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Influence of the physical properties of river sediments on the proliferation of Chlorella vulgaris

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ABSTRACT

Many physical factors affect the actions of microalgae besides chemical factors in the real environment. In this paper, the physical effects of river sediment on the growth of Chlorella vulgaris (Cv) are simulated by pure quartz sand. Three factors (particle size, solid-liquid ratio and flow velocity) and three levels of factorial experimental design are adopted. Orthogonal results reveal that, the physical effect intensity of each factor on algae is solid-liquid ratio > flow velocity > particle size. However, the inhibition of Cv proliferation becomes stronger with finer particle size at higher flow velocity and one-sized solid-liquid ratio.

KEY WORDS: Factorial designs; physical effect; river sediment; chlorella vulgaris.

INTRODUCTION

The discharge of large amounts of nitrogen (N) and phosphorus (P) leads to eutrophication in rivers and lakes, which is the main cause of algal blooms (Lake and Coolidge, 2007; Leaf and Chatterjee, 1999; Mayers and Flynn, 2014; Young and Morse, 1999). For the proliferation process of water body, most studies focus on the effects of chemical factors such as dissolved oxygen (DO), pH, nutrients and light. S.P.Singh et al. revealed that the optimal growth temperature and illumination of green algae is 20-30 °C and 33 - 400 $umol \cdot m^{-2} \cdot s^{-1}$, respectively (Singh and Singh, 2015). Kazem Darvish Bastamia et al. studied the removal efficiency of nutrient TP and TN in wastewater by Chlorella vulgaris (Cv) is 100% and 80%, respectively (Znad and Al Ketife, 2018).

In environmental applications, microalgae has been used in wastewater treatment because of good adsorption capacity for heavy metal ions (Ahmad and Bhat, 2018; Lalhmunsiama and Gupta, 2017; Wan Maznah and Al-Fawwaz, 2012). S.V. Gokhale et al. showed that the adsorption efficiency of 1g/L spirulina platensis reached 73.6% in 100ppm Cr (VI) solution (Gokhale and Jyoti 2008). In bio-energy, microalgae is utilized to product biodiesel due to the strong lipid accumulation ability (Lv and Cheng, 2010). Yusuf Chisti et al. discussed the competitiveness of biodiesel and mineral oil, and believed that microalgae has a broader outlook than mineral oil (Chisti, 2007).

However, hydrodynamic conditions play a key role in algal blooms(Long and Wu, 2011; Song and Zhang, 2018). There are some physical factors that affect the actions of microalgae in the actual environment, except for the influence of chemical factors (such as pH, DO and illumination) (Lecina and Nadal, 2016). For example, the large difference of water flow (solid-liquid ratio) and flow velocity between dry and wet season may change the efficiency of microbial utilization of nutrients. Meanwhile, these conditions can indirectly cause chemical interactions between microorganisms and water. QianYu et al. found that the eutrophic lake had a significant influence on the river downstream (Yu and Chen, 2017), which means that physical conditions (such as water flow rate and velocity) may have a significant impact on algae growth because flow velocity represents the power to supply nutrients. The change of water flux may cause pollution release from sediments, Lake, Bjorn A et al. investigated the relative importance of P sequestration by aluminum hydroxide ($Al(OH)_3(s)$), and ferric (oxy)hydroxide ($Fe(OH)_3(S)$) dissolution with subsequent P release (Lake et al., 2007), we think the solid-liquid ratio is one of the important factors in the above studies.

At present, chemical factors are concerned in all studies of microbe, while ignoring the direct effects of physical properties. In this study, quartz sand is used to simulate the physical effects of river sediment on the growth of Cv, orthogonal experiments is carried out and used to analyze the physical effects of flow rate, particle size and solid-liquid ratio.

EXPERMENTAL DESIGN

In batch experiments, three-level and three-factor (Flow rate, Particle size and Solid-liquid ratio, L9-3-3) are designed in the orthogonal test, and the experiment (3^3=27 groups, parallel samples 27 groups) is operated. The classical distribution of orthogonal point from L9-3-3 is shown in Table 1. Figure 1 shows a batch experiments.

The orthogonal experiment is divided into three batches (each batch contains three particle size and three solid-liquid ratios). The experimental procedure of a batch is as follows: pre-culture of microalgae is diluted to initial concentration by BG-11 medium (initial pH=7), the pure quartz sand of different particle size and quality is weighed into the 20 conical flasks (250 mL) for experimentation (9×2 (experimental group and parallel sample) + 2 (blank control) =20), then 200 mL suspension is added to each erlenmeyer flask, the new medium is incubated under constant temperature and light for a week (25°C, 5000lux, day/night of 12h/12h, shock speed is set in batches). The standard growth curve of microalgae is determined by the mounted bio-optical microscope (40×) and chlorophyll fluorometer (680 nm). Biomass (cell/mL) = $4.17\times10^5\times OD^{680}$. The value of the range and average is depicted via orthogonal analysis assistant (V3.1 professional edition).

Table 1 The classical distribution of orthogonal point from L9-3-3

Factor	Flow velocity (r/min)	Particle size (meshes)	Solid-liquid ratio (g/mL)
Test 1	50	5-7	0.025
Test 2	50	10-20	0.125
Test 3	50	30-40	0.25
Test 4	100	5-7	0.125
Test 5	100	10-20	0.25
Test 6	100	30-40	0.025
Test 7	150	5-7	0.25
Test 8	150	10-20	0.025
Test 9	150	30-40	0.125

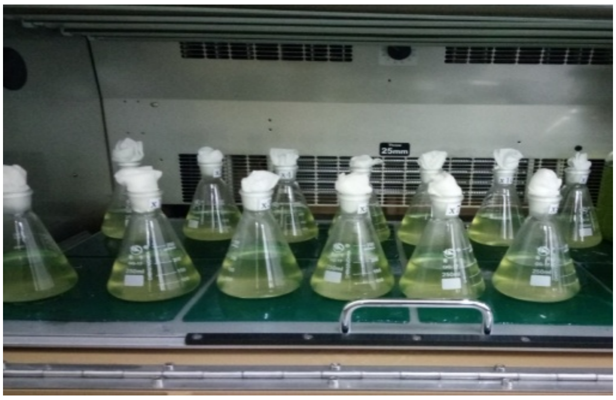

Figure 1 Batch experiment are done separately

RESULTS AND DISCUSSION

The Change of Biomass

The flow of water is thought of as a dynamic force that carries nutrients in rivers and lakes. Since the velocity of water can affect the migration efficiency of nutrients in water, this physical factor also directly or indirectly act on the growth of microalgae. The suspended Cv in the water is a dynamic process relative to the river sediment, so the absorption of nutrients or the proliferation of algae may be limited by the size of sediment particles. However, the degree to which solid-liquid ratio promotes or hinders the process of change to some extent.

The change of biomass in orthogonal experiments is shown in Figure 2 according to Table 1. Different combinations of rotating speed, particle size and solid-liquid ratio make significant difference in biomass production under the same chemical conditions (light, pH, initial biomass and nutrients). The combinated conditions of 150r/min, 5-7mesh and 0.25g/ml from c are most favorable for the growth of Cv, but this does not give us the best of all the conditions. Higher flow velocity is conducive to the growth of Cv in Figure 2, but the biomass is particularly weak at 30-40 mesh, 0.25g/mL solid-liquid ratio in Figure c, which also implies the potential effect of particle size and solid-liquid ratio on Cv production.

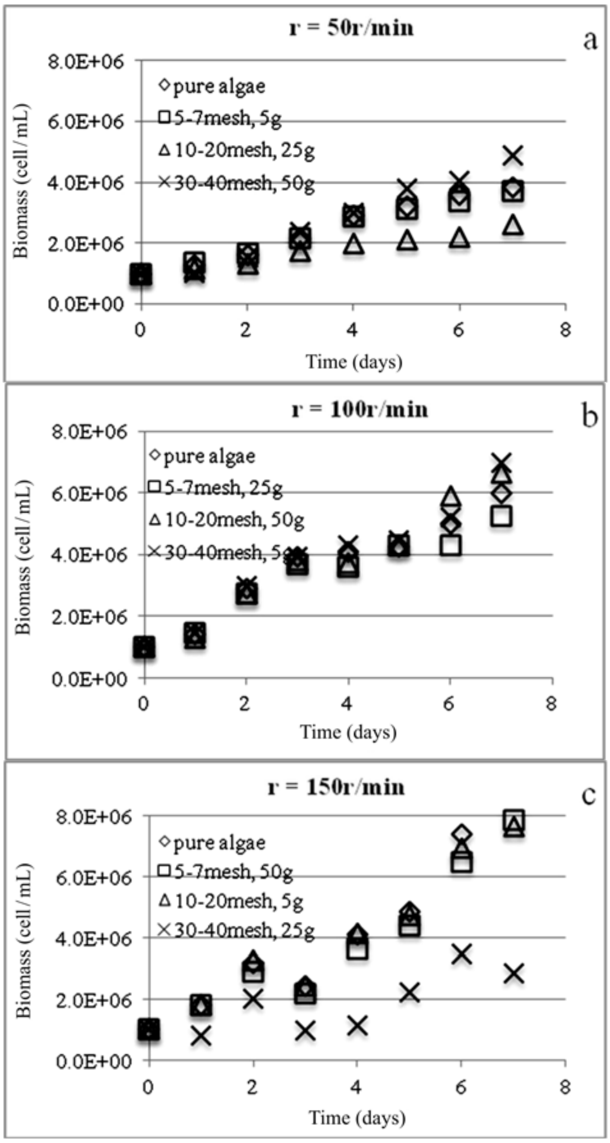

Figure 2 The change of biomass in orthogonal experiments (three-level and three-factor). The rotating speed of a, b and c are 50 r/min, 100 r/min and 150 r/min, respectively

The Range Analysis

The range(R_j) in the orthogonal analysis is the greatest indicator to analysis the degree of factorial influence, the factor becomes more important with the lager R_j. R_j was calculated by formula (1). However, the only drawback of the analysis is that it is not sure what's the cause of the gap(the indicator or experimental error). Here, some error can be removed by averaging the parallel samples in our work. Table 2 shows the average and range of orthogonal results, the importance of the three factors of algae growth is arranged as Solid-liquid ratio > Flow velocity > Particle size according to the value of R_j.

Table 2 The range and average of orthogonal results from L9-3-3

	Flow velocity	Particle size	Solid-liquid ratio
Average value K_1	3.74×10^6 ($\overline{K}_{11}$)	5.62×10^6 ($\overline{K}_{21}$)	6.12×10^6 ($\overline{K}_{31}$)
Average value K_2	6.31×10^6 ($\overline{K}_{12}$)	5.64×10^6 ($\overline{K}_{22}$)	3.58×10^6 ($\overline{K}_{32}$)
Average value K_3	6.14×10^6 ($\overline{K}_{13}$)	4.92×10^6 ($\overline{K}_{23}$)	6.48×10^6 ($\overline{K}_{33}$)
Range R_j	2.57×10^6	7.2×10^5	2.89×10^6

$$R_j = \max\,(\overline{K}_{j1}, \overline{K}_{j2}, \overline{K}_{j3}) - \min\,(\overline{K}_{j1}, \overline{K}_{j2}, \overline{K}_{j3}) \quad (1)$$

The optimal level (m) of factor (j) and the lever combination of various factors can be judged through $\overline{K}_{jm}$ calculation. $\overline{K}_{jm}$ is the sum of the test indicators corresponding to the j-th factor and m-th level. The optimal values are selected as $\overline{K}_{31}$, $\overline{K}_{12}$ and $\overline{K}_{33}$ at each level, so, it is determined that the best combination of the three factors for microalgae growth are 150r/min (Rotating speed), 10-20mesh (Particle size) and 0.25g/ml (Solid-liquid ratio).

The Effect of Single Factor

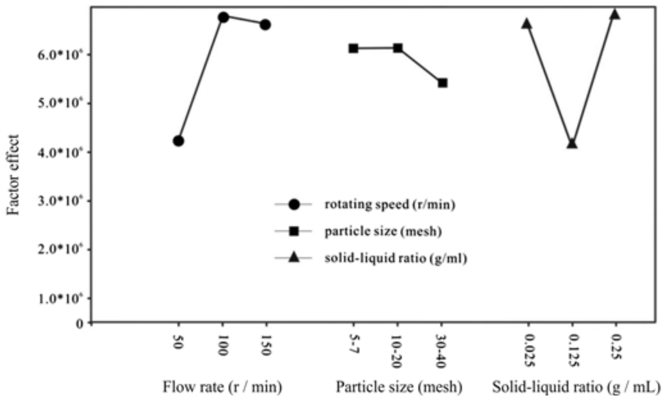

Fig 3 Factor effect curve of orthogonal results

The influence of single factor can be investigated by the effect curve in orthogonal experiments. The advantage of the effect curve is that the single factor is considered while offsetting the influence of other factors. The disadvantage is that it cannot find the best combination of multiple factors. Figure 3 is the effect curve of three factors in the orthogonal experiment, the effect of flow velocity, particle size and solid-liquid ratio on the growth of Cv can be observed separately. It can be seen that the influence of three-factors on the growth of Cv is completely different. The flow velocity reflects a strong positive effect from 50, 100 to 150r/min, because faster-growing Chlorella dominates in the relatively turbulent water (Yu and Liu, 2018). For the sand particle size, the finer the particle size tends to produce a negative effect, and the change is relatively weak. However, the solid-liquid ratio has an appropriate amount of influence with the strong positive and negative effects, and the solid-liquid ratio of 0.125 g/ml is most unfavorable for the growth of Cv.

VI CONCLUSION

Physical factors has obvious impacts on the growth of Cv. The importance of the three factors for Cv growth is arranged as solid-liquid ratio > flow velocity > particle size according to the range R_j in the orthogonal experiment. The change of biomass is more dramatic through the effects of sand particle size and solid-liquid ratio at higher flow rate. The growth of Cv at higher flow rates became more prosperous, the phenomenon may be caused by increased circulation of nutrient. However, biomass production is significantly blocked at 150r/min and over 10mesh (particle size < 830um), which may be due to sand retarding algae migration into overlying water and physical damage to Cv. According to the orthogonal analysis, the best combination of the three factors for microalgae growth is determined as 150r/min (Rotating speed), 10-20mesh (particle size) and 0.25g/ml (Solid-liquid ratio).

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