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## **Study of light requirements of a Photobioreactor**

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**Abstract.** *Photobioreactors are used to grow photosynthetic cell cultures. Many factors influence the growth of cell cultures such as light, carbon dioxide, and nutrients. To influence the photobioreactor an understanding of the light requirements for microalgae is necessary. It is known that photosynthetic microalgae need light, but the intensity and length of exposure is not known. Efficient PBR design requires that light be provided at the required intensities, duration and wavelength based on pigments present in the microalgae. An excessive intensity may lead to photoinhibition and photooxidation while low intensities may not promote algal growth. Long exposure to light may lead to minor damage of algal collection antenna that can be quickly repaired by the cell if placed in the dark. Photosynthesis is a process that takes time and once the process is initiated, additional photon energy collected will be lost as thermal energy. Photons that are collected, but not at the appropriate energy (wavelength) will be inefficient for photosynthesis. If the photon has too little energy, it will be likely lost as thermal energy. Temperature of medium is also influences the light intensity requirements for optimal growth. Factors effecting selection and design of light source are addressed.*

**Keywords.** Photobioreactors, photoinhibition, photosynthesis, green algae, blue-green algae, temperature, light intensity, LED, light source, photons, light energy.

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## **Introduction**

A photobioreactor (PBR) is a system, which provides an artificial environment to grow phototrophic cell cultures. Many factors influence the design of a successful photobioreactor such as light, carbon dioxide and nutrients. Light is often considered to be one of the most important factors, Richmond, 2004. In the past, algal growth has been limited by light (photon flux density). Over the past fifty years scientists and engineers have been trying to develop a viable photobioreactor for large-scale algal production. But due to inefficiencies in the design procedures and lack of understanding of various factors which influence the design of an efficient photobioreactor no widely accepted PBR designs have been developed resulting in little or no wide spread adaptation of PBR technology. To efficiently design a photobioreactor, a detailed understanding of the influence light has on the microalgal culture as well as how light interacts with other factors to influence the culture must be developed by the designer.

Pharmaceuticals, chemicals, health foods, animal feed, and human food are examples of products that can be made algae biomass. Table 1 from Benemann, et.al. (1987) and Table 1 from Grima, et. al. (1999) are a comprehensive list of possible products from algae biomass. The two tables show the possible products that could be developed if algal cultures could be produced on an industrial scale.

## **Factors effecting selection and design of the light source(s)**

The design of an efficient light source for a PBR requires knowledge of the various aspects of light that influence microalgae needs to be developed. Some factors that influence the light requirements of an algal culture are:

1. Type of algae culture.
2. Type of light source.
3. Intensity of light source.
4. Effect light source has on cell development in the algae culture.
5. Dark period requirement of the algal culture.

### ***Type of algae culture***

Different types of algae cultures need different light and nutrient sources. The light requirement for algae is dependent upon the major pigments present in the algal cell. The algae of interest are blue-green and green algae. Various pigments present in blue-green and green algae are shown in Table 1 which were derived from Hoek et. al 1995. As it can be seen from Table 1 Chlorophyll a and  $\beta$ -carotene are common in both types of algae. The main pigment in these two types of algae is Chlorophyll a. Chlorophyll a is located as a part of core and reaction center protein complexes and in the light-harvesting antenna. Other important pigments such as chlorophyll b, carotenes, and phycobilins act as supplementary pigments for light harvesting (Richmond, 2004). A detailed explanation of pigments and their importance is discussed in Kommareddy and Anderson, 2003. Kommareddy and Anderson (2003) also discuss how different light wavelengths that are absorbed are converted to energy for the photosynthetic process.

Different pigments absorb/harvest different regions of visible light energy, see Figure 1. Figure 2, shows the penetration depth of light spectra in *Nannochloropsis* sp. (a green algae) as a function of cell density. The important pigments of *Nannochloropsis* algae as listed in Table 1

for green algae are Chlorophyll a, Chlorophyll b and  $\beta$ -carotene. By comparing Figures 1 and 2 it is observed that the light wavelengths corresponding to the absorption range of these pigments (approximately 400-500nm and 600-700nm) also corresponds to the light wave lengths with the least penetration depth because they are absorbed by algae. Another interesting aspect, which can be noted from Figure 2, is that when the concentration of algae in  $gL^{-1}$  is small, there is low absorption by the supplementary pigments. This suggests that supplementary pigments are not used to harvest light until there is a deficiency of light in the wavelengths absorbed by Chlorophyll a. This suggests that individual algae and the algal culture have to be considered when identifying important pigments for light harvesting that dictate the design of the lighting system for a PBR producing this type of algae.

Table 1. Pigments for Blue – Green algae and Green algae (derived from Hoek et. al. 1995).

<b>Cyanophyta (Blue – Green algae)</b>		
<b>Pigments group</b>	<b>Important Pigments</b>	<b>Pigments present or occur rarely or occur in small quantities</b>
<b>Chlorophylls</b>	<b>Chlorophyll a</b>	
<b>Phycobilins</b>	<b>Phycocyanin Allophycocyanin Phycoerythrin Phycobilisomes</b>	
<b>Carotenes</b>	<b><math>\beta</math>-carotene</b>	
<b>Xanthophylls</b>	<b>Zeaxanthin Echinenone Canthaxanthin Myxoxanthrophyll Oscillaxanthin</b>	<b><math>\beta</math>-cryptoxanthin Isocryptoxanthin Mutachrome</b>
<b>Chlorophyta (Green algae)</b>		
<b>Pigments group</b>	<b>Important Pigments</b>	<b>Pigments present or occur rarely or occur in small quantities</b>
<b>Chlorophylls</b>	<b>Chlorophyll a Chlorophyll b</b>	<b>Chlorophyll <math>c_1</math> Chlorophyll <math>c_2</math> Chlorophyll <math>c_3</math></b>
<b>Carotenes</b>	<b><math>\beta</math>-carotene</b>	<b><math>\alpha</math>-carotene <math>\gamma</math>-carotene</b>
<b>Xanthophylls</b>	<b>Lutein Violaxanthin Neoxanthin</b>	<b>Zeaxanthin Echinenone <math>\beta</math>-cryptoxanthin Antheraxanthin Siphonein Siphonoxanthin</b>

### ***Type of light source***

After identifying the type of algae culture to be grown, it important to identify the right type of light source (appropriate wave lengths) to achieve a high level of photosynthetic efficiency. Kommareddy and Anderson (2003) discussed energy produced by different light sources in the visible spectrum. Electricity is used in closed loop photobioreactors to produce light making it essential to ensure that the light source is optimized relative to algae light use and cost of

production so that a high level of electrical efficiency is obtained. The efficiency at converting electricity into light varies with different light sources. The efficiency of light sources to convert electricity to light is further compounded when wavelength of light is considered. Light sources with descending order of efficiency are light emitting diodes (LEDs), grow flux / fluorescent lights and incandescent/halogen lamps. Since LEDs are the most efficient light source for converting electricity in to light with the desired wavelength, they should be given high priority for use. However, LEDs don't produce light in a broad white light spectrum which may make it necessary to use a combination of light sources or combination of LEDs.

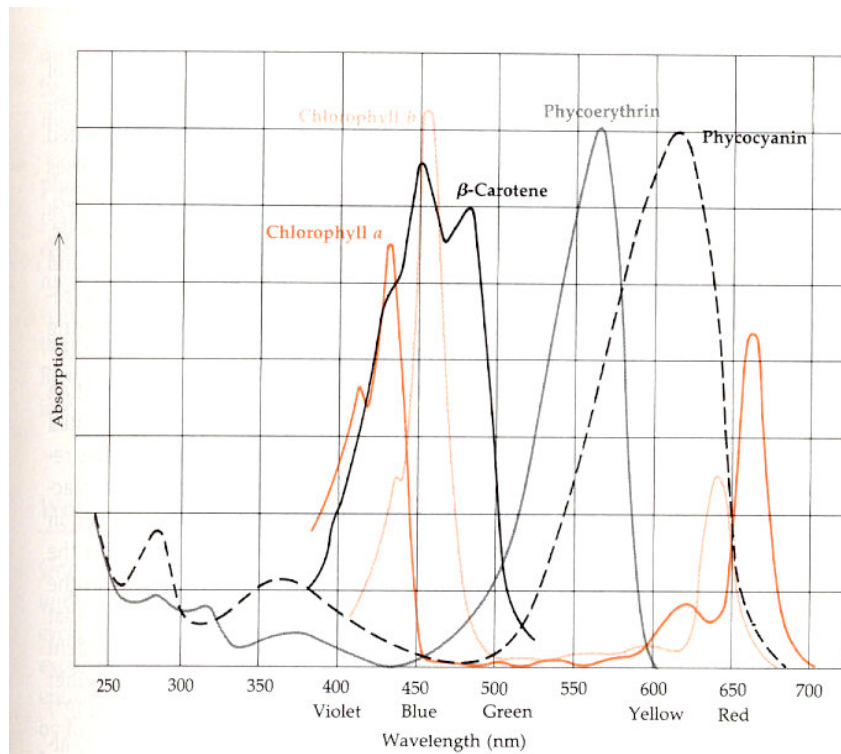


Figure 1. Absorption spectrum of different pigments (Purves and Orians, 1983).

Essentially any type of light sources which produces light between 400 nm – 500 nm and 525 nm to 680 nm should support the growth of blue-green algae. But as described in Kommareddy and Anderson (2003), higher energy photons are absorbed from light with wavelengths of 400nm –500 nm and 525 nm to 630nm have to loose energy as heat before they can be used in photosynthetic reaction center which needs 680 nm and 700 nm. The best way to achieve such high concentration of photons of red light close to 680 nm and 700 nm is by using LEDs with peak wavelengths close to 680 nm. Since green algae have Chlorophyll a, Chlorophyll b, and  $\beta$ -carotene are light harvesting pigment present, any light source which can produce wavelengths ranges of 400 nm to 500nm and 620 nm to 680 nm should be able to support growth of algae. But with the same argument discussed above we can conclude that LEDs should provide the required photos with least amount of energy input.

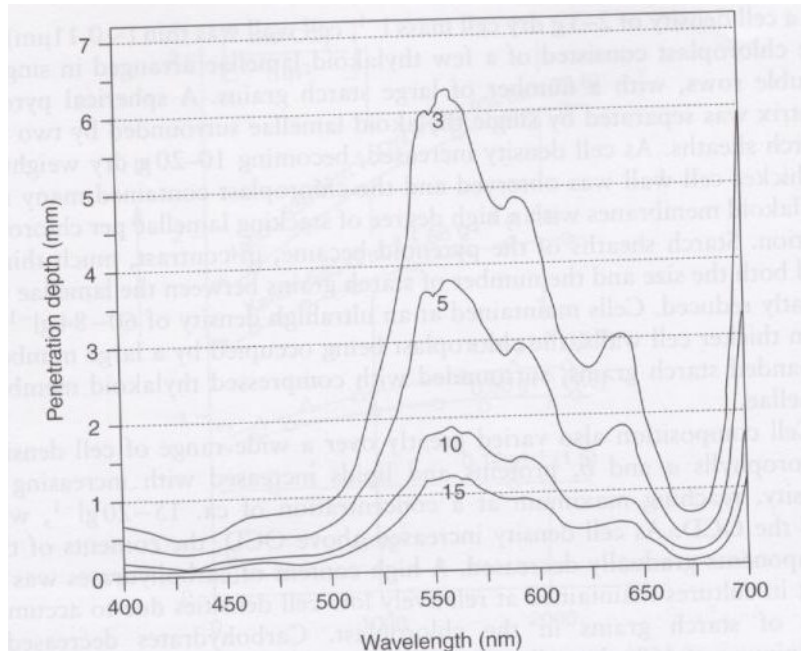


Figure 2: Penetration depth spectra in *Nannochloropsis* sp. as a function of cell density (Richmond, 2004)

### ***Intensity of light source***

Intensity of a light source gives the number of photons that are available for photosynthetic process. The energy associated with photons with a wavelength of 680nm is the energy level required by Chlorophyll a to initiate photosynthesis. Light with a wavelength of 680nm is near the longest wavelength of visible light. Therefore, most of the visible light has sufficient energy to support photosynthesis. However, if the wavelength is small the energy associate with the wavelength is high. For a given light source, Figure 3 shows the effect light intensity has on photoautotrophic growth of photosynthetic cells. As it can be seen from the figure when light intensity is very small there is no cell growth. Once cell growth has begun, higher light intensities result in increased cell growth up to a light intensity where growth stops increasing with light intensity. The light intensity at which cell growth begins is known as the compensation light intensity ( $I_c$ ) while the light intensity at which no further increase in growth takes place with increased light intensity is known as light saturation ( $I_s$ ). Further increase in light intensity does not increase the specific growth rate, but does not hinder growth. The point at which increased light intensity decreases the specific growth rate is the point where photoinhibition begins ( $I_d$ ). Each type of algae has a specific curve that describes the specific growth rate relationship with light intensity. The relationship of specific growth rate with light intensity is temperature dependent, Richmond (2004). Generally, as the temperature increases, the saturation intensity increases which results in a higher specific growth rate. Three strains of algae are derived from Kim et. al. (2002). Table 2 gives the compensation and saturation light intensities of the three strains; *Anacystis nidulans*, *Nostic muscorum*, and *Chlorella huxleyi*. The light intensities listed by Kim et. al. (2002) are in lumens. The lumen values were converted to  $W/m^2$  with the conversion factors listed in Langhans and Tibbitts, 1997. Table 2 shows that the compensation and saturation light intensities for tungsten and fluorescent lights is much higher than for LEDs with a 680nm wave length. This may be in part due to the large amount of energy produced by tungsten and fluorescent lights either in the infrared range and/or in the visible light range with

wavelengths and thereby energy content greater than the 680nm needed by Chlorophyll a. The excess energy in the shorter wavelength is converted to thermal energy and lost.

Table 2: Light intensity and algal growth ( data from Kim, et. al. 2002)

Species	Strain	Type of light	$I_s$ (klx)	$I_c$ (klx)	$I_s$ (W/m <sup>2</sup> )	$I_c$ (W/m <sup>2</sup> )
<b>Cyanobacteria (blue-green algae)</b>	<b>Anacystis nidulans</b>	<b>Tungsten</b>	<b>3.50</b>	<b>0.30</b>	<b>13965.00</b>	<b>1197.00</b>
	<b>Nostic muscorum</b>	<b>Florescent</b>	<b>3.10</b>	<b>0.11</b>	<b>9083.00</b>	<b>322.30</b>
<b>Green algae</b>	<b>Chlorella huxleyi</b>	<b>680 nm</b>	-	-	<b>13.70</b>	<b>1.46</b>

Figure 4, shows that light intensity and specific growth rate ( $\mu$ ) decreases exponentially from the wall of the photobioreactor. The decrease is due principally to the absorption of photons by the algal biomass in dense cultures. Light attenuation in less dense cultures is a function of the absorption of photons by the water in the algal growth medium as well as the biomass suggesting that blue light would penetrate deeper in less dense algal cultures. However, Figure 2 shows that algal cultures with a density of 3g/L effectively absorb all blue light (300-400nm). For blue light to have a greater penetration depth, the culture density must be less than 3g/L. The attenuation of light in the algal culture is a function of the extinction coefficient. Molina et. al. (2001) suggest using 0.0369m<sup>2</sup>/g while Ogabonna and Tanaka (1997) use 200m<sup>2</sup>/kg (0.2m<sup>2</sup>/g). The extinction coefficient is likely wavelength and algae absorption dependent. Neither extinction coefficient addresses light wavelength or algal pigment. For maximum penetration of 680nm light the surface light intensity may be set at or just below the photoinhibition intensity which is 600W/m<sup>2</sup>. It would be desirable to maintain growth throughout the lit region of the PBR so the minimum light intensity would be set at 1.46W/m<sup>2</sup>. The resulting lit region would be for a algal density of 10g/L is 1.1m (0.0369m<sup>2</sup>/g) and 0.21m (0.2m<sup>2</sup>/g). The high light intensity near the surface of the PBR may result in a large waste of energy since the specific growth rate is the same between 13.7 and 600W/m<sup>2</sup>. A balance between light penetration and energy loss needs to be established.

Another method to increase the efficient use of the photons provided is to move the algae closer to the light source when it needs photons for photosynthesis and farther away when it is performing photosynthesis and needs no additional photons (mixing). Once a photon is absorbed, it needs 6s to reset itself (perform photosynthesis) so that it is ready to receive another photon (Merchuk and Wu, 2003). Figure 5, shows the time scale of photosynthesis given by Merchuk and Wu, 2003. Figure 5 shows that the time required to capture a photon is as little as 10<sup>-14</sup> to 10<sup>-10</sup> s, which is less than time required to transfer an electron (10<sup>-10</sup> to 10<sup>-2</sup> s) and fix a carbon atom into a molecule of carbon dioxide (10<sup>-2</sup> to 10<sup>2</sup> s) for cell growth. Richmond (2004) on the other hand states that the rate of photosynthesis is governed by the turn over of electron transport that takes 1-15ms. It is further asserted that an algal culture that has adapted to a high light intensity may require only 2ms. This short dark period would make it difficult to move algae into and out of the lit region of a PBR while the 6s dark period is more manageable. Absorption of a photon is almost instantaneous making the amount of time that the algal cell needs to be in the lit region of the PBR a function of how long it takes a photon to impact the absorbing pigment in the antenna. This is a function of the light intensity (photons/m<sup>2</sup>/s) at the depth the cell is in the PBR and the relative portion the absorbing pigment is of the antenna region. Becker (1994) states that chlorophyll comprises 0.5-1.5%, carotenoids comprise 0.1-2%, and phycobiliproteins comprise 1% of the algal dry biomass. Evaluating Figure 1 with the smaller weight percentages would suggest distributing 2000 $\mu$ mol/m<sup>2</sup>/s in quantities of 625, 125,

and  $1250\mu\text{mol}/\text{m}^2/\text{s}$  in the wave length ranges of 400-500nm, 500-600nm, and 600-700nm respectively for blue-green algae, see Table 1. However, it should be re-emphasized that the smaller the wavelength is, the more energy that is required to create the photon. A balance between energy used and likelihood that photons will be absorbed more quickly needs to be established. Mixing, light intensity for depth penetration, and light wavelengths used need to be evaluated and a compromised established that gives high growth with the least energy input.

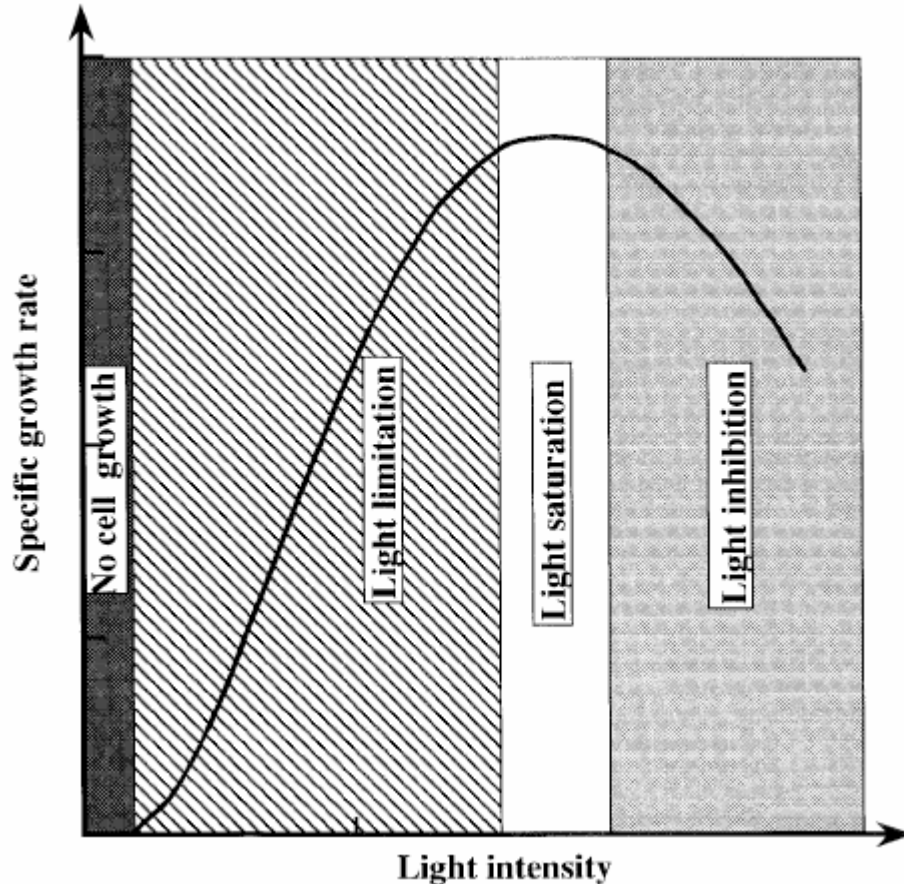


Figure 3: Effect of light intensity on photoautotrophic growth of photosynthetic cells (Ogbanna and Tanaka, 2000).

### ***Effect light source has on cell development in the algae culture***

When red LEDs are used to grow *Chlorella vulgaris* (green algae) it was found that the size of algae is half of what it was when grown under white light ( $30$  versus  $60\mu\text{m}^3/\text{cell}$ ), but twice the number of cells of the smaller algae were produced essentially keeping the biomass same (Lee and Palsson, 1996). It was also noted that the DNA distribution is narrower and had smaller size for the algae grown under red LEDs than when grown under fluorescent light (Lee and Palsson, 1996). This would be a positive result if the DNA distribution has to be kept small so that the new DNA is close to the original DNA. Lee and Paulsson (1996) observed that the size of the algae grew to the normal size ( $60\mu\text{m}^3/\text{cell}$ ) once they were exposed to fluorescent light after they were grown under LED. These results suggest that large numbers of algae could be produced under red light and then their size (biomass) increased if they were transferred to white light.

Light serves many purposes other than photosynthesis, which are wavelength dependent. Specific wavelengths stimulate flowering, stem growth, germination, etc. It has been suggested that blue light in the amount of 5-10% of total photons may be required if red light is used for purposes other than photosynthesis (Lee and Palsson, 1996; Shotipruk, et. al. 1999). Small amounts of non-photosynthetic light should be provided. The question is how much. At this time a small amount of white light may be needed in the PBR to provide for non-photosynthetic needs.

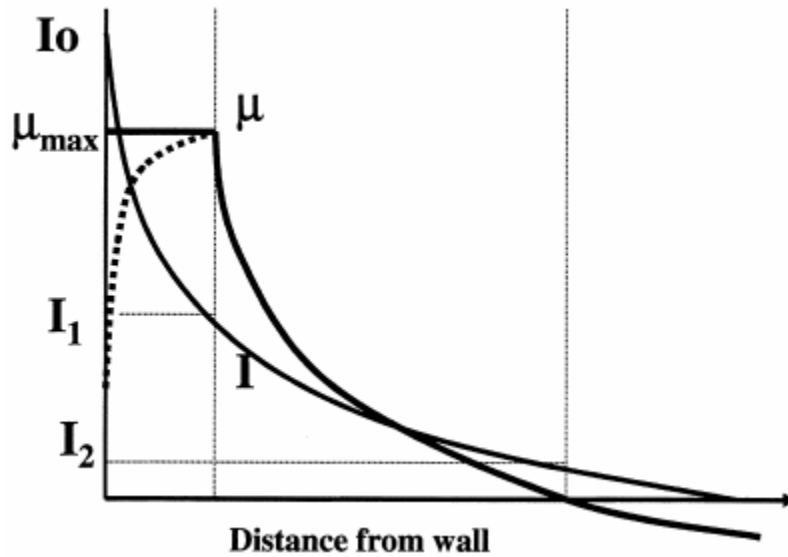


Figure 4: Illumination and growth rate as a function of distance from illuminated wall.

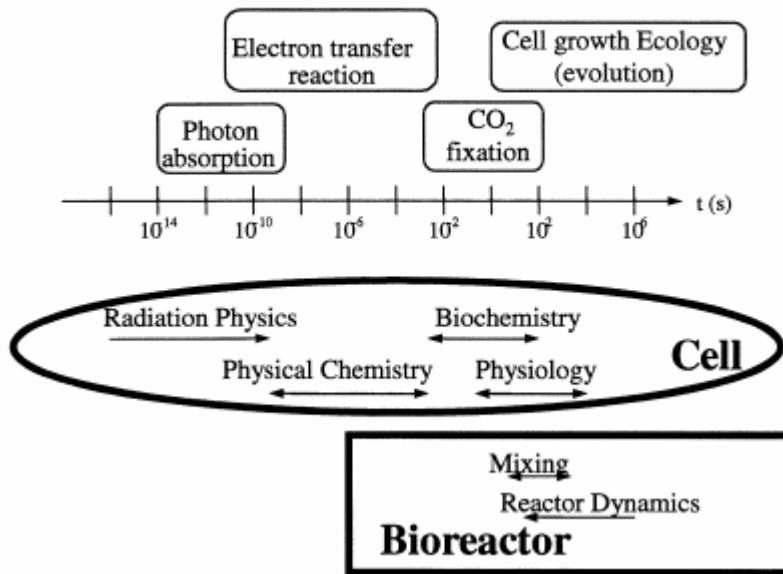


Figure 5: Time scale of photosynthesis (Merchuk and Wu, 2003)

## ***Dark period requirement of the algal culture***

The effect of duration of light exposure on the cell is not well understood. Very little information is available as to how much time the cell should be in the light and dark. Researchers have used some growth conditions that have a small dark period, some have used no dark period, and some have used dark periods up to 18 hours (Kim, et. al. 2002). Merchuk and Wu (2003) suggest an appropriate dark period is 6 s. High illumination of the antennas may damage them necessitating longer dark periods to repair the damage (Wu and Merchuk, 2001). There is no consensus on what is an appropriate light/dark cycle. Since naturally grown algae have dark times (nighttime), it is assumed by many researchers that dark periods are required. Long dark periods generally resulted in biomass loss as well as a decline in growth rates because the algae under go photorespiration and consume oxygen and carbohydrates (Molina, et. al. 2001; Merchuk and Wu, 2003).

## **Summary**

Light requirements of a PBR are driven by many complex relationships.

1. The algal culture influences light demand through antenna pigments in the cell, cell size desired, and culture density. The first step in designing light source for algae is identifying light harvesting pigments present.
2. Energy is required to produce photons in a closed PBR. Wavelengths should tend to be long as possible for photosynthesis to reduce energy demand but this needs to be balanced against depth of light penetration in to the PBR and amount of each photon capturing pigment in cells.
3. Light source intensity affects the number of photons penetrating to a given depth in the PBR but high intensities may not increase growth rate near the light source. Light penetration, photo inhibition, and biomass produce influence light intensity.
4. Photosynthesis is a time dependent process. Once it has started additional photons are not used for photosynthesis allowing for a dark period. Also antennas, which are damaged, need a recovery time. Mixing, light intensities and culture density interact to influence dark/light cycles.
5. Light/dark cycle appropriate for a particular species of algae has to be determined.

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