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Rapid optimization of process conditions for cultivation of transgenic *Laminaria japonica* gametophyte cells in a stirred-tank bioreactor

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Abstract

Batch cultivation for transgenic kelp gametophyte cells was investigated in an online controlled 5 L stirred-tank photo-bioreactor to rapidly optimize the process conditions by monitoring the rate of increase of pH. The transgenic kelp gametophytes with heterologous gene encoding hepatitis B surface antigen (*HBsAg*) could rapidly grow in the bioreactor. Optimal temperature and agitation rate for bioreactor cultivation of gametophytes were 15 °C and 200 rpm. Optimal incident light intensities depended on the initial cell densities.

Keywords: Gametophyte cells; *HBsAg*; *Laminaria japonica*; pH; Process conditions; Stirred-tank bioreactor

1. Introduction

Seaweeds, referred to as marine macro-algae, have been a rich and diverse source of pharmaceutical and biomedical compounds [1] and are now being developed by genetic engineering [2]. To avoid problems of contamination of culturing transgenic marine plants in open systems [3], transgenic seaweeds need to be cultured in a bioreactor. Process conditions can then be applied to maximizing biomass production and production of transgenic proteins. However, in these bioreactor experiments [4–7], process conditions were optimized by measuring dry cell weight or chlorophyll *a*, which not only need to kill the algae and consume much more time but also lacked the accuracy. Because seaweeds are photolithotrophic, the photosynthesis is the sole source of all of energies, which means that the photosynthesis activity directly influences the culture growth of seaweeds. As we know, the photosynthesis can consume CO_2 and produce O_2, so the rate of increase of pH in the culture can be a tool for monitoring the culture growth.

Our laboratory has successfully established a transformation model for *Laminaria japonica* [8,9] and produced transgenic kelp expressing *HBsAg* [10]. In this present work, we have chosen transgenic *L. japonica* for bioreactor operation and optimized the process conditions in the stirred-tank bioreactor by the rate of increase of pH which can be online recorded.

2. Materials and methods

2.1. Cell culture and maintenance

Transgenic gametophytes of kelp expressing *HBsAg*, produced by our laboratory [10], were used in all of the experiments in the present study. Cell suspensions were cultured in a modified seawater, containing 4 mM $NaHCO_3$, 1.5 mM $NaNO_3$ and 0.17 mM NaH_2PO_4, and maintained at 15 °C under a low light density of 18 $\mu mol\, m^{-2}\, s^{-1}$ provided by 28 W cool white fluorescent lamp with a photoperiod of 14-h light:10-h dark.

2.2. Stirred-tank bioreactor cultivation

An online stirred-tank bioreactor was used in all of culture experiments. The reactor consisted of a 5 L flat bottomed cylindrical glass vessel and a stainless steel head plate with several ports for holding the stirrer engine and rotating shaft, baffles, aeration gas inlet, gas outlet, inoculum pipe, sample pipe, harvesting pipe, dissolved oxygen electrode, pH electrode and temperature electrode. The illumination stage consisted of

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two light banks positioned on opposite sides of the glass reactor vessel. Each light bank consisted of two 20 W fluorescent tube lamps. Different incident light intensities were supplied by changing the distance between the lamps and the reactor vessel surface. Incident light intensities were measured in units of uE m^{-2} s^{-1} (μmol photon m^{-2} s^{-1}) with a LI-190SA quantum sensor (Beijing LiGaoTai Tech. Co. Ltd.). For the stirred-tank bioreactor incident light intensity measurement, the light sensor was positioned on the inside surface of the vessel to obtain the true incident light intensity to the culture. The pH was measured with a JK-F407 pH sensor (Shanghai JingKong Industry & Trade Co. Ltd.). The temperature in the bioreactor was monitored by a 3TC-100 temperature sensor (Shanghai TianDa Co. Ltd.) and auto-controlled by an online controlled heat and cooling system. Cooling water from a low-temperature circulator was pumped through the glass vessel jacket to maintain different temperatures. Air was pumped into the culture until the pH was fixed before each experiment. Then, the lamps were turned on and the pH was measured and recorded online for 1 h.

2.3. Dry matter measurement

Dry weight determined by filtering a 50.00 mL cell suspension sample through a pre-dried and pre-weighted filter paper with a pore size of 0.2 μm. The filter paper was then washed thoroughly with dematerialized water and dried for 48 h at 80 °C. The filter papers were then weighed, enabling determination of dry matter retained in the filter paper.

3. Results and discussion

3.1. Effect of temperature on the culture growth

Temperature is a key process factor in the design of algal photo-bioreactors [11]. In general, the effect of temperature on algal culture growth is very significant [12]. The effect of temperature on transgenic kelp gametophytes growth was conveniently assessed by the rate of increase of pH measurements as showed in Figs. 1 and 2. The optimal temperature of culture growth of transgenic kelp gametophyte cells was 15 °C not only at the low initial cell density of 135.6 mg DW L^{-1} but also at the high initial cell density of 273.6 mg DW L^{-1} when the incident light intensity was 138.5 uE m^{-2} s^{-1}. Only at 15 °C, the rate of increase of pH almost remained constant, which means the photosynthesis activity is the highest. However, when the gametophytes were cultivated at 10 or 20 °C, the rate of increase of pH decreased and even reached zero.

3.2. Effect of incident light intensity on the culture growth

Incident light intensity is an important process variable for photolithotrophic culture growth because it can provide the necessary energy for algal photosynthesis. Optimal incident light intensity was extremely relative to the initial cell density as showed in Figs. 3 and 4. At the high initial cell density of 273.6 mg DW L^{-1}, the culture was light limited when it was

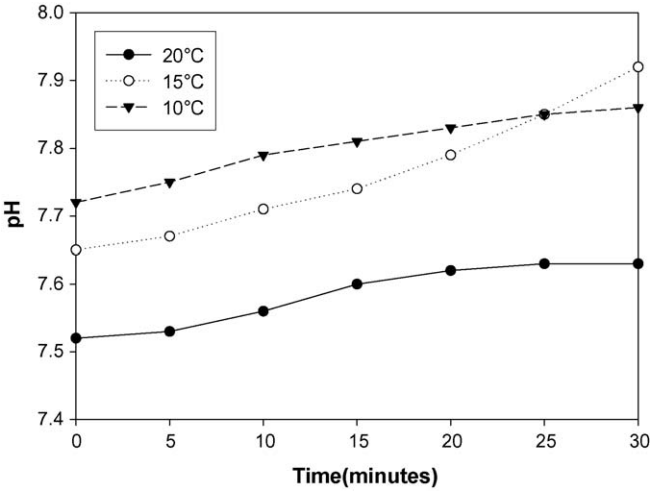

Fig. 1. Effect of different temperatures on the pH at the initial cell density of transgenic *Laminaria japonica* gametophyte cells (135.6 mg DW L^{-1}).

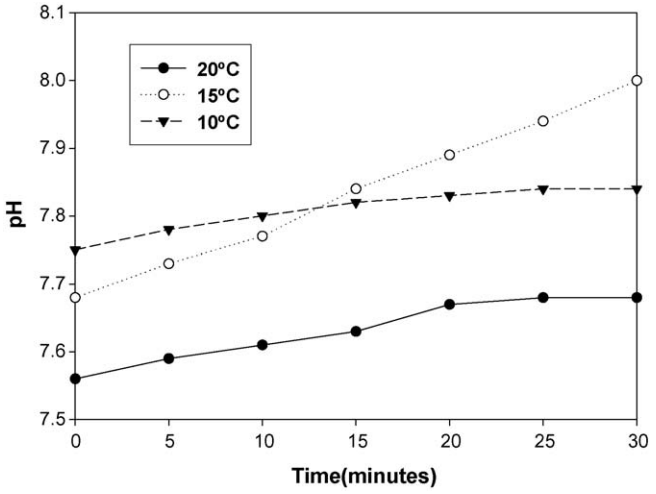

Fig. 2. Effect of different temperatures on the pH at high initial cell density of transgenic *Laminaria japonica* gametophyte cells (273.6 mg DW L^{-1}).

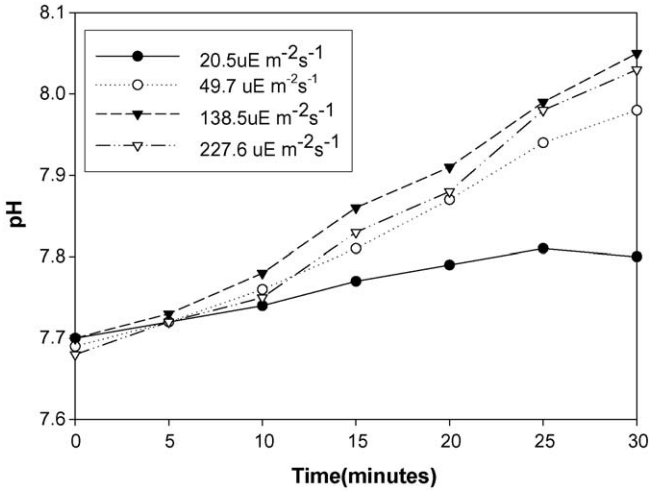

Fig. 3. Effect of incident light intensity on the pH at the low initial cell density of transgenic *Laminaria japonica* gametophyte cells (135.6 mg DW L^{-1}).

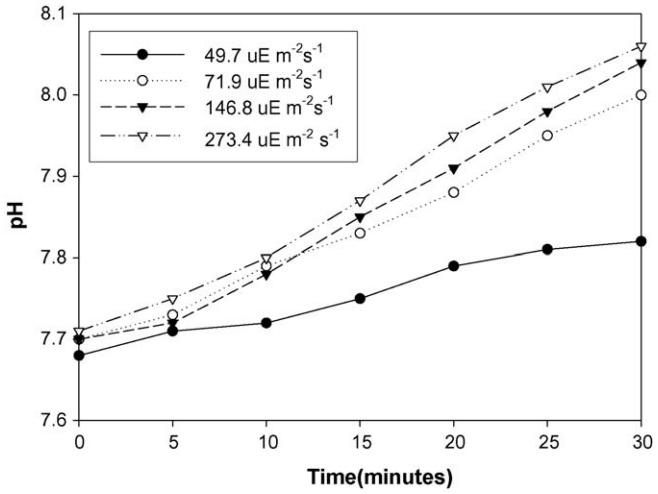

Fig. 4. Effect of incident light intensity on the pH at the high initial cell density of transgenic *Laminaria japonica* gametophyte cells (273.6 mg DW L^{-1}).

exposed to a high incident light intensity of 49.7 uE m^{-2} s^{-1}, which did not limit the culture at the low initial cell density of 135.6 mg DW L^{-1}. When the initial cell density is 135.6 mg DW L^{-1}, the culture was light limited when it was exposed to a low incident light intensity of 20.5 uE m^{-2} s^{-1}. The rate of increase of pH obviously increased with increasing the incident light intensity from 49.7 to 227.6 uE m^{-2} s^{-1} (Fig. 3) and from 71.9 to 273.4 uE m^{-2} s^{-1} (Fig. 4). So in a word, optimal incident light intensity can be reached only at a given initial cell density to maximize the culture growth.

3.3. Effect of agitation rate on the culture growth

It is very clear that algal cells, particularly filamentary cells, may be affected by the shear rate in the surrounding liquid. So agitation rate is an extremely important parameter for bioreactor cultivation of transgenic kelp gametophytes. Optimal agitation rates can produce an excellent liquid circulation pattern, which maintains a uniform distribution of macro-algal cells within liquid phase of the stirred-tank bioreactor and provides all the cells with an even exposure to the mean light intensity and necessary nutrients in the culture. In this study, cultivation experiments were carried out at agitation rate ranging from 100 to 250 rpm at a constant incident light intensity of 138.5 uE m^{-2} s^{-1} and an initial cell density of 135.6 mg DW L^{-1}. Fig. 5 showed the rate of increase of pH changed with different agitation rates. The optimal agitation rate was 200 rpm at given culture conditions. At the agitation rates below or above 200 rpm, the corresponding rates of increase of pH are relatively lower than that of 200 rpm. High agitation rate can create excessive turbulence and promote hydrodynamic shear damage of the cells.

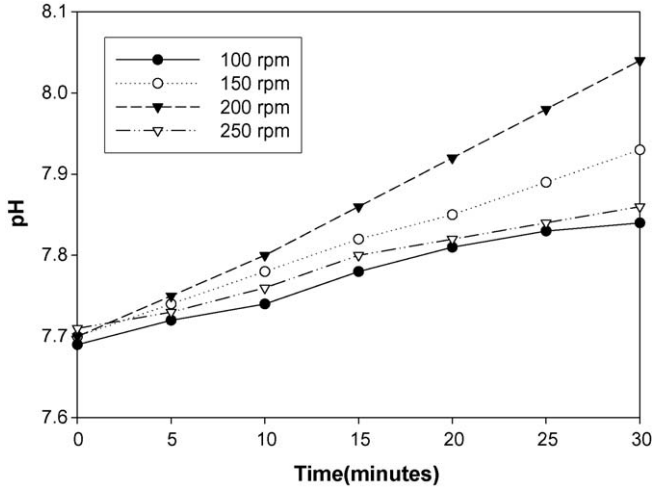

Fig. 5. Effect of the agitation rate on the pH when transgenic *Laminaria japonica* gametophyte cells were cultivated (temperature, 15 °C; incident light intensity, 138.5 uE m^{-2} s^{-1}; initial cell density, 135.6 mg DW L^{-1}).

4. Conclusions

Batch cultivation of transgenic *L. japonica* gametophyte cells was feasible in a stirred-tank photo-bioreactor. Several important process conditions can be optimized rapidly by measuring the pH online. The method is more sensitive and quicker than the commonly used growth parameters such as dry weight, chlorophyll *a* concentration. Optimal temperature and agitation rate for gametophytes growth were 15 °C and 200 rpm. However, optimal incident light intensities varied with different initial cell density cultures. High incident light intensities can be applied to high initial cell density culture. The study is valuable for the development of bioprocess engineering of cell cultures for transgenic seaweeds.

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