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WORD COUNT 2945
TIME SUBMITTED 22-JAN-2022 11:08AM
PAPER ID 81673343

IOP Conference Series: Earth and Environmental Science

The effect of metal ion Cd(II) concentration on the growth of *Spirulina* sp. cultured on BG-11 medium

The effect of metal ion Cd(II) concentration on the growth of *Spirulina* sp. cultured on BG-11 medium

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Abstract. Research on the growth rate of *Spirulina* sp. carried out in BG-11 medium with UV light intensity of 3000 lux and a temperature of 25 ˚C with the addition of metal ion Cd(II) concentration was carried out. An initial abundance of *Spirulina* sp. in the medium is 10.10^3 ind./L with the concentrations of Cd(II) added to the culture medium of 0, 1, 3, and 5 mg/L, respectively. *Spirulina* sp. growth rate in the control media continued to increase until the exponential phase on the seventh day with growth rate of growth rate of $4.3x10^3$ ind/L/day, while the medium with the addition of Cd(II) with concentrations of 1, 3, and 5 mg/L experienced a decrease in growth rate of $3.9x10^3$ ind/L/day, 3.8 $x10^3$ ind/L/day, and $0.07x10^3$ ind/L/day, respectively. The concentration of Cd(II) 5 mg/L in the culture medium of *Spirulina* sp. decreased the growth significantly. So that it can be concluded the potential use of *Spirulina* sp. as an adsorbent of Cd(II) metal can be effective at concentrations up to 3 mg/L.

1. Introduction

Pollution by cadmium (Cd) is very hazardous for the environment and living things because Cd is a non-degradable heavy metal and can accumulate in the environment [1]. The accumulation of this heavy metal often settle at the bottom of the waterbody to form complex compounds with organic materials that can increase the level of toxicity [2]. Microalgae are known as one of the parameters for determining water quality [3]. Among them is cyanobacteria which is often found in a typical freshwater ecosystem [4].

Spirulina sp. as one of the microalgae has the potential as a heavy metal adsorbent [5]. *Spirulina* sp. has high biosorption capacity, bind metal ions from solution and able to adsorb heavy metals [6] because the algae dry biomass are containing functional groups such as -OH, -C = O, -CH and CO which can act as active sites for ionic binding of heavy metal ions [7]. However, the absorption capacity of microalgae as a direct adsorbent of heavy metals by fresh microalgae also a promising tool for heavy metal remediation [8,9,10]. This study aims to determine the concentration of heavy metal Cd which can still be tolerated by *Spirulina* sp. by observing the growth rate. In this study the BG-11 medium was used as a breeding medium as well as a direct interaction medium for microalgae *Spirulina* sp. under various concentrations of Cd metal ions.

2. Methodology

2.1. Chemicals

Analytical grade reagents for the adsorption studies were used namely aquadest, BG-11 Medium, and the cadmium standard solution traceable to SRM from NIST $Cd(NO_3)_2$ in HNO_3 0.5 mol/L 1000 mg/l Cd Certipur® from Merck Millipore. Biosorbent of *Spirulina* sp. is a spiral blue green algae. cultured from swamp ecosystem in the vicinity of Palembang, South Sumatera according to Zulkifli *et al* [11].

2.2 Equipment

Glassware and bottles are sterilized before use. All equipments are first washed with liquid soap and rinsed thoroughly, then put into the autoclave at 121°C with a pressure of 15 lbs for 15 minutes. The solution of Cd(II) concentrations were measured using Atomic Absorption Spectroscopy model AA-7000 from SHIMADZU.

2.3 Stock culture of Spirulina *sp. in BG-11 Medium*

Propagation of *Spirulina* sp. carried out by culturing *Spirulina* sp. in the culture bottles with BG-11 medium up to 1000 mL. Pure culture *Spirulina* sp. as was put into the medium much as 100 mL. Each bottle is mounted with an aerator and illuminated with a 3.000 lux of 36 watt fluorescent lamp from Philips.

2.4 Treatment Stage Spirulina sp.

Each treatment is conducted with 6 replication bottles of culture filled with BG-11 medium. In addition to control, 18 bottles of culture medium were prepared with the addition of Cd(II) ion at various concentrations i.e. 1, 3, and 5 mg/L. Initial density of *Spirulina* sp. was $1x10^4$ ind/L in each culture bottle (Fig. 1). Growth rate were measured every day until the 7th day.

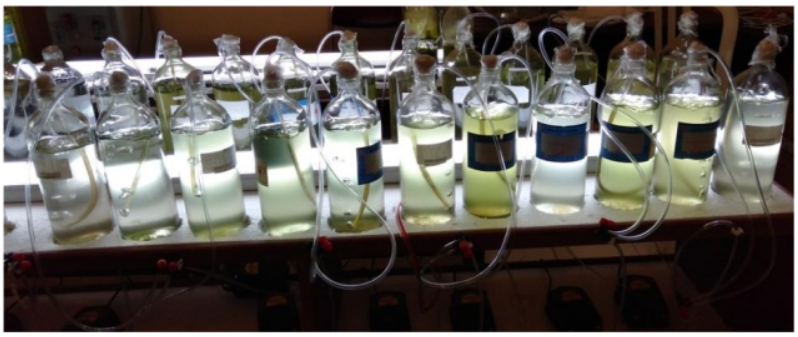

Figure 1. The *Spirulina* sp. culture and Cd^{2+} treatment in BG-11 medium

2.5 Calculation of Decreased Cd(II) Ion Concentration

The Cd(II) metal absorption data by *Spirulina* sp. carried out by determine the concentration of Cd(II) metal ion remaining in the solution medium was measured using AAS model AA-7000 from SHIMADZU.

3. Results and Discussion

Research on *Spirulina* sp. performed in BG-11 medium with an initial abundance of $1x10^4$ ind./L. The study was conducted for 7 days with the calculation of abundance every day using the Sedgweich Rafter Counting Cell (SRCC) under a microscope with a magnification of 10x. The growth rate of *Spirulina* sp. in the control media can be seen from the graph in Fig. 2.

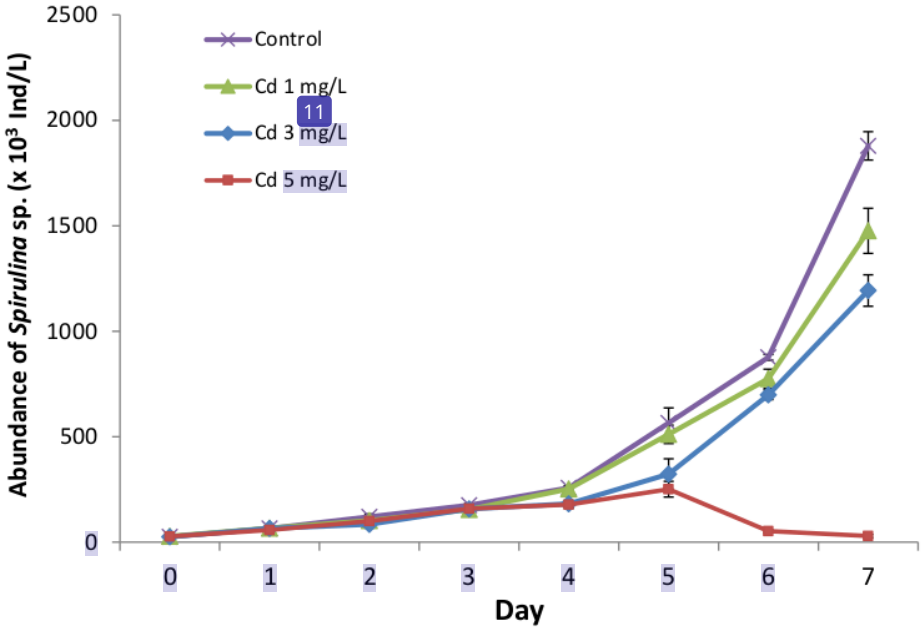

Figure 2. The abundance of *Spirulina* sp. during observation

The increase amount of cell number is due to active algae cells proliferating and the formation of proteins and components of the plasma cells that are needed in growth [12]. Based on Fig. 2 the number of cells in the controlled media continues to increase gradually from the first day to the seventh day. The *Spirulina* sp. in control media was not seen to have an adaptation phase but directly enters the exponential phase. It does not require a long time to adapt, *Spirulina* sp. continues to experience growth until the exponential phase on the sixth day to the seventh day with an abundance of $1.8x10^6$ ind/L. The ability of microalgae growth is influenced by organic or inorganic compounds or substances present in the media which are a source of nutrition and can also be a limiting nutrient for *Spirulina* sp [13]. The treatment with Cd 1 mg/L and Cd 3 mg/L continued to experience growth with adaptation phase occurred on the first day where there has not been an increase in the cell number of *Spirulina* sp. The exponential phase occurs from the sixth day to the seventh day with the cell number of *Spirulina* sp. increase significantly from $0.7x10^6$ ind/L to $1.4x10^6$ ind/L for treatment with Cd 1 mg/L and $0.6x10^6$ ind/L to $1.2x10^6$ ind/L for treatment with concentration of Cd 3 mg/L, respectively. At this condition *Spirulina* sp. still able to tolerate the concentration of Cd 1 mg/L and Cd 3 mg/L in the medium. Treatment of higher concentration of Cd 5 mg/L resulted in higher an abundance of $2.5x10^2$ ind/L at day fifth (Fig. 2).

Based on Figure 3, the growth rate on the control and treatment with Cd 1 mg/L and Cd 3 mg/L continued to increase until 7th day and had the highest growth rate of $4.3x10^3$ ind/L/day, indicating that the environmental conditions at the control medium were better than the other three treatments condition added with cadmium. The growth rate of *Spirulina* sp. which treatment with Cd 1 mg/L and Cd 3 mg/L had lower growth rates than control of $3.9x10^3$ ind/L/day and $3.8x10^3$ ind/L/day, respectively. The cell growth will be influenced by the availability of the main elements in the culture in the form of C, H, O, N, P, K, S, Ca, Fe, Mg and the presence of micro-nutrient elements [14]. The death phase is characterized by no more cells reproducing or doing division, causing the number of cells to decrease and the growing media does not provide the nutrients the cells need to grow [15].

Factors affect the growth of *Spirulina* sp. including i.e. temperature, light, pH, and aeration. The green blue algae grow well at pH 7 and tolerate more alkaline conditions than acidic conditions because they

are able to utilize carbon dioxide at low concentrations, and the optimal temperature for growth of blue-green algae ranges between 20-30˚C [16].

The growth rate in the treatment of 1 mg/L cadmium is lower than the growth rate of the control and higher than the Cd treatment of 3 mg/L and 5 mg/L but growth continues to increase until the 7th day. The Cd metal has properties which is similar as zinc (Zn) in the algae so that Cd can replace the Zn function in the enzyme reaction [17] and change the structure of the enzyme and affect its activity in the amount of Zn which plays very little in the growth of microalgae so that the ability of the metal Cd can replace the Zn function synthesizes the carbonic anhydrase enzyme that produces ions hydrogen and is used for cell division, causing growth to continue to increase.

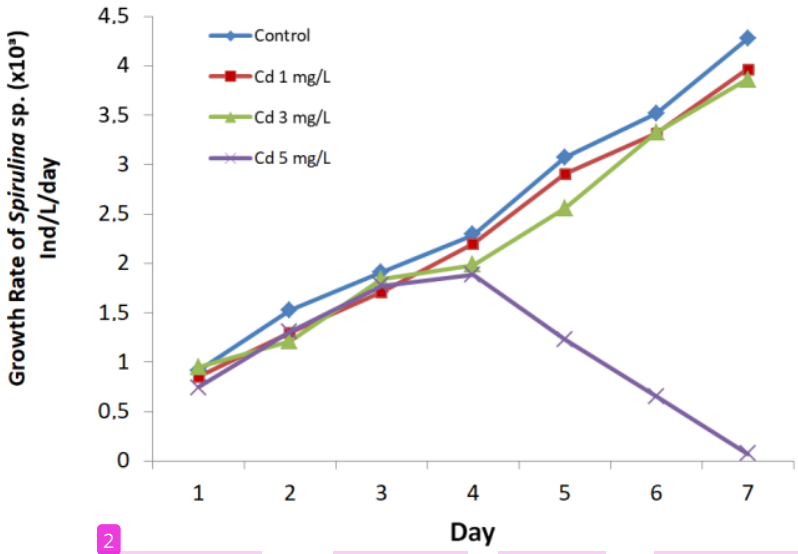

Figure 3. The effect of Cd initial concentration in the growth rate of *Spirulina* sp.

The growth rate of the treatment concentration of Cd 3 mg/L increased but the growth was lower than the control and Cd 1 mg/L. Concentration of Cd 3 mg/L inhibits metabolism of *Spirulina* sp. due to the toxic properties of metal ions at the time of absorption or active metabolism. The active metabolism of binding of metal ions is possible accompanied by toxic symptoms. When toxin is already in the cell, metal ions bound to cell organelles or with proteins such as metallothionein will increase membrane permeability thereby inhibiting cell intracellular interactions.

The highest concentration of Cd 5 mg/L cause death of *Spirulina* sp. This heavy metals with the high concentrations are already toxic to *Spirulina* sp. so that it affects pigment and lipid biosynthesis and chloroplasts ultrastructure so that it negatively affects photosynthetic efficiency [18,19]. Metal Cd in high concentrations can cause imbalances in protein ion charge because one amino acid group in the polypeptide (protein) chain, namely the carboxyl group is negatively charged will bind the Cd^{2+} metal ion with positively charged to form metal-ligand bonding. The death cell due to poisoning begins with the process of chloroplast damage [20]. Chloroplast damage causes inhibitation of the photosynthesis process and it will decrease the essential organic carbon needed by the *Spirulina* sp [21]. Apart from causing chloroplast damage, high concentration of Cd metal ion is thought to cause mitochondrial damage [22].

Table 1. The concentration of Cd(II) metal ion remaining in the solution medium

Initial concentration of Cd(II) (mg/L)	Sample repetition			Concentration of Cd(II) remain in BG- 11 medium (mg/L)
	1	**2**	**3**	
1	0.56	0.51	0.54	0.54±0.02
3	2.71	2.79	2.75	2.75±0.04
5	4.89	4.86	4.87	4.87±0.01

Base on the data in Table 1, the higher initial Cd concentration of 5 mg/L in the treatment of *Spirulina* sp. the lowest absorption occurs of 0.13±0.01 mg/L and *vice versa* for smaller initial concentrations resulting in higher absorption. The initial concentration of 1 mg/L and 3 mg/L results the amount of Cd(II) absorbed were 0.46±0.02 mg/L, and 0.25±0.04 mg/L, respectively. The heavy metals can inhibit the respiration of microalgae to photosynthesize, in addition to the sensitivity of microalgae to heavy metals that vary each species interferes with physiological processes and inhibits photosynthesis. Pollutants that enter mesophyll cells will have an influence at the molecular level causing changes in the stomata response, chloroplast structure, CO_2 fixation [23].

4. Conclusion

The growth rate in the control media of *Spirulina* sp. continued to grow until the exponential phase on the seventh day with an abundance of $4.3x10^3$ ind./L, while the medium with the addition of Cd(II) with concentrations 1, 3, and 5 mg/L experienced a decrease in the number of abundances of $3.9x10^3$ ind./L, $3.8x10^3$ ind./L, and $2.5x10^2$ ind/L, respectively. The maximum concentration of Cd(II) in the culture medium of *Spirulina* sp. is 3 mg/L while in higher concentration of Cd(II) decreased the growth significantly, so potential use of *Spirulina* sp. as an adsorbent of Cd(II) metal can be effective at concentrations up to 3 mg/L. The higher growth rate of *Spirulina* sp. on culture BG-11 medium the higher amount of Cd(II) absorbed.

Acknowledgement

The authors wish to acknowledge gratefully to the University of Sriwijaya through PNBP Hibah Profesi No. SP DIPA-042.01.2.400953/2019, by contract number 0014/UN9/SK.LP2M.PT /2019 for supports this research.

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