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Influence of solar irradiance levels on the formation of microalgae-bacteria aggregates for municipal wastewater treatment

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ABSTRACT

Light intensity is considered an important factor in the production of extracellular polymeric substances and the formation of microalgae-bacteria aggregates. The influence of the solar irradiance level on the formation of microalgae-bacteria aggregates was studied, considering the settling properties and the removal of organic matter and nutrients. Three different solar average irradiance levels of 6213, 2741 and 3799 Wh m^{-2} d^{-1} were studied in an 80 L outdoor high rate algae pond (July to November 2015) operating at a hydraulic retention time of 10 days and treating municipal wastewater. The highest irradiance level (6213 ± 1186 Wh m^{-2} d^{-1}), showed a poor wastewater treatment performance related to low removal efficiencies of TN (36 ± 12%), total COD (50 ± 8%). However, the removal efficiency of P-PO_4^{3-} evidenced the highest values (92 ± 1%). Furthermore, low settling velocity (Sv) and settleability (4×10^{-3} m h^{-1} and 26 ± 11%, respectively) were associated with a poor aggregation formation in the system. In contrast, low irradiance levels (< 3800 Wh m^{-2} d^{-1}) promoted the formation of microalgae-bacteria flocs and granules with high settling velocity and settleability (18 m h^{-1} and 85%, respectively). Moreover, under low irradiance levels, high removal efficiencies for TN (60 ± 5%), total COD (89 ± 3%) and P-PO_4^{3-} (28 ± 7%) were observed. Nitrification mechanism was only detected at low irradiance levels, which contributed, on average, to 30% of the TN removal from the influent. A relevant factor in the overall good performance of the microalgae-bacteria systems was EPS formation.

1. Introduction

Aerobic processes driven by microalgae-bacteria associations can be seen as a feasible alternative for wastewater treatment. Microalgae produce the required oxygen that allows bacteria to remove pollutants, generating the CO_2 needed by bacteria [1,2]. Furthermore, the mixotrophic characteristic of the microalgae growth has been considered as another mechanism for the removal of organic matter in the systems of wastewater treatment [3]. This conglomerate of photosynthetic and heterotrophic organisms has made the microalga-bacteria system efficient in high rate algal ponds (HRAP) used to treat different types of wastewater, including municipal [4,5], piggery [6,7] and industrial effluent [8]. An important issue in HRAP systems is the dominance of microalgae, such as *Scenedesmus* spp. and *Chlorella* spp., with low settling properties (usually < 3.6×10^{-3} m h^{-1}), which makes their separation from water very difficult [9,10]. The low settling velocity observed in microalgae is an obstacle for biomass harvesting and therefore can limit the cost-effectiveness of biomass recovery and the expansion of microalgae-bacteria to commercial scale [11]. The formation of microalgae-bacteria flocs and aggregates has been studied to overcome settling problems [8,12–14]. To induce the formation of microalgae-bacteria aggregates, and reach high settleability, different factors such as hydraulic and solid retention times (HRT and SRT, respectively) have been evaluated [12–15]. Aside from the microalgae-bacteria floc system, the generation of granular structures in batch systems has been another strategy to promote highly efficient recovery of biomass [16]. However, it has been observed that solar irradiance has an adverse impact on granular stability because of decreases in both extracellular polymeric substance (EPS) production and nutrient removal [17].

Studies of microalgae-bacteria aggregates, for both flocs and granules, have emphasized the relevance of the production of EPS by microalgae and bacteria as the key factor for forming the aggregates. Nevertheless, the presence of EPS is affected not only by operative factors, such as hydraulic retention time (HRT) and solids retention time (SRT) but also by environmental factors such as temperature and light intensity, which affect the algae growth kinetics of both planktonic and biofilm structures.

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Species such as *Microcystis aeruginosa*, *Arthrospira platensis*, and the cyanobacterium *Nostoc* sp. show a positive correlation between EPS production and light intensity in temperature conditions at approximately 30 °C. These species achieved the maximum content of EPS at a high light intensity between 100 and 180 μmol $m^{-2} s^{-1}$ [18–20]. On the other hand, *Graesiella* genus presents different behavior, where the largest production of EPS is related to low light intensity (40 μmol $m^{-2} s^{-1}$) [21]. Nevertheless, when high irradiance levels are present (environmental solar irradiance ≥ 1000 W m^{-2}), the relation with EPS still needs to be assessed. Currently, the control parameters for microalgae-bacteria flocs and granules are related to operative factors (HRT and SRT), and most of the experiments have been carried out in SBR-mode. However, the experiments in continuous operative mode have also shown the formation of these microalgae-bacteria flocs, using anoxic-aerobic algal-bacterial photobioreactor (AA-ABPh) and HRAP under laboratory conditions [14,22]. Environmental parameters such as light intensity and its influence on EPS production need to be assessed to determine their effect on microalgae-bacteria aggregates formation in continuous flow systems treating wastewater. This study aimed to evaluate the influence of solar irradiance on the formation of microalgae-bacteria aggregates, considering the settling properties and the organic matter and nutrient removal in a continuous HRAP treating municipal wastewater.

2. Materials and methods

2.1. Experimental design

The experimental setup consisted of a continuous HRAP made of fiberglass with a total capacity of 80 L (50 L of working volume) and surface area of 0.26 m^2. The HRAP system was operated outdoors from July to November 2015 with an HRT of 10 days. The measurements and the experiment were carried out in Queretaro, Mexico (20° 42 N, 100° 26 W), situated at 1900 m above the sea level. HRAP mixing was assured by a six-blade paddle wheel driven by a motor engine at 10 rpm (Cole Parmer, USA), resulting in a liquid velocity of 0.2 m s^{-1}. The effluent was settled using a 3 L gravity settler. The start-up, acclimatization, and stability of the microalgae-bacteria system were accomplished as previously reported by Arcila and Buitron [14]. Three different solar irradiance levels were evaluated: 6213 ± 1186, 2741 ± 667 and 3799 ± 373 Wh $m^{-2} d^{-1}$, namely, high irradiance level (HIL), low irradiance level (LIL) and medium irradiance level (MIL), respectively. To provide different solar irradiance levels, the HRAP was covered with several layers of greenhouse nylon screen that provided different shade levels. The nylon material did not show selective wavelength absorption, maintaining the spectral composition of sunlight invariant during all experiment stages (Fig. 1S, Supplementary material). The HRAP under the different solar irradiance levels were operated until steady state was reached and the steady state was maintained for at least two HRTs.

Samples of 300 mL were taken from two points, namely at the HRAP reactor and at the settler. Parameters such as total and soluble chemical oxygen demand (COD), total and volatile suspended solid (TSS and VSS), nitrogen sources as ammonium ($N\text{-}NH_4^+$), nitrate ($N\text{-}NO_3^-$) and nitrite ($N\text{-}NO_2^-$), as well as phosphorus ($P\text{-}PO_4^{3-}$), and settling velocity (Sv) were taken twice a week. Proteins and carbohydrate in the extracellular polymeric substance (EPS) and the total Kjeldahl nitrogen (TKN) were evaluated by triplicate once a week. The solar irradiance in the HRAP reactor was measured on the liquid surface. Additionally, the environmental solar irradiance and temperature data were obtained from the Geosciences Center meteorological station at the UNAM [23]. There was no external addition of carbon dioxide to the HRAP except for that naturally introduced by the paddles. There was no biomass recycling, and hence, the SRT equals the HRT during all experimental phases.

Table 1
Municipal wastewater characterization. Values of means ± standard deviation have been calculated using weekly data obtained for all the three periods studied (n: 17).

Parameter	Mean ± SD
CODt (mg L^{-1})	816 ± 129
CODs (mg L^{-1})	591 ± 92
VSS (mg L^{-1})	135 ± 23
pH	7.4
$N\text{-}NH_4^+$ (mg L^{-1})	64 ± 10
$N\text{-}NO_3^-$ (mg L^{-1})	3 ± 2
$N\text{-}NO_2^-$ (mg L^{-1})	2 ± 1
TKN (mg L^{-1})	105 ± 30
TN (mg L^{-1})	110 ± 16
$P\text{-}PO_4^{3-}$ (mg L^{-1})	15.3 ± 1.3

2.2. Microorganisms

The HRAP was inoculated with a mixture of 833 mg volatile suspended solids (VSS) L^{-1} of microalgae biomass and 2740 mg VSS L^{-1} of activated sludge obtained from the aeration tank of a municipal wastewater treatment plant. The microalgal biomass was composed of mixed microalgae collected from an aquatic environment in Queretaro State, Mexico and propagated in Bold medium [24]. *Scenedesmus* sp. was microscopically identified as the dominant genus.

2.3. Wastewater source

The municipal wastewater was collected from a wastewater treatment plant located in Santa Rosa Jauregui, Queretaro, Mexico. Samples were taken after primary treatment (coarse and fine screening and primary sedimentation). Before feeding the HRAP, the wastewater was passed through a sieve (Tyler No 65) and stored in a cold room at 4 °C for a maximum of seven days. The average quality of the municipal wastewater influent is shown in Table 1.

2.4. Analytical procedures

TSS and VSS concentrations, TKN and sludge index volume (SIV) were analyzed according to APHA standard methods (APHA, 2005). $N\text{-}NH_4^+$ and COD, both total and soluble, were determined through the 8,000 HACH and 10,031 HACH colorimetric methods. Nitrate and nitrite were measured following the 10,237 and 10,206 HACH colorimetric methods. Likewise, $P\text{-}PO_4^{3-}$ was determined by a colorimetric method (10,127 HACH). OD and pH were measured in situ (YSI Model 50B and Sensorex pH 5450C, respectively). All the soluble parameters were filtered through 0.45 μm pore size membranes (Whatman glass microfiber filters, Grade GF/A). For the identification and quantification of microalgae, a LEICA DM500 microscope with an image acquisition system (LEICA ICC50 HD) and a stereoscopic lens (Zeiss Stemi DV4) was used. The microalgae genera were identified according to Wehr and Sheath [25]. The quantification was assessed according to microscopic methods for quantitative phytoplankton analysis using the counter chamber method in a Sedgewick-Rafert counting slide [26]. Five samples were collected during the steady state period for each irradiance level for microbiological analysis and granular structure determination. Samples were homogenized by sonication (500 watts) for 60 s and then counted. Six random fields composed of sixteen squares were counted per slide. Chlorophyll (a) was determined according to Strickland et al. [27]. The settling velocity (Sv) of the sludge and granules were measured according to the methodologies of Zhaowei et al. [28] and Yu et al. [29], respectively. Granular structures were extracted from the broth culture, and the Sv of granules was measured separately. The settleability percentage was calculated based on Eq. (1). The diameter of the granules was determined by using a stereomicroscope (Zeiss Stemi DV4, Germany) and the image software analyzer.