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Principles for Efficient Utilization of Light for Mass Production of Photoautotrophic Microorganisms

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ABSTRACT

Outdoor production of microalgae could be set on a sound industrial basis if solar energy were utilized at a much higher efficiency than presently obtained. Many types of photobioreactors have been developed in the past in an attempt to answer this challenge, but their photosynthetic efficiency has been rather similar to the basically inefficient open raceway commonly used today. Efficient utilization of the oversaturating solar energy flux mandates that reactors should have a narrow lightpath to facilitate ultra-high cell densities, be maximally exposed to sunshine, and have an efficient mixing system to create strong turbulent streaming to affect dark–light cycles of the highest possible frequency.

Index Entries: Photoautotrophs; mass production; photobioreactors; ultra-high cell density; mixing rate.

INTRODUCTION

A central issue in mass cultivation of photoautotrophic microorganisms concerns the mode of cultivation by which to utilize light energy for growth most effectively. The concept of using sunlight energy to mass-produce microalgae for various economic purposes is based on a premise that microalgae may be so cultured as to be limited by light only. Microalgae, however, are usually light-saturated at 5–10% of the solar energy flux available in midday, and therefore, abundant solar light can be only partially used for the production of biomass. Several theoretical approaches have been suggested to address this difficulty, e.g., Kok and

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Van Dorschot *(1)*, who suggested improving outdoor productivity by using strains of algae with a higher capacity to use strong irradiance for photosynthesis. More recently, Sukenik et al. *(2)*, following this line of thought, suggested algae should be improved to increase their photosynthetic capacity at light saturation by amplification of the carboxylation enzyme relative to the electron transport complexes. Phillips and Myers *(3)* suggested a different approach. Observing that light of high intensity may be used with higher efficiency if presented in short flashes separated by long dark periods, they proposed that utilization of high-intensity light would be enhanced by inducing turbulent streaming in culture suspension. They showed that a dense algal culture in sunlight exhibited a significant increase in growth when the algal cells were moved in and out of the high light intensity region at the front surface, in rates that produced flash times of 1–100 ms *(3)*.

Thus far, the practical expression of the principles governing the utilization of sunlight for photoproduction of algal mass has been the open raceway, which has become the major reactor available for mass production of microalgae outdoors. This device has both enhanced and impeded the development of algal biotechnology *(4)*, as shall be elucidated in what follows. In this work, we present principles by which to utilize high-intensity light, such as sunlight, in high efficiency.

MATERIALS AND METHODS

Microorganism

The cyanobacteria *Spirulina platensis* was grown in Zarouk medium *(5)* in which the $NaNO_3$ concentration was raised to 5.0 gL^{-1}. Culture temperature was 35 °C and pH was 9.5. Culture supernatant was replaced with fresh growth medium once a day by filtering the algal suspension through a 300-mesh screen.

Photobioreactor

A flat-plate bioreactor made of glass consisting of 2.6- or 1.3-cm wide flat tanks equipped with a perforated tube extending along the bottom through which a stream of compressed air was passed to affect stirring. The tanks were immersed in a water jacket for temperature regulation. The total illuminated surface of each tank was 0.1 m^2, and mixing and CO_2 were provided by continuous supply of air enriched with 2% CO_2 injected through the perforated tube. Turbulent flow was controlled by adjusting the rate of air passing through a gas flow meter, being expressed in terms of L air/L culture suspension/min (L L^{-1} min^{-1}). Illumination was provided by 1500-W halogen lamps, and light intensity was modified by manipulating the distance between the light source and the reactor.

Measurements and Analytical Methods

Output rate of biomass was estimated by measuring changes in biomass concentration at 4-h intervals as reflected in the dry weight, a procedure repeated at least four times. PFD was measured with a Li-Cor model Li-185A, and the oxygen production rate (OPR) was measured according to Guterman et al. *(6)*. Dry weight was determined in duplicates using 5-mL samples *(7)*.

RESULTS AND DISCUSSION

Our early experimentations in outdoor cultures were all carried out in open raceways—shallow pans with a divider in the middle to form a raceway in which the algal suspension is caused to flow at 20–40 cm $\cdot$ s^{-1} by a paddle wheel. This reactor requires simple technology to construct and to maintain, but does not meet the major requirements for efficient utilization of light: its water level must be maintained at a height of 12–15 cm, resulting in very dilute (e.g., a few hundred mg of dry wt/L) algal suspensions. Also, the paddle wheel cannot practically affect in large areas the extent of turbulent streaming required to create a high frequency of the dark/light cycle algal cells undergo when growth is light-limited. It is a particularly poor device for light utilization in winter owing to lack of temperature control *(8)*. As a result of all its drawbacks, the open raceway yields low outputs of algal mass (e.g., 20–40 t $\cdot$ ha^{-1} $\cdot$ y^{-1}) and requires handling very large amounts of algal suspension, which often become readily contaminated as well as increasing the cost of harvesting. The inadequacy of the open raceway prompted research to develop more efficient modes of production with which to facilitate a more effective use of the high photon flux density (PFD) existing outdoors. In continuous cultures, productivity (P) at steady state is the product of uxv, u representing the specific growth rate, x the concentration of algal mass, and v the culture volume. Therefore, the only practical method by which to increase P per unit area is to increase x without lowering u as a result of the enhancement in mutual shading and the ensuing rise in light limitation. We suggest achieving this by employing photobioreactors in which the lightpath has been greatly reduced and optimal cell concentrations (defined as that algal density that would result in the highest output rates under the given environmental circumstances) have been greatly increased, resulting in higher output rates (Fig. 1). Many enclosed photobioreactors facilitate an increase in cell concentration of up to twice one order of magnitude compared with the open raceway. Most of these new devices, however, have not increased the efficiency by which light is utilized for production of chemical energy. We suggest this failure stems from inadequate optimization of the short lightpath in relation to cell density and the extent of mixing. In what follows, these basic relationships will be examined in some detail.

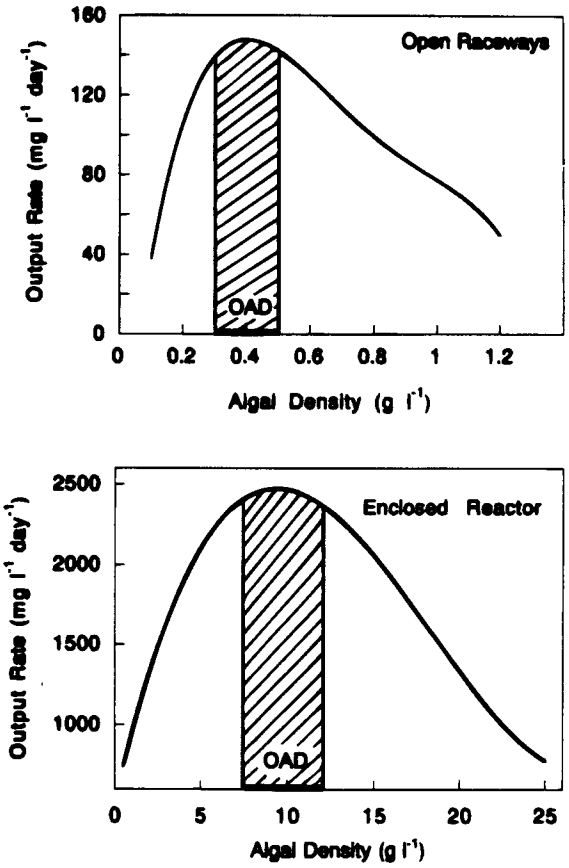

Fig. 1. The optimal algal density and output rates in an open raceway and an enclosed photobioreactor.

The interrelationships between the length of the lightpath and the optimal algal density in a photoautotrophic culture is shown in Fig. 2. As the lightpath is reduced, the optimal density increases because of the exponential increase in the availability of light to the cells in the culture. In addition, Hu et al. *(9)* discovered recently that the areal output rate also increases with reduction in the lightpath (Fig. 2), indicating that as the lightpath is reduced, photosynthetic efficiency increases, reflecting a more efficient utilization of the light source.

The other important mode by which the efficiency of light utilization is increased concerns the rate of mixing of the culture *(10)*. This is clearly evident in Fig. 3, describing the relationships between the population density and the output rate as affected by varying rates of mixing, under a relatively "low-" and "high-"incident light.

A low mixing rate (0.6 L air/L algal suspension/min) in low light resulted in an optimal algal density (OAD) of ca. 2 g/L^{-1}. As the rate of mixing was increased, the OAD shifted up (to ca. 5 g/L^{-1}), and as appro-

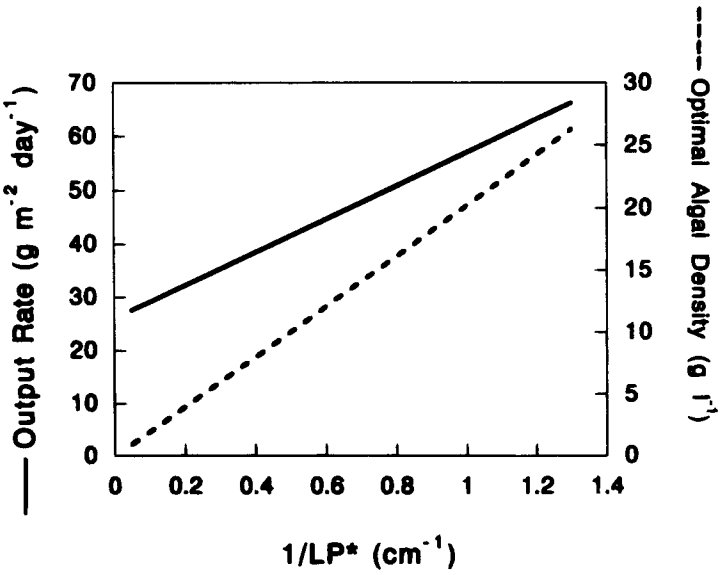

Fig. 2. The optimal algal density and output rate as affected by the length of the lightpath.

priate, the output rate increased significantly from 70 (at the minimal mixing rate) to 100 mg dry wt/L^{-1}/h^{-1} *(10)*. Two aspects concerning the relationship between the output rate and cell density at this low light intensity deserve attention: when cell density was below 2 g/L, there was no difference in output rate in response to a wide range of mixing rates (i.e., 0.6–4.2 $L/L^{-1}/min^{-1}$), indicating the rate of mixing was not limiting productivity under these conditions. Indeed, the magnitude of the effect exerted by mixing was strictly dependent on the strength of the light source as well as on culture density. Thus, as the PFD was increased to 1800 $\mu mol/m^{-2}/s^{-1}$ (the order of magnitude of the energy level existing outdoors at noon), the output rates of biomass obtained at this energy flux indicated a sensitive response to the rate of mixing, an increase in aeration rate from 0.6–4.2 L/L^{-1} resulting in doubling the output rate. A further increase in aeration rate to 6.3 $L/L^{-1}/min^{-1}$ was harmful *(10)*. Clearly, the higher the intensity of the light source, the higher the optimal population density would become and the more significant the degree to which the rate of mixing affects the output rate would be. The role of stirring in affecting maximal light utilization is evidenced from monitoring the photosynthetic efficiency (PE): First, mutual shading becomes ever more severe as the optimal population density is increased in adjustment to the reduction in lightpath. Second, assuming the nutritional requirements are satisfied and the environmental conditions optimized, the light regime to which the individual cells are exposed becomes the predominant parameter affecting productivity in cultures of

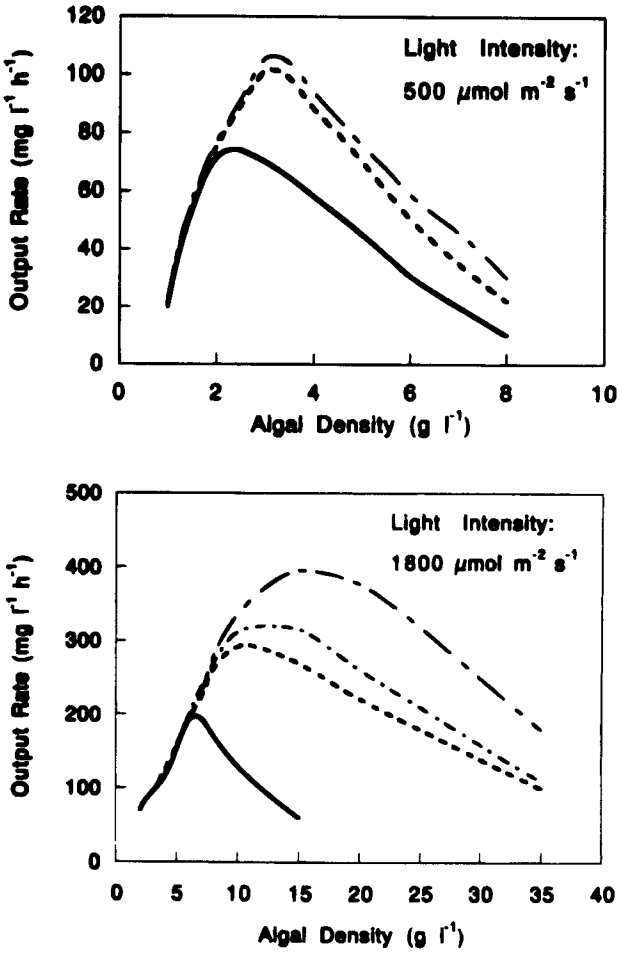

Fig. 3. Interrelationships among light intensity, algal density, and output rate as affected by the rate of mixing.

ultra-high cell densities. The light regime for the average cell in the culture is governed by several factors, among which are the intensity of irradiance at the reactor surface and the average duration of exposure of the cells to the photic as well as the dark volumes of the reactor, i.e., the frequency of fluctuating between these zones, which creates "light–dark cycles" (L–D cycle), well analyzed by Terry *(11)*. The frequency of the L–D cycle to which cells in a photobioreactor are exposed is a function of the lightpath across which axis the algal cells are moved back and forth from the lit to the dark volume in the reactor, as well as the extent of turbulence, which affects the rate of such movement. The estimated average duration of the L–D cycle at a rate of flow of ca. 250 mm/s^{-1} in relation to the length of the lightpath is portrayed in Fig. 4, indicating the smaller the lightpath (i.e., the width of the reactor), the higher the frequency of the L–D cycle.

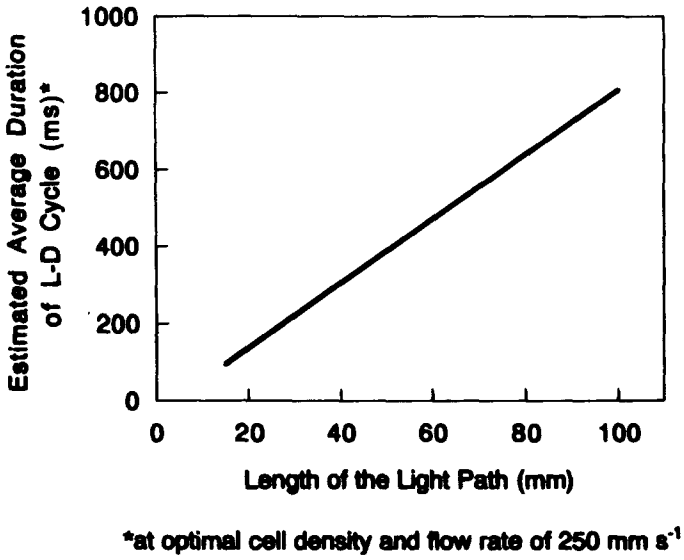

Fig. 4. The relationship between the length of the lightpath and the L–D cycle.

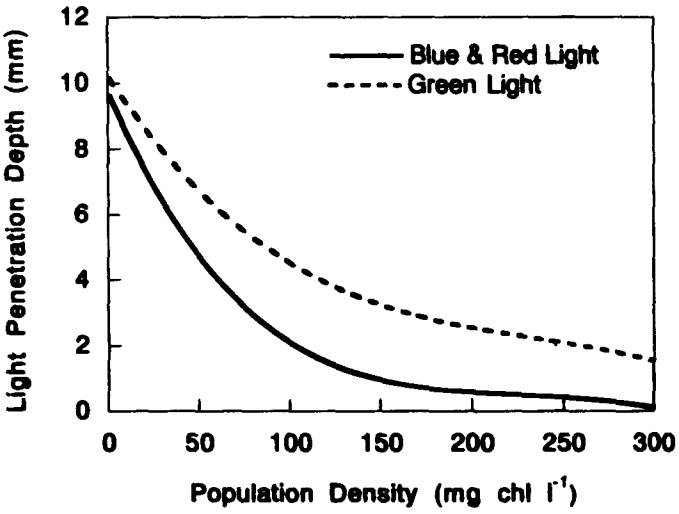

Fig. 5. Blue and red, and green light penetration into the algal suspension as affected by the population density.

The rate of flow of the algal suspension, however, has no effect on the ratio between the duration of the light and that of the dark phase in the L–D cycle. This ratio is determined only by the relative width of the photic zone as determined by the intensity of the light source and the population density. Gitelson et al. *(12)* have measured the penetration depth of light energy in the range of photosynthetic active radiation (PAR) in relation to algal density (expressed in mg chlorophyll; Fig. 5). Light penetration depth (defined as the depth at which down-welling irradiance is decreased to

10% of incident irradiance) differed for blue and red lights compared with green light (Fig. 5), highlighting the complexity of the photic volume in ultra-high-density cultures. Nevertheless, the general relationship between light penetration depth and population density is such that light penetration (and hence the photic volume) are strictly dependent on the population density. In ultra-high population densities, the photic zone may be ca. 1–4 mm deep in the flat plate reactors used in this study. When light was irradiated on one reactor surface only, the photic volume occupied up to 10% of the total reactor volume. Therefore, the ratio of the light to the dark phase in the L–D cycle would be ca. 10, found by Kok *(13)* as well as by Phillips and Myers *(3)* to be conducive to efficient utilization of light. In reactors with a lightpath of 15–25 mm, the frequency of the L–D cycles under our experimental conditions was roughly estimated to range between 100 and 200 ms. The basic premise is that the higher the frequency in which cells in a severely light-limited culture are exposed to short pulses of light, the more efficient the utilization of a high flux of over saturating light would be. Surprisingly perhaps, the strategy by which to utilize the high PFD existing outdoors at the highest efficiency is by "diluting" it, converting in effect direct beam to diffuse light. Such "conversion" is affected by a method developed by Pulz *(14)*. Accordingly, flat plate panels are placed outdoors in close proximity to one another, resulting in a large algal volume spread, in effect, over a small ground area. Obviously, light available for the culture in such mutually shading plates will be essentially low-energy diffuse light. Therefore, the optimal population density in such plates would necessarily be greatly reduced to ca. 1/5 or less of that maintained in flat plates exposed to direct beam radiation. Since, however, photosynthetic efficiency involved in utilizing low-energy light could be as much as five times higher than that of the high-energy rate provided by direct beam radiation, the output rate per ground area of such a system would be the highest attainable, yielding the highest efficiency possible for strong light utilization (Fig. 6). Record areal output rates reflecting highest PE, however, do not impart economic significance on this system: The volumetric yield (i.e., the amount of cell mass per unit of reactor volume) is necessarily much reduced in such reactor arrangement, and the capital investment involved in stacking expensive photobioreactor hardware to yield low volumetric yields represents a financial burden that seems to counteract the mere benefit of utilizing PAR in a most efficient manner. Indeed, economic constraints in microalgal biotechnology favor high volumetric yields, reflecting the advantage of a high photobioreactor efficiency in terms of output rate per unit reactor volume.

We suggest that a promising method for effective utilization of solar energy outdoors is to focus on efficient distribution of high light flux to the individual cells in ultra-high-density cultures, rather than on strategies based on spatial dilution of direct beam. We place this assertion on the basis of our results obtained by growing *S. plantensis* in flat plate reactors

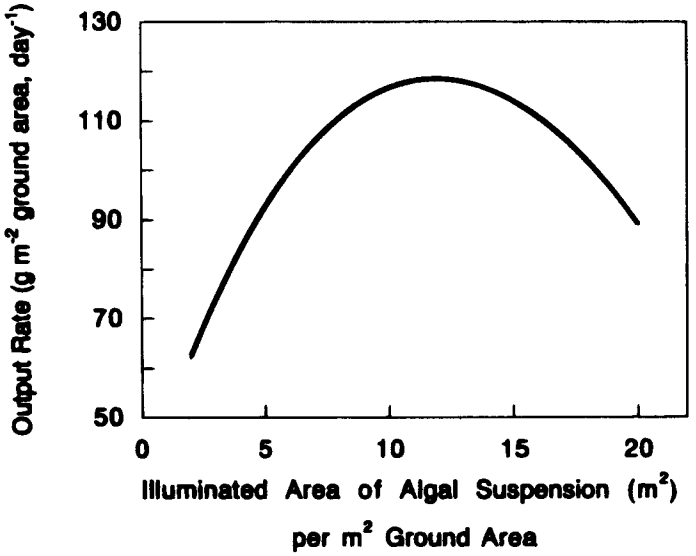

Fig. 6. The areal output rate as related to the total surface area of cultures in vertical flat plate reactors placed varying distances apart.

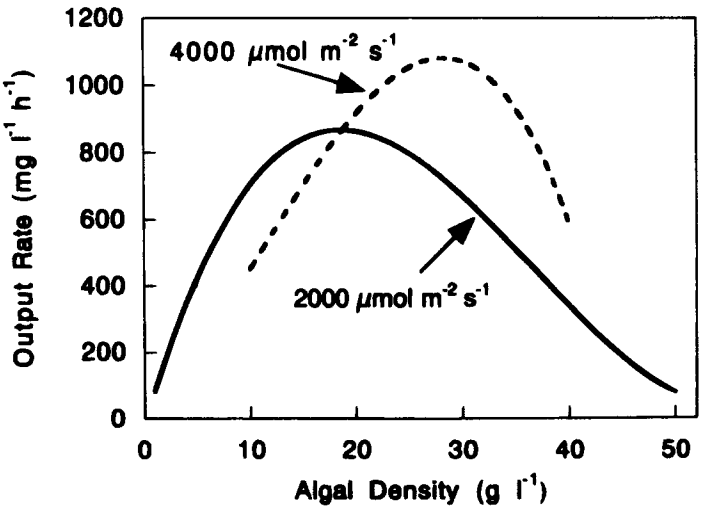

Fig. 7. The effect of the photon flux density on the output rate of cell mass at the optimized population density and mixing rate.

under laboratory conditions, in which light was applied continuously to both reactor surfaces *(10)*. Cultures responded well to an increase in light intensity provided the population density and mixing rates were optimized with added light increment (Fig. 7). At a light flux of 2000 µmol photons/m^{-2}/s^{-1} applied on each side of the flat plate reactor, i.e., ca. twice the light flux available outdoors (at noon), the optimal population density ranged between 18 and 22 g dry wt/L^{-1} and the maximal output rate was

900 mg/L^{-1}/h corresponding to ca. 7.8 g dry wt of algal mass/m^2/h—a record output. This output approaches the value predicted by Raven *(15)*, who calculated the maximal output rate of photosynthetic activity in a hypothetical culture exposed to 2000 μmol photons/m^{-2}/s^{-1} as being ca. 11 g/L^{-1}/h^{-1} assuming the quantum demand was 16 (mole photons per mole co_2 fixed in cell mass), cell content was 50% carbon and all incident photons absorbed by the algal suspension (Fig. 7). The latter condition seems to be well answered in our ultra-high-cell density cultures, in which measurements of the oxygen production rate (OPR) reveal that no saturation of light takes place, i.e., the OPR in our flat plate reactor is linearly related to light intensity, provided cell concentration and mixing rate are optimized. Indeed, when the culture was exposed to 4000 μmol photons, the range of the optimal population density steadied between 30 and 38 g/L^{-1} and the output rate was increased to 1150 mg dry wt of cell mass/L/h. As expected, this surge in output rate was achieved with a much reduced photosynthetic efficiency, declining from ca. 15–10% (not shown).

In conclusion, we propose that the key for obtaining high output rates of photoautotrophic cell mass at high PE under supersaturating PFD rests in reactors with a narrow lightpath, in which the culture may be maintained at ultra-high, carefully optimized algal density by providing mixing at the maximal rates permissible without cell damage.

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