# Cultivation of an *Arthrospira platensis* with digested piggery wastewater

Rui Liu, Qingqing Guo, Wei Zheng, Lüjun Chen and Jinfei Luo

## ABSTRACT

An *Arthrospira platensis* strain ZJWST-S1 was isolated in Jiaxing City, China, which proved able to proliferate quickly in undiluted digested piggery wastewater (DPW), and the protein content in the algal biomass was high. Single factor experiments showed that the strain was able to quickly grow in a Zarrouk medium as the dosage of sodium bicarbonate, nitrate-nitrogen and phosphate-phosphorus was not less than 4.0 $\text{mg}\cdot\text{L}^{-1}$, 40 $\text{mg}\cdot\text{L}^{-1}$ and 10 $\text{mg}\cdot\text{L}^{-1}$, respectively. No growth inhibition was observed when the culturing medium contained nitrite nitrogen of 0–120 $\text{mg}\cdot\text{L}^{-1}$ and ammonium nitrogen of below 20 $\text{mg}\cdot\text{L}^{-1}$. Five runs of semi-continuous cultivation with DPW as the culturing medium in a 250 L raceway pond showed that the biomass yield in a 9-day semi-continuous culturing was up to 45.2–64.7 $\text{g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, higher than the yields obtained by other researchers, and the crude protein content in biomass was over 50%, meeting the national animal feed grade standard. Total nitrogen (TN) and total phosphorus (TP) were removed from DPW at a rate of 10.9–14.0 $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ and 1.3–1.8 $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$, respectively. The mass balance revealed that 80–93% of TN and 84–98% of TP reduced from DPW were converted to *A. platensis* biomass.

**Key words** | *A. platensis*, digested piggery wastewater, feed grade protein, nitrogen, phosphorus

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## INTRODUCTION

Pig husbandry is a major industry in many rural areas of China. The national stocks of pigs are about 500 million, causing an annual discharge of more than 650 million tons of piggery wastewater that contains at least 27,000,000 tons of chemical oxygen demand (COD), 13,500,000 tons of total nitrogen (TN) and 1,440,000 tons of total phosphorus (TP) (Qiu *et al.* 2013). Technologies are urgently needed for the efficient removal of nitrogen and phosphorus from piggery wastewater. However, the commonly used biological treatment methods, such as sequencing batch reactor (SBR) or anaerobic-anoxic-oxic ($A^2O$) processes, require high operation costs, which are not affordable by farmers in rural areas.

*Arthrospira*, an ancient microalgae, rich in protein, amino acids, vitamins and bioactive components, has been acknowledged worldwide as an effective immune-enhancer and feed additive that can prevent disease, promote animal growth and improve the appearance of animal products and nutritional values (Ajayan *et al.* 2012). In recent years, *Arthrospira* has been cultivated globally on a large scale due to its fast growth, high photosynthetic efficiency, and high economic profits. However, *Arthrospira* biomass is mainly used for food, although the potential demand for animal feed grade *Arthrospira* biomass is very high. The main reason is that the conventional cultivation methods require the addition of large amounts of sodium bicarbonate and nutrient fertilizers, which makes the operation costs too high to be affordable by farms for animal feed use (Duerr *et al.* 1997).

Digested piggery wastewater (DPW) contains not only high concentrations of nutrients (nitrogen, phosphorus and potassium), but it is also rich in 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) (Xia 2011). Using DPW to cultivate *Arthrospira* not only removes nitrogen and phosphorus from DPW, but also reduces the cultivation cost of *Arthrospira*. The harvested algae powder can be applied as animal feed, given that the quality reaches the national standard for animal feed. Canizares & Dominguez (1993) cultured *Arthrospira* in aerobically biodegraded piggery wastewater, and obtained algae

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powder containing 36% protein. Li *et al.* (2011) cultured *Arthrospira* in 50 mL of DPW with 10% Zarrouk medium as the supplement, and obtained an *Arthrospira* powder yield rate of 7.5 $g \cdot m^{-2} \cdot d^{-1}$ with a protein content of 30–40%. Chung *et al.* (1978) cultured *Arthrospira* in Erlenmeyer flasks containing 10% DPW that was supplemented with sodium bicarbonate and other inorganic nutrients, and obtained an algae biomass of 5 $g \cdot m^{-2} \cdot d^{-1}$ with a protein content of 55–61%. Chaiklahan *et al.* (2010) cultured *Arthrospira* in an outdoor raceway pool with a capacity of 100 L which contained five times diluted DPW supplemented with 4.5 $g \cdot L^{-1}$ of sodium bicarbonate and 0.2 $g \cdot L^{-1}$ of nitrogen phosphorus compound fertilizer, and a biomass growth rate of 12 $g \cdot m^{-2} \cdot d^{-1}$ was achieved. The above studies suggest that it is possible to cultivate *Arthrospira* with DPW as the culturing medium; however, most of the studies used diluted DPW which was supplemented with nitrogen and phosphorus fertilizers. Moreover, the growth rate and protein content of *Arthrospira* cultured in DPW are lower than those when culturing in a Zarrouk medium.

In this study, an *Arthrospira platensis* strain ZJWST-S1, which is strongly adapted and resistant to high concentrations of DPW, was selected for cultivation in undiluted DPW. Its favorable requirement for carbon, nitrogen and phosphorus and its tolerance limits to ammonium and nitrite were confirmed by single factor analysis. Five runs of indoor raceway pond tests were then carried out, and the algae yield and water quality were studied.

## EXPERIMENTAL METHODS AND MATERIALS

DPW was taken from a large piggery farm in Jiaxing City, China. The wastewater was pretreated with iron-carbon internal electrolysis followed by an aerobic membrane bioreactor. The treated DPW contained 133 ± 28 $mg \cdot L^{-1}$ COD, 1031 ± 49 $mg \cdot L^{-1}$ TN, 6.1 ± 0.8 $mg \cdot L^{-1}$ ammonia nitrogen, 69 ± 11 $mg \cdot L^{-1}$ TP. This treated effluent was used for *Arthrospira* cultivation after pH was adjusted to 8.0 or higher with 0.1 $g \cdot L^{-1}$ sodium bicarbonate.

Local *A. platensis* ZJWST-S1 was isolated from a DPW storage pool in Jiaxing City, and inoculum enlargement was carried out in Zarrouk liquid medium (Zarrouk 1966). *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, DLMM S2 were the strains which are commonly used in China for commercial cultivation.

### Flask tests on growth behaviors and nutrient requirements

Flask tests were carried out in order to compare the growth behaviors of the local strain ZJWST-S1 with the three commercial strains Ns-90020, DLMM S6, DLMM S2, and to investigate the nutrient requirement of ZJWST-S1. The *A. platensis* strains were filtered through a 400 mesh filter, washed with a Zarrouk medium which did not contain nitrogen and phosphorus, and then inoculated into different types of culturing media or DPW in 250 mL Erlenmeyer flasks. The flasks were then placed in an illuminating incubator at 25 (±2) °C for 9 days. The photo-period was set at 12 h light (6000 ± 2000 lux) and 12 h dark. The solution was mixed manually once a day.

All experiments were carried out in triplicate. The influence of inorganic carbon, TN and TP on the growth of ZJWST-S1 was studied by adjusting the concentrations of $NaHCO_3$, $NaNO_3$ and $K_2HPO_4$ in the Zarrouk medium, respectively. The inhibition by ammonia and nitrite was investigated by dosing different concentrations of ammonium sulfate and sodium nitrite into the Zarrouk medium.

### Semi-continuous cultivation in a raceway pond

Five runs (Runs 1–5) of semi-continuous cultivation tests were carried out indoors in a raceway pond whose capacity was 250 L (1.8 m in length × 0.4 m in width × 0.3 m in depth). ZJWST-S1 in the logarithmic growth phase was inoculated into the pretreated, undiluted DPW and the initial $OD_{560}$ was approximately 0.25. Water temperature was maintained at 20–25 °C. The light intensity was 5,000 lux and the light-dark ratio was 15 h:9 h. The system was continuously stirred with a paddle agitator at 30 rpm/min. DPW in the pond was renewed by 1/5, 2/5, 3/5, 4/5 and 100% at the end of Runs 1–5 after 9 days of cultivation.

### Analytical items

*Arthrospira* cell concentration in the culturing medium was represented with the Chlorophyll *a* concentration, which was measured as the absorbance at 560 nm ($OD_{560}$) with a 722s spectrophotometer (Precision Instruments Co. Ltd, Shanghai, China). The dry weight of *Arthrospira* biomass was measured at the end of each run by collecting all the *Arthrospira* with double-layer filter paper and drying at 80 °C for 2–4 h. A linear relationship was derived between the dry weight and $OD_{560}$ ($R^2 > 0.997$). The crude protein content of algae (%) was determined by the Coomassie brilliant blue method (Sedmak & Grossberg 1977). Water quality was analyzed according to the standard methods (Standard Methods for Water and Wastewater Monitoring and Analysis 2002). Nitrogen and phosphorus contents in *Arthrospira* cells were estimated according to the method of Thorbergsdóttir & Gíslason (2004), who expressed *Arthrospira* cell components with a formula of $C_{106}H_{263}O_{110}N_{16}P$, hence the weight percentage of C, N and P being 35.8%, 6.3% and 0.87%, respectively. The conversion efficiency of TN or TP from DPW to *A. platensis* biomass synthesis was calculated as follows:

$$\text{Conversion efficiency of TN or TP } (\%) = \frac{\text{Net increase in dry weight of } \textit{Spirulina}}{\text{Net reduce of TN and TP from DPW}} \times \text{percentage of TN or TP in the cell of } \textit{Arthrospira}$$

## RESULTS AND DISCUSSION

### Growth of *A. platensis* in DPW and Zarrouk medium

The 9-day productivity of *A. platensis* ZJWST-S1, Ns-90020, DLMM S6 and DLMM S2 is compared in Figure 1. In the Zarrouk medium, the productivity of ZJWST-S1 was similar to that of DLMM S6 and DLMM S2, and much higher than that of Ns-90020. The crude protein contents in dry algal biomass were 62.8 ± 6% for ZJWST-S1, slightly higher than but not significantly different from those of DLMM S2 ($p = 0.123$)and DLMM S6 ($p = 0.135$), and lower than those of Ns-90020 ($p = 0.041$). As the medium was changed to DPW, only ZJWST-S1 had fast growth. DLMM S6 grew a little, while Ns-90020 and DLMM S2 did not grow at all. The yield of ZJWST-S1 in DPW was little different from that in a Zarrouk medium ($p = 0.396$). The crude protein content in dry biomass of ZJWST-S1 slightly decreased to 59.4 ± 2%, but was much higher than those of DLMM S6 (45.1 ± 5%).

### Growth of ZJWST-S1 under different nutrient conditions

Inorganic carbon, nitrogen and phosphorus are principal nutrients for algae growth. Single factor experiments were carried out with flask tests and the growth curves of ZJWST-S1 were studied in a Zarrouk medium whose carbon, nitrogen and phosphorus concentrations were adjusted by dosing different concentrations of sodium bicarbonate, $NaNO_3$ and $K_2HPO_4$, as shown in Figures 2–4.

Growth curves in Figure 2 show an initial lagging phase of 3 days in the test without sodium bicarbonate, but 2 days in other tests. ZJWST-S1 in the tests with sodium bicarbonate of 2.0 $g{\cdot}L^{-1}$ or less appeared yellowish green and the growth rate was significantly slower than in other tests. The 9-day productivity was greatly increased as the sodium bicarbonate concentration was increased from 2.0 to 4.0 $g{\cdot}L^{-1}$, but little increase was found with the further increase of sodium bicarbonate concentrations. The result was inconsistent with the literature (Hu *et al.* 2012), which also reported that *Arthrospira* can gradually adapt itself to low concentrations of inorganic carbon and was able to

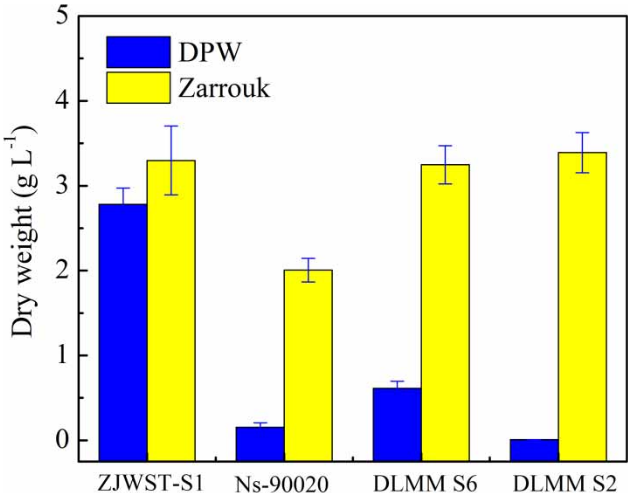

**Figure 1** | The productivity of different *Arthrospira* strains in Zarrouk and DPW medium after 9 days' cultivation.

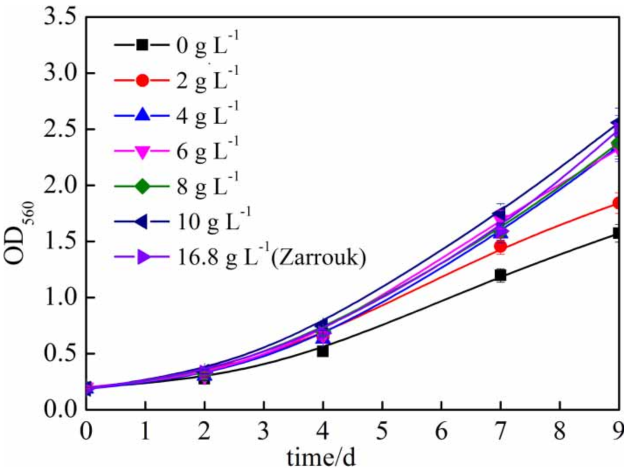

**Figure 2** | Growth curves of *Arthrospira* ZJWST-S1 in a Zarrouk medium with different concentrations of sodium bicarbonate.

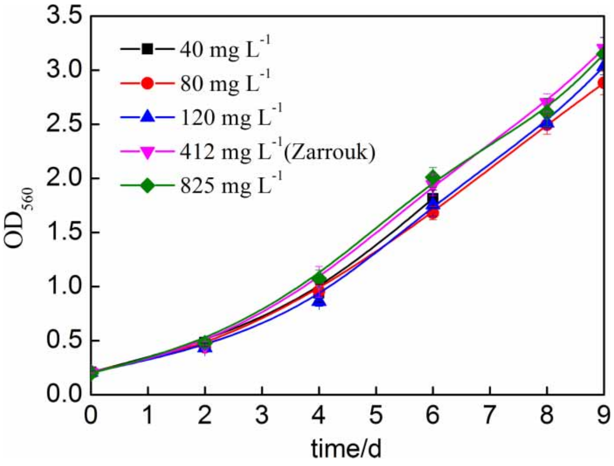

**Figure 3** | Growth curves of *Arthrospira* ZJWST-S1 in a Zarrouk medium with different concentrations of nitrate nitrogen.

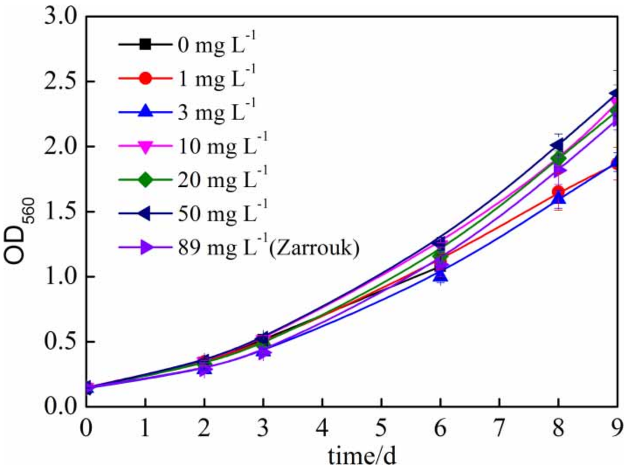

**Figure 4** | Growth curves of *Arthrospira* ZJWST-S1 in a Zarrouk medium with different concentrations of phosphorus.

achieve high productivity even if the dosage of sodium bicarbonate was reduced to one-quarter of the Zarrouk medium.

Growth curves in Figures 3 and 4 did not show significantly different growth rates among tests containing nitrogen of 40–825 mg·L$^{-1}$ and phosphorus of 10–89 mg·L$^{-1}$. However, ZJWST-S1 in the medium containing 0–3 mg·L$^{-1}$ phosphorus turned yellowish and settled to the bottom of the flasks. This suggests that the critical phosphorus concentration required for algal fast growth is between 3 and 10 mg·L$^{-1}$. Phosphorus concentration of 10 mg·L$^{-1}$ or above is of benefit for algal growth, however, the algal growth rate would not significantly increase with the increase in the phosphorus concentration. The same high grow rate of *Arthrospira* was also achieved by Shi & Chen (1989) in a medium with only one-sixteenth of the $K_2HPO_4$ dosage (TP of 5.6 mg/L) as in the Zarrouk medium. However, they found the growth rate of *Arthrospira* began to slow down after 5 days with the exhaustion of phosphorus.

Ammonium and nitrite are common inorganic nitrogens in DPW. They are considered to be toxic to algae growth, although traces of them may be beneficial (Markou & Georgakakis 2011). Nitrite and ammonium were supplemented into the Zarrouk medium, and their influence on the algal growth is shown in Figures 5 and 6. The results show that nitrite did not inhibit the growth of ZJWST-S1 in all tests. On the contrary, it seemed to slightly promote the growth, especially in the groups with nitrite nitrogen concentrations of 30–60 mg·L$^{-1}$ (Figure 5). The stimulation of nitrite on the growth of *A. platensis* was also reported by Markou & Georgakakis (2011). Although the algal growth was not affected by ammonia nitrogen at a

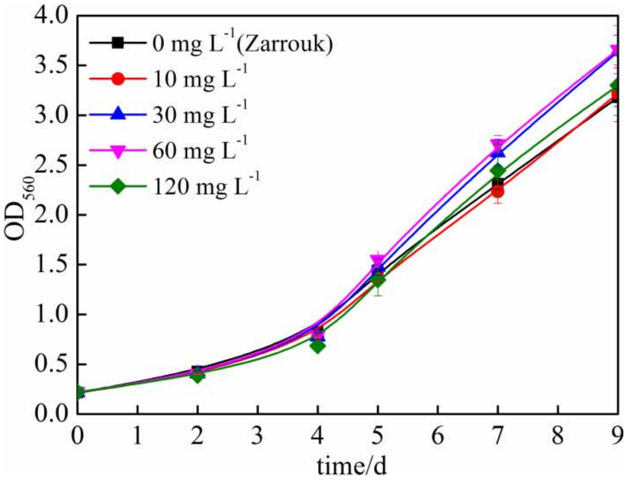

**Figure 5** | Growth curves of *Arthrospira* ZJWST-S1 in a Zarrouk medium with different concentrations of nitrite nitrogen.

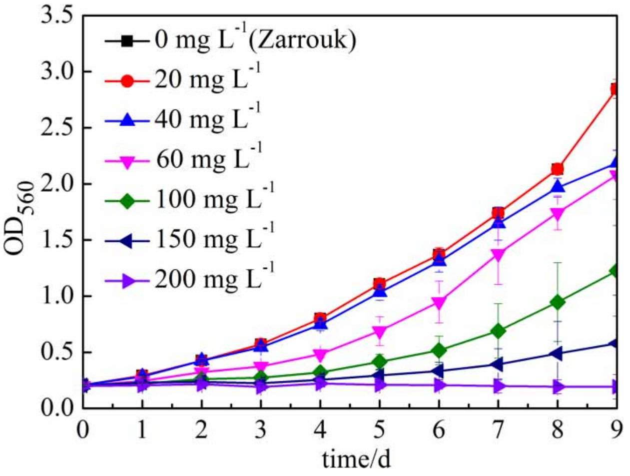

**Figure 6** | Growth curves of *Arthrospira* ZJWST-S1 in a Zarrouk medium with different concentrations of ammonia nitrogen.

concentration of 20 mg·L$^{-1}$, the growth rate slightly declined at a concentration of 40 mg·L$^{-1}$, and obviously reduced at 60 mg·L$^{-1}$ or above. The inhibition of ammonia nitrogen on the algal growth is inconsistent with the literature. Converti *et al.* (2006) reported that the maximum ammonia nitrogen concentration able to sustain the batch growth of *A. platensis* without inhibition was 1.7 mM (24 mg·L$^{-1}$), while 10 mM (140 mg·L$^{-1}$) significantly inhibited the algal growth. Soletto *et al.* (2005) mentioned that the optimum ammonia nitrogen concentration for the growth of *A. platensis* was 1.1 mM (15 mg·L$^{-1}$), while inhibition was observed at 1.7 mM (24 mg·L$^{-1}$).

## Raceway pond tests on productivity of ZJWST-S1 in DPW

As shown in Figure 7, during the five runs of raceway pond tests, *A. platensis* ZJWST-S1 in DPW showed logarithmic growth in the first 7 days and steady growth afterwards. The net productivity at Runs 1–5 by subtracting the initial inoculum amount was 64.7, 48.8, 45.2, 36.3 and 52.8 g·m$^{-2}$·d$^{-1}$, respectively, higher than 19.9 g·m$^{-2}$·d$^{-1}$ reported by Chaiklahan *et al.* (2010). In addition, the crude protein contents in biomass in the five runs were 63.2, 61.1, 59.7, 58.3 and 63.8%, respectively, higher than the requirement of not less than 50% in the National Animal Feed Grade Standard of *Spirulina* Powder (1998). Both the productivity and the protein content tended to decrease with the ongoing of the runs, which might be attributed to the accumulation of secondary metabolites in the pond (Keating 1977). A sudden increase in the productivity in Run 5 might be due to the fact that four-fifths of DPW was renewed, and the accumulated secondary metabolites were thereby much diluted.

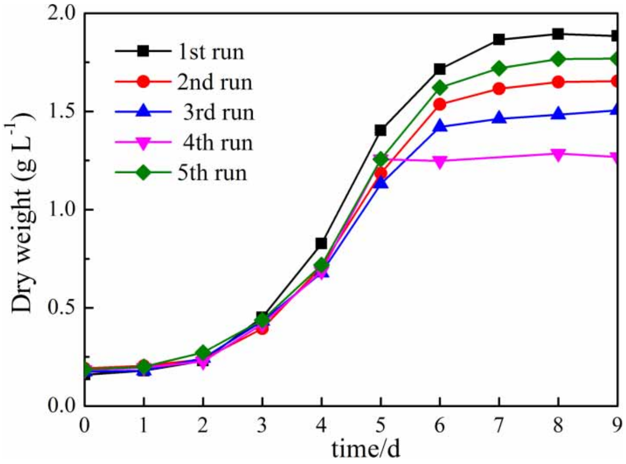

**Figure 7** | Biomass concentration profiles of *Arthrospira* ZJWST-S1 during semi-continuous culture in DPW.

## Nutrient removal from DPW and their conversion rates to *A. platensis* cells

Water quality parameters were measured before and after algae harvest at each run of the raceway pond test, as shown in Table 1. During each run for 9 days, 98–126 mg·L$^{-1}$ of TN and 12–16 mg·L$^{-1}$ of TP were removed from DPW. The daily removals of TN and TP were calculated as 10.9–14.0 mg·L$^{-2}$·d$^{-1}$ and 1.3–1.8 mg·L$^{-2}$·d$^{-1}$ on average, and the corresponding removal rates of TN and TP in DPW were, respectively, 9.4–11.6% and 19.5–22.2%. COD was almost reduced by 50%, perhaps due to the partial heterotrophic growth of *A. platensis* and the adsorption onto the algae surface. The decrease of COD was also observed by Mezzomo *et al.* (2010), who considered that the COD utilized by the heterotrophic growth of *A. platensis* should be small since no linear relationship could be derived between the decrease of COD and the growth of *A. platensis*.

The mass balance analysis reveals that 80–93% of TN and 84–98% of TP reduced from DPW were converted to *A. platensis* biomass. Namely, most of the TN and TP in DPW had been utilized by microalgae.

## CONCLUSIONS

The *A. platensis* strain ZJWST-S1 was able to proliferate quickly in undiluted DPW, and the protein content in the

**Table 1** | Water quality at each run of the raceway pond test

| | **Influent (mg·L$^{-1}$)** | | | | **Effluent (mg·L$^{-1}$)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Run** | **COD** | **Ammonia nitrogen** | **TN** | **TP** | **COD** | **Ammonia nitrogen** | **TN** | **TP** | **TN removal (%)** | **TP removal (%)** |
| 1 | 171 | 6.0 | 1087 | 74 | 97 | 5.0 | 961 | 58 | 11.6 | 21.6 |
| 2 | 118 | 5.4 | 999 | 63 | 52 | 4.2 | 887 | 49 | 11.2 | 22.2 |
| 3 | 97 | 5.3 | 965 | 54 | 48 | 4.9 | 858 | 42 | 11.1 | 22.2 |
| 4 | 133 | 6.2 | 1042 | 72 | 76 | 5.1 | 944 | 57 | 9.4 | 20.8 |
| 5 | 147 | 7.3 | 1062 | 82 | 92 | 6.7 | 954 | 66 | 10.2 | 19.5 |

algae biomass was high. Single factor experiments showed that the pre-treated DPW containing high concentrations of inorganic carbon, nitrogen and phosphorus can be used as a natural culturing medium for producing ZJWST-S1 at a low cost. Using DPW to culture ZJWST-S1 can not only help harvest high-profit animal feed grade proteins, but also helps remove nitrogen and phosphorus nutrients from DPW. The application of the technology would benefit sustainable development of the pig husbandry industry.

Compared to industrial wastewater, DPW contains little toxicants and is relatively eco-safe. However, exogenous chemicals, in particular heavy metals and antibiotics, are sometimes present in DPW since these chemicals are widely employed in intensive livestock farming as feed additives or therapeutic drugs to promote growth or prevent disease. These exogenous chemicals should be avoided, or removed, before DPW is used for algae cultivation. Moreover, suspended solids, bacteria and parasitic ovum in DPW should also be removed because these pollutants would contaminate algae and thereby decrease the quality of the algal products. Conventional biological treatment systems have limitations in the efficient removal of these contaminants. A membrane bioreactor, which combines membrane separation with biological wastewater treatment, is strongly recommended.

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