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The potentiality of *Arthrospira platensis* microalgal species for mining wastewater bioremediation by biosorption removal of heavy metals (Zn^{2+}, Cu^{2+}, Pb^{2+}, Fe^{2+})

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ABSTRACT

Arthrospira platensis has very high absorption by photosynthesis processes of CO_2 fixation and release of oxygen. It was becoming significant microalgae for biological wastewater treatment because of their bodies' accumulation of nutrients, heavy metals, organic and inorganic compounds used as the main source of their life production in wastewater. The objective of this study was to determine the ability of *Arthrospira platensis* for bioremediation by biosorption of heavy metals present in mining wastewater. Microalgae culture condition parameters were: temperature fixed at 30°C–35°C; the light was 1,000–2,000 lux set for 12 h d^{-1} and 12 h $night^{-1}$; pH; turbidity was 25–40 NTU. Biosorption was influenced by sorbent concentration (5–60 g L^{-1}), pH 2.0–9.0, and contact time (60 min) for heavy metals removal. It was related to nutrients consumption from wastewater with microalgae growth production by biosorption and takes place with separation of micronutrients (Zn, Cu, and Fe) and toxic metal such (Pb) from microalgae samples in a solution using centrifugation. The photosynthesis process was very suitable, that why chlorophyll productivity was very high. At low light intensity (1,000–1,400 lux), *Arthrospira platensis* consumed a high amount of nutrients (COD, TP, TN, ammonium) for growth. At high light intensity (2,000 lux), cell productivity, biomass productivity, growth rate, protein content, and energy value were very high. The effect of biosorbent on heavy metals removal from wastewater provides the following results: zinc adsorbed decreased from 4.08 to 2.1216 mg g^{-1}; iron from 6.03 to 0.6 mg g^{-1}; copper from 5.02 to 0.75 mg g^{-1}; and lead decreased from 7.04 to 0.98 mg g^{-1}. The effect of pH on metals biosorption was: sulfates (SO4) 93%, iron Fe 99%, lead Pb 95%, zinc Zn 52%, copper Cu 94%) removal efficiency with pH above 7.1. Heavy metals biosorption from wastewater was depended on sorbent concentration, pH, and contact time. The presence of multi-metal in culture systems decreases zinc adsorption, compared to the single metal one. The previous study was *Arthrospira platensis* biomass removed 50% of zinc from wastewater, which means that higher concentrations of other metal ions present in the wastewater don't affect the removal of zinc ions from the wastewater. The removal of zinc ions from the mining wastewater was about 52% in the current study.

Keywords: Absorption; Heavy metals; Mining wastewater; Micronutrients; Bioremediation

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1. Introduction

Arthrospira platensis is a filamentous cyanobacterium that growth in tropical, subtropical climates and alkaline lakes. It is known as a food additive to beat malnutrition in some developing countries. Microalgae *Arthrospira platensis* is the most widely used algae in the world and the most cultivated of the microalgal species that existed. Usually, *Arthrospira platensis* is cultivated in shallow, mixed ponds or semi-closed, tubular photobioreactors and it is well adapted in all culture parameters conditions for wastewater treatment [1]. This microalgal species is also known for its ability of bioremediation for biomass production by biosorption of heavy metals from mining wastewater [2]. *Spirulina* photosynthesis metabolism occurs through the presence of 1% chlorophyll-a inside their cell, one of nature's highest levels [3]. *Arthrospira platensis* have become significant microorganisms for the biological purification of wastewater since they can accumulate nutrients, heavy metals, organic and inorganic compounds in their bodies. Those components are the main source of their life. It also has essential amino acids responsible for the building blocks of proteins. Protein makes up about 60%–70% of *Spirulina* dry weight. Those amino acids, lipids, and glucose inside *Spirulina* can be converted into oil, which means bioenergy.

To carry out this research, lab-scale experimental was realized in 6-month duration. The sampling method was about the collection of water samples from the surface and underground water in the field study. The research location was in Huangshaping, Hunan Province, China. The water samples were brought to the laboratory for experiments. *Arthrospira platensis* was provided by Wuhan Hydrobiology, Chinese Academy of Sciences. The culture has been done with mining wastewater inside an enclosed system. Experiments setup culture have realized in the enclosed ST40 feed tank of 22 L with mining wastewater inside and 2 PVC foils of 1,000 mL volume. Various parameters were adopted to enhance the growth rate and suitability of *Arthrospira platensis* such as nutrient composition, pH, light effect, temperature, turbidity, inoculation size, air pump pressure, biochemical oxygen demand, chemical oxygen demand, dissolved metals, water quality, and nutrients [4]. Biosorption was influenced by sorbent concentration in contact time of 60 min. Microalgae biosorption for their growing production took place with the separation of micronutrients (Zn, Cu, and Fe) and toxic metal such (Pb) from microalgae samples in a solution using centrifugation. Wastewater and medium were pumped continuously from their recipients to the cultural tank system. After a few days, the output treated water was collected at the tip of the tank. The input Wastewater and output treated water were analyzed respectively before and after treatment. This is done to determine how much water is being purified during a specific time.

Due to mining exploitation, the surrounding area was contaminated by the major sources of pollution in groundwater such as the underground water gushing, the tailings ponds, water seepage. The existence of lead–zinc tailings and iron–zinc tailings wastes in the site is adequate for rainfall. After rainwater landing, the Suspended solids and amount of major pollutants affect the surface water. Therefore, the mining wastewater has a very high concentration of micronutrients Zn, Cu, Pb, and Fe. The zinc, iron, copper, sulfates and lead tailing zone are the most containing water in the study area. Knowing that the mining wastewater is really difficult to purify and costs very expensive to treat with the treatment plan. It takes a long procedure to achieve and obtain purified water from a mining exploration site. That's why it is very important to use biological treatment by using microalgae to treat mining wastewater in this study case. The ability of *Arthrospira platensis* culture production on wastewater was very necessary for the treatment of mining wastewater on a small scale treatment [5]. *Arthrospira platensis* is a great accumulator. It's able to be adsorbent with high selectivity for pollutants and can be generated with high alkalinity which is very essential for the precipitation of heavy metals. This species also has great potential efficiency for heavy metals removal in acid mining drainage [6]. To better understand this study, the explanation below will give the details.

2. Materials and methods

2.1. Study areas

2.1.1. Study area localization and description

The research area was localized in Huangshaping surface and groundwater. The study was used some databases originally from fieldwork research. It was done to estimate the mining contamination in Huangshaping groundwater. The residents' groundwater utilization is more traditional. The main sources of use for living water are irrigation and industrial enterprise. Huangshaping town is 9 km from the county town of Guiyang. As shown in Fig. 1, mining site geographical coordinates: longitude 112°40′20″ ~ 42′20″ North latitude 25°38′59″ ~ 41′27″ East [7]. According to the survey of the site visit, the town of Huangshaping drinking water source which is Fangyuan reservoir as well as the east river is associated with the Huangshaping lead-zinc mine groundwater. According to the previous research, all used water dressing workshops and dressing production are outside exploitation areas. The dressing wastewater recycling in the region has no function of groundwater remediation. The aquifer reservoir in the district of Fangyuan is ranges about 3.8 km from the Huangshaping mine. But the springs and rivers are very close to the mining area.

2.1.2. Sampling procedure on site

In this study, all the sites are located around JA Bridge, Tai Hang Road, the implemented wastewater treatment plant, and Xinmin Village. The study sampling sites were divided into six parts: S1: site one (Tailings South of the Drilling Zk53); S2: site two (Tailings North of the Drilling Zk1); S3: site three (Six Springs Pool); S4: site four (Well Dairies Residents); S5: site five (Wo Mau Village Springs); S6: site six (Xiàojiāquán Point).

The amount of 70 L of wastewater was collected for each sampling period three times. Wastewater sampling took place when mine is depositing [8]. Samples were collected from the tailings dam and transported to the environmental laboratory in an ice chest containing ice cubes for analysis within 2–4 h after collection [9]. In this study, the

sampling preparation, sampler cleaning, and sampling method have been referred to as procedures described in American Public Health Association [59]. All the chemicals used for biosorption experiments were purchased from the tailing zone and were of analytical grade. The wastewater containing zinc (Zn), iron (Fe), copper (Cu), and lead (Pb) in concentration 45 mg L^{-1} (pH 6.5) was taken from electroplating units [10]. Current treatment schemes of industrial effluent for metal removal include a complex of chemical methods [11].

It was carried out through the cleaned plastic bottles using detergent 10% HNO_3, triple-rinsed with distilled water, and finally triple-rinsed with the sample. Samples for heavy metals analysis were filtered and acidified with 0.1 mol of HNO_3 before storing it in a refrigerator [12]. The following factors should be taken into consideration to decide when wastewater samples are collected in the receiving environment:

- When the concentration in the exposure area of contamination is expected to be highest.
- When the biological factors are being conducted.
- The samples were collected and stored under cooling to 4°C.

2.1.3. Description of the microalgal studied species (Arthrospira platensis)

Blue-green multicellular filamentous microalgae (about 8 μm in diameter). It is grown in a tropical and subtropical zone, with a temperature optimum above 35°C. It is a rich source of vitamins, especially vitamin B12, minerals, protein, and carotenoids. In wastewater, it has the ability for biomass production, nutrients removal, and heavy metals removal. The *Spirulina platensis* (13 mL for each) strain was provided by the Wuhan Institute of Hydrobiology, Chinese Academy of Sciences.

2.1.4. Classification and biochemical composition of Arthrospira platensis

- Classification:
  Domain: Bacteria
  Phylum: Cyanobacteria
  Class: Cyanophyceae
  Order: Oscillatoriales
  Family: Microcoleaceae
  Genus: *Arthrospira*
  Species: *Arthrospira platensis*
- Biochemical composition:

Arthrospira platensis is very rich in protein contents ranging from 53% to 68% by dry weight. Its protein harbors all essential amino acids. *Arthrospira platensis* has very high amounts of polyunsaturated fatty (PUFAs) and the total lipid content is about 5%–6%. The γ-linolenic acids, the essential Omega-6 fatty acid were found in those PUFAs. Besides, *Arthrospira platensis* have some ingredients such as vitamins, minerals, dietary supplement, and photosynthetic pigments.

2.2. Experimentation methods

2.2.1. Mining wastewater treatment flu chart

The wastewater is about 6liters were fed into the feed tank and by gravity and it will be fed into the rectangular reactor. The reactor was operating at room temperature. These parameters following were tested respectively: light, pH, temperature, heavy metals, and organic compounds [13]. Experiments were carried out in the ST40 tank of 22 L (5.81179 gallons) and 2 PVC foils of 1,000 mL volume. The total cultivation volume in the tank was 12 L and 900 mL for each foil (Fig. 4). The volume was maintained through the daily addition of wastewater and medium to replace water lost by evaporation [14]. 2.57 g L^{-1} of NH_4–NO_3 was added daily as a nitrogen source during 20 d of the cultivation period. The effect of biosorbents concentration on Zn, Fe, Cu, and Pb removal from wastewater was examined by varying from 5 to 60 g L^{-1}. To determine the contact time required for the sorption equilibrium experiment, samples were withdrawn at predetermined time intervals 5, 15, 30, 45, and 60 min.

2.2.2. Medium preparation

Medium cultivation has a great impact on biomass microalgal productivity and other compounds of interest [15]. The source of nitrogen in this research was ammonium nitrate (NH_4–NO_3) because it's has a great effect on *A. platensis* productivity [16].

There are 10 types of mother solution with their dosage: $NaHCO_3$ (13.61 g L^{-1}), Na_2CO_3 (4.03 g L^{-1}), K_2HPO_4 (0.50 g L^{-1}), $NaNO_3$ (2.50 g L^{-1}), K_2SO_4 (1.00 g L^{-1}), NaCl (1.00 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.20 g L^{-1}), $CaCl_{12}{\cdot}2H_2O$ (0.4 g L^{-1}), $FeSO_4{\cdot}7H_2O$ (0.01 g L^{-1}), and A5 (1 mL L^{-1}).

Each component of the medium was put into 100 mL of filtered water to give mother solutions. 1 mL of each mother solution was versed into 990 mL of filtered water to give 1 L of the medium.

A5 composition with their concentration was 2.86 g L^{-1} dH_2O of H_3BO_3; 1.86 g L^{-1} dH_2O of $MnCl_2{\cdot}4H_2O$; 0.22 g L^{-1} dH_2O of $ZnSO_4{\cdot}7H_2O$; 0.39 g L^{-1} dH_2O of $Na_2MoO_4{\cdot}2H_2O$; 0.08 g L^{-1} dH_2O of $CuSO_4{\cdot}5H_2O$; and 0.05 g L^{-1} dH_2O of $Co(NO_3)_2{\cdot}6H_2O$.

A5 (trace metal solution) preparation: 2.86 g of H_3BO_3 dissolved in 300 mL dH_2O; 1.86 g of $MnCl_2{\cdot}4H_2O$ dissolved in 200 mL dH_2O; 0.22 g of $ZnSO_4{\cdot}7H_2O$ dissolved in 25 mL dH_2O; 0.39 g of $Na_2MoO_4{\cdot}2H_2O$ dissolved in 25 mL dH_2O; 0.08 g of $CuSO_4{\cdot}5H_2O$ dissolved in 25 mL dH_2O; 0.05 g of $Co(NO_3)_2{\cdot}6H_2O$ dissolved in 25 mL dH_2O

To get 600 mL of A5, 540 mL of distilled water are added 30 mL of H_3BO_3; 20 mL of $MnCl_2{\cdot}4H_2O$ and 2.5 mL of each $ZnSO_4{\cdot}7H_2O$, $Na_2MoO_4{\cdot}2H_2O$, $CuSO_4{\cdot}5H_2O$, $Co(NO_3)_2{\cdot}6H_2O$ solutions. After preparation of the medium, it must be sterilized for 20 min in an autoclave incubator, allowed to be cool and put in the fridge.

2.2.3. Optimum culture conditions

The cultural conditions are as follows:

After receiving algae seed, shake the algae liquid in the test tube, transfer directly into a triangular glass bottle under

sterile operation (25–50 mL). Seal the bottle and place it in a light incubator for 2–3 d under weak light.

The culture temperature was 25°C–35°C, the light condition was 1,000–2,000 lux, and the time was set at 12 h d^{-1}/12 h $night^{-1}$; and the pH was: 2.0–8.0. The humidity was 25–40 [17].

Prepare the corresponding medium, take 5–10 mL of algae liquid and add 10–20 mL fresh medium, and culture it in a sterile triangular bottle for 20–30 d (the time of algae seeds with a long growth cycle to be extended), the algae seed are growth in good condition and biomass have increased significantly, which can be transferred again at a ratio of 1:5 (algal liquid = medium).

The microalgae culture was started from 30 mL inside a 100 mL flacon. Feed algal culture by adding the quantity of evaporated water every 3 d [18]. After 10–15 d, separate the culture in another 2 foils of 250 mL. Add 50 mL of algal liquid into 100 mL of the fresh medium inside two 250 mL foils. The culture was made from small to big and bigger. When the algal solution <500 mL was cultured, the flask could be shaken twice a day. When the algal solution >500 mL was cultured, it could be aerated or the shaker used to aid the culture [19].

2.2.4. Physicochemical parameters

The temperature, pH, electrical conductivity (EC), and total dissolved solids (TDS) were measured by a portable waterproof HANNA – pH/EC/TDS/temperature meter (HI9813-6).

2.2.5. Preparation of adsorbent

Arthrospira platensis biomass purchased from culture systems was dried in an oven at 80°C for 24 h. Then the biomass was homogenized in a homogenizer at 600 rpm for 10 min and afterward used in the experiments [20].

2.2.6. Dry weight determination

The biomass obtained was washed twice with distilled water. *A. platensis* sample filtration through dried Whatman filter (pore size 0.42 μm) with carefully up to 0.0001 g level has been realized for the dry weight biomass determination [21]. The difference between the initial and final weight was taken as the dry weight of *A. platensis* biomass. The dry weight was expressed in terms of g L^{-1} [22].

2.3. Kinetic parameters calculation

2.3.1. Maximum specific growth rate (μm)

The maximum growth of microalgal where occurred with the use of nutrients combines with the light intensity of 60 μmol photons m^{-2} s^{-1}. The distance between light and culture was 30 cm up. The temperature was at 27°C, and cell concentration was achieved 1,322 mg L^{-1}. Minimum use of the medium in the light intensity of 10 μmol photons m^{-2} s^{-1} and the temperature was 30°C. Cell concentration has been achieved 687 mg L^{-1}. The maximum specific growth rate was calculated as follows:

$$\mu m = \frac{1}{X}\left[\frac{dX}{dt}\right] \tag{1}$$

where (dX/dt) represents the microalgal growth rate [23,24].

2.3.2. Cell productivity (P_X)

Data analysis of cell growth was collected through a turbidimeter. The FEMTO® spectrophotometer gives the transmission reading of suspension at 630 nm. The correlations of the calibration curve were (5, 24). The dry mass per liter of suspension was expressed. The cell productivity rate of *Arthrospira platensis* was calculated as the following formula:

$$P_X = \frac{X_{max} - X_i}{T_{max}} \tag{2}$$

where P_X = cell productivity (mg L^{-1} d^{-1}); X_i = initial cell concentration (mg L^{-1}); X_{max} = maximum cell concentration (mg L^{-1});

Table 1
Experiments conducted under different light intensities, starting from 2,000 lux and reducing to 1,000 lux, with the medium using NH_4NO_3 as the nitrogen source

Parameters \ Experiments	Test 1[a]	Test 2[a]	Test 3[b]	Test 4[b]	Test 5[c]	Test 6[c]	Test 7[d]	Test 8[d]
Final cellular concentration (X_f)	687	792	1,505	1,408	1,103	1,181	1,370	1,322
Cultivation time T^e (d)	16	16	15	14	11	11	15	15
Chlorophyll biomass content (mg g^{-1})	15.3	12.8	6.7	9.3	13.4	11.2	11.0	11.3
Protein biomass content (%)	62.5	60.1	60.5	59.8	63.2	62.8	60.2	61.7
Lipid biomass content (%)	15.8	14.6	14.7	14.1	15.6	14.8	19.2	15.2
Total chlorophyll per cultivation (mg)	52.6	50.7	50.4	65.5	73.9	66.1	75.4	74.7
Chlorophyll productivity P_{Cl} (mg L^{-1} d^{-1})	0.6	0.6	0.6	0.9	1.3	1.2	1.0	1.0
Cell productivity P_X (mg L^{-1} d^{-1})	46.4	39.8	97.0	97.0	95.7	102.8	88.0	84.8
Nitrogen-cell conversion factor $Y_X = N$ (g g^{-1})	2.1	1.8	4.1	3.8	3.0	3.2	3.7	3.6

[a]Experiment carried out at 1,000 lux; [b]Experiment carried out at 2,000 lux; [c]Experiment carried out with light intensity reduction on 9th day; [d]Experiment carried out with light intensity reduction on the 13th day; [e]Corresponding time for the highest total chlorophyll production; [f]Considering 7 L of cultivation.

T_{max} = cultivation time related to the maximum cell concentration (d).

2.3.3. Biomass chlorophyll productivity

The maximum cell concentration was corresponding with determined chlorophyll produced in the sample. That occurred in 10 mL of cultivation medium filtered for vacuum extraction of cells at 1.0 mm porosity polytetrafluorethylene membranes. The material was acquiescing to extraction by using acetone. Chlorophyll a was measured in spectrophotometric with calibration curve (13, 16, 24, 25, 29, and 41) as standard for chlorophyll a.

Biomass chlorophyll productivity was calculated as follows:

$$P_{Cl} = \left[Cl\right] \cdot \frac{X_m}{T_e} \tag{3}$$

where P_{Cl} = chlorophyll productivity (mg L^{-1} d^{-1}); [Cl] = chlorophyll a content in biomass (mg g^{-1}); X_m = maximum cell concentration (mg L^{-1}); T_e = cultivation time related to maximum cell concentration (d).

2.3.4. Nitrogen-cell conversion factor

In fed-batch runs using NH_4NO_3, the medium preparation was adjusted from the original nitrogen source ($NaNO_3$) protocol [25]. The feeding times were 20 d for adding NH_4NO_3. The nitrogen source was added twice per day. The light intensity was adjusted depending on the experimental conditions, as shown in (Table 1).

The nitrogen-cell conversion was calculated as the following formula:

$$Y_{X/N} = \frac{\left(X_m - X_1\right)V}{N_t} \tag{4}$$

where $Y_{X/N}$ = nitrogen-cell conversion factor (g g^{-1}); X_i = initial cell concentration (mg L^{-1}); X_m = maximum cell concentration obtained (mg L^{-1}); V = cultivation volume (L); N_t = Total nitrogen quantity added (mg).

2.3.5. Nutrients removals analysis

At the beginning and the end of each test experimentation, the deduction of nutrients (COD, ammonium, and phosphorous) were accomplished [26]. The COD and phosphorous removal were calculated as shown by the equation below:

$$\text{Removal}\left(\%\right) = \left[\frac{x_i - x_f}{x_i}\right] \times 100 \tag{5}$$

where x_i = COD or phosphorous before the biomass growth and x_f = COD or phosphorous after the biomass growth.

2.3.6. Data analysis

Data were detected by using statistical analyses (Statistica 9.0, StatSoft Inc., Tulsa, OK, USA) and exposed to one-way analysis of variance (ANOVA) [27]. The presentation of data was in terms of the mean (±standard deviation), proximate composition (%, dry matter) and energy value (kcal). Data analysis was made by using Statistical differences between treatments means were established using the Fisher LSD (Least Significant Difference) test [28]. Multiple range tests were used to compare treatment averages of the microalgae characteristics. Statistical significance occurred when all analyses were calculated at $p \leq 0.05$ value.

2.3.7. Microalgal growth comparing ammonium nitrate and urea as a nitrogen source

Arthrospira platensis was cultivated in mine simple wastewater under ammonium nitrate as a source of nitrogen. Cultivation was realized in laboratory room weather conditions for 20 d. The absorbance (Abs) was measured at Abs 600 nm and the optical density readings of *Arthrospira platensis* were achieved significantly high ($p < 0.05$) with ammonium nitrate as a source of nitrogen [29]. As shown in Fig. 3a, the culture under using ammonium nitrate has a great effect on the growth absorbance rate than using urea. For all nitrogen sources measurement, at exponential growth phase (23.8 ± 0.27 μm, 23.4 ± 0.17 μm for ammonium nitrate, and 17.6 ± 0.46 μm for urea), the average cell diameter was larger than at the stationary phase (21.4 ± 0.22 μm,

Table 2
Arthrospira platensis cultivation under different light intensities with accurate parameters

Light intensity (lux)	1,000	1,200	1,400	1,600	1,800	2,000
Cell productivity P_X (mg L^{-1} d^{-1})	159.61	167.2	171.42	171.50	176.2	183.57
Exponential phase duration (d)	10	4	4	4	4	4
Linear growth phase duration (d)	10	8	8	8	8	8
μ_{max} (h^{-1})	0.0222	0.0228	0.0234	0.0243	0.0257	0.0264
X_{max} (mg L^{-1})	2,284	2,391	2,479	2,458	2,446	2,643
Protein content (%)	51	53	56	59	63	65
(P_{Cl}) chlorophyll content (%)	0.27	0.24	0.21	0.18	0.15	0.12
Nutrients removal (%)	92	87	82	75	61	49
$Y_{X/N}$ (mg mg^{-1}), with dilution rate: N_0 = 0.5 to 5 mmol L^{-1}	50	46	43	35	30	32

20.3 ± 0.76 μm for ammonium nitrate, and 17.6 ± 0.46 μm for urea). The average diameter of cells grown with urea was significantly lower (ANOVA, $p < 0.001$) than the cells growing on ammonium nitrate [30].

3. Results and discussion

3.1. Arthrospira platensis fed-batch and tank reactor culture grow rate

In this current study, no light photoinhibition was found for the range of intensities tested. Productivity and cell concentration were maximal for 2,000 lux and reached 183.57 of cell productivity and maximal growth rate at 2,643 mg L^{-1}. As the differences between 1,000–1,400 lux were not so high in terms of productivity and protein contents, the use of 1,000 lux should be recommended for lower production costs [31]. However, nutrients removal was very high at decent light intensity (1,000–1,400 lux). Table 2 shows that at 1,000 lux high content of chlorophyll was observed. Nutrients removal was also very high. It means that *Arthrospira platensis* have the ability of photosynthetic regulation to adapt to its favorable environment. At low light intensity, cell productivity was very low. But it is very convenient for *Arthrospira platensis* via photosynthesis by consuming nutrients it uses to their growth [32].

The influence of the dilution rate and ammonium nitrate concentration had impacts on biomass concentration (X_{max}), biomass productivity (P_X), and nitrogen-to-cell conversion factor ($Y_{X/N}$). It was demonstrated that X_{max} had a decreasing behavior when the dilution rate value increased. Dilution rate had a positive influence on biomass productivity (P_X), considering the runs in which steady-state condition was achieved. On the other hand, the nitrogen-to-biomass conversion factor ($Y_{X/N}$) decreased as the dilution rate value increased [33].

3.2. Influence of light intensity on biomass chlorophyll productivity and algal growth rate

In fact, in this continuous cultivation by using NH_4–NO_3 as a nitrogen source, the results observed that there are higher bioenergy yields at low light intensity (1,000 lux). That light effect was discovered in both cultures fed-batch and tank reactor culture and also nitrogen source (medium) [34]. Biological assimilation generally takes place during the biomass growth phase, and once the biomass concentration reaches a threshold level, chemical precipitation may predominate. Taking into consideration the experiment at 2,000 lux to promote cellular growth and after that was reduced (at 9th or 13th days of cultivation) to increase the chlorophyll biomass content [35]. Referred to previous works studies, it was verified that the utilization of a light intensity rate of 2,000 lux has the best growth result, and the utilization of a light intensity rate of 1,000 lux issued the largest chlorophyll content in the biomass [36]. And also the temperature was 30°C. It can be observed in Fig. 2a that the 9th and the 13th days of cultivation correspond approximately to the middle and the end of the logarithmic phase of the growth, respectively. Therefore, these days were chosen to perform the reduction of the light intensity from 2,000 to 1,000 lux [37].

3.3. Nutrient removal rate of Arthrospira platensis

Arthrospira platensis was isolated and maintained at an optimum temperature of 30°C. The cultures were ready

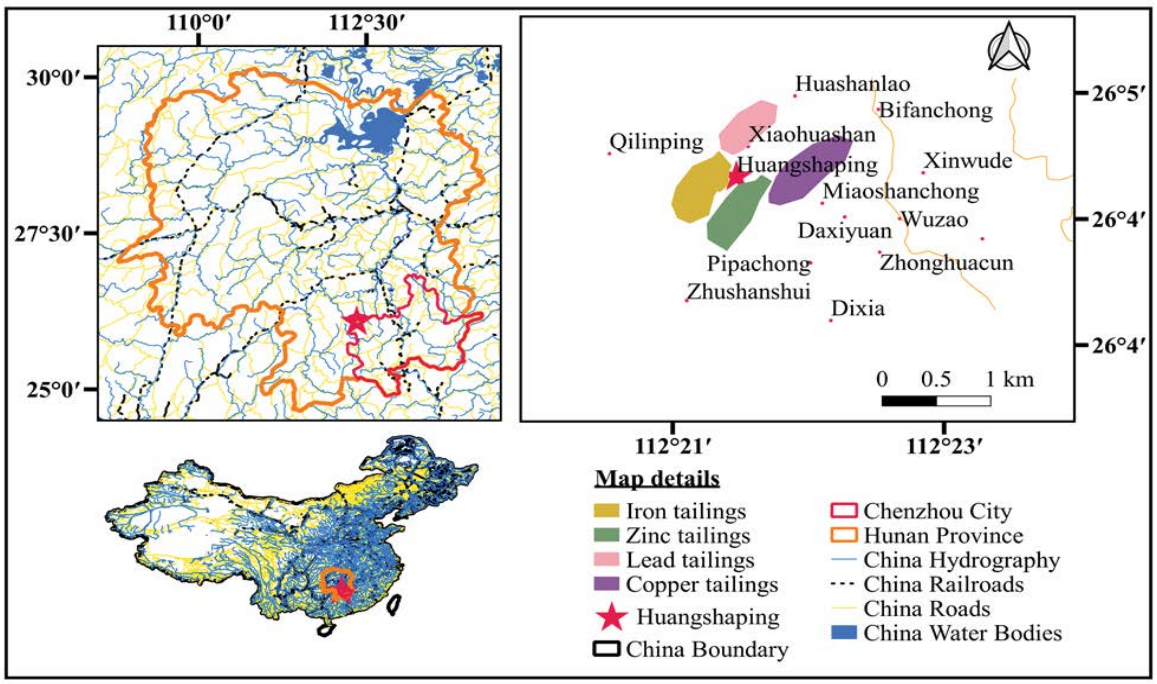

Fig. 1. Map showing mining tailings in Huangshaping in Chenzhou City of Hunan Province, P.R. China.

for inoculation when they reached the exponential growth phase [38]. Photosynthesis processes were very suitable at 1,000–1,400 lux and indicate high chlorophyll content. The consummation of nutrients in wastewater is necessary for the growth of *Arthrospira platensis*. The growth rate had a very close connection with light intensity, pH, the abundance of nutrients, nitrogen source, and temperature [39]. Nutrient consummation and growth medium nitrogen sufficient stipulate the increase of total chlorophyll content during the cultivation period [40]. The experiment showed that from the 9th day of cultivation, nutrients removal was increased until the peak of production [41]. Ammonium was 95.18% removed from the culture; total nitrogen was 60.49% of removal; total phosphorus had 90.92% removal, and COD was 61.80% removed for each 24 h of analysis [42] (Fig. 2b and c).

3.4. Arthrospira platensis biomass composition and energy value

Microalgae biomass was harvested from 10 to 20 d of cultivation because before 10th-day culture has only focused on inducing for adaptation and on optimum culture condition. The harvest processes have a scale of one day [43]. Protein, aldehyde (CHO), fiber, and lipid were at the highest rate on the 20th day because of cell productivity maximal concentrates on that period of culture [44]. The energy content was more abundant in the 13th and 17th days due to the stable light and pH during this culture period [45]. Contrary to ash and moisture compositions were respectively high on the 10th day and 12th day.

According to energy values, *Spirulina* showed higher energy and higher apparent metabolizable energy corrected for nitrogen conversion and removal. *Spirulina* has a relatively high content of protein, contains all essential amino acids and other components as shown in (Fig. 5). The biomass of microalgae was abundant from the culture [46].

3.5. Heavy metals biosorption from wastewater

3.5.1. Effect of pH on biosorption

The pH of the culture solution is a very important factor affecting the biosorption of heavy metals, because of its impact on the surface functional groups of biosorbents and the chemistry of the heavy metal in water. In the current study, the pH value was measured from 2 to 8 (Fig. 3b). At pH value 2, heavy metals removals efficiency was very low respectively 32% for Zn, 39% for Cu, 36% for Pb, 38% for SO_4, and 38% for Fe [47]. The sulfate was used for the oxidation of metals to increases the number of bonds to oxygen [48]. The low pH conditions allow hydrogen and hydronium ions to compete with heavy metals binding sites on the biomass, that causing poor heavy metals uptake. At pH value above 7.1, the biomass biosorption was very high and biosorption capacity was the same, corresponding to SO_4 93%, Fe 99%, Pb 95%, Zn 89%, Cu 94% removal efficiency. An increase in adsorption capacity with increasing solution pH is explained by the availability of negative charged binding sites thus increasing attraction of metal ions and allowing biosorption onto the microalgal cell surface [49]. In this study, it has been demonstrated that when there is no existence of hydroxide precipitation, the pH study was more reliable.

3.5.2. Effect of biosorbent concentration on heavy metals removal from wastewater

The effect of the biosorbents concentration on Zn, Cu, Pb, and Fe biosorption from solutions was shown the removal efficiency very high [50]. Thus, heavy metals removal efficiency from solution with an initial concentration of 10 mg L^{-1} varied from 85% to 90% at biosorbent concentrations increase from 5 to 60 g L^{-1} (Fig. 3c). To increase the efficiency removal from wastewater different amount of

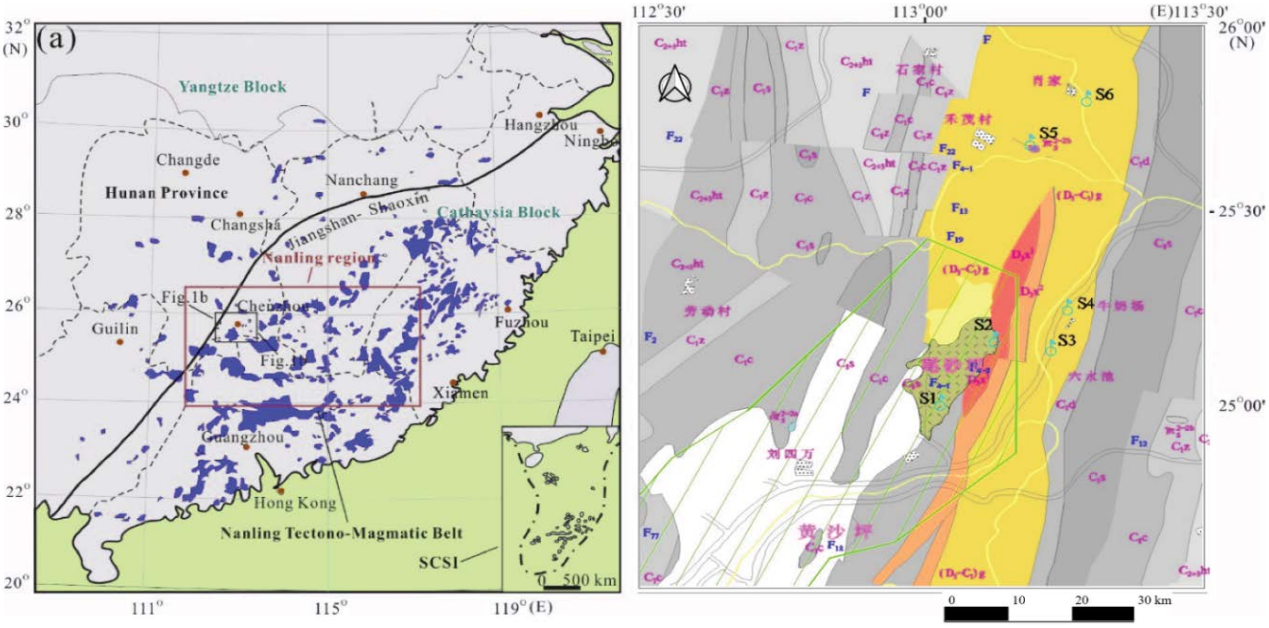

Fig. 2. Sampling site localization map.

biosorbent was used in the experiment. It can be attributed to the increase in the surface area resulting from the increase in sorbent mass, thus increasing the number of active biosorption sites [51]. Beyond a certain value of 40–60 g L^{-1}, the percentage removal reaches almost a constant value. Therefore, the difference in biomass removal capacity at sorbent dosage 40 and 60 g L^{-1} was about 3%. After all, the use of a 40 g L^{-1} biosorbent dose is justified for economical purposes [52]. However, the amount of zinc adsorbed decreased from 4.08 to 0.4 mg g^{-1}, iron from 6.03 to 0.6 mg g^{-1}, copper from 5.02 to 0.75 mg g^{-1}, and lead decreased from 7.04 to 0.98 mg g^{-1}. This is because, at higher adsorbent concentrations, the solution's ion concentration drops to a lower value and the system reaches equilibrium at lower values of '*q*' indicating that the adsorption sites remain unsaturated [53]. The maximum removal of Zn 85.5%, Cu 88.5%, Pb 90%, and Fe 89% was observed at the adsorbent dosage of 60 g L^{-1}.

3.5.3. Heavy metals removal from wastewater compared to time hardness

The kinetic profile of micronutrient heavy metals (Fe, Zn, and Cu) and toxic heavy metal (Pb) removal from wastewater by *Arthrospira* biomass is shown in (Fig. 6). Biosorption of these heavy metals was increased in the function of time. For maximal biosorption efficiency, the required time was 30–60 min, because the solutions equilibrium time was achieved after 30 min [54]. Fe was at 3.23 mg g^{-1}, which corresponds to 69% of removal. Zn was 2.1216 mg g^{-1}, which corresponds to 52% of removal. Cu was at 4.92 mg g^{-1}, which corresponds to 77% of removal. Pb was at 3.71 mg g^{-1}, which corresponds to 73% of removal. The time required for maximum heavy metals removal from model solution and wastewater remained almost the same until the end of the experiment. Lower heavy metals removal efficiency can be explained by their higher concentration in wastewater [55]. When the concentration of another metal biosorption in multi-metal systems increase, zinc adsorption capacities of *Arthrospira platensis* significantly decreased in binary and ternary systems in comparison with a single metal one, which means that, at low metal level, only Zn^{2+} adsorption was strongly affected by the co-metal presence [56]. Various combinations of metal ions would give insight into metal-metal about metal-microbe interactions. In this present study, the concentrations of other metal ions present in the wastewater were so high, that why it affects the

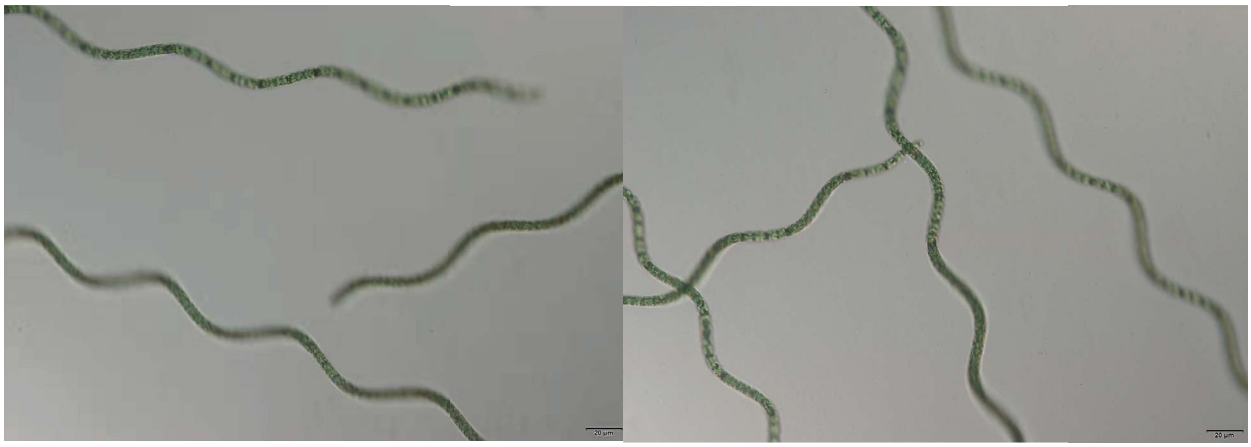

Fig. 3. Microscopic view of *Arthrospira platensis*.

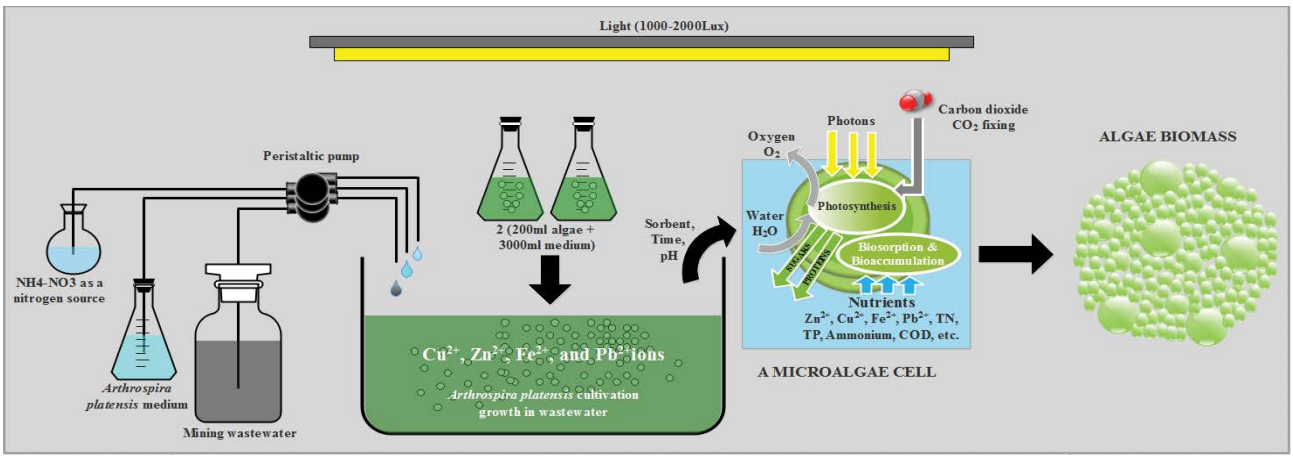

Fig. 4. Microalgae culture growth by biosorption and bioaccumulation for biomass production.

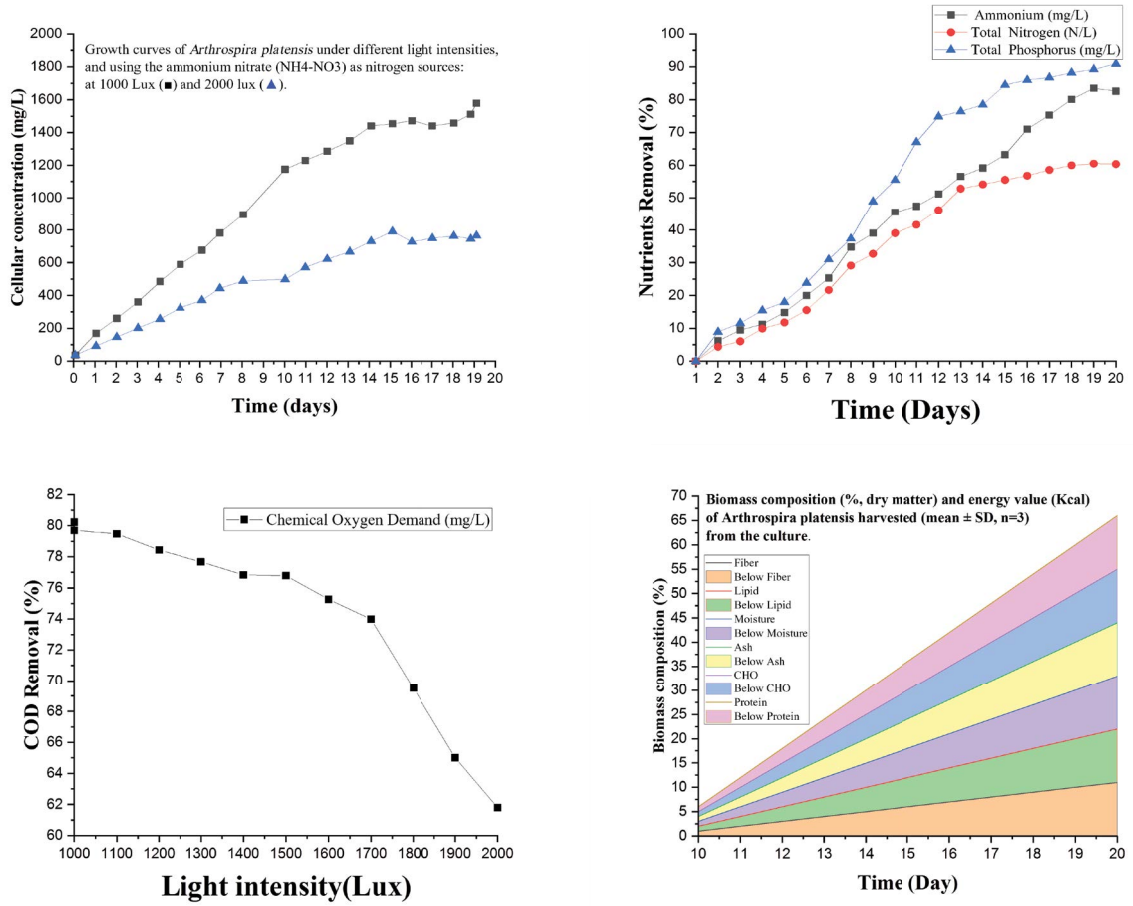

Fig. 5. *Arthrospira platensis* growth, nutrients removal, and biomass composition in the function of time and light intensity.

removal of zinc ions from the wastewater was *Arthrospira* biomass removed 52% of zinc from wastewater, containing beside zinc iron, and another metal [57–59].

4. Conclusions

This current research was realized in lab-scale experimentation culture inside mining wastewater. The wastewater samples were collected from surface and underground water in the field study (in Huangshaping, Hunan Province) and were bringing to the laboratory for experimentation to be treated. *Arthrospira platensis* is a type of microalgae that can be adapted to the high alkalinity and toxicity of micronutrients. The growth rate efficiency was also very high. Its cultivation in mining wastewater was demonstrated the potential ability for biomass production, nutrients removal, and heavy metals removal. *Arthrospira platensis* can be an alternative to abet in wastewater treatment by reducing the environmental impact.

The parameters of optimum culture conditions were temperature: 25°C–35°C; light intensity 1,000–2,000 lux set at 12 h d^{-1}/12 h $night^{-1}$; pH 2.0–8.0. The humidity was 25–40. The microalgae culture seeds were 20–30 d with a long growth cycle to be extended. In this study, ammonium nitrate (NH_4–NO_3) was used as a nitrogen source and was compared to urea as a nitrogen source. The biosorbent concentrations have a significant effect on biosorption capacity and removal efficiency of heavy metals. Sorbent concentration, pH, and determining time have an influence on heavy metals biosorption from wastewater.

At high light intensity (2,000 lux), cell productivity, protein content, and growth rate were very high. Contrarily for the decent light intensity (1,000 lux–1,400 lux), chlorophyll content, nitrogen conversion, and nutrients removals were very high. This may cause by the suitable photosynthesis and consummation of nutrients for microalgae growth. That is why, ammonium was 95.18% removed from the culture; Total nitrogen was 60.49% of removal; Total phosphorus had 90.92% removal, and COD was 61.80% removed for each 24 h of analysis. Nutrient consummation and growth medium nitrogen sufficient stipulate the increase of total chlorophyll content during the cultivation period. Also, the influence of the dilution rate and ammonium nitrate concentration had impacts on biomass concentration (X_{max}), biomass productivity (P_X), and nitrogen-to-cell conversion factor ($Y_{X/N}$). The nitrogen-to-biomass conversion factor ($Y_{X/N}$) decreased as the dilution rate value increased. The energy content was more abundant in the 13th and 17th days due to the stable light and pH during this culture period. The culture under using ammonium nitrate has a great effect on

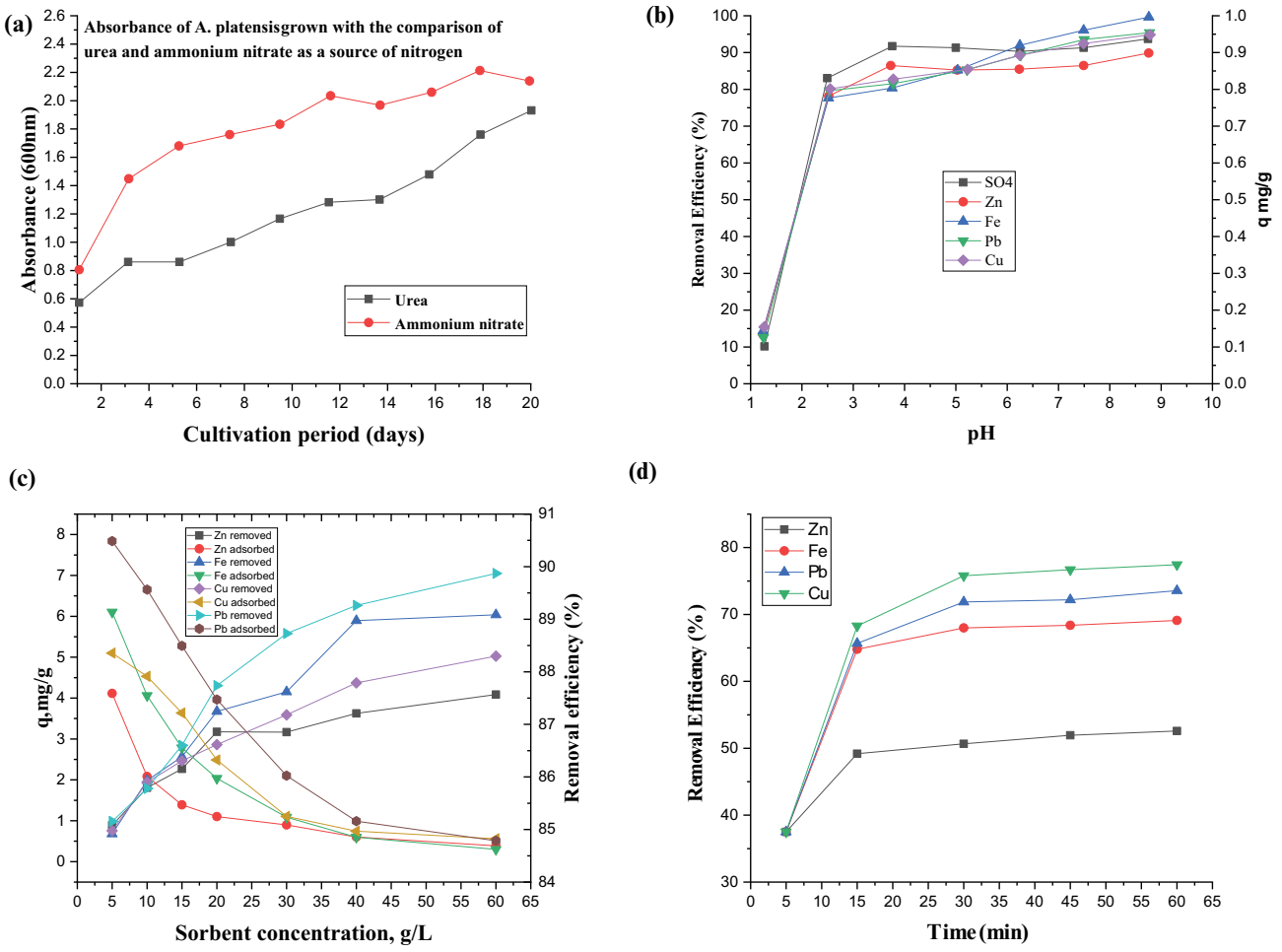

Fig. 6. The absorbance of *Arthrospira platensis*, the effect of pH, biosorbent concentration, and contact time on heavy metals biosorption from wastewater (T 20°C; C_i 45 mg L^{-1}; pH 6.5; sorbent concentration 10 g L^{-1}).

the growth absorbance rate than using urea. However, the average diameter of cells grown with urea was significantly lower than the cells growing on ammonium nitrate.

Heavy metals biosorption from wastewater was depended on sorbent concentration, pH, and contact time. At pH value above 7.1, the biomass biosorption was very high corresponding to SO4 93%, Fe 99%, Pb 95%, Zn 89%, Cu 94% removal efficiency. For the effect of biosorbent concentration on heavy metals removal from wastewater, the maximum removal was Zn 85.5%, Cu 88.5%, Pb 90%, and Fe 89%, observed at the adsorbent dosage of 60 g L^{-1}. About the effect of contact time on heavy metals removal, the maximum removal was Fe 69%; Zn 61%; Cu 77%; Pb 73%. The presence of multi-metal in culture systems decreases zinc adsorption compared to the single metal one. In the previous study *Arthrospira* biomass, 50% of zinc was removed from wastewater, which means that higher concentrations of other metal ions present in the wastewater don't affect the removal of zinc ions from the wastewater.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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