

The phycoremediation of textile wastewater discharge by *Chlorella pyrenoidosa* H. Chick, *Arthrospira platensis* Gomont, and *Chaetoceros calcitrans* (Paulson) H. Takano

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Abstract. Textile wastewater discharge is one of the big aquatic pollution problem in Indonesia. Although treatment had been applied to reduce the concentration of pollutants, the textile wastewater discharge often still keep the concentration of pollutant that is above the limit for the usage of the river water. Phycoremediation, the use of microalgae to clean up the wastewater, had been developed and applied successfully according to several research. This study was conducted in order to compare the ability of three species of microalgae from different divisions, i.e. *Chlorella pyrenoidosa*, *Arthrospira platensis*, and *Chaetoceros calcitrans* on removing total nitrogen (TN), total phosphorous (TP), heavy metal of Cr, Cu, Pb, and Cd from wastewater, and their accumulation. Batch mode was used to compare their ability of wastewater remediation. The results show that *C. pyrenoidosa* has excellent performance on removal Cr, Cu, Pb, and Cd more than 80%, and has Bio Concentration Factor (BCF) higher than *S. platensis* and *C. calcitrans*. According to our study, *C. pyrenoidosa* can be suggested as a very good phycoremediator of Cd from textile wastewater discharge. The establishment of phycoremediation for the textile industries can make the discharge treatment process easier and cheaper. Nevertheless, the biomass production from phycoremediation process can be converted into biofuel. This way, a sustainable environmental friendly textile industry will work together with producing alternative energy.

Key Words: phycoremediation, textile, Bio Concentration Factor, *Chlorella pyrenoidosa*, *Arthrospira platensis*, *Chaetoceros calcitrans*.

Introduction. The textile industry was one of the main pollution source worldwide. Indonesia is the second country among the countries with highest levels of pollutants that contributed more than 20% of the registered levels for water pollution caused by textile industry after Turkey across G20 countries (Paraschiv et al 2015). During the technological process, there are chemical steps like sizing, desizing, scouring, mercerizing, bleaching, dyeing, printing, and finishing which consume large quantities of freshwater. In a textile industry, a number of dyes chemicals and auxiliary chemicals are used to impart desired quality in the fabrics. In the dyeing process 100 L water is required to process 1 kg textile materials, resulting in high amount of wastewater (Sasikala & Sudha 2015). The dyeing process produces deeply coloured discharged wastewater that might contain heavy metals, dyes, pigments, organic and inorganic pollutants (Kamaruddin et al 2013). Long term exposure to textile pollutants from the waterways may promote cancer, acute toxicity, skin disease, allergenic and mutagenic effects (Firmino et al 2010; Gnanapragasam et al 2010; Gomaa et al 2012).

Various physical and chemical remediations have been applied to treat textile wastewater, such as using of penton reagents, ozonation, photochemical, NaOCl, electrochemical destruction, activated carbon, membrane filtration, ion exchange, or electrokinetic coagulation with the drawbacks on the sludge generation and concentrated sludge production, short half life, release of aromatic amines, and high cost (Robinson et al 2001). Adsorption process was the most acceptable technique for purification of textile

wastewater rather than the other physico chemical processes (Kamaruddin et al 2013). There is a great demand to find out solutions for remediation methods that are effective in removing or reducing pollutants in large volume but with low cost (Robinson et al 2001).

Many studies have been developed on using microroganisms to remediate dyes in wastewater, such as the use of bacterial consortium of *Pseudomonas* (Jadhav et al 2010; Phugare et al 2011), fungus *Coriolus versicolor* (Hai et al 2006), *Aspergillus niger*, *A. japonicus*, *Rhizopus nigricans*, *R. arrhizus* (Kumari & Abraham 2007), biofilm of *Irpex lacteus* for rotating biological contactor (Malachova et al 2013); yeasts: *Saccharomyces cerevisiae* (Kumari & Abraham 2007), *Rhodotorula mucilaginosa* (Onat et al 2010).

In Indonesia, phytoremediation using *Chrysopogon zizanioides* had been applied to aquaculture wastewater, primarily to lower nitrogen concentrations in the ponds with mesohaline salinity (Raharjo et al 2015). Aquatic plant (*Ipomoea aquatica*) performed as phytoremediation for freshwater crayfish wastewater quality by reduced ammonia (NH₃) until 84.6%, nitrate (NO₃) until 34.8%, and oorthophosphates underwent of 44.4% reduction (Effendi et al 2015). The advantages of phytoremediation is that it is a green technology and environmentally friendly in treating wastewater and is a natural recycling method. The final product can be used, for example, as animal feed and ingredients for organic farming (Raharjo et al 2015).

Biological remediation has started to develop and promise low cost and low chemical inputs. Phycoremediation is the application of macro and microalgae to remove or transform the pollutants, including nutrients and toxic chemicals from waste water (John 2000; Olguin 2003; Mulbry et al 2008; Olguin & Sanchez-Galvan 2012), but many researchers used term of phycoremediation for the application of microalgae to remediate pollutants (Ahmad et al 2013; Soeprbowati & Hariyati 2013, 2014a, 2014b). Phycoremediation is one of the best methods to remove or reduce the pollutant from the environment (Brar et al 2017). Phycoremediation comprises six applications, i.e. to remove nutrient, particularly organic pollutants from municipal wastewater and effluents; to remove nutrient and toxic compounds; to remediate acid and metals; to sequestrate CO₂; to transform and degrade xenobiotics; and to detect toxic compounds (Olguin 2003).

The main advantages of algae for remediation of pollutants are low cost of the operation, no secondary pollution (Mulbry et al 2008); no carbon need for nutrient removal, releasing oxygen into the atmosphere (Pacecho et al 2015); accumulate and converse harvested algae from nutrient remediation into fertilizer (Olguin 2003; Brar et al 2017); the biomass can produce biogas through anaerobic digestion, bioethanol by carbohydrate fermentation, and bio-oils by high temperature conversion (Park et al 2011); bioaccumulation and biosorption related to the flexible metabolism (Zinicovscaia & Cepoi 2016; Ayangbenro & Babalola 2017).

The microalgae species that had been used as bioremediator are *Spirogyra* sp. (Venkata Mohan et al 2015); *Chlorella vulgaris* not only reduces the pollutant load (COD, NH₄-N, and PO₄-P) but also the color during remediation of textile wastewater (Lim et al 2010). *Chlorella pyrenoidosa* was used to study the phycoremediation of textile wastewater, being able to reduce nitrate, phosphate, and organic load by 62%, 87%, and 81% respectively (Pathak et al 2014). *Aphanotheca* sp., *Gloeocapsa* sp., and *Phormidium* sp. shown useful in decolourisation of most of the dyes (Sasikala & Sudha 2015).

This research was conducted in order to compare the population growth and the ability of three species of microalgae from different divisions: *C. pyrenoidosa* – Chlorophyta, *Arthrospira platensis*, previously known as *Spirulina* – Cyanobacteria, and *Chaetoceros calcitrans* – Bacillariophyta to remediate textile wastewater discharge.

Material and Method

Collection of textile wastewater samples. After preliminary investigation in Semarang, Indonesia, it was choosen one textile industry that has a high heavy metals concentration in the wastewater that discharge into the river. Wastewater samples were

collected from textile wastewater discharge to the river. The composite sample was filtered to remove coarse particles.

Population growth in the batch. *C. pyrenoidosa*, *A. platensis*, and *C. calcitrans* used in this study were obtained from Main Center Water Aquaculture Development, Jepara - Indonesia. The experiment work was carried out in the Laboratory of Ecology, Faculty of Science and Mathematics, Diponegoro University, Semarang, Indonesia, in batch mode for 15 days with three replications on October 2013. All equipments have been sterilized with Chlorox 150 ppm for one hour and washed with boiled water. One liter of sea water with a salinity of 15 ppt that was enriched with Walne fertilizer was used as a culture medium. For batch of *C. calcitrans* silicate was added to provide its growth requirement. Regarding the different size of microalgae, the initial population of *C. pyrenoidosa* was 1,000,000 cell mL⁻¹, *A. platensis* was 10,000 cell mL⁻¹, and *C. calcitrans* was 1,000,000 cell mL⁻¹. During the experiment, the algae population growth was calculated every day for 15 days. Algae cultured in the Walne medium without textile wastewater served as a control. All experiments were performed in triplicates. During the treatments, pH, temperature, salinity, and light intensity were maintained to be stable on 7-8, 28-32°C, 32-34 ppt, and 4,200 lux, respectively.

Analysis of water quality. The analysis of total nitrogen (TN), total phosphorous (TP), heavy metals - chromium (Cr), copper (Cu), lead (Pb), and cadmium (Cd) - as well as chlorophyll-*a*, were measured at the initial day of the experiment (D0), and D3, D7, D10, and D15. The TN, TP, and chlorophyll-*a* were measured with spectrophotometer Shimadzu double beam 1300, and the heavy metals concentrations in media culture and in the microalgae were measured with AAS Perkin Elmer 3110.

Bio concentration factor (BCF). The reduction of heavy metals (percentage of removal) was calculated as well as microalgae population. Bio concentration factor (BCF) was calculated to determine the accumulation of heavy metals in the microalgae. BCF is a comparison between heavy metal concentrations on the microalgae with the concentration on the aqueous environment (Ivanciuc et al 2006):

$$BCF = C_{org} / C_{media}$$

where: C_{org} = heavy metals concentration in the microalgae;

C_{media} = heavy metals concentration in the batch media

Results. The preliminary study showed that the concentration of Cr, Cu, Pb, and Cd did not exceed limitation for industrial wastewater standard as stated in the Regulation of the Ministry of Environment No 3 (2010). The concentration both for Pb and Cr from industrial wastewater discharge was maximally 1 mg L⁻¹, Cd was 0.1 mg L⁻¹, and Cu was 2 mg L⁻¹. However, the concentration of Pb, Cd, Cr, and Cu from textile industry wastewater exceeded the water quality standard for sources of drinking water (Class 1), tourism, husbandaries, fisheries, and garden watering (Class 2), husbandaries, fisheries, and garden watering (Class 3), and garden watering (class 4) as stated in the Government Decree No 82 (2001) (Table 1).

C. pyrenoidosa, *A. platensis*, and *C. calcitrans* have been cultured in the textile wastewater. Overall, the algae required three days for adaptation before exponential growth (Figure 1). *C. pyrenoidosa* was able to grow in the textile wastewater although its growth was below the control. Peak time of population growth occurred in the day 8 and 12. Meanwhile in control the population increased by the time. *A. platensis* was able to live in the textile wastewater although its population sharply decreased since the first day. Peak time population growth occurred in the 5, there was a constant growth until the day 12, then it increased until the end of the experiment on day 15 (Figure 1). This condition also occurred on the *C. calcitrans* batch, that required three days for adaptation, followed by slow growth until the day 8, and exponential growth in the day 9-14, then reduced growth.

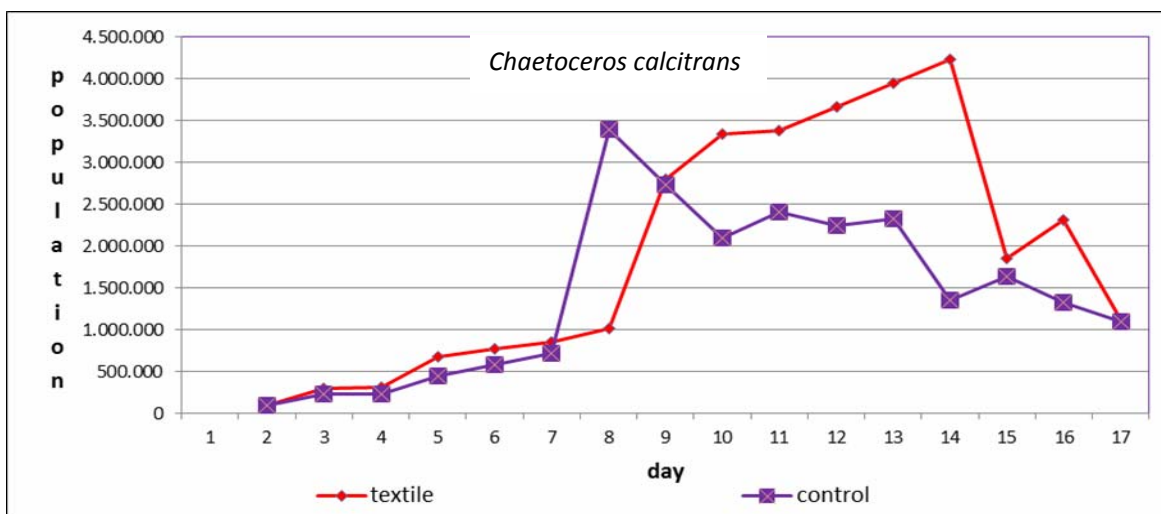
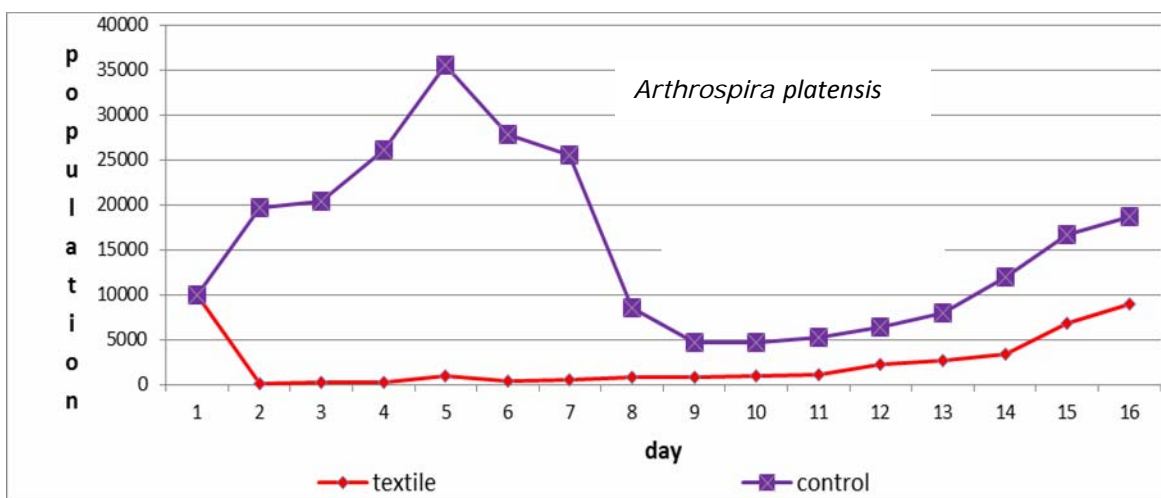
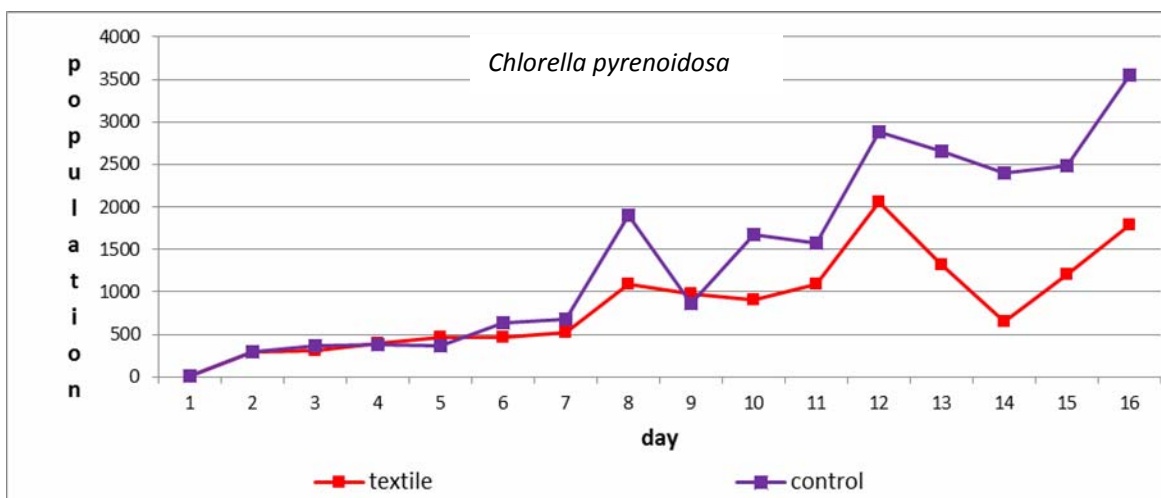


Figure 1. Population growth (cell L⁻¹) of *C. pyrenoidosa*, *A. platensis*, and *C. calcitrans*.

Table 1

The concentration of Pb, Cd, Cr, Cu, Total Nitrogen (TN), and Total Phosphorous (TP) from textile industry

No	Parameter	Textile wastewater	Water quality standard (Government Decree No 82 2001)				Industrial wastewater standard The Regulation Ministry of Environment No 03 2010
			Class I	Class II	Class III	Class IV	
1	Pb (mg L ⁻¹)	0.725	0.03	0.03	0.03	1	1
2	Cu (mg L ⁻¹)	0.65	0.02	0.02	0.02	0.2	2
3	Cd (mg L ⁻¹)	0.44	0.01	0.01	0.01	0.01	0.1
4	Cr (mg L ⁻¹)	0.318	0.05	0.05	0.05	1	1
5	TN (mg L ⁻¹)	0.779	0.5				20 (NH ₃ -N)
6	TP (mg L ⁻¹)	10.45	0.2	0.2	1	5	

In line with algae population growth, there was a trend of reducing heavy metals concentration in the culture batch. The percentage of decreasing concentration of heavy metals increased by time. *C. pyrenoidosa* has the highest bioremoval ability compared to *A. platensis* and *C. calcitrans*. *C. pyrenoidosa* has ability to reduce Cr<Cu<Pb<Cd (Figure 2). In the day 7 the bioremoval was more than 50%, and in the day of 15 was > 75%. *A. platensis* has the bioremoval of heavy metals Cd<Pb<Cr<Cu. The bioremoval of *A. platensis* for Cu was more than 50% at day 7 and 80% at day 15. The bioremoval of *C. calcitrans* were Cr<Cd<Pb<Cd that was reach more than 50% at day 10 and >80% only for Cd (Figure 2).

The highest bioremoval and bioaccumulation at day 15 was found for *C. pyrenoidosa*. The highest BCF was 10.39 by *C. pyrenoidosa* for Cd. BCF by *A. platensis* were Cu>Pb>Cr>Cd, respectively. Although BCF of *A. platensis* were lower than *C. pyrenoidosa*, but the percentage of accumulation Cr was more than 10X, whereas for *C. calcitrans* was 17.86 X for Cd accumulation (Figure 3).

Discussion. The concentration of Cr, Cu, Pb, and Cd from textile wastewater discharge in this research was below the permissible limits of Ministry of Environment No 3 (2010). However, the values exceeded the water quality standard for sources of drinking water (Class 1), tourism, husbandaries, fisheries, and garden watering (Class 2), husbandaries, fisheries, and garden watering (Class 3), and garden watering (class 4) as stated in the Government Decree No 82 (2001). Therefore, such discharged waters can not be used for irrigation purpose or released into water bodies.

At the laboratory scale research, it was found that population growth of *Chlorella* sp. is influenced by nitrates, phosphates, temperature, pH and salinity (Aprilliyanti et al 2016). The textile wastewater contains organic carbon and other compounds, such as a high nitrogen and phosphorous that can be used for cultivation of microalgae. The population growth of microalgae in the textile wastewater depends also on critical variables such as pH, temperature, availability of light, CO₂ and O₂, and therefore, in this research, they were kept uniform.

The presence of heavy metals (Table 1) in textile wastewater discharge in our study inhibited algae population growth below the control (Figure 1). Hence, it was not only the effect of heavy metals, but might also be the availability of toxic organic compounds or other biotic factors, such as bacterial pathogens and zooplankton that are not covered by this research.

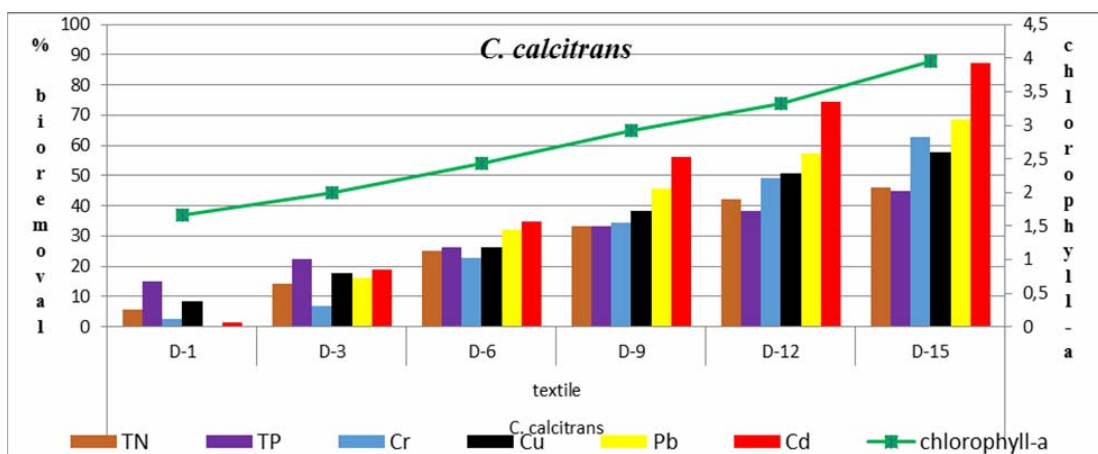
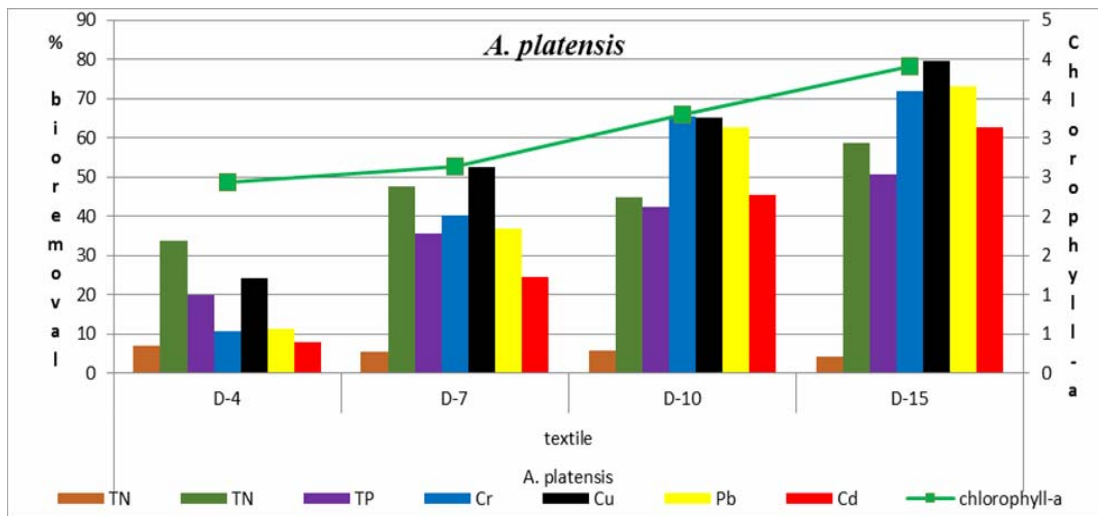
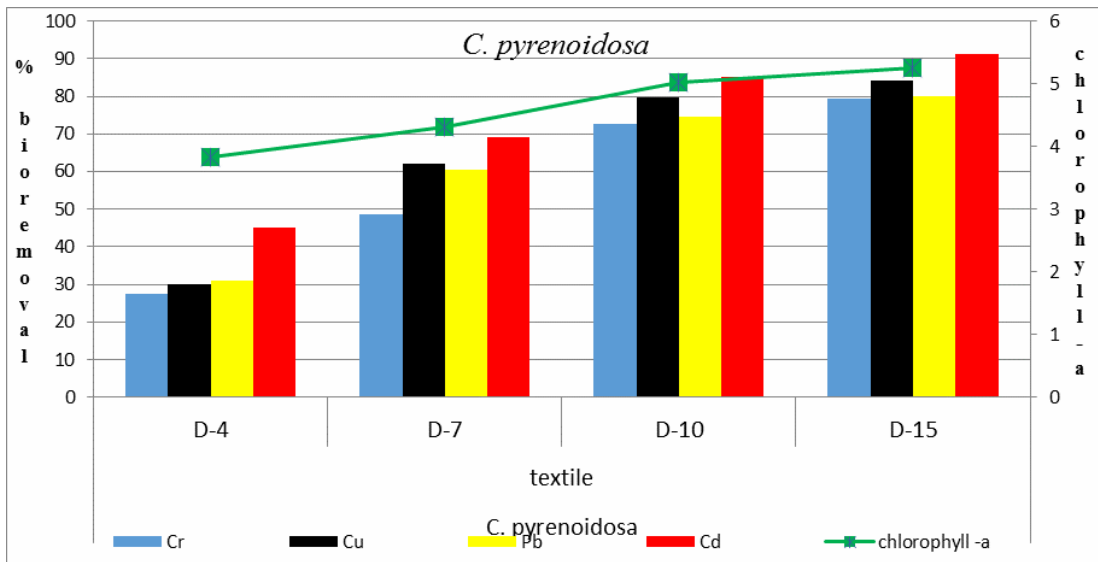


Figure 2. The percentage of bioremoval of TN, TP, heavy metals Cr, Cu, Pb, and Cd, in accordance with chlorophal-a content.

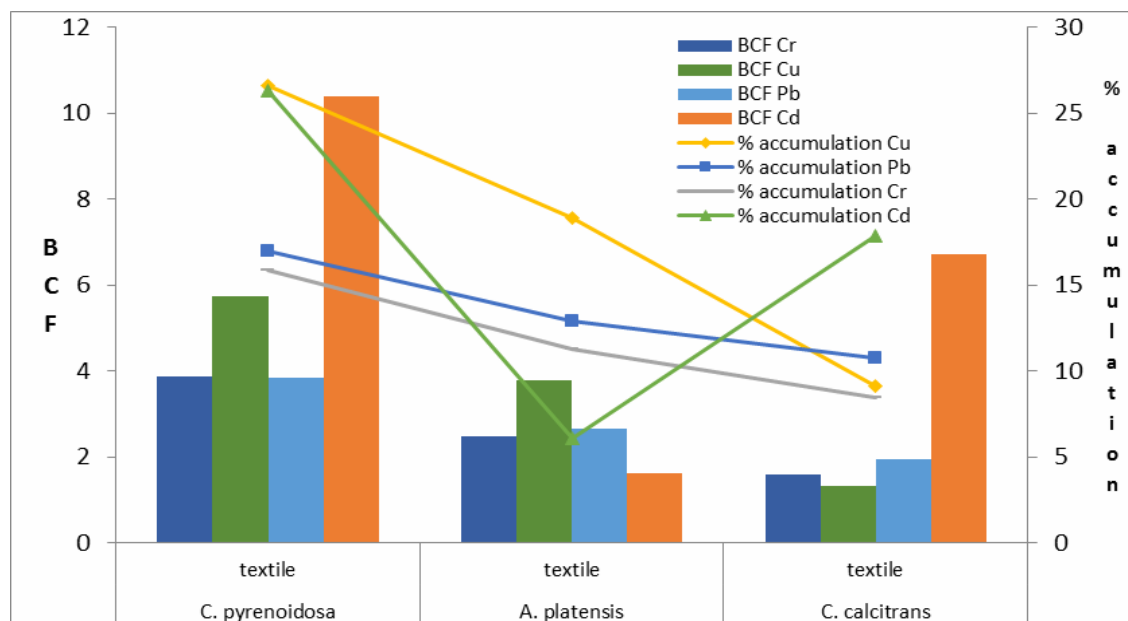


Figure 3. BCF and percentage increase of accumulation by *C. pyrenoidosa*, *A. platensis*, and *C. calcitrans* in the batch of textile wastewater.

Microalgae utilize carbon for the synthesis of biomass in three modes, namely autotrophic, mixotrophic, or heterotrophic (Chang et al 2011). Several microalgae such as *Chlorella*, *Scenedesmus*, *Tetraselmis*, and *Nitzschia* have the ability to switch between autotrophic and heterotrophic mode of assimilation, depending on the environmental condition. The heterotrophic mode can occur both in the presence of light or absence of light, and the population growth of algae remains stable.

Phycoremediation is cost effective, environmental friendly, and a safe process. During our study it reduced the concentration of TN and TP in the discharge textile wastewater by about 40% within 15 days (Figure 2). Based on this research the remediation of heavy metal Cr, Cu, Pb, and Cd were much more higher than TN and TP. Ahmad et al (2013) found out that *Chlorella vulgaris* is able to reduce total Kjeldahl nitrogen (93.1%), total phosphorus (98.0%), nitrate (98.3%), phosphate (98.6%) of sewage waters, much more higher than mix culture of *Microspora* sp., *Navicula* sp., *Lyngbya* sp., *Cladophora* sp., *Spirogyra* sp. and *Rhizoclonium* sp. Pathak et al (2014) found that *C. pyrenoidosa* removed nitrate and phosphate from the effluent textile industry by 62 and 87%, respectively.

Within 15 days, the bioremoval of heavy metals were more than 70% by *C. pyrenoidosa*, more than 60% for *A. platensis*, and more than 50% for *C. calcitrans* (Figure 2). According to Purnamawati et al (2013), within 26 days, *C. vulgaris* has better accumulated Pb rather than Cd. A high concentration of Cd had prolonged peak population growth, and had induced chlorosis. *C. pyrenoidosa* was able to reduce 44.83% of Cu from plastic wastewater discharge within 8 days (Sari et al 2014). In our research, *C. pyrenoidosa* was shown the best performance for Cd (> 80%) in the day 10 and 15 (Figure 2).

This indicated that the heavy metals were removed from the medium and were accumulated by microalgae. However, not all heavy metals removed from the medium were absorbed and accumulated by microalgae. Bioremoval of heavy metals was increased by time that was also in line with an increase of chlorophyll-a in the cultivation batch for all three species in this research. The highest bioremoval of heavy metals by *A. platensis* was 80% for Cu, followed by Pb, Cd and Cd (Figure 2). A similar result was found by Al-Homaidan et al (2014) where the maximum biosorption was in solution containing 100 mg L⁻¹ Cu with 0.05 mg dried biomass of algae under 90 minute contact

time. Adsorption of Cu ions by *A. platensis* has gradually increased along with decreasing of biomass concentration. *Arthrospira* sp. adsorbs 74% Pb and its biosorption capacity was estimated to be 0.62 mg Pb/10⁵ alga cells (Chen & Pan 2005). In another study, the concentration of 3 mg L⁻¹ Pb had induced population growth of *A. platensis* that was higher than control, and able to remove 15% Pb from the medium within 8 days (Prambodo et al 2016). The concentration of 1 mg L⁻¹ heavy metals may induce the population growth of *A. platensis*, due to improvement of enzymatic processes, the concentration of 3 mg L⁻¹ heavy metals inhibits population growth, and the concentration of 5 mg L⁻¹ heavy metals is toxic for *A. platensis*, therefore, *A. platensis* can be used as a metal absorbent and can be cultivated from wastewaters after phycoremediation process for other uses such as fertilizer, with the note that heavy metal contamination in waste water should not exceed 1 mg L⁻¹. It will be better if *A. platensis* will be applied for 15 days in contaminated wastewater to get the highest removal of heavy metals (Soeprbowati & Hariyati 2014a). Zinicovscaia & Cepoi (2016) also found that *A. platensis* has the ability to absorb the ions of heavy metals Cu²⁺, Cd²⁺, Cr³⁺, Cr⁶⁺. Native cyanobacteria *Nostoc muscorum* and *Anabaena variabilis* have an active role in the reduction of pollution level from textile wastewater effluent (Talukder et al 2015).

In a previous study, the concentration of 1 mg L⁻¹ heavy metals may induced the population growth of *C. calcitrans*, the concentration of 3 mg L⁻¹ heavy metals inhibited the population growth, and a concentration of 5 mg L⁻¹ heavy metals was toxic for *C. calcitrans*. This was in line with a reduction of heavy metals in the culture medium. During 7 days, *C. calcitrans* was able to decrease the Cu concentration with 20% (Fitriyanto et al 2016). Phycoremediation of Pb by *C. calcitrans* was better when applied for 14 days (92% reduction) rather than 9 days (48% reduction). *C. calcitrans* was phycoremediator for Cu and Cr for 9 days treatment, but was Pb > Cu > Cr > Cd for 14 days treatment, respectively (Soeprbowati & Hariyati 2014b). Some microorganisms from the group Bacillariophyta, commonly known as diatom, are excellent bioindicator of water quality (Soeprbowati et al 2016a, 2017) and had been used to reconstruct past environmental condition (Soeprbowati et al 2012, 2016b). However, *C. calcitrans* is also such a phycoremediator.

The BCF can be used as a tool to describe the bioconcentration of heavy metals in the polluted aquatic environment (Burada et al 2014). The highest BCF of *C. pyrenoidosa* was 10.39 for Cd. Although BCFs of *A. platensis* were lower than *C. pyrenoidosa*, the percentage of Cr accumulation was more than 10X (Figure 3). *A. platensis* has the ability to uptake and accumulate metal in its cells and is known as one of the most efficient microalgae in this process although little information is available to support this subject (Akbarnezhad al 2016). In batch mode in the laboratory study, *A. platensis* was found having high tolerance to Pb²⁺, Cd²⁺, Cu²⁺ and Cr³⁺ at low concentration. It was suggested that for phycoremediation process, using *A. platensis* is more appropriate to be applied for low concentration of heavy metals in contaminated wastewaters. In the field study, the plankton presents a great capacity for accumulation of Cd and Zn.

This research has proved that at the laboratory scale phycoremediation is one alternative to solve water quality problems. So, this has to be confirmed under absolute field conditions since there are many factors which affect microalgal growth. There are many challenges in the up scaling cultivation of microalgae in wastewater that requires to be addressed. For example, at the laboratory scale studies, Walne fertilizers were added to trigger population growth, while on large scale additional supplementation has to be developed. The addition of carbon source has to be considered at large scale to induce population growth. However, on the large scale of bioreactor with the major requirement of light which is needed to raise a high population density in photo-heterotrophic mode of nutrition the optimal functionality is hard to maintain (Venkata Mohan et al 2015). Behind those problems, this method is still cost-effective, easy and simple to support large scale autotrophic algal cultivation (Brar et al 2017).

The other problems that are usually associated with large scale phycoremediations are constraint in control, contamination, lost of water due to evaporation, and irregular productivities (Brar et al 2017). Therefore, the design of a bioreactor as well as the chosen species that can grow efficiently in low cost open system with no impact of

contamination with toxic compounds have to be well prepared. The research on phycoremediation needs to conquer limitation before any application at the large scale. After remediation process, biomass harvesting requires to be urgently converted into biofuel. Integrated technology of wastewater remediation and energy crisis are both important challenges in the near future.

Conclusions. This study has successfully documented the baseline data for *C. pyrenoidosa*, *A. platensis*, and *C. calcitrans* as phycoremediators for textile wastewater discharge. Although the population growth of microalgae in the medium of wastewater discharge was below the control, the microalgae had reduced heavy metals concentration. *C. pyrenoidosa* had excellent performance on removal of Cr, Cu, Pb, and Cd more than 80%, and had BCF higher than *A. platensis* and *C. Calcitrans*. Thus it can be suggested as a very good phycoremediator of Cd from textile wastewater discharge. The establishment of phycoremediation for the textile industries can make the discharge treatment process easier and cheaper. Nevertheless, the biomass production from phycoremediation process can be converted into biofuel. This way, a sustainable environmental friendly textile industry will work together with producing alternative energy.

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