Variable photosynthetic characteristics in waste stabilisation ponds

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Abstract Algae play several key roles in waste stabilisation ponds. A model has been developed to predict algal concentration in waste stabilisation ponds, in which the relationship between photosynthesis and underwater light availability is central. One equation was selected from several alternative expressions that describe this relationship. The selected equation consisted of four photosynthetic parameters. A field sampling programme was designed to investigate the relationships between the photosynthetic parameters and the pond environment. Although initial regression analyses were unsuccessful, distinct diurnal variations were revealed in two key photosynthetic parameters, related to an inverse variation in chlorophyll a concentration. This led to the derivation of a dynamic feedback hypothesis which challenges the classic assumption in algal modelling of constant photosynthetic parameters.

Keywords Algae; photosynthesis; stabilisation

Introduction

The role of algae in waste stabilisation ponds

Most waste stabilisation ponds are designed on the basis of BOD (biochemical oxygen demand) loading or pathogen removal rates, and take the algal activity for granted. Algae play several key roles: providing oxygen for efficient bacterial degradation of organic load, minimising odour emission, and providing conditions for enhanced pathogen reduction and ammonia nitrogen removal. Pond failure can occur if the algal population is insufficient. The ability to predict and ultimately control algal concentration would therefore be a useful tool for designers and operators.

Algal models

In natural environments, photosynthetic activities are often used as a measure of algal growth (Post et al., 1985). Algal concentration is assumed to decline in proportion to the respiration rate during the night and to increase during the day in proportion to the difference between the light dependent gross photosynthetic rate and the light independent respiration rate. Much of the research on algal production applies to marine and freshwater situations, where nutrients and light are both important considerations. In the case of waste stabilisation ponds, nutrients are usually provided in excess by the influent sewage, and algal concentrations, and the resulting rate of light attenuation with depth, tend to be much higher than in lakes and oceans.

A model to predict algal concentration in waste stabilisation ponds was developed (Weatherell, 2001). This combined the relevant techniques and knowledge from the large body of marine and freshwater algal research (particularly Talling, 1957; Platt et al., 1980; Huisman, 1999) with consideration of the issues peculiar to waste stabilisation ponds. Inspiration for this aspect was drawn from research on high rate algal ponds (particularly...
Grobbelaar, 1981; Fallowfield and Martin, 1988), for which more attempts at algal modelling have been made than for conventional ponds.

The model is applicable to conventional facultative and maturation ponds in climates where algae can play a key role, and to high rate algal ponds. The algal population in the pond is represented by chlorophyll $a$ concentration. The key functions within the model are as follows: underwater light availability is dependent on chlorophyll $a$ concentration, photosynthetic rate is dependent on underwater light availability, and rate of increase in chlorophyll $a$ is in turn dependent on photosynthesis.

The relationship between light and photosynthesis

The functional relationship between light intensity and photosynthesis forms the basis of most models of algal production (Platt et al., 1977). Most researchers have found this relationship to follow the same general pattern for a wide range of algal populations and conditions. This general shape is illustrated by the curves in Figure 1 (which relates also to the Results section).

The curves are characterised by an initial linear slope, which then begins to bend over and eventually levels off. The initial linear slope in the light limited region is a result of photosynthetic rate increasing with light availability. The rate of increase gradually becomes smaller in the transition phase. The curve becomes horizontal when the sample reaches a maximum or saturated photosynthetic rate, which remains the same regardless of further increases in light intensity. A reduction in the photosynthetic rate is sometimes observed with further increases in light intensity.

Mathematical expressions used to describe the relationship between photosynthetic rate ($P$) and light intensity ($I$) are referred to as PI equations. Since there is much debate about the best expression to describe the relationship between photosynthetic rate and light intensity, ten potential PI equations were evaluated using field and laboratory data (Weatherell, 2001). The selected equation consists of four photosynthetic parameters: light-saturated photosynthetic rate ($P_{\text{max}}$), light-limited photosynthetic efficiency ($\alpha$), a convexity parameter ($\chi$) and a photoinhibition parameter ($\beta$). Referring back to the curves in Figure 1, $P_{\text{max}}$ defines the highest point on the curve, $\alpha$ defines the initial gradient, $\chi$ defines the sharpness of transition from initial slope to saturation, and $\beta$ defines the downturn of the curve at high light intensities.

Issues addressed in this paper

This paper describes a sampling programme initially designed to investigate relationships between photosynthetic parameters and the pond environment, including sunlight, temperature, pH, chemical oxygen demand (COD) and dissolved oxygen concentration (DO). In the light of the results observed, the paper goes on to discuss the relationship between diurnal variations in photosynthetic parameters and diurnal variations in chlorophyll $a$ concentration.
Methods

Field site
The sampling programme was carried out between 13/05/99 and 10/06/99 in the waste stabilisation system at the University of Dar es Salaam, Tanzania (6°48’S, 39°13’E), which is a relatively small pond serving a population of around 5,000. Ponds 1, 2 and 3 were sampled, where Pond 1 is a primary facultative pond, flowing to Ponds 2 and 3 in series. The ponds were 1.61 to 1.92 m deep, 2,883 to 5,124 m² in area, and had retention times of 18.8 to 23.2 days. The estimated average influent BOD to Pond 1 during the sampling period was 150 mg/l, and the estimated average surface loading rate was 125 kg ha⁻¹ d⁻¹. This information was derived from data provided by Kayombo et al. (1999) and the Department of Civil Engineering, University of Dar es Salaam.

Sampling
In each pond, sampling was carried out in the morning (between 7:25 and 8:05 am) and again in the afternoon (between 13:15 and 14:40 pm), on four separate days, approximately one week apart. Pond 1 was also sampled twice daily on consecutive days, from the afternoon of 13/05/99 to the afternoon of 16/05/99.

Samples were taken from the entire pond depth at three points across the pond, using a column sampler. A single 2 m length of 2 to 2.5 cm diameter plastic tube was slowly lowered vertically to the pond bottom, raised again slightly to avoid uptake of settled sludge, then a rubber bung was pushed into the top end of the tube. As the tube was carefully raised, the water was held in the tube by capillary action, and another bung was inserted into the bottom end of the tube just before it reached the water surface. The tube was then placed over a suitable container and the sample dispensed, mixed, and transferred into sample bottles.

Sample analyses
Temperature, DO and pH readings were taken in the sample bottles immediately after collection using calibrated portable probes (Model 95 DO meter, YSI Inc., Dayton, USA; model 3051 pH meter, Jenway Ltd, Essex, UK). Within an hour of sampling, triplicate sub-samples of the mixed pond sample were analysed for chlorophyll a by the method similar to that of Pearson et al. (1987). The COD concentrations of triplicate filtered and unfiltered sub-samples were measured by the Closed Reflux Titrimetric Method (APHA, 1995).

Weather data
Light intensity observations (in the 400–700 nm range) just above the water surface were made at the time of sampling, using a Li-193SA spherical quantum sensor (Li-Cor Inc., Lincoln, USA). 9 am and 3 pm air temperature readings, and daily sunshine cards were obtained from a weather station at the University of Dar es Salaam. Daily air temperature was estimated as the average of the morning and afternoon readings. The number of hours of bright sunshine per day were estimated by measuring the burnt trace along the sunshine cards, as described by the UK Meteorological Office (1982). In order to provide data describing the slightly longer term environment, the average temperature and total sunshine hours were recorded the day before each sampling day, and also over the seven days preceding each sampling day.

Light attenuation
Light intensity observations were also made at 5 cm intervals throughout the pond depth, and values of the overall vertical light attenuation coefficient were estimated according to Beer’s Law. Secchi depth, a simple alternative measure of light penetration, was also
measured. A white polystyrene disc, 30 cm in diameter, attached to a graduated stick, was lowered into the pond until invisible.

**Estimation of photosynthetic parameters**
A “PI method” was developed to measure photosynthetic rate (P) of field samples under a prescribed range of light intensities (I) and controlled laboratory conditions (Weatherell, 2001). As soon as possible after sample collection, the PI method was used to produce a set of photosynthetic rate and light intensity measurements for the sample. The photosynthetic rate was measured as the rate of production of DO per unit chlorophyll \( a \). The PI equation from the model was fitted to the data by the method of least squares in order to estimate the value of the photosynthetic parameters (\( P_{max} \), \( \alpha \), \( \chi \) and \( \beta \)) of each sample.

**Results and discussion**

**Statistical analyses**
Best subsets regression and multiple regression were used in an attempt to identify relationships between the photosynthetic parameters of the pond algae and various environmental factors, including sunlight, temperature, pH, COD and DO. No simple relationship, with a reasonable mechanistic explanation, could be identified.

**Diurnal variation in \( P_{max} \) and \( \alpha \)**
In addition to the regression analyses, the photosynthetic parameter data were examined for possible explanations of the variations observed in photosynthetic behaviour. The set of data in consecutive samples from Dar es Salaam Pond 1 show a distinct and consistent difference in the \( P_{max} \) and \( \alpha \) values of samples taken in the morning from those taken in the afternoon (Table 1), as illustrated in Figure 1.

Light-saturated photosynthetic rate (\( P_{max} \)) and light-limited photosynthetic efficiency (\( \alpha \)) are the parameters most frequently used to characterise photosynthetic behaviour, and are the most significant to total photosynthetic activity. Values of both \( P_{max} \) and \( \alpha \) were higher in morning samples than afternoon samples.

The values of the photosynthetic parameters (\( P_{max} \), \( \alpha \), \( \chi \) and \( \beta \)) were derived from the instantaneous relationship between photosynthetic rate and light intensity. It is accepted that they can vary with environmental conditions, and that despite its traditional notation, the \( P_{max} \) value measured for an alga is not the absolute maximum potential rate for that alga under any circumstances.

Nevertheless, it was considered reasonable to assume that the photosynthetic parameters of a mixed sample, consisting of several column samples across the pond, would be representative of the photosynthetic characteristics over just one day within a single pond. According to Kirk (1994), it is commonly assumed that the values of \( P_{max} \) and \( \alpha \) observed at midday apply throughout the day. The large short term fluctuations seen for Pond 1 in Table 1 provide evidence that this assumption is invalid. The repeated daily pattern of variation in \( P_{max} \) and \( \alpha \) suggests an inherent link with the diurnal light cycle. Such a consistent link with sampling time was not observed for the convexity (\( \chi \)) or photoinhibition (\( \beta \)) parameters.

**Deviations from diurnal variation of \( P_{max} \) and \( \alpha \)**
Table 1 also includes the values of \( P_{max} \) and \( \alpha \) found for the other 22 weekly samples from three different ponds. Values of \( P_{max} \) were again generally higher in morning samples than afternoon samples taken from the same pond on the same day. The variation between morning and afternoon values of \( \alpha \) was not as great or consistent as the variation in \( P_{max} \).

In four cases the value of \( P_{max} \) for an afternoon sample was greater than for the morning
An inherent link with the diurnal light cycle is therefore too simplistic an explanation for the large short term variations in $P_{\text{max}}$ and $\alpha$. Probably the most obvious explanation for the four exceptions is variation in cloud cover over the day. In addition to the surface light observations and qualitative notes on the weather at the time of sampling, the daily sunshine cards were re-examined for the number of hours bright sunshine within the four hours preceding sampling. No consistent relationship was found to explain the exceptions. Correlation analysis (method as described below) confirmed that there was no significant linear association between any of the photosynthetic parameters and these two quantitative short term measures of light.

Table 1 also shows that the variations between ponds and between weekly samples could be greater than between morning and afternoon. For example, the value of $P_{\text{max}}$ might be higher for a morning sample than for the afternoon sample on the same day, but much lower than the value of $P_{\text{max}}$ for an afternoon sample taken in the same pond a week later. This indicates that there is another source of variation that is sometimes more significant than the diurnal light cycle.

### Table 1

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<th>Sample date</th>
<th>Time of day</th>
<th>$P_{\text{max}}$</th>
<th>$\alpha$</th>
<th>Chlorophyll a (µg/l)</th>
<th>Light attenuation coefficient (m$^{-1}$)</th>
<th>Secchi depth (cm)</th>
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$P_{\text{max}}$ units: mg DO s$^{-1}$ (µg chl a)$^{-1} \times 10^{-6}$
$\alpha$ units: mg DO s$^{-1}$ (µg chl a)$^{-1}$ (µmol m$^{-2}$ s$^{-1}$)$^{-1} \times 10^{-8}$

* missing observations
# outliers identified according to Grubb’s test, described by Rohlf and Sokal, 1995
Correlation of $P_{\text{max}}$ and $\alpha$ with chlorophyll $a$ concentration

The chlorophyll $a$ data had previously been omitted from the regression analyses, but are shown in Table 1 together with the corresponding values of $P_{\text{max}}$ and $\alpha$. On inspection of the first few rows of chlorophyll $a$ concentrations, large variations were observed between morning and afternoon samples, and the variations appeared to be inversely related to the diurnal variations in $P_{\text{max}}$ and $\alpha$.

Following this qualitative examination of trends, correlation analysis was carried out to test the full data set (excluding outliers identified according to Grubb’s test, described by Rohlf and Sokal, 1995) for statistical associations. Statistical software (Minitab for Windows, version 13.1, Minitab, USA) was used to produce a table of correlation coefficients and p-values. A coefficient of zero indicates that there is no linear relationship between two variables. The p-values indicate whether the correlation coefficient is significantly different from zero. A correlation is judged significant if the p-value is less than or equal to the level of significance. A significance level of 0.05 was used, i.e. a probability of error of 5%.

The correlation analysis confirmed that chlorophyll $a$ concentration had a more significant linear association with both $P_{\text{max}}$ (p-value = 0.000) and $\alpha$ (p-value = 0.016) than any other pond factor. The correlations between chlorophyll $a$ concentration and the other photosynthetic parameters ($\chi$ and $\beta$) were found to be insignificant even at the 20% significance level (i.e. p-values $\geq 0.2$).

The correlations of $P_{\text{max}}$ and $\alpha$ with chlorophyll $a$ concentration were negative. Any direct influence of $P_{\text{max}}$ and $\alpha$ on the chlorophyll $a$ concentration is expected to be positive. It is therefore more likely that it is some influence of the chlorophyll $a$ concentration on $P_{\text{max}}$ and $\alpha$ that is leading to the negative correlation.

Correlation of chlorophyll $a$ with underwater light intensity

Due to the strong negative influence of chlorophyll $a$ concentration on underwater light availability of waste stabilisation ponds (Curtis et al., 1994), the inverse correlation of $P_{\text{max}}$ and $\alpha$ with chlorophyll $a$ concentration intuitively suggests that the variation in $P_{\text{max}}$ and $\alpha$ might be positively related to the underwater light availability. This is the reason for including light attenuation coefficient and Secchi depth observations in Table 1. Bearing in mind that the light attenuation coefficient is an inverse measure while Secchi depth is a positive measure of relative light availability, the inspection of the first few rows of these data appear to comply in general with the theory that $P_{\text{max}}$ and $\alpha$ are related to the underwater light availability.

Correlation analysis (method as described earlier) confirmed the significant positive linear correlation of column average chlorophyll $a$ concentration with light attenuation coefficient (p-value = 0.000), and the significant negative correlation with both of these with Secchi depth (p-values = 0.000). However, the only significant linear correlation between either the light attenuation coefficient or the Secchi depth and the photosynthetic parameters was between the light attenuation coefficient and $P_{\text{max}}$, and this was not as significant (p-value = 0.028) as the correlation between chlorophyll $a$ concentration and $P_{\text{max}}$ (p-value = 0.000).

A possible reason behind the stronger correlation of $P_{\text{max}}$ and $\alpha$ with chlorophyll $a$ concentration than with direct measures of underwater light attenuation, is that chlorophyll $a$ concentration, in addition to providing a measure of the underwater light attenuation relative to the surface, is also a function of the past underwater light availability, which takes into account the past surface light intensity. Chlorophyll $a$ concentration is therefore a useful surrogate for underwater light history, while the light attenuation coefficient and Secchi depth are only relevant to the light attenuation at the sampling time itself. The latter are
also subject to large measurement error due to cloud and water movements during in situ observation.

**Dynamic feedback hypothesis**

The significant inverse correlation of $P_{\text{max}}$ and $\alpha$ with chlorophyll $a$ concentration, coupled with the general diurnal trends initially observed, and the strong association of light attenuation with chlorophyll $a$ concentration, led to the conception of the following hypothesis.

*The key photosynthetic parameters ($P_{\text{max}}$ and $\alpha$), describing the instantaneous relationship between photosynthetic rate and light intensity in waste stabilisation pond algae, vary in response to the underwater light availability, which is inversely related to the column average chlorophyll $a$ concentration.*

**Validity of the dynamic feedback hypothesis**

It must be noted that this hypothesis was derived from a single sampling programme of one pond system in Dar es Salaam. However, there is some evidence to support the hypothesis in other relevant research on algal photosynthesis and waste stabilisation ponds. Several studies have reported diurnal or light-cycle related variations in $P_{\text{max}}$ (and again to a lesser extent $\alpha$) of a scale comparable to those found in this study (Platt et al., 1980; Harding et al., 1982; Neale and Marra, 1985). Contributing to the hypothesis were the surprisingly large diurnal variations observed in column chlorophyll $a$ concentrations. Discounting other explanations (such as spatial variation, dilution, motility, buoyancy and passive sinking; discussed by Weatherell, 2001), the large variation implies very high growth rates, and similarly high rates of overnight decline. The credibility of these rates requires consideration.

$P_{\text{max}}$ and $\alpha$ have been measured in this research in the popular form of dissolved oxygen production per unit of chlorophyll $a$. Further research is required to determine whether the diurnal variation in $P_{\text{max}}$ and $\alpha$ applies when measured per unit biomass or cell.

**Conclusions**

According to the algal model described in the introduction, chlorophyll $a$ concentration is a function of photosynthetic rate, which is a function of the underwater light availability and the photosynthetic parameters at any given instant. In addition to the influence of chlorophyll $a$ concentration on the photosynthetic rate via underwater light availability, it is now suggested that there is an additional dynamic feedback mechanism of chlorophyll $a$ concentration on the photosynthetic parameters via the short term underwater light history. While recognising the limitations of this study, the results challenge the classic assumption in algal modelling of constant photosynthetic parameters.

**References**


