Temperatura del cultivo mixto de *chlorella vulgaris* a cielo abierto: incidencia en la concentración de biomasa

Temperature of the mixed culture of chlorella vulgaris to open sky: incidence in biomass concentration

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Resumen

La temperatura del cultivo es proporcional a la producción de microalgas, alcanzando valores óptimos para cada especie. Por encima de esta, aumenta la respiración y la fotorrespiración, reduciéndose la productividad. En este trabajo se ha evaluado la incidencia de la temperatura de suspensión en la biomasa del cultivo mixto de *Chlorella vulgaris* en residuales porcinos a cielo abierto. Se realiza un análisis multivariado para obtener las variables ambientales con mayor influencia en la temperatura del cultivo, observándose qué todos los factores son estadísticamente significativos pero la irradiación solar es el que tiene mayor influencia, mostrando que el 72,8 % de la variabilidad de la temperatura depende de la irradiación solar. A través de un análisis de regresión no lineal y aplicando el modelo cinético logístico de Verhulst se analizan tres corridas a tres temperaturas promedios diarias (28°C, 29°C, 30°C) obteniéndose los mejores ajustes a 30°C donde se alcanza una máxima concentración de biomasa de20, 8 g.L^{-1}.

Palabras clave: *Chlorella vulgaris;* temperatura, biomasa, residuales porcino

Abstract

The culture temperature is proportional to the production of microalgae, reaching optimal values for each species. Above this optimal value, the respiration and photorespiration increases thus reducing productivity. In this work, the incidence of culture temperature in the biomass concentration of the mixed cultivation of *Chlorella vulgaris* has been evaluated in porcine residuals in an open system. A multivariate analysis is carried out to obtain the environmental variables with the greatest influence on the algae crop temperature, although all the factors are statistically significant, the solar irradiation has the greatest influence, showing that 72,8% of the temperature variability depends on solar irradiation. Through a non-linear regression analysis and by applying the logistic kinetic model of Verhulst, three runs are analyzed at three average daily temperatures obtaining the best fitting at 30°C where a maximum of biomass concentration of 20,8g.L^{-1} is reached.

Keywords: *Chlorella vul*garis; temperature; biomass; porcine residuals.

Introduction

Microalgae culture acquire a great protagonism, being considered eco-friendly, since they efficiently recycle nutrients from liquid and gaseous media, incorporating them into their metabolism for biomass generation [1].

They rank among the most important biological resources that have the capacity to adapt to different environments, even in severe and extreme climatic conditions. Some are extremophile in terms of temperature (thermophilic), pH (acidophilic and alkalophilic). They represent the fastest growing plants on Earth; they can grow a hundred times faster than trees, so much that their weight can be doubled daily [2].

Microalgal biotechnology has gained importance in recent years due to the development of new environmental technologies, given that its growth requires low cost substrates and can be effective for obtaining high added value compounds [3].

In general, microalgae are grown in wastewater to obtain carbohydrates, lipids and proteins that are mainly focused on the production of fuel and animal feed. However, it is known that microalgae produce high value bioactive compounds, which may have different pharmaceutical and cosmetic applications [4].

They have the potential to reduce greenhouse gases (GHG), especially carbon dioxide emissions and the ability to assimilate nitrogen and phosphorus from wastewater[1, 5, 6].This efficient photosynthetic system is capable of converting solar radiation into chemical energy accumulated in the biomass with an efficiency comparable to higher yielding plants, such as sorghum, sugar cane, elephant grass and corn, in addition to the biomass It is usable and contains between 40 and 50% of proteins, not counting the content of vitamins, fats and other biofactors that act favorably on human health [7].

The current production of microalgae biomass is low, only 9,000 tons of dry matter per year; the price of biomass is high, from 30 to 300 € kg^{-1}, however, the size of these markets grows considerably [8].

The large-scale cultivation of microalgae seeks to obtain important quantities of a valuable product; therefore, the productivity of the system must be maximum. Understanding of the factors that determine optimal growth is critical: culture medium, gas exchange, pH, temperature and lighting regime. Temperature is a very important environmental factor due to its great influence on the development of microalgae. The effect of temperature on the biochemical composition mainly affects two different mechanisms: the rate of temperature dependence of chemical and biochemical reactions, and the temperature dependence for photosynthetic carbon fixation in several types of macromolecules, such as example proteins, carbohydrates and lipids [9].

The temperature is proportional to the production of microalgae, reaching optimal values for each species. Above this, respiration increases and photorespiration reduces overall productivity. The optimal values, in general, are between 28 °C and 35°C [1].

When it is a closed farming system, the temperature can be controlled easily by several mechanisms but in an open system, it is more difficult to control, since it depends on the meteorological conditions of the region [10].

The objective of this work is to evaluate the incidence of the suspension temperature in the biomass concentration of the mixed *Chlorella Vulgaris* culture in an open culture system existing in the pork hatchery "El Brujo" in Santiago de Cuba.

Materials and methods

Microorganism and general experimentation conditions

1- Cebadero Porcino
2– Criba de mallas
3– Sedimentador
4– Trampa de fibras
5,9,12– Tanques
6, 8– Bombas
7– FOTOBIORREACTOR
10- Gasómetro
11– Digestor Anaeróbio
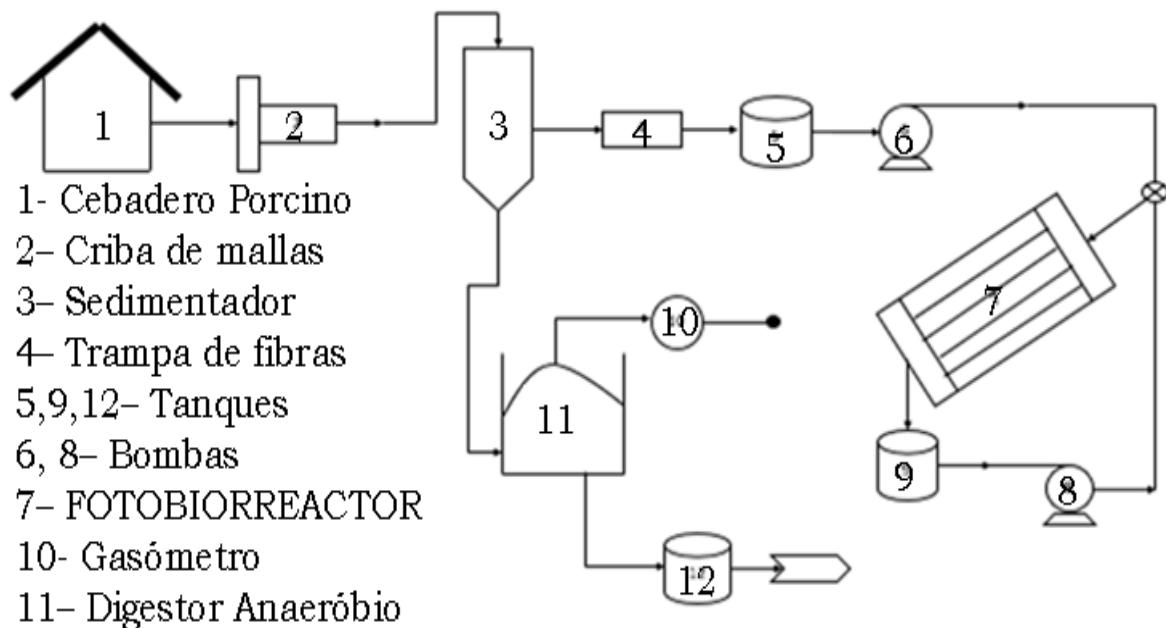
Fig. 1. Technological scheme of the process.

The cultivated species is the microalga Chlorella vulgaris, conserved in the collection of microalgae of the CIES at an average temperature of 25 °C.

The statistical data was taken from experiments carried out in a thin-film photobioreactor of 3500 m^2 that is part of an integral waste treatment system located in El Brujo porcine feedlot areas in Santiago deCuba, (figure1). The other experimental conditions are referred by Alfaro and collaborators [7].

Meteorological Variables

The values of temperature, solar radiation, wind speed and relative humidity were taken fromActinometric station,coupled to a data acquisition system (Data logger MP-110) from the Solar Energy Research Center.

Measuring the crop temperature

The determination of the temperature of the medium was made with a Jenco portable meter model 6072 (Japan).

Statistical analysis

The statistical package Statgraphics Centurion XV (Stat Point, Inc., USA) and Microsoft Excel 2016 (Microsoft, USA) were used to perform the statistical analysis of the data. The graphics were made in the Origin ®6.1 version 6.1052 (B232)

Results and discussion

In an open culture system, the meteorological variables of the geographical location where it is located influence the temperature of the culture medium. To assess the incidence of the environmental parameters Relative humidity (Hr), Incidental solar irradiation (Irr), Ambient temperature (Ta) and Wind speed (W), in the culture temperature (Ts), multiple linear regression analysis is performed, (table 1).

Table1

Results of the linear regression analysis

Parameter	Estimate	Standard Error	Statistical T	Value-P
CONSTANT	-8,35225	0,66082	-12,6392	≤0.01
Hr	0,14683	0,00318	46,1446	≤0.01
Irr	0,00812	0,00008	103,2160	≤0.01
Ta	-0,96478	0,01099	-87,7656	≤0.01
W	0,88836	0,01715	51,8044	≤0.01

The multiple correlation coefficient, R^2, obtained was 0,882, indicating that 88,2 % of the variability of the response is caused by the effect of the factors.

The analysis of variance (table 2), determines that all the factors are statisticallysignificant ($p < 0,05$), that is, they influence the value of the response.

Table 2

Results of variance analysis

Source	Sum of squares	Gl	Middle Square	Reason-F	Value-P
Model	9884,97	4	2471,24	5091,54	≤0.01
Residue	1321,16	2722	0,48536		
Total (Corr.)	11206,10	2726			

The adjusted model equation that describes the relationship between the temperature of the crop and the 4 independent variables is : .(Equation 1)

$$Ts = -8{,}35225 + 0{,}14683*Hr + 0{,}00812*Irr - 0{,}96478*Ta + 0{,}88836*W$$

(1)

To assess the incidence of the environmental parameters Relative Humidity (Hr), Incidental Solar Irradiation (Irr), Ambient Temperature (Ta) and Wind Speed (W), in the Cultivation Temperature (Ts), a multivariate analysis is performed. Table 3 shows Pearson's product moment correlations between each pair of variables. The range of these correlation coefficients ranges from -1 to +1, and they measure the strength of the linear relationship between the variables. The P-value tests the statistical significance of the estimated correlations.

Table 3

Pearson'sproduct moment correlations

	W(m/s)	Ta(^{o}C)	Irr(w/m^2)	Hr(%)
Ts(^{o}C)	0.4958	-0.0660	0.7276	-0.3853
value-P	≤0.01	≤0.01	≤0.01	≤0.01

Values (p <0, 05), indicate correlations significantly different from zero, with a confidence level of 95 %, observing that all the factors are statistically significant, that is, they influence the value of the response. Of these, solar irradiation is the one with the greatest influence, showing that 72, 8 % of the temperature variability depends on solar irradiation.

The statistical summary of the analyzed data was in table 4. The values of average temperature in the crop oscillate in the range of 29 ± 2 ^{o}C.

Table 4

Statistical summary

	Ts (^{o} C)	*W(m/s)*	*Ta (^{o} C)*	*Irr (W/m^2)*	*Hr (%)*
Count	2500	2500	2500	2500	2500
Average	29,00	3,01	30,33	571,68	62,10
Standard deviation	2,00	1,54	1,60	241,12	7,79
Coefficient of variation	6,89%	51,04%	5,29%	42,18%	12,54%

Biomass concentration at different average temperature values

By means of a non-linear regression analysis, adjusting the data to the logistic or Verhuls growth model the maximum concentrations obtained are analyzed by comparing three values of average daily temperature (28ºC, 29ºC, 30ºC), under the same growing conditions,(figure 1). The results show that the maximum concentration of biomass is 20, 8 g.L^{-1} corresponding to the day of 30 º C of average temperature.

In a recent review of the optimal conditions of Chlorella cultivation Daliry and collaborators [11] report that the optimum temperature for Chlorella vulgaris is approximately 30°C, at which maximum productivity is reached. The range of temperature values reported by the authors as optimal coincides with the values obtained in this experiment, validating that the climatic conditions of the study region in Santiago de Cuba are favorable to achieve high productivities of mixed biomass of microalgae and bacteria.

Masojídek and collaborators [12] report studies carried out in a thin layer cascade photobioreactor in environmental conditions similar to ours (22 ºC-36 ºC), obtaining high productivities in a range of biomass concentration of 6,5-13,5g.L^{-1} and estimate that sunlight was used efficiently. The low productivity, they suppose, was related to photoinhibition or photolimitation. The values reported in the present study coincide with these authors, reaching an optimum range of biomass concentration of (6,5-11) g.L^{-1} to obtain high biomass productivities.

Niela González Muñoz, Orlando Gines Alfaro Vives, Harold Crespo Sariol, Rosa María Pérez Silva, Abdiel Jover Capote, p. (568-578)
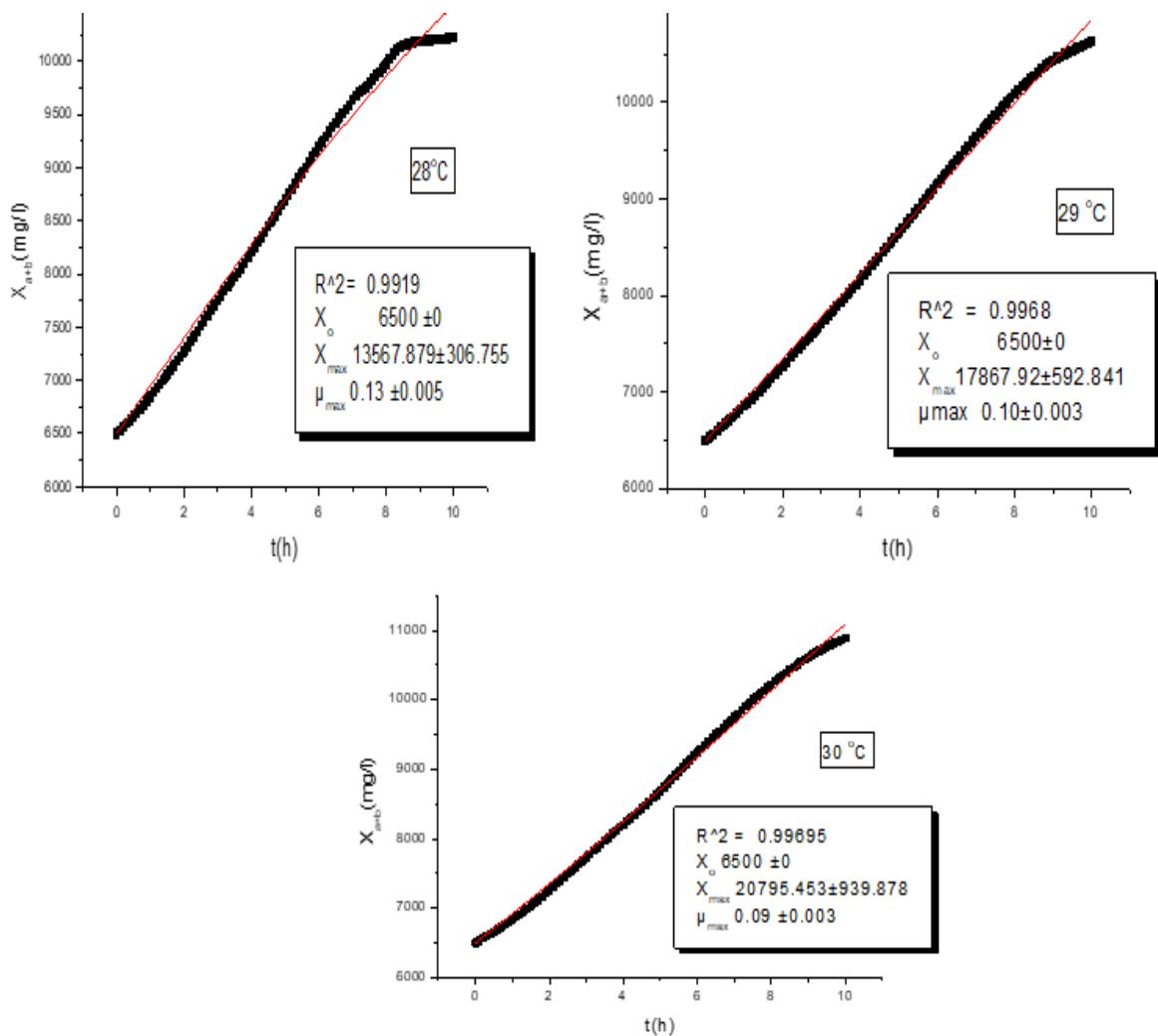
Fig.1. Adjust to the microalgae and bacteria concentration data (Xa + b) to the kinetic model of Verhulst at three average temperatures in the crop (28°C, 29°C, 30°C)

Behavior of solar irradiation

As the intensity of the specific radiant light depends on the direction of observation, it is advisable to use the integrated variables in all spatial directions and the visible spectrum in the modeled mathematical formulation. By integrating the solar irradiation data, the area under the curve can be visualized during the daytime. (Equation 2)

$$I = \int_{0}^{10} Irr \, dx \qquad (2)$$

The attenuation of light in photobioreactors creates a heterogeneous radiation field within the crop, affecting local kinetics. Therefore, it is necessary to take

into account the effects on the available growth in each point of the reactor.Figure 2 shows the behavior of solar irradiation at 10 o'clock on the day of maximum biomass concentration. The data obtained show the gradual increase until reaching the maximum values at noon, corresponding to 875,17W/m^2. The Accumulated solar intensity in the day was 6226, 12 W/m^{2}h.
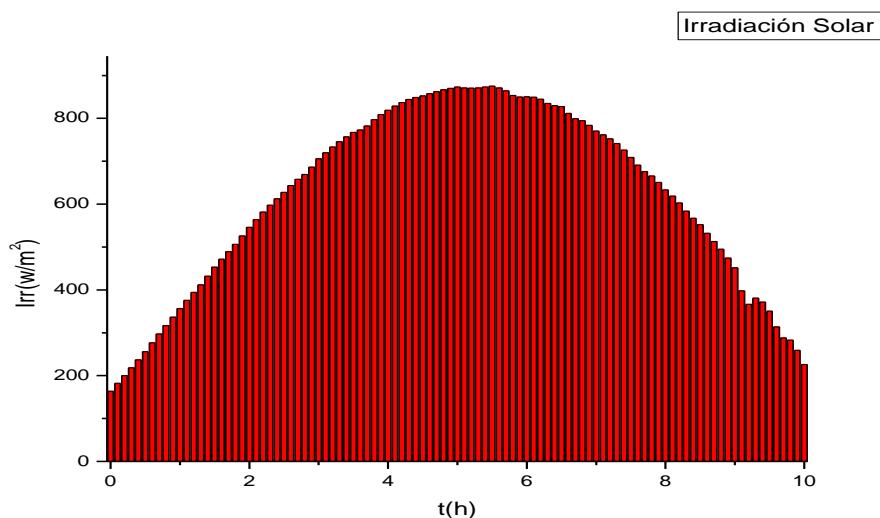
Fig. 2- Example of the behavior of solar irradiation in a day of cultivation.

Area	Peak at	Width	Height
6226,12W/m^{2}h	5,5 h	7,6 h	875,17/m^2

Conclusions

The values of temperature in the crop are influenced by the meteorological variables of the locality, being the solar irradiation the one that has greater incidence reaching its peak values at noon.

The values of temperature in the culture medium are in the optimum range to obtain high concentrations of biomass.

The maximum biomass concentration value (20,8 g.L^{-1}) was obtained on the day analyzed at 30 ^{O}C average temperature.

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