Can microalgae cultivated in modified mariculture wastewater become live feeds in aquaculture?

Lijie Zhang [a], Haiyan Pei [a, b, *]Liqun Jiang [a], Qingjie Hou [a], Zhigang Yang [a], Ze Yu [a], Xiaodong Wang [a],
[a] *School of Environmental Science and Engineering, Shandong University, 27 Shanda Nan Road, Jinan, 250100, China.*
[b] *Shandong Provincial Engineering Centre on Environmental Science and Technology, 17923 Jingshi Road, Jinan, 250061, China.*
Zhanglj1104@163.com

Introduction

Microalgae have great potential for the production of high amount of lipids, proteins, carbohydrates, pigments etc. (Ansari *et al*., 2015; Suganya *et al*., 2016). Thereby, microalgae are natural food of aquaculture ecosystem and widely used for larvae, crustacean and mollusks (Reitan *et al*., 1997), which is because microalgae based feed can provide proteins, essential amino acids, fatty acids, pigments etc. required for high quality aquaculture produce (Ansari *et al*., 2017).

Nevertheless, so far, live feed in aquaculture have mainly concentrated on normal medium-grown microalgae, which infers a long road ahead for aquaculture commercialization as first feeds of fish owing to the freshwater shortage. With the rapid development of aquaculture, huge amount of aquaculture wastewater brought several negative impacts to the environment and economics (Ansari *et al*., 2017). Aquaculture and microalgae could form a promising biorefinary where the wastewater generated in aquaculture and generated biomass can be directed towards the aquaculture feed production. Use of aquaculture wastewater minimizes the dependency of microalgae cultivation on fresh water and chemical nutrients. Due to low contents of nitrogen and phosphorus in aquaculture wastewater, this proposal needs a little help from nutrients addition. Two measures were taken: on the one hand, we add $NaNO_3$ & K_2HPO_4 to aquaculture wastewater, attaining the level in BG11 medium; on the other hand, monosodium glutamate (MSG) as a flavor enhancer is extensively used in food products. During MSG production, there is a residue after glutamate extraction and bacteria removal, and it is always rich in nitrogen and phosphorus, with TN of 62–72 g/L and TP of 657–686 mg/L. The residue (MSGR) may be an economical and acceptable nutrient source for microalgae biomass production from aquaculture wastewater.

Following from the aforementioned concerns, we chose mariculture wastewater as our source of water and minerals for algal biomass production, and MSGR and $NaNO_3$ & K_2HPO_4 as the source of macroelements (nitrogen, phosphorus) to culture *Chlorella sorokiniana* SDEC-18. The main goals in this study comprised: (1) observing the growth and biocomposition of microalgae in mariculture wastewater; (2) understanding the effects of nutrients addition on the growth and biocomposition of microalgae; (3) analyzing whether the nutritional value of the algae cultivated in modified mariculture wastewater could satisfy the recommendation of WHO/FAO.

Material and methods

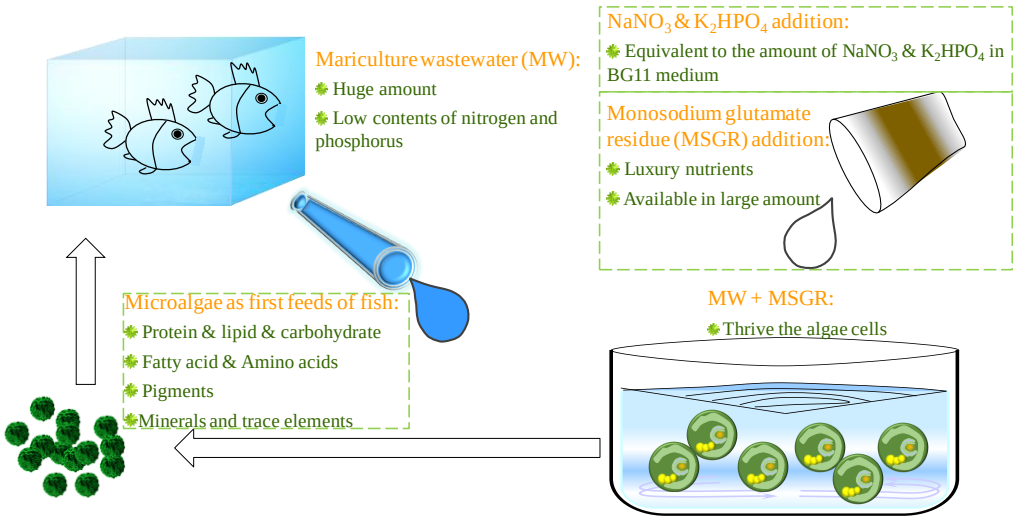

Fig.1. Abstract graph of the experimental design.

The freshwater microalgal strain used was *Chlorella sorokiniana* SDEC-18 isolated previously from the local environment, namely Quancheng Lake in Jinan, China (Song *et al*., 2014).

The mariculture wastewater (MW) was collected from The Manage Committee of Qingdao Aquatic Product's Germchit Industrialization Base of State Oceanic Science Research Center in Qingdao, China, and filtered through 0.22μm membranes before use.

The nutrient MSGR is a residue remaining after glutamate extraction and bacteria removal during MSG

production, and was supplied by Liangshan Linghua Gourmet Powder factory (Jining, China). MSGR was used directly for algal cultivation and presented the following characteristics: COD_{Cr} 18–22 g/L, BOD_5 10–11 g/L, TOC 6.9–7.0 g/L SO_4^{2-} 190–210 mg/L, TN 40–50 g/L, and TP 400–500 mg/L.

The experiment was carried out in triplicate in 1 L Erlenmeyer flasks. Monosodium glutamate residue (MSGR) of 1% and $NaNO_3$ & K_2HPO_4 equivalent to BG11 medium were applied to modify MW, as well as BG11 medium and pure MW were used as controls, simultaneously. Cells were cultivated at 25±1 ℃ under 81 μmol $m^{-2}s^{-1}$ of continuous illumination. Flasks were manually shaken thrice a day to avoid adherence of the cells to the surface of the flasks. The algae were grown over a 10-day period, and the physiological and biochemical changes in the morphology, biomass production, pigment contents and amino acids, fatty acids were evaluated. The whole process was shown in Fig. 1.

Results and discussion

As depicted in Fig. 2(a), microalgae nearly cannot grow in pure MW, but exhibited faster biomass accumulation in MW supplemented with nutrients, especially MSGR — namely 0.51 g/L — was 1.5 times higher than in BG11 medium. And algal growth in MW-containing medium suffered from retardation compared to that in BG11media, which ascribed to high salinity in MW (composed of brackish groundwater with salinity equivalent to seawater and bait as nutrient source). The cell size distribution is shown in Fig. 2(b), in MW-containing medium, relatively large cells gradually dominated the population, and markedly increase (p<0.05) in cell size was presented, even reaching 8.65 μm of mean diameter in MSGR addition medium. Besides chlorophyll a, carotenoids are another important pigmentation of microalgae for protecting the photosynthetic system. Used in the food industry, carotenoids are unique constituents of a healthy diet, playing an important role in improving antioxidant status and preventing cancers (Singh et al., 2017). MW-containing medium could enhance carotenoids production of microalgae (Fig. 2(c)), a non-enzymatic antioxidants, which could improve the fish quality. As shown in Fig. 2(d), the protein was markedly accumulated in MSGR addition medium, the productivity of 23.2 mg/L/d, nearly twice higher than in BG11 medium, which attributed to rich nitrogen and glutamic acid in MSGR.

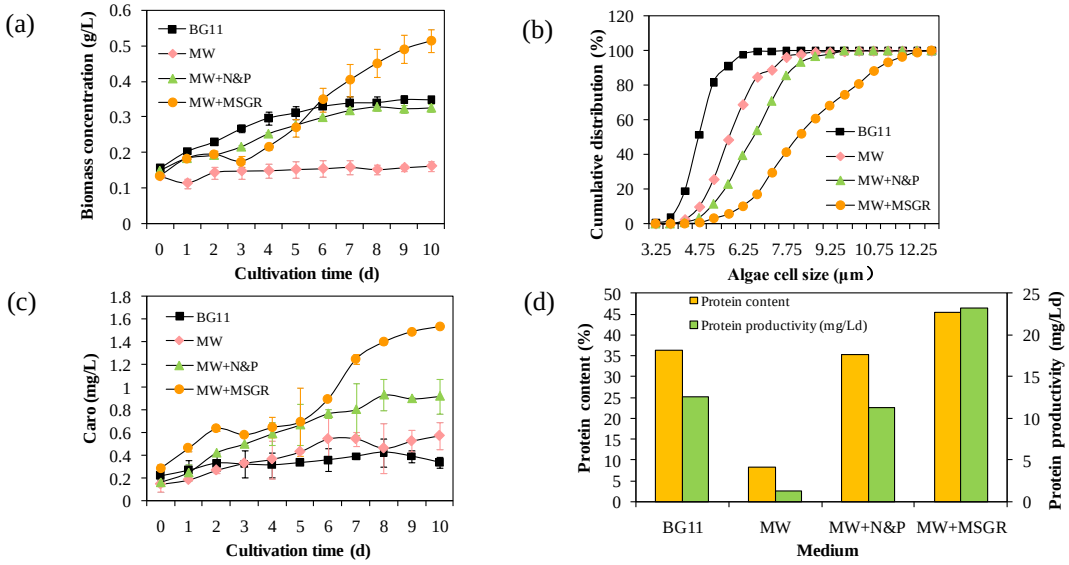

Fig.2. (a) Growth curves; (b) cell size distribution; (c) carotenoids content changes; (d) protein content & productivity of *Chlorella sorokiniana* SDEC-18 in BG11, MW, MW+N&P and MW+MSGR.

Conclusions

Nutrients addition really aids mariculture wastewater cultivation of microalgae for live feeds in aquaculture, especially monosodium glutamate residue (MSGR). MSGR addition improved the productivity and nutritional value of the algae, satisfying the recommendation of WHO/FAO, which built a recycling system to achieve freshwater saving and aquaculture commercialization, a win-win benefit in environment and economy.

References

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