Raceway pond cultivation of a new *Arthrospira* sp. ZJWST-S1 in digested piggery wastewater treated by MBR and ozonation

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Abstract: A new *Arthrospira* strain named *Arthrospira* sp. ZJWST-S1 was isolated from a local digested piggery wastewater (DPW) storage pool in Jiaxing City, Zhejiang Province, China. It possessed good stain resistance against contaminants in DPW, which was pretreated with a membrane bioreactor (MBR). The strain was identified as *Arthrospira platensis* (*A. platensis*) based on its morphological characteristics and the 16S rDNA sequencing analysis. The effect of chrominance on growth of *A. platensis* ZJWST-S1 was investigated in a field raceway pond filled with MBR effluent or MBR effluent decolorized with ozonation. After ozonation, the chrominance of MBR effluent was decreased from 700 mg Pt/L to 150 mg Pt/L. Two runs of cultivation showed that *A. platensis* ZJWST-S1 grew faster in the ozone decolorized MBR effluent, the averaged biomass concentration being 0.907 g/L after 10 days of cultivation, close to that in a Zarrouk medium (0.969 g/L). By comparison, the biomass grew much slower in the non-decolorized MBR effluent (0.624 g/L). The pollutant removal was also benefited from the accelerative growth of *A. platensis* ZJWST-S1 in the decolorized MBR effluent. Almost all ammonium, 61.2% of nitrate and 68.1% of phosphate were removed by the *A. platensis* ZJWST-S1 in the decolorized MBR effluent, much higher than the corresponding values of almost all ammonium, 25.4% of nitrate and 36.5% of phosphate in the MBR effluent. Furthermore, the *Arthrospira* biomass harvested from the ozone decolorized MBR effluent after 10 d cultivation was with crude protein content of 59.1%±3.5% in dry algae powder. The content of Pb, As, Cd and Hg in biomass was also low enough to meet the Chinese *Arthrospira* Standard for Animal Feed (GB/T 17243-1998). This study showed that the new strain *A. platensis* ZJWST-S1 possessed potential to be used for producing animal feed and simultaneous removal of nitrogen and phosphorus in DPW.

Keywords: digested piggery wastewater (DPW), ozone decolorization, *Arthrospira platensis*, 16S rDNA, nitrogen and phosphorus recovery

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1 Introduction

Digested piggery wastewater (DPW) is characterized by high concentration of pollutants while low ratios of carbon to nitrogen. Wastewater like this is hard to remove COD, nitrogen and phosphorus with traditional

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biological treatment methods, especially, without external carbon addition[1]. The still high concentrations of nitrogen and phosphorus, trace elements (such as zinc and magnesium), bioactive substances (such as protein, amino acids, sugars, indole acetic acid and ribose) and growth regulators (such as vitamins and growth hormone) remained in the biologically-treated effluent would result in severe eutrophication of rivers and lakes[2].

Microalgae-based wastewater treatment methods have been increasingly researched since being put forward by Oswaldand Gotaas in 1957[3-5]. The main advantages of the process are high algae biomass yield as well as the simultaneous inorganic nutrients removal from wastewater. In recent years, *Arthrospira* cultivated in DPW has been extensively investigated due to its fast growth, high economic profits and easy harvesting[6]. *Arthrospira*, an ancient microalgae which contains 50%-70% of protein and is rich in vitamins, amino acids and bioactive substances, has been widely used as food and feed supplements for immune enhancement and growth improvement[7]. However, the traditional large-scale cultivation requires a large amount of baking soda, nitrogenous fertilizer and phosphate fertilizer, which account for almost 20%-30% of the total cost of *Arthrospira* powder production[8]. Cultivating *Arthrospira* in DPW not only greatly reduces the cultivation cost and recovers nutrient from DPW, but also brings in extra income when the harvested algae powder is processed to product (animal feed protein for example). However, some technical bottlenecks have been encountered during the cultivation of *Arthrospira* in DPW, where in the most critical one is the poor resistance of common *Arthrospira* strains to the complex contaminants in DPW, especially the high concentration of ammonium and chrominance. The concentration of ammonium in DPW is usually 500-1500 mg/L in China. Few *Arthrospira* can grow fast in ammonium concentration of over 60 mg/L, though some of them are isolated from ammonium-contained wastewaters[9-12]. Dilution is commonly carried out in order to decrease the ammonium concentration in lab-scale studies. However, dilution is not preferred in large-scale application, because a lot of clean water is used and nutrients in the wastewater are remarkably diluted. As for the high chrominance in DPW, its negative effect on the growth of *Arthrospira* will significantly increase with the scale up of cultivation. Another critical technical bottleneck of culturing *Arthrospira* in DPW, as well as in other wastewater, is that few studies have been conducted to harvest *Arthrospira* with crude protein content of over 50%[13-15].

In this study, a new *Arthrospira* sp. strain (ZJWST-S1) was identified which was isolated from a DPW storage pool located in Jiaxing City, China, and had demonstrated a much higher growth rate in DPW than other three commercial *Arthrospira* strains in our previous study[9]. In addition, the chrominance in DPW after treatment with a membrane bioreactor (MBR) was removed by ozone oxidation, and the increase in the biomass yield was studied in raceway pond cultivation. The quality of the harvested biomass was analyzed, and the pollutant removal in the DPW was investigated.

2 Methods

2.1 *Arthrospira* strains

The *Arthrospira* strain used in this study was named ZJWST-S1, which demonstrated fast growth in DPW pretreated by a MBR in our preliminary study[15]. The strain was isolated from a DPW storage pool in Jiaxing City, China. The pool receives DPW from a piggery farm 0.15 km away where the DPW was taken for this study. *A. platensis* Ns-90020 was provided by Wuhan Botanical Garden, Chinese Academy of Sciences. *A. platensis* DLMM S6 and DLMM S2 were provided by School of Life Sciences, Xiamen University. *A. platensis* Ns-90020, DLMM S6 and DLMM S2 are commercially employed in *A. platensis* large-scale cultivation in China[16-18].

2.2 Digested piggery wastewater

DPW used in the study was collected from a large piggery farm in Jiaxing City, China. The water was removed suspended solids (SS) with sedimentation, removed organics, ammonium and microorganisms with a MBR, and decolorized with ozone oxidation. The physicochemical characteristics of MBR effluent and ozone decolorized MBR effluent are shown in Table 1.

Table 1 Physico-chemical characteristics of the MBR effluent and ozone oxidated MBR effluent

Parameter	Means and standard deviations	
	MBR effluent	Ozone oxidated MBR effluent
pH	8.50±0.20	8.25±0.20
COD/mg·L^{-1}	280±14	230±11
NH_4^+-N/mg·L^{-1}	60.5±0.5	45.0±0.3
NO_3^--N/mg·L^{-1}	160.8±3.6	168.1±6.5
TP/mg·L^{-1}	45.0±1.2	38.9±0.8
PO_4^{3-}P/mg·L^{-1}	39.5±0.8	35.1±1.1
Chrominance/mg Pt·L^{-1}	700±50	150±20

2.3 *Arthrospira* cultivation experiments

Lab-scale cultivation experiments of ZJWST-S1 and other three commercial *A. platensis* strains (DLMM S6, DLMM S2 and Ns-90020) were carried out in 250 mL Erlenmeyer flasks which had been pre-filled with 90 mL MBR effluent. *Arthrospira* strains were cultured for 10 days in an illuminating incubator (GZP-450, Jinghong Laboratory Instrument Co. Ltd, Shanghai, China). The initial inoculum should be controlled at OD_{560} of 0.3. The culturing temperature was maintained at (30±1)°C. The photoperiod was 12 h light (6000±2000 lx, 7:00 am - 7:00 pm) and 12 h dark (7:00 pm - 7:00 am). OD_{560} of the mixed liquor in the flasks was measured after shaked by hand once per day. Meanwhile, mixed liquor in the flasks were sampled at intervals to observe the algae cell morphology. All of the above cultivation experiments were conducted in triplicates.

Field cultivation of ZJWST-S1 was carried out in a raceway pond (2.0 m × 0.6 m × 0.25 m) which were prefilled with about 130 L MBR effluent, ozone decolorized MBR effluent or Zarrouk medium. ZJWST-S1 in the logarithmic growth phase was inoculated into the aforementioned three medium and the initial biomass concentration was approximately 0.25 g/L, with a suspension depth of 12 cm. Water temperature was maintained at 25°C-35°C. The average light intensity was 50 000 lx and the light-dark ratio was approximately 13 h:11 h. The system was continuously stirred with a paddle agitator at 30 r/min. Two runs, adding up to 20 d, were carried out in order to investigate the effect of chrominance on biomass production. $NaHCO_3$ with a final concentration of approximately 16.8 g/L was added into the MBR effluent and ozone decolorized MBR effluent so as to insure adequate carbon source for *Arthrospira* growth.

2.4 Analysis

2.4.1 Morphological identification

Arthrospira sp. ZJWST-S1 and other three commercial *Arthrospira* strains were identified morphological features including width of trichome, cell size, shape of the helix, gas vesicles and calyptra on the end cells with a microscope (400×magnification; Olympus, Japan). The microalgae mixed liquor was sampled from the cultivation flasks, washed with deionized water, re-suspended in deionized water, then put about 0.1 mL on the center of a microslide for subsequent morphological identification. All of the morphological identification experiments were carried out in triplicate.

2.4.2 Molecular analysis of 16S rDNA

16S rDNA was sequenced in order to identify the new *Arthrospira* strain. DNA of *Arthrospira* sp. ZJWST-S1 was extracted according to the protocol in literature[19]. The purity of extracted DNA was tested by 0.7% agarose gel electrophoresis (Liuyi Instrument, China) which showed that the extracted DNA fragments had a length of over 10 kb for strain ZJWST-S1 as well as for *A. platensis* Ns-90020, DLMM S6 and DLMM S2. The ratio of OD_{260}: OD_{280} of extracted DNA was all over 1.8, suggesting that the DNA extracted be pure[20]. The purified products were then digested with FastDigest ECORI (Thermo Scientific, USA) and linked by T4 DNA Ligase (Sangon Biotech, China) with restriction enzymes. PCR pre-amplification reactions and selective amplification reactions were performed according to the published protocols[21].

Selective amplification products were separated with a 6% agarose gel electrophoresis, recovered with a Gel Extraction Mini Kit (Sangon Biotech, China), and sequenced by Life Technologies Corporation (China). Sequencing results were analogously searched with Blast 2.2.21 (NCBI Genebank). The multiple sequence alignment analysis of nucleotide sequences was carried out with MEGA v.4.0[22]. The phylogenetic tree was structured by the neighbor-joining method and the genetic distance was calculated using the published method[23]. The composition of 16S rDNA bases, conserved sites,

variable sites, parsimony informative sites and singleton sites were calculated during the analysis of the primary structure of 16S rDNA. The secondary structures of 16S rDNA sequence for *Arthrospira* sp. ZJWST-S1 and *A. platensis* strains DLMM S6 were predicted online with 4SALE v.1.5[24], which were read and exported by Structure View.

2.4.3 General analysis

The algae cell number was determined with a blood-cell counting method[25]. The cell concentration in the mixed liquor was indicated with the chlorophyll *a* concentration, by measuring the absorbance at 560 nm with a spectrophotometer (Precision Instruments, China). Biomass was evaluated using the method of dry cell weight, by filtering the mixed liquor samples through a 400 mesh filter, washing the solids with acidified water (pH 4, carboxylic acid solution) to eliminate salt precipitates and alkalinity, and finally drying the washed solids in an oven at 60°C for 4 h and weighing. The dry cell weight showed a linear relationship with OD_{560}, as follows: y=0.7178x+0.0025 (R^2>0.997), in which Y (g/L) is the dry cell weight; x is the absorbance at 560 nm. The crude protein content of dry algae biomass (%) was determined with a Coomassie brilliant blue method[26]. The moisture content in the algae powder were determined after washing algae biomass with acidic water, and then drying at 105°C; The ash content in the algae powder was measured after burning the dry biomass at 600°C for 4 h. Heavy metals (Pb, As, Cd and Hg) in the algae powder were determined with an atomic absorption spectrophotometer (Agilent Technologies, USA) after microwave digestion (Sineo, China). Bacteria, coliforms, molds and pathogens were determined with plate counting methods.

3 Results and discussion

3.1 Identification of the local *Arthrospira* sp. ZJWST-S1

Arthrospira is a type of microalgae characterized by corkscrew-shaped filament whose species include *A. platensis, A. subsalsa* and *A.maxima*. *A. platensis* is characterized by trichome width of 5-6 μm, cell length of 2-6 μm, round helix end and round end cell, evident calyptra on end cells and granular cytoplasm containing gas vacuoles. However, what makes the difference of *A. maxima* and *A. subsalsa* from *A. platensis*is are that they do not have calyptra on end cells and gas vesicles. Moreover, compared with *A. platensis*, *A. subsalsa* has slimmer trichome (1.5-2.5 μm), *A. maxima* has wider trichome (6-8 μm) and longer cells (8-12 μm) [27].

In order to get a preliminary species identification on the locally isolated *Arthrospira* strain and investigate its morphology change after MBR effluent cultivation, the morphological characteristics of ZJWST-S1 were observed together with other three known *Arthrospira* strains (DLMM S6, DLMM S2, Ns-90020) before and after cultivation in MBR effluent for 10 days. As shown in Figure 1a and Table 2, the four *Arthrospira* strains all had blue-green corkscrew-shaped filaments, which fitted the taxonomy definition of *A. platensis* and were different from the morphological characteristics of *A. maxima* and *A. subsalsa*. The morphological characteristics of the four *Arthrospira* stains cultured in the MBR effluent for 10 d are shown in Figure 1b.

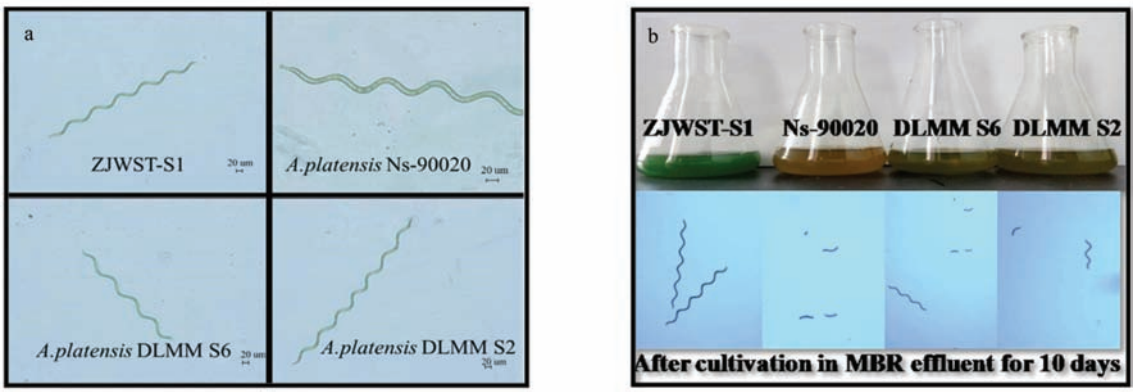


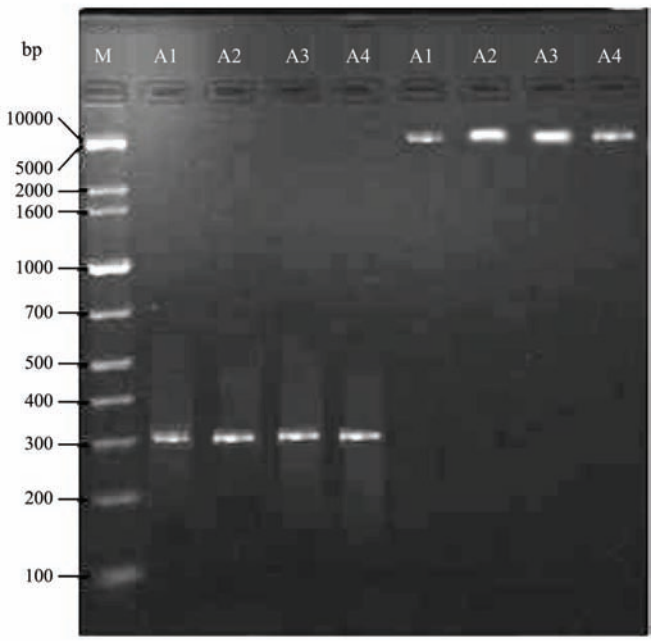
Figure 1 Microscopic and macroscopical image characteristics of *Arthrospira* sp. ZJWST-S1, Ns-90020, DLMM S6 and DLMM S2 before (a) and after (b) 10 d cultivation in MBR effluent

Table 2 Morphological characteristics of *Arthrospira* sp. ZJWST-S1 and three typical *A. platensis* strains

Strains	Width of trichome/μm	Shapes of the helix end and the end cell	Calyptra on end cells	Gas vesicles	Cell length/μm
*Arthrospira*sp. ZJWST-S1	5.5-6.0	Both ends rounded	Present	Present	4-5
A.platensis Ns-90020	5.3-5.7	Both ends rounded	Present	Present	5
A.platensis DLMM S6	5.5-5.8	Both ends rounded	Present	Present	4
A.platensis DLMM S2	5.8-6.0	Both ends rounded	Present	Present	4
A.platensis essential features[28]	5-6	Both ends rounded	Present	Present	2-6
A.maxima essential features[28]	6-8	Slightly diminished at one end	Absent	Absent	8-12
A.subsalsa essential features[28]	1.5-2.5	Slightly diminished at both ends	Absent	Absent	2-5

A. platensis ZJWST-S1 changed little in color and algae morphology. However, the filaments of other three strains became shorter, lost spiral characteristics and even broken into pieces, which might be attributed to the high stress from the high concentrations of NH_4^+-N and other contaminants in the DPW medium. The above differences also suggest that ZJWST-S1 possesses stain resistance against high concentrations of NH_4^+-N and other contaminants in the undiluted DPW.

Furthermore, 6% agarose gel electrophoresis (Figure 2) showed that 16S rDNA fragments of all strains had a length of 310 bp. *Arthrospira* ZJWST-S1 demonstrated the smallest genetic distance of 0.5679 to *A. platensis* DLMM S6, and the largest distance of 0.7881 to *A. platensis* DLMM S2 (Table 3). *Arthrospira* ZJWST-S1 was therefore considered to have the closest genetic similarities to *A. platensis* DLMM S6.



Note: M: Marker; A1: *Arthrospira* sp.ZJWST-S1; A2: *A. platensis* Ns-90020; A3: *A. platensis* DLMM S6; A4: *A. platensis* DLMM S2.

Figure 2 16S rDNA extraction products and enzyme digested fragments of four *Arthrospira* strains

Table 3 Genetic distance between *Arthrospira* sp. ZJWST-S1 and three typical *A. platensis* strains

Strain	ZJWST-S1	Ns-90020	DLMM S6	DLMM S2
Ns-90020	0.7881	-	-	-
DLMM S6	0.5679	0.8516	-	
DLMM S2	0.8649	0.6342	0.9204	-

All the known Genbank sequence of *Arthrospira* was aligned, and a phylogenetic tree was structured by a neighbor-joining method with *Microcystis aeruginosa* as an out group (Figure 3). The 16S rDNA of 21 *Arthrospira* species in the phylogenetic tree can be classified into three clades. Clade A consists of all *A. maxima* strains with the posteriori probability of 0.99[29], Clade B consists of all *A. platensis* strains with the posteriori probability of 0.86, and Clade C consisted of all *A. subsalsa*s trains with the posteriori probability of 0.96[30]. Clade A (*A. maxima*) and Clade B (*A. platensis*) fell into one branch while Clade C fell into another branch. The locally isolated *Arthrospira* sp. ZJWST-S1 was located in Clade B, as an *A. platensis* strain closely related to *A. platensis* DLMM S6 with the posteriori probability of 0.98.

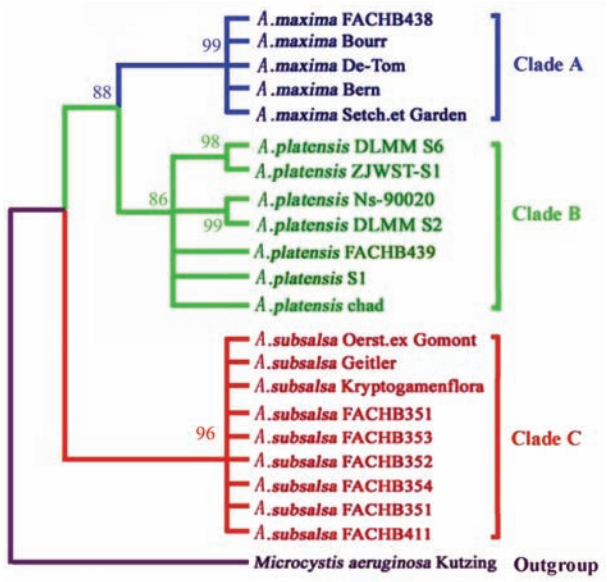


Figure 3 Topological tree inferred from the alignment of 16S rDNA sequences of *Arthrospira* sp.

A. platensis ZJWST-S1 and DLMM S6 demonstrated the highest genetic similarity as well as the most similar morphological characteristics. No significant difference was observed in the primary structure of the two strains, as shown in Table 4. 16S rDNA of *A. platensis* ZJWST-S1 had 252 conservative sites, 21 variable sites, 25 parsimony informative sites and 35 singleton sites. Variable sites and parsimony informative sites in 16S rDNA of *A. platensis* ZJWST-S1 accounted for 6.77% and 8.06% of the total sites, respectively. The above portions were similar to those in *A. platensis* DLMM S6, which were 7.09% and 6.77%, respectively. Two microalgae strains are considered to be same if the difference between their Gas Chromatograph (GC) contents is less than 2%, to be homogeneous but different if the GC contents differ in the range of 2%-5%, to be heterogeneous but in the same genera if the difference of GC contents ranges 5%-15%, to be in different genera and families if the GC contents differ over 15%[31]. Table 4 shows that the content of G+C was 45.1% for *A. platensis* ZJWST-S1, close to the value of 43.3% for *A. platensis* DLMM S6. The difference of GC contents between *A. platensis* ZJWST-S1 and DLMM S6 was only 3.99%, suggesting that the two strains be different but homogeneous.

The difference between the locally isolated strain *A. platensis* ZJWST-S1 and *A. platensis* DLMM S6 was further studied by carrying out the secondary structure analysis of their 16S rDNA. The secondary structural pattern observed in the chlorophytes reflects the dynamic folding process as it is synthesized 5′-3′. The localized hairpin loops with limited long-range pairing interactions illustrate a compaction of the secondary structural pattern portion of the primary rDNA transcript as proposed by Venkateswarlu[32]. Previous research has revealed that kinetic processes play an important role in the formation of functional structures in DNA[33]. Some differences were also observed between the secondary structures of *A. platensis* ZJWST-S1 and DLMM S6 (Figure 4). Both strains had five helixes in the secondary structures of the 16S rDNA. *A. platensis* ZJWST-S1 had one helix in the position typically occupied by Helix I but *A. platensis* DLMM S6 did not, because positions 38 base had caused base unmatchable substitution (namely from A:T to T:T). *A. platensis* ZJWST-S1 had no helixes in the position typically occupied by Helix III but *A. platensis* DLMM S6 had one, because positions 103 base had caused base matchable substitution (C:A to G:T).

Table 4 16S rDNA sequence base compositions of *Arthrospira* sp. ZJWST-S1 and *A. platensis* DLMM S6

	Length/bp	Cs	V	Pi	S	Nucleotide content/%				
						T	C	A	G	G+C
ZJWST-S1	310	252	21	25	35	28.4	20.6	26.5	24.5	45.1
DLMM S6	310	250	22	21	41	30.1	17.2	26.6	26.1	43.3

Note: Cs: Conservative sites; V: Variable sites;Pi: Parsimony informative sites; S: Singletonsites.

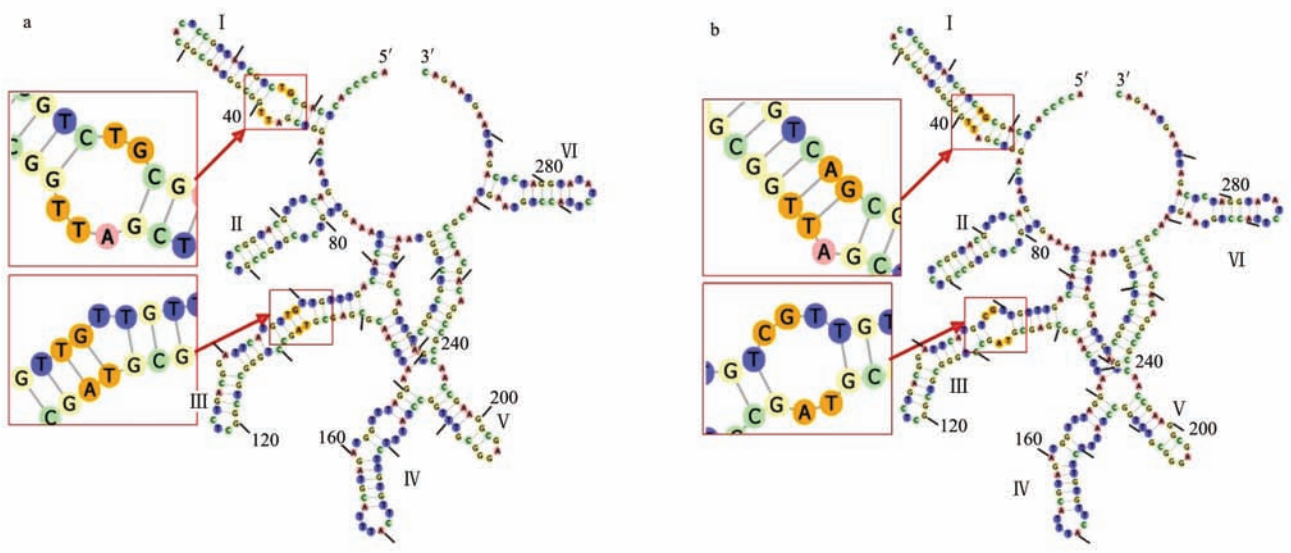


Figure 4 16S rDNA secondary structures of *A. platensis* ZJWST-S1 (a) and DLMM S6 (b)

The environment conditions, which have been previously hypothesized to associate with rRNA primary transcript processing, are involved in ribosome biogenesis[34,35]. Different geographical sources and natural conditions can lead to some changes in the DNA secondary structure, which might finally lead to a change in gene expression and regulation information and then bring in differences in the physiological and biochemical characteristics between *A. platensis* ZJWST-S1 and *A. platensis* DLMM S6.

3.2 Influence of ozone decolorization on biomass production of Arthrospira sp. ZJWST-S1 in raceway pond cultivation

A. platensis ZJWST-S1 has demonstrated good stain resistance against MBR effluent as compared with other commercial strains. However, its high growth rate in the lab-scale cultivation could be partially attributed to the shallow suspension depth in the flask, which ensured adequate illumination for algae cultivating even decolorized MBR effluent. As the cultivation scale was enlarged, however, the negative effect of chrominance would become increasingly significant. Raceway pond cultivation of *A. platensis* ZJWST-S1 was carried out for two runs in MBR effluent, ozone decolorized MBR effluent and Zarrouk medium, as shown in Figure 5. In run 1, the biomass productivity and the biomass concentration after 10 d cultivation were 9.5 g/(m^2·d) and 1.016 g/L in Zarrouk medium, 4.5 g/(m^2·d) and 0.602 g/L in MBR effluent, and 8.3 g/(m^2·d) and 0.912 g/L in the ozone decolorized MBR effluent. Compared to the growth in Zarrouk medium, ZJWST-S1 growth in the ozone decolorized MBR effluent was slightly decreased while that in the MBR effluent was greatly decreased. The MBR effluent culture group whose chrominance was not removed at all showed biomass productivity of about 46% lower than the ozone decolorized group.

Run 2 was continued in the original medium of run 1 without any other treatment after biomass harvest. Growth of ZJWST-S1 in this run performed similar to run 1.ZJWST-S1 still grew fast in the ozone decolorized MBR effluent and Zarrouk medium, with biomass productivity of 7.7 g/(m^2·d) and 7.8 g/(m^2·d) respectively. The growth was still much decreased in the MBR effluent, with biomass productivity of 4.7 g/(m^2·d). The similar growth curves of *A. platensis* ZJWST-S1 in the two runs also suggested that the biomass growth might be not limited by the nutrients.

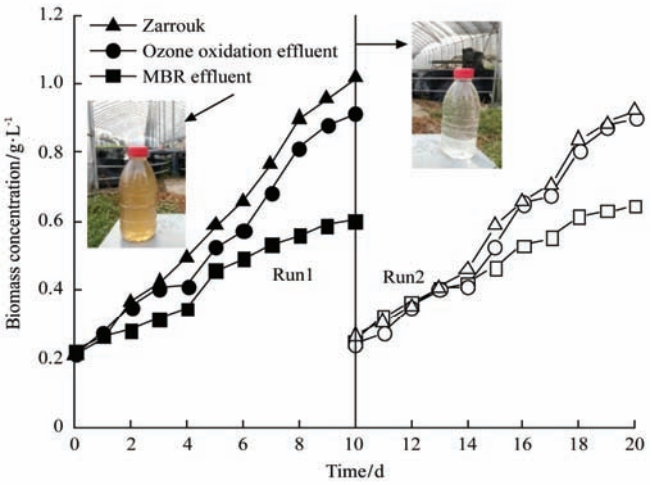


Figure 5 Growth curves of *A. platensis* ZJWST-S1 in Zarrouk medium, MBR effluent and ozone oxidated MBR effluent for two runs of field raceway pond cultivation (run 2 was operated in the corresponding original medium after harvest of run 1)

3.3 Contaminants removal and *Arthrospira* biomass analysis

The contaminants (nitrogen, phosphorus etc.) in the effluent, which are also nutrients for the growth of *A. platensis* ZJWST-S1, were removed during the biomass production process. In this study, nitrogen (NH_4^+-N, NO_3^--N) and phosphorus (PO_4^{3-}-P) concentrations in the ozone decolorized MBR effluent and MBR effluent were analyzed during *Arthrospira* cultivation, as shown in Figure 6. The ammonium concentration in the two effluents both sharply decreased until exhausted at 6th day and 12th day respectively. The nitrate performed just the opposite: the concentration decreased slowly in the early period and the decreasing was accelerated afterwards. The removal of ammonium in the effluent taking precedence over nitrate was because ammonium was utilized preferentially by *Arthrospira*[36]. On the whole, the removal of pollutants (NH_4^+-N, NO_3^--N and PO_4^{3-}-P) was much more efficient in ozone decolorized MBR effluent than in MBR effluent, as be attributed to the accelerative growth of *A. platensis* ZJWST-S1 in the ozone decolorized medium. In particular, after 20 days cultivation (two runs), the ammonium, nitrate and phosphate in the ozone decolorized MBR effluent was

decreased from 45 mg/L, 168.1 mg/L and 35.1 mg/L to 0, 65.3 mg/L and 11.2 mg/L, respectively, with removal rates of 100%, 61.2% and 68.1%. However, those in MBR effluent decreased from 60.5 mg/L, 160.8 mg/L and 39.5 mg/L to 0, 120 mg/L and 25.1 mg/L, respectively, with removal rates of 100%, 25.4% and 36.5%. It also confirmed that the nitrogen, phosphorus, etc. in the ozone decolorized MBR effluent and MBR effluent was still adequate after two runs of cultivation.

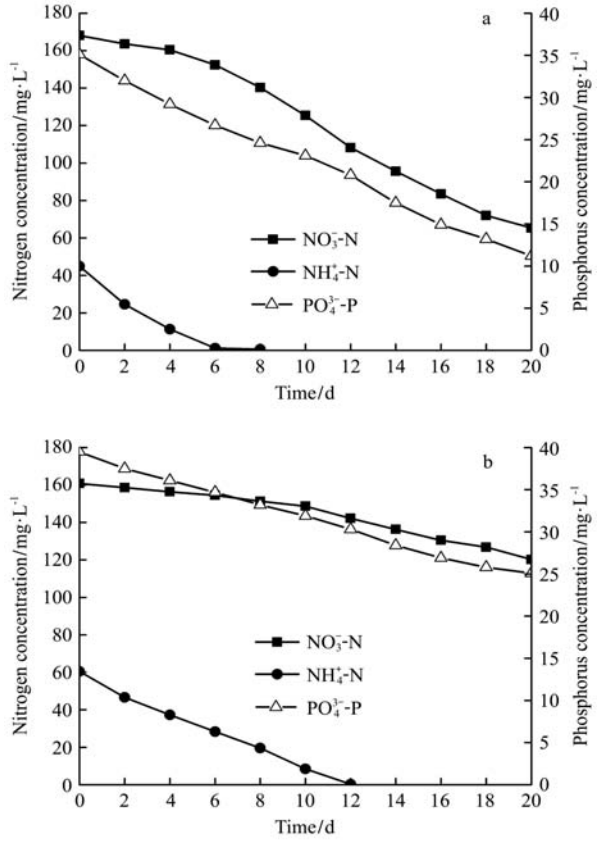


Figure 6 Concentrations of NH_4^+-N, NO_3^--N and PO_4^{3-}-P in ozone oxidated MBR effluent (a) and MBR effluent (b) during cultivation of *A. platensis* ZJWST-S1 for 20 days

Compared to industrial wastewater, DPW contains little toxicants and is relatively eco-safe. However, the suspended solids, bacteria and parasitic ovum in DPW which would contaminate algae and thereby decrease the quality of the algae products should also be removed. The MBR used in this study that combined membrane separation with biological wastewater treatment, could efficiently remove antibiotics, heavy metals, suspended and microorganisms[37,38]. Advanced treatment with ozonation could further improve the effluent quality. In addition, we selected a large farm without illegal addition of heavy metals. The concentrations of Pb, As, Cd and Hg in the harvested *A. platensis* ZJWST-S1 biomass were (0.073±0.019), (0.87±0.04), (0.090±0.011) and (0.070±0.005) mg/kg dry biomass, respectively, which were lower than the Chinese *Arthrospira* Standard for Animal Feed Grade (GB/T 17243-1998). The other parameters such as moisture and crude protein etc. all met the Standard as shown in Table 5. As for these measured items, the new strain has the potential to be used for producing high quality feed *Arthrospira* protein from DPW. The use of ozone decolorized MBR effluent as the culturing medium to produce animal feed protein with the locally isolated *A. platensis* ZJWST-S1 strain would reduce the *Arthrospira* biomass manufacturing cost, increase the income of the farmers, and provide a sustainable alternative for DPW management.

Table 5 Quality of *A. platensis* ZJWST-S1 powder produced in ozone decolorized MBR effluent

Properties	*Arthrospira* powder cultivated in DPW	Feed grade standard[a]
Moisture/%	6.27±0.23	≤7
Crude protein/%	59.10±3.48	≥50
Ash/%	7.15±0.89	≤10
Pb/mg·kg^{-1}	0.073±0.019	6.0
As/mg·kg^{-1}	0.87±0.04	1.0
Cd/mg·kg^{-1}	0.090±0.011	0.5
Hg/mg·kg^{-1}	0.070±0.005	0.1
Total plate count/cells·g^{-1}	$1.7\times10^3\pm0.2\times10^3$	5×10^4

Note: [a]: the Chinese *Arthrospira* Standard for Feed Grade (GB/T 17243-1998).

4 Conclusions

A new *Arthrospira* strain (ZJWST-S1), which was isolated from a DPW storage pool and possessed good stain resistance against contaminations in undiluted DPW, was identified as *A. platensis* according to its morphological characteristics and the 16S rDNA sequencing analysis. The decolorization of MBR effluent by ozone oxidation could significantly increase the growth rate of ZJWST-S1. In the two runs of raceway pond cultivation, the average area biomass productivity was increased from 4.6 g/(m^2·d) (in MBR effluent) to 8.0 g/(m^2·d) (in ozone decolorized MBR effluent) with a 74% promotion. Meanwhile, the removal of contaminants from the effluent was also benefited from the accelerative growth of *A. platensis* ZJWST-S1 by decolorization. The *A. platensis* ZJWST-S1 removed almost all ammonium, 61.2% nitrate

and 68.1% phosphate from the ozone decolorized MBR effluent, whereas it only removed almost all ammonium, 25.4% nitrate and 36.5% phosphate from the MBR effluent. In addition, the *Arthrospira* biomass harvested from the ozone oxidation effluent was analyzed. Its crude protein content in dry mass was 59.1%±3.5%. The contents of Pb, As, Cd and Hg in biomass met the Chinese *Arthrospira* Standard for Animal Feed (GB/T 17243-1998). This study shows that the new strain *A. platensis* ZJWST-S1 has the potential to be used for producing animal feed protein by recovering nitrogen and phosphorus from undiluted DPW.

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