. Pan. A Universal method for flocculating harmful algal blooms in marine and fresh waters using modified sand. Environ. Sci. Technol. 2013.47: 4555—4562.

[11] Lone, Y., R.K. Koiri, and M. Bhide. An overview of the toxic effect of potential human carcinogen Microcystin-LR on testis. Toxicol. Rep. 2015.2 : 289–296.

[12] Ma, J., J.D. Brookes, B. Qin, H.W. Paerl, G. Gao, P. Wua, W. Zhang,J. Deng , G. Zhu, Y. Zhang, H. Xu, and H. Niu. Environmental factors controlling colony formation in blooms of the cyanobacteria *Microcystis* spp. in Lake Taihu, China. Harmful Algae. 2014.31: 136–142.

[13] National Environmental Board. *Surface Water Quality Standards, issued under the Enhancement and Conservation of National Environmental Quality*. The Royal Government Gazette, Bangkok. 1994:234-240

[14] Paerl, H.W., H. Xu, M. J. McCarthy, G. Zhu, B. Qin, Y. Li, and W.S. Gardner. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): The need for a dual nutrient (N&P)

management strategy. Water Res. 2011.45:1973-1983.

[15] Pan, G., B. Yang, D. Wang, H. Chen, B.H. Tian, M.L. Zhang, X.Z. Yuan, and J. Chen. In-lake algal bloom removal and submerged vegetation restoration using modified local soils. Ecol. Eng. 2011.37 :302-308.

[16] Pan, J.R., C. Huang, S. Chen, and Y.C. Chung. Evaluation of a modified chitosan biopolymer for coagulation of colloidal particles. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 1998.147:359–364.

[17] Park, M.H., B.H. Kim, I.M. Chung, and S.J. Hwang. Selective bactericidal potential of rice (*Oryza sativa* L. var.japonica) hull extract on *Microcystis* strains in comparison with green algae and zooplankton. Bull. Environ. Contam Toxicol. 2009.83: 97–101.

[18] Park, M.H., M.S. Han, C.Y. Ahn, H.S. Kim, B.D. Yoon, and H.M. Oh. Growth inhibition of bloom-forming cyanobacterium *Microcystis aeruginosa* by rice straw extract. Lett. Appl. Microbiol. 2006.43:307-312.

[19] Park, M.H., S.J. Hwang, C.Y. Ahn, B.H. Kim, and H.M. Oh. Screening of seventeen oak extracts for the growth inhibition of the cyanobacterium *Microcystis aeruginosa* Kütz. em. Elenkin. Environ. Contam. Toxicol. 2006.77: 9–14.

[20] Patria, A. Production and characterization of chitosan from shrimp shells waste. AACL Bioflux. 2013.6: 339-344.

[21] Pei, H.Y., C.X. Ma, W.R. Hu, and F. Sun. The behaviors of *Microcystis aeruginosa* cells and extracellular microcystins during chitosan flocculation and flocs storage processes. Biores. Technol. 2014.151 : 314–322.

[22] Puschner, B., and A. Roegner, *Cyanobacterial (blue-green algae) toxins.* In R.C. Gupta (ed) Veterinary Toxicology. Academic Press, London. 2012:953-965.

[23] Puschner, B., and J.F. Humbert. *Cyanobacterial (blue-green algae) toxins.* In R.C. Gupta (ed) Veterinary Toxicology. Academic Press, London. 2007:714-724

[24] Rashid, N., S.U. Rehman, and J.I. Han. Rapid harvesting of freshwater microalgae using chitosan. Process Biochem. 2013.48: 1107–1110.

[25] Wang, Z., C. Wang , P. Wang, J. Qian, J. Hou, Y. Ao, and B. Wu. The performance of chitosan/ montmorillonite nanocomposite during the flocculation and floc storage processes of *Microcystis aeruginosa* cells. Environ. Sci. Pollut. Res. 2015. 22: 11148–11161.

[26] Watson, S.B., B.A. Whitton, S.N. Higgins, H.W. Paerl, B.W. Brooks, and J.D. Wehr. *Harmful Algal Blooms*. In J.D. Wehr R.G. Shesth J.P. Kociolek (ed)

Freshwater Algae of North America. Academic Press, London. 2015: 873-920.

[27] Wen, S., J.A. HAGSTRÖM, J. Yuhong , L. Yaping, and K. Fanxiang. Effects of rice straw on the cell viability, photosynthesis, and growth of *Microcystis aeruginosa*. Chin. J. Oceanol. Limnol. 2014. 32 : 120-129.

[28] Wichelen, J.V., P. Vanormelingen, G. A. Codd, and W. Vyverman. The common bloom-forming cyanobacterium *Microcystis* is prone to a wide array of microbial antagonists. Harmful Algae. 2016.55:97–111.

[29] Xiao, X., Y.X. Chen, X.Q. Liang, L.P. Lou, and X.J.Tang. Effects of Tibetan hulless barley on bloom-forming cyanobacterium (*Microcystis aeruginosa*) measured by different physiological and morphologic parameters. Chemosphere. 2010. 81 : 1118–1123.

[30] Xu, Y., S. Purton, and F. Baganz. Chitosan flocculation to aid the harvesting of the microalga *Chlorella sorokiniana*. Biores. Technol. 2013.129: 296–301.

[31] Yan, Q., Y. Yu,W.Feng, G. Pan, H. Chen, J. Chen, B. Yang, X. Li, and X. Zhang. Plankton community succession in artificial systems subjected to cyanobacterial blooms removal using chitosan-modified soils. Microb. Ecol. 2009.58: 47–55.

[32] Peerapornpisal, Y., and S. Niwasabutra. *Phycology Laboratory.* Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai. 1985:103-119.

[33] Zou, H., G. Pan, H. Chen, and X. Yuan. Removal of cyanobacterial blooms in Taihu Lake using local soils. II. Effective removal of *Microcystis aeruginosa* using local soils and sediments modified by chitosan. Environ. Pollut. 2006.141: 201-205.

[34] Yisa, J. and Jimoh, T. Analytical Studies on water quality index of River Landzu. Am. J. Appl. Sci. 2010.7(4): 453–458.

[35] Sanap, R.R., Mohite, A.K., Pingle, S.D. and Gunale, V.R. Evaluation of water qualities of Godawari River with reference to physicochemical parameters, district Nasik (M.S.), India. Pollut. Res. 2006.25(4): 775–778.

[36] Hagström, J.A., Sengco, M.R., and Villareal, T.A. Potential methods for managing *Prymnesium parvum* blooms and toxicity, with emphasis on clay and barley straw: a review. J. Am. Water. Resour. Assoc. 2010.46 (1): 187-198.

[37] APHA., AWWA., and WPCF. *Standard Methods for the Examination of Water and Wastewater*. 18th ed. American Public Health Association, Washington D.C. 1992.

[38] Lochmatter, S. and C. Holliger. Optimization of operation conditions for the startup of aerobic granular sludge reactors biologically removing carbon, nitrogen, and phosphorous. Water Res. 2014.59: 58–70.

[39] Smith, V.H., Tilman, G.D., and Nekola, J.C. Eutrophication:impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ. Pollut. 1999.100: 179–196.

[40] Wetzel, R.G. *Limnology*. 3rd ed. Academic Press, New York. 2001.