Algal Research 41 (2019) 101569

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Algal wastewater treatment: Photoautotrophic vs. mixotrophic processes

N. Nirmalakhandan[a,*], T. Selvaratnam[b], S.M. Henkanatte-Gedera[a], D. Tchinda[a], I.S.A. Abeysiriwardana-Arachchige[a], H.M.K. Delanka-Pedige[a], S.P. Munasinghe-Arachchige[a], Y. Zhang[a], F.O. Holguin[c], P.J. Lammers[d]

[a] Civil Engineering Dept., New Mexico State University, Las Cruces, NM, USA
[b] Civil & Environmental Engineering Dept., Lamar University, Beaumont, TX, USA
[c] Plant & Environmental Sciences Dept., New Mexico State University, Las Cruces, NM, USA
[d] Arizona Center for Algae Technology & Innovation, Arizona State University, Phoenix, AZ, USA

ARTICLE INFO

Keywords:
Autotrophy
Heterotrophy
Mixotrophy
Galdieria sulphuraria
BOD removal
Nutrient removal
Pathogen removal

ABSTRACT

Photoautotrophic algal systems have been investigated as potentially greener and more sustainable alternatives to the traditional bacteria-based wastewater treatment (WWT) systems. This paper presents mixotrophic algal systems as better suited for WWT. Since the literature is void of mixotrophic WWT systems, a brief review of the laboratory results from the literature comparing the different types of algal metabolisms is presented first. Details of a mixotrophic WWT system driven by an extremophilic alga, *Galdieria sulphuraria* (pH = 1 to 4; temperature = 25 to 56 °C) are presented next. Results from pilot scale version of this mixotrophic system (700 L) are summarized to show that it can reduce biochemical oxygen demand (BOD) and nutrients in primary-settled wastewater in a single step to yield discharge-ready effluent in < 3 days of fed-batch processing. Average volumetric removal rates of BOD_5 (16.5 ± 3.6 mg L^{-1} d^{-1}) and ammoniacal nitrogen (6.09 ± 0.92 mg L^{-1} d^{-1}) in this mixotrophic system were found to be not different from those reported for the photoautotrophic high rate algal ponds (significance level of 0.05); but, that of phosphates (1.40 ± 0.57 mg L^{-1} d^{-1}) is greater than that reported for the photoautotrophic high rate algal ponds (significance level of 0.05). Additionally, the extreme culture conditions in this particular mixotrophic system enabled concurrent reductions of *E. coli* (> 5 log) and other pathogenic bacteria.

1. Introduction

According to the American Society of Civil Engineers (ASCE), the nation's Publicly Owned Treatment Works (POTWs) that are mandated to collect and treat municipal wastewaters are the most basic and critical infrastructure facilities to protect public health and the ecosystem [1]. To meet the mandated treatment levels, POTWs rely on a series of processes, each designed to remove a specific pollutant in the wastewaters. Although effective in their respective removals, these processes are energy- and resource-intensive [2]. Most of these processes were developed in the 1900s, with little regard to their carbon-footprint or life-cycle impacts [3]; their benefits of improved sanitation and ecosystem services were valued to be much greater [4].

A recent EPA report has acknowledged that, new technologies are needed for WWT to reduce greenhouse gas emissions and to recover nutrients at substantially less cost and with reduced carbon footprint [3]. ASCE has also recommended replacement of the ageing, energy-intensive WWT systems with newer technologies that could recover energy and other valuable resources from wastewaters [1]. It is now recognized that rehabilitation of the nation's ageing wastewater infrastructure is a critical need; and new technologies are becoming available for doing so [3].

1.1. Algal wastewater treatment

Among the emerging WWT technologies, algal-based systems hold promise as greener and sustainable alternatives to the current practice. Metabolic capabilities of algae enable them to grow in wastewaters, ingesting their organic- and nutrient-contents. In contrast to the current energy-intensive technologies, algal processes can be driven by energy derived either from sunlight or from the wastewater itself. Oswald and co-workers were among the first to engineer algal systems for energy-efficient, low-cost WWT [5,6]. Their work has led to the development of the high rate algal pond (HRAP) that has been widely adopted primarily

* Corresponding author.
E-mail address: nkhandan@nmsu.edu (N. Nirmalakhandan).

https://doi.org/10.1016/j.algal.2019.101569
Received 21 March 2019; Received in revised form 16 May 2019; Accepted 1 June 2019
Available online 12 June 2019
2211-9264/ © 2019 Published by Elsevier B.V.

for algal biomass production to derive value-added products rather than for WWT [7].

Renewed interest in algal-based technologies has emerged recently for varied applications including WWT. Algal WWT enables pollution control as well as generation of energy- and nutrient-rich algal biomass from which, biofuels, fertilizers, animal food supplements, and other high value products could be recovered [8]. Using energy from sunlight and/or from the wastewater for WWT can conserve fossil fuels used by traditional WWT processes and abate associated emissions. Recovering fertilizers from the biomass grown in wastewaters can conserve the energy currently used in fertilizer manufacture [2]. Algal WWT technologies also can benefit from solar disinfection, decreasing final disinfectant demand and the potential for formation of harmful disinfection byproducts (DBPs). Thus, algal WWT systems have the potential to turn POTWs into resource recovery facilities for sustainable utility service.

In this paper, we compare two algal pathways for WWT: a conventional system based on photoautotrophic metabolism versus an emerging one, based on mixotrophic metabolism. First, we present an overview of metabolism of algal systems and their suitability for WWT. Details of a mixotrophic process, utilizing an extremophile, *Galdieria sulphuraria*, and its capability in single-step WWT are summarized next, including laboratory results and pilot scale results. To the best of our knowledge, *Galdieria sulphuraria* has not been evaluated previously by any other groups for WWT. Performance of this *G. sulphuraria*-based mixotrophic WWT process is then compared with literature data on the traditional photoautotrophic WWT process.

1.2. Algal metabolism

Major metabolic requirements of all algal systems include i) a source of carbon for anabolism; ii) a source of energy for catabolism; and iii) availability of major and minor nutrients. Depending on their metabolic choice of sources of carbon and energy, algal systems are classified as photoautotrophic, heterotrophic or mixotrophic; heterotrophs are subclassified as photoheterotrophs and chemoheterotrophs (Table 1).

Photoautotrophic and chemoheterotrophic systems have been adopted in many applications including WWT. However, applications of mixotrophic systems are currently limited [9] even though they can be seen to be the most suitable ones for WWT from a metabolic perspective. Wang et al. have presented a review of the current status and prospects of the three major algal systems [9]. The three metabolisms and their applications in WWT are summarized next.

1.2.1. Photoautotrophy

Photoautotrophic cultures use inorganic carbon as the carbon source for growth; they rely on light as the exclusive energy source, which is converted by the photosynthetic machinery to chemical energy required for growth. This process has been engineered in two major approaches for municipal WWT.

In one approach, photoautotrophic algae and heterotrophic bacteria are employed symbiotically to remove BOD and nutrients from municipal wastewaters [5,6]. The premise of this approach is that the photoautotrophic algae would photosynthetically produce the oxygen necessary for oxidative assimilation of BOD in the wastewater by the heterotrophic bacteria; and the carbon dioxide produced during BOD oxidation would serve as the inorganic carbon source for the photoautotrophic algae. In this manner, the energy needs for aeration in the classical activated sludge process for BOD removal could be averted.

In another application, photoautotrophic algae are used as a tertiary process following the activated sludge process for nutrient removal [10]. The heterotrophic bacteria in the activated sludge by itself cannot remove N and P in the primary effluent to the discharge standards. The reason being the mismatch in the C:N:P ratio between the primary effluent and the activated sludge biomass. As such, photoautotrophic algae are used in a follow-up process to reduce the residual N and P to their respective discharge standards. In this case, the photoautotrophic algal reactor will need and external source of inorganic carbon; typically, the reactor is sparged with gaseous CO_2 to serve this need.

In both the above applications, the photoautotrophic process is often implemented in open raceways driven by paddlewheels. These systems are designed to utilize natural sunlight as the energy source; as such, the biomass density and the depth of the water column in the raceway have to be maintained low to ensure adequate sunlight penetration. But, low biomass densities translate to low volumetric removal rates of BOD, N, and P; and, shallow depths translate to large surface area causing high evaporative water loss and increased potential for invasion by predators, competitors, and parasites [11]. At the same time, shallow depths and lower biomass densities can, at times, result in photoinhibition and photolysis.

Since the pH in photoautotrophic systems is often high (> 9), significant amounts of NH_3 ($> 75\%$) [12] would be lost by volatilization and phosphates, by precipitation [13]. Findings of Garcia [14] and Picot et al. [15] confirmed volatilization as a major mechanism of total nitrogen removal in HRAPs. These losses could reduce biomass growth and the potential for downstream recovery of energy and nutrients from the biomass [16]. In the second application where gaseous CO_2 is sparged to provide the inorganic carbon, the gas-to-liquid transfer efficiency of CO_2 suffers because of the short bubble detention time at shallow depths. Poor transfer efficiency results in loss of CO_2, lower growth rates, and low volumetric pollutant removal rates.

1.2.2. Heterotrophy

Chemoheterotrophic cultures obtain their carbon solely from organic chemicals; they obtain their energy needs also from organic chemicals. Most green algae are capable of heterotrophic growth [17]. Since chemoheterotrophic systems do not depend on light as photoautotrophs do, heterotrophic WWT systems can be operated at much higher biomass densities to achieve high volumetric removal rates of pollutants. Higher biomass yields are possible with chemoheterotrophs because, the energy density of organic chemicals (e.g. glucose, $\Delta H = -2801\ \mathrm{kJ\ mole^{-1}}$) is higher than that of carbon dioxide ($\Delta H = -395\ \mathrm{kJ\ mole^{-1}}$). However, they have to be provided with external supply of oxygen. Photoheterotrophic cultures obtain their carbon solely from organic chemicals, but their energy needs from light.

A concern with heterotrophic systems is the possibility of contamination/competition due to the high-energy carbon substrates. Most industrial heterotrophic systems have resorted to enclosed reactor configurations to maintain axenic cultures. To take full advantage of the higher biomass densities that can be achieved in the heterotrophic mode, most applications rely on highly engineered bioreactors; such systems are deemed too expensive for application in WWT where the liquid volume is large and the concentrations of organic substrate is low [18]. In a review of the literature on heterotrophic metabolism and its applications, heterotrophic WWT systems were mentioned only as a possibility with limited laboratory results supporting their viability [18].

1.2.3. Mixotrophy

Mixotrophic cultures are versatile in that, they can obtain their carbon needs either from organic or inorganic chemicals. Likewise, they

Table 1
Classification of algal metabolism based on carbon and energy sources.

Metabolism	Carbon source	Energy source
Photoautotrophic	Inorganics	Light (obligatory)
Heterotrophic		
Chemoheterotrophic	Organics	Organics
Photoheterotrophic	Organics	Light (obligatory)
Mixotrophic	Organics and/or inorganics	Organics and/or light

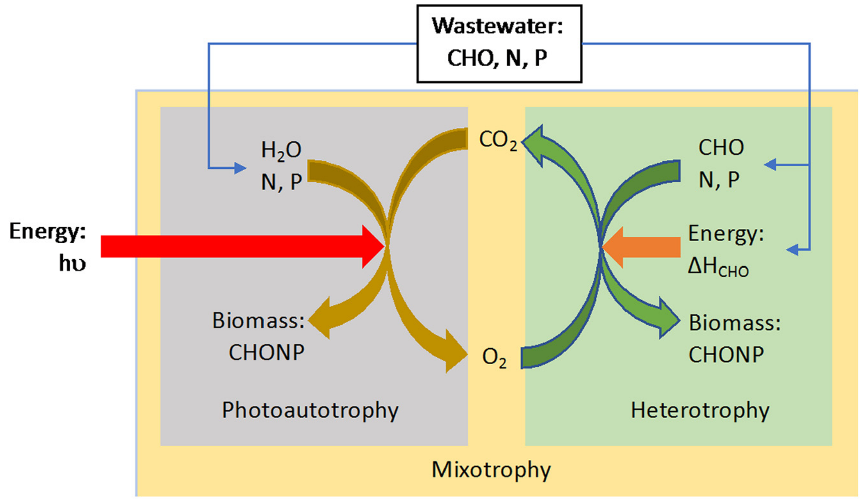

Fig. 1. Schematic of mixotrophic process for utilizing pollutants in wastewater (CHO, N and P) with energy from light [hυ] in photoautotrophy and/or from organics [ΔH] in heterotrophy.

can obtain their energy needs from organic or inorganic chemicals, depending on the ratio of the energy available from the organic chemical source to that from the light source. It can be considered as a combination of photoautotrophic and chemoheterotrophic processes as illustrated in Fig. 1; each process can occur independent of the other resulting in accumulation/depletion of CO_2 and O_2.

While similarities can be noted among the reactants and products in the mixotrophic and HRAP systems, a major distinction is that, the former relies on heterotrophic algae and the latter, on heterotrophic bacteria. The difference between mixotrophs and photoheterotrophs is that, mixotrophs can grow on single-constituent metabolism; i.e. they can grow exclusively on light photosynthetically if organic carbon is not available; or, they can grow exclusively on organic carbon in dark; whereas, photoheterotrophs require both light and organic chemicals to grow. Additionally, mixotrophs have the ability to utilize organic carbon and inorganic carbon simultaneously as carbon source; and utilize light and organic carbon also simultaneously as energy source. This unique metabolic flexibility of mixotrophs makes them an ideal candidate for round-the-clock wastewater treatment at scale.

Considering the mixotrophic process as a combination of photoautotrophy and heterotrophy as shown in Fig. 1 and assuming simple stoichiometry, the biomass growth under mixotrophy, X_m [gr], in a batch processing time of t* [hr] can be estimated from:

$$X_m = I_{abs(t)}\ A\ [Y_a]\ t^* + (-\Delta H)_{org}(S_{t=0} - S_{t=t^*})V\ [Y_h] \tag{1}$$

where, I_{abs} is the light energy absorbed by the cells [$kJ\,cm^{-2}\,h^{-1}$]; A is the light incident area [cm^2]; Y_a is the biomass yield under autotrophic mode [g cell kJ^{-1}]; $(-\Delta H)_{org}$ is the enthalpy of the organic substrate [$kJ\,kg^{-1}$]; S is the concentration of the organic substrate [$g\,L^{-1}$]; V is the culture volume [L]; and Y_h is the biomass yield under heterotrophic mode [g cell kJ^{-1}].

Previously, it was found that mixotrophic growth with acetate as carbon source could increase biomass yield, cell density and daily productivity of *Chlorella sorokiniana* cells compared to autotrophic growth while the production of lipid, protein and starch were also increased [19]. De novo transcriptome assembly and gene expression analysis suggested that carbon loss due to acetate oxidation is reduced by upregulation of phosphoenolpyruvate carboxylase through an alternative carbon fixation pathway under mixotrophy conditions [19]. Another study revealed that mixotrophic growth of *C. sorokiniana* with glucose could cause increased expression level of accD gene (heteromeric acetyl-CoA carboxylase beta subunit) for fatty acid synthesis, which most likely triggered increased lipid content in stationary phase of mixotrophic growth [20]. These findings suggest that the superior performance of mixotrophic cultures is due to their ability to maximize recovery of carbon atom lost by organic oxidation.

1.2.4. Advantages of mixotrophy

Many studies have evaluated mixotrophy as a function of algal strains and pure substrates, mostly under laboratory conditions. Majority of them have concluded that the mixotrophic growth rate to be greater than the heterotrophic and photoautotrophic growth rates, and to be approximately equal to the sum of the two, provided the cultures are light-limited [17,21]. This conclusion agrees with the simple result indicated by Eq. (1). For example, Marquez et al. evaluated growth of *Spirulina platensis* on glucose and found autotrophic growth rate of 0.45 d^{-1}; heterotrophic growth rates of 0.2, 0.2, and 0.2 d^{-1} with glucose concentrations of 1.0, 2.0, and 3.0 $g\,L^{-1}$, respectively; and corresponding mixotrophic growth rates of 0.61, 0.66, and 0.65 d^{-1} [21]. Growth rates of *Chlorella sorokiniana* under mixotrophic mode at three concentrations of glucose and at two temperatures (25 °C and 37 °C) were found to be greater than the sum of its growth rates under heterotrophic and photoautotrophic modes [22] as illustrated in Fig. SI-1 included in the Supplement section.

Zhang et al. reported that mixotrophic biomass yield on glucose was > 60% than that for heterotrophic cultures; and the enhancement effect of light under mixotrophic cultivation was 7.35-fold [23]. Cheirsilp and Torpee studied the growth of a freshwater *Chlorella* sp., a marine *Chlorella* sp., *Nannochloropsis* sp. and a *Cheatoceros* sp. in a synthetic medium under the three modes and concluded that all four strains achieved highest biomass production in the mixotrophic mode [24]. Cerón Garcí et al. studied growth of *Phaeodactylum tricornutum* on glycerol and found 9-fold higher biomass concentrations; 8-fold higher biomass productivities; and 10-fold higher eicosapentaenoic acid (EPA) production in the mixotrophic mode than in photoautotrophic mode [25]. Li et al. evaluated mixotrophic cultivation of *Chlorella sorokiniana* in a growth medium supplemented with 4 $g\,L^{-1}$ of glucose and reported growth rates and maximum biomass densities 1.8- and 2.4-fold higher than those in heterotrophic cultures; and 5.4- and 5.2-fold higher than in photoautotrophic cultures, respectively [22].

Another advantage of mixotrophic growth is the higher lipid content in the resulting algal biomass and increased lipid productivity in certain strains. Higher biomass production and higher lipid contents result in higher lipid productivity translating to higher energy recovery, making the process greener. Liang et al. investigated growth of *Chlorella vulgaris* under the three growth conditions with acetate, glucose, and glycerol, and reported that biomass and lipid productivities were highest under the mixotrophic mode [26]. For example, with 1% glucose, volumetric biomass productivity increased from 151 mg L^{-1} day^{-1} in the heterotrophic mode to 254 mg L^{-1} day^{-1} in the mixotrophic mode; and, lipid productivity increased from 35 to 54 mg L^{-1} day^{-1}. Li et al. found that *Chlorella sorokiniana* growing in glucose achieved lipid content of 45% under mixotrophic mode compared to only 13% in the heterotrophic mode [22]. The study by Cheirsilp and Torpee which compared the growth of freshwater *Chlorella* sp., marine *Chlorella* sp., *Nannochloropsis* sp. and *Cheatoceros* sp. on glucose under photoautotrophic, heterotrophic and mixotrophic modes had recorded much higher lipid production in the mixotrophic mode than in the photoautotrophic and heterotrophic modes for all four cultures [24].

Mixotrophic growth can also be advantageous over photoautotrophic growth due to higher tolerance to photoinhibition and photooxidative damages, especially in closed photobioreactors where oxygen could accumulate. Photoinhibition was observed in photoautotrophic culturing of *Spirulina* sp. at light intensity of 50 W m^{-2}, whereas, it was able to endure light intensities of 0–65 W m^{-2} under mixotrophic mode [27]. While they also found the mixotrophic growth rate to be greater than autotrophic and heterotrophic growth rates, the notion of simple addition growth rates was not found to be valid in this case. Lower sensitivity of mixotrophic cultures to wide range of light intensities can be beneficial in WWT in acclimating to and recovering from diurnal light changes, in supporting high cell densities, and in accommodating high turbidity levels.

Although all the above findings of recent reports are based on laboratory studies on pure substrates, they are promising and provide a strong motivation for evaluating the mixotrophic pathway for WWT. In a review of heterotrophic and mixotrophic culturing, Perez-Garcia and Bashan concluded that, with the current knowledge, mixotrophic systems may be the most appropriate ones for sustainable WWT [28]. Our previous reports have described the development of a mixotrophic WWT system and documented its ability in removing BOD, nutrients, and pathogens from primary-settled wastewaters in a single step [29,30]. In this paper, we summarize results from long term operation of a pilot scale version of this mixotrophic WWT system deployed at a local wastewater treatment plant and, compare its performance against that of the more common HRAP system.

2. Materials and methods

The proposed mixotrophic algal-based WWT system is distinct from traditional algal systems in that, it employs an extremophilic microalga. This strain, *Galdieria sulphuraria* (hereafter *G. sulphuraria*), has been isolated from geothermal springs, acclimated to pH of 1.0–4.0 and temperatures of 25–56 °C. To the best of our knowledge, this study was the first one to demonstrate i) the feasibility of cultivating *G. sulphuraria* in primary effluent under field conditions; ii) the ability of the system to reduce the organic- and nutrient-content of primary effluent to secondary discharge standards; and iii) the potential for inactivating coliform and pathogenic bacteria to non-detectable levels. The selection of the extremophilic alga, *Galdieria sulphuraria*, in our efforts for WWT was based on several factors such as ability to resist invasion by predators and competitors; minimize ammonia loss by volatilization and phosphate loss by precipitation; potential for pathogen reduction; and, deployment in hot/humid climates in enclosed reactors.

2.1. Laboratory studies

Goals of our laboratory studies were to demonstrate the mixotrophic capability of *G. sulphuraria* and to assess its ability to grow in primary settled wastewater as it had not been reported on previously. These laboratory studies were run in 16 mm borosilicate glass tubes in a Tissue Culture Roller Drum Apparatus (New Brunswick Scientific, Eppendorf, CT, USA) rotating at 16 rpm. The tubes were inoculated with 6 mL of the culture and placed in the outer rim of the roller drum housed in an incubator (Percival, IA, USA) maintained at 40 °C with a 14 h/10 h light/dark cycle. The incubator environment was kept constant at a CO_2 level of 2–3% (vol vol^{-1}). Growth of *G. sulphuraria* in glucose and in primary-settled wastewater was evaluated against that in the standard growth medium in terms of OD at 750 nm and ash-free dry weight.

2.2. Pilot scale studies

Based on our laboratory results, a pilot scale system utilizing *G. sulphuraria* was deployed at a local wastewater treatment plant. The bioreactor developed in this study is also uniquely different from those in previous studies in that, it is engineered as an enclosed raceway, fabricated of clear polyethylene, enabling natural illumination and heating by sunlight (Fig. SI-2, Supplement Section). A motor-driven paddlewheel provided mixing and circulation of the culture. This enclosed configuration enabled trapping of solar heat to maintain the cultures at above-ambient temperature of 35–45 °C. The headspace of the bioreactor was supplied with CO_2-enriched air (2% vol vol^{-1}). The pH of the feed was adjusted to 2.0 by adding sulfuric acid; the cultures self-maintained the operating pH at 4.0.

This cultivation system overcomes many of the limitations in the traditional open raceway configuration; it eliminates evaporative losses and odor emissions; and minimizes invaders and predators. The low pH along with solar insolation contribute to high degree of deactivation of native pathogens, beneficial in minimizing final disinfectant demand and the potential for formation of malignant disinfection byproducts. Low pH also minimizes ammonia loss by volatilization, resulting in higher biomass production and hence, maximizing volumetric pollutant removal rates and downstream recoveries. Because of mixotrophy, the system is not light-limited; as such, higher biomass densities and culture depths could be supported to improve volumetric pollutant removal rates. The enclosed headspace in this bioreactor minimizes loss of CO_2 ensuring adequate CO_2 supply for autotrophic growth during photoperiod.

The pilot scale system was initiated with 3 × 300 L reactors and subsequently scaled up to 2 × 700 L. The 700 L system has been operational for over 2 years in batch mode and then in fed-batch mode for over 1 year with stable performance. In this paper, results from the fed-batch operation, conducted in two identical bioreactors (R1 and R2) are summarized. The active volume of the bioreactors was 700 L and the culture depth was 20 cm. Fed-batch cycles were initiated with 300 L of the preadapted culture of *G. sulphuraria* mixed with 400 L of primary effluent. Each fed-batch cycle was terminated when the concentrations of BOD_5, ammoniacal-nitrogen (N), and phosphates (P) in the reactor were reduced to their respective secondary discharge standards (of 30 mg L^{-1} BOD; 10 mg L^{-1} N; and 1 mg L^{-1} P). Upon reaching all three discharge standards, the paddlewheel was switched off for 24 h allowing the biomass to settle. Thereafter, 400 L of the supernatant were discharged, and the bioreactor was recharged with 400 L of fresh primary effluent to start a new cycle. The accumulated algal biomass was harvested every 5-consecutive fed-batch cycles before initiating the next set of 5 cycles. Six sets of cycles were completed over 120 days, with 5 cycles in each set (total of 30 cycles). Samples from the reactors were analyzed for N and P on a daily basis; and for BOD_5, every two days. Samples were filtered through 0.45 μm membrane filters and placed on mEndo and mFC nutrient agar mediums at 35 °C and 44 °C respectively, to enumerate total and fecal coliforms.

3. Results and discussions

3.1. Laboratory demonstration of mixotrophic WWT by G. sulphuraria

Initial assessment of the mixotrophic nature of *G. sulphuraria* was done under laboratory conditions by comparing its growth on nutrient-rich, carbon-free standard growth medium against that in glucose-enriched medium [30]. As reported in the literature for mixotrophic growth of other strains, by *G. sulphuraria* also grew at a faster rate in the mixotrophic mode ($0.340 \pm 0.022\,g\,L^{-1}\,d^{-1}$) than in the photoautotrophic mode ($0.250 \pm 0.058\,g\,L^{-1}\,d^{-1}$). Mixotrophic growth rates of *G. sulphuraria* on sterilized ($0.186 \pm 0.010\,g\,L^{-1}\,d^{-1}$) and unsterilized ($0.175 \pm 0.028\,g\,L^{-1}\,d^{-1}$) primary-settled wastewater were found to be 35% greater than the photoautotrophic growth rate in the carbon-free standard growth medium ($0.134 \pm 0.010\,g\,L^{-1}\,d^{-1}$). These results have also confirmed 50% higher biomass yields under mixotrophic mode than in the pure heterotrophic mode (0.63 vs. 0.42 g biomass g glucose^{-1}) [30].

In an outdoor test, growth of *G. sulphuraria* in two identical reactors in autophototrophic mode was measured to be $0.035\,g\,L^{-1}\,d^{-1}$; when one of the reactors was enriched with 25 mM of sucrose after two weeks operation, growth rate in that reactor increased 8-fold to $0.282\,g\,L^{-1}\,d^{-1}$ confirming the improvement under mixotrophic conditions. Continuing our preliminary studies with pure substrates [29], we have demonstrated, for the first time, *G. sulphuraria's* ability for simultaneous removal of BOD and nutrients from primary effluent to produce discharge-ready effluent in 3–5 days of batch-processing. Volumetric removals of ammoniacal nitrogen ($4.7–5.0\,mg\,L^{-1}\,d^{-1}$) and phosphate ($1.2–1.7\,mg\,L^{-1}\,d^{-1}$) recorded in these studies were comparable to those reported in the literature for other strains [31,32].

3.2. Field demonstration of WWT by G. sulphuraria

Long term testing of the 700 L reactors in batch and fed-batch modes have been documented. Fig. 2 summarizes results from two identical pilot scale reactors R1 and R2 fed with primary effluent and operated in fed-batch mode over the 120-day period as indicated earlier. During this period, culture temperature ranged 27 to 46 °C (average = 34.9 ± 4.3 °C) and dissolved oxygen ranged 5.1 to $7.8\,mg\,L^{-1}$ (average = $6.2 \pm 0.5\,mg\,L^{-1}$). Variations of concentrations of NH_4-N, P, and BOD_5, measured on day 0 and day 2 of each fed-batch cycle are summarized in Fig. 3-a to c. It can be noted that the system can achieve the discharge standards for all three pollutants in < 3 days, irrespective of the day-to-day variation in the influent concentrations of NH_4-N (average = $23.4 \pm 3.5\,mg\,L^{-1}$), P (average = $4.2 \pm 1.5\,mg\,L^{-1}$), and BOD_5 (average = $55.4 \pm 9.7\,mg\,L^{-1}$). Based on the performance of the two reactors depicted in Fig. 2, no statistical difference could be found between the two, indicating that the system is reproducible and stable.

Variations of inlet concentrations of BOD_5, PO_4, and NH_4-N and the processing times required, t* [day] to meet all three discharge standards in the laboratory studies and the pilot scale studies (300 L batch; 700 L batch and 700 L fed-batch) are summarized in Fig. 3. The shorter process time in the case of the fed-batch operation is due mainly to accumulation of the biomass over the 5 fed-batch cycles in each set. Further process optimization is planned to reduce the process time so that the footprint of this mixotrophic system could be reduced. Options to reduce the process time includes increasing the culture depth and the initial biomass density at each fed-batch cycle.

The pilot scale system was able to reduce total coliform counts (2.29×10^7 CFU 100 mL^{-1}) and fecal coliform counts (1.41×10^7 CFU 100 mL^{-1}) in the primary effluent to non-detectable limits within one day of batch processing [33]. In the fed-batch mode, 1.68 log reductions of total bacteria were recorded in 5 days. Copies of *Enterococcus faecalis* (7.13×10^5 copies/100 mL) and *Escherichia coli* (2.77×10^5 100 mL^{-1}) in the primary effluent were reduced to non-detectable limits in 5 days resulting in 5.44 and 7.44 log reductions, respectively [33].

3.3. Performance of mixotrophic vs. photoautotrophic WWT systems (HRAP)

In this section, results from the fed-batch trials of the mixotrophic WWT system utilizing *G. sulphuraria* are compared against the performance of the more common photoautotrophic HRAP systems compiled from the literature. A direct comparison is difficult because the literature reports varied by strains used, types of wastewaters assessed, the influent concentrations, modes of operation of the HRAP systems, the types of analysis followed, and the data reported. Nevertheless, we present these results for a holistic comparison to show that the mixotrophic processes warrant further investigations. We have selected the volumetric removal rates (VRR) of BOD, N, and P as the basis of comparison irrespective of mode of operation (batch, semi-continuous, continuous) and environmental conditions (temperature, season, location). When VRR results were not reported directly in the literature, we used the reported meta data to calculate the VRR; or estimated data from the plots included in the reports to estimate the VRR.

3.3.1. BOD reduction

Volumetric removal rates of BOD from our studies (30 data points from 30 cycles) are compared with similar results compiled/calculated from the literature for HRAPs from 15 literature reports. Details of the data sources are included in the Supplement section (Table SI-1). Some reports had reported COD instead of BOD_5; we have listed the reported COD values in those cases. VRR values for the 45 cases are plotted against the respective initial BOD_5 (or COD) concentrations in Fig. 4. As can be noted from Fig. 4, the VRR found in our studies varied linearly with the initial concentration over the range of concentrations encountered during the run, with a correlation coefficient of $r^2 = 0.993$; n = 30. All but one of the 15 data points representing HRAPs fall below

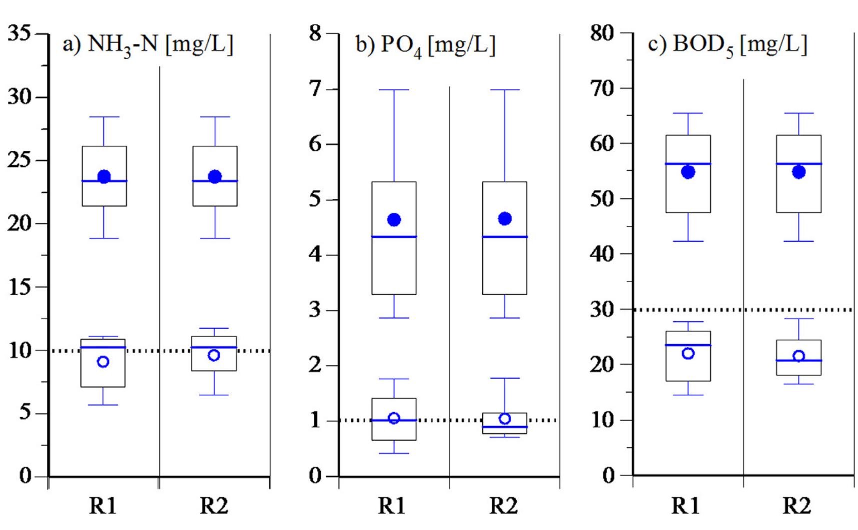

Fig. 2. Summary of results from 30 consecutive fed-batch cycles of 4 days each, run over 120 days in two parallel reactors R1 and R2, with harvesting of accumulated biomass every 5 cycles. Ranges of concentrations on Day 0 (●) and Day 2 (○) of: ammoniacal nitrogen (a); phosphates (b); and biochemical oxygen demand (c). Dashed lines indicate discharge standards.

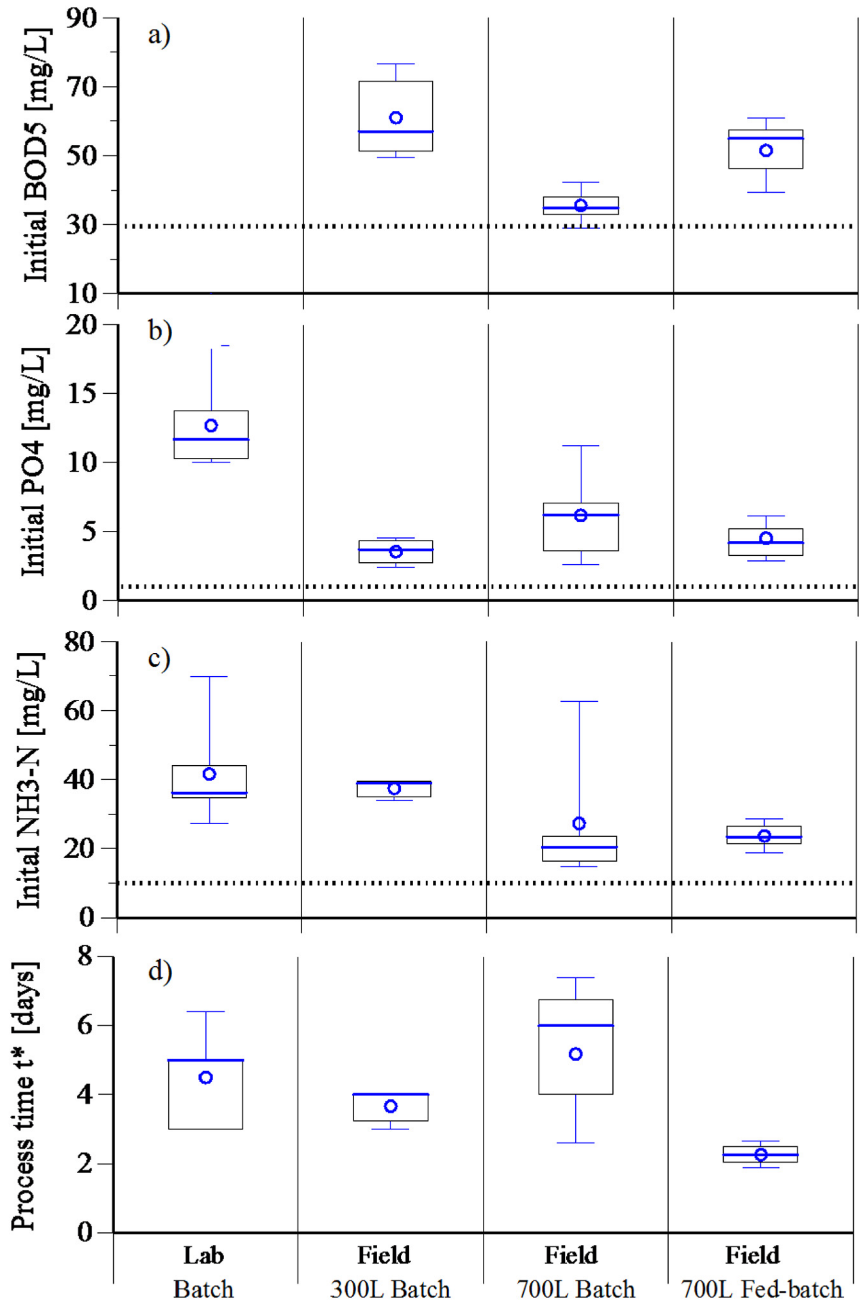

Fig. 3. Ranges of initial concentrations of: biochemical oxygen demand, BOD_5 (a); phosphates, PO_4 (b); ammoniacal nitrogen, NH_4-N (c); and process time required, t* (d) to meet their respective discharge standards of 30 mg L^{-1}, 1 mg L^{-1}, and 10 mg L^{-1} in laboratory batch tests; 300 L batch operation; 700 L batch operation; and 700 L fed-batch operation. Dashed lines indicate discharge standards. (BOD_5 data were not collected during these lab tests).

this correlation line; in the case of the single outlier, the reported data was in terms of COD rather than of BOD_5. Average volumetric removal rate of BOD_5 in our mixotrophic system (16.5 ± 3.6 mg L^{-1} d^{-1}) was found to be not different from the average of the reported values for the photoautotrophic HRAPs (significance level of 0.05).

3.3.2. Nitrogen reduction

Volumetric removal rates of NH_4-N from our studies (30 data points from 30 cycles) are compared with similar results compiled/calculated from the literature for HRAPs from 25 literature reports. Details of the data sources are included in the Supplement section. Some reports had reported total nitrogen (TN) instead of NH_4-N; we have listed the reported TN values in those cases. VRR values for the 25 cases are plotted against the respective initial NH_4-N (or TN) concentrations in Fig. 5. As can be noted from Fig. 5, the VRR found in our studies varied linearly with the initial concentration over the range of concentrations encountered during the run, with a correlation coefficient of r^2 = 0.991; n = 30. Since all but two of the 25 data points representing HRAPs fall below this correlation line, it follows that the mixotrophic process affords better removal of nitrogen than HRAPs; in the case of the two outliers, the reported data were in terms of TN rather than of NH_4-N. Average volumetric removal rates of NH_4-N in our mixotrophic system (6.09 ± 0.92 mg L^{-1} d^{-1}) was found to be not different from the average of the value reported for the photoautotrophic HRAPs (significance level of 0.05).

Since VRR is calculated from the initial and final liquid phase concentrations of NH_4-N, the above values include removal of N by algal uptake as well as by other abiotic mechanisms. As mentioned

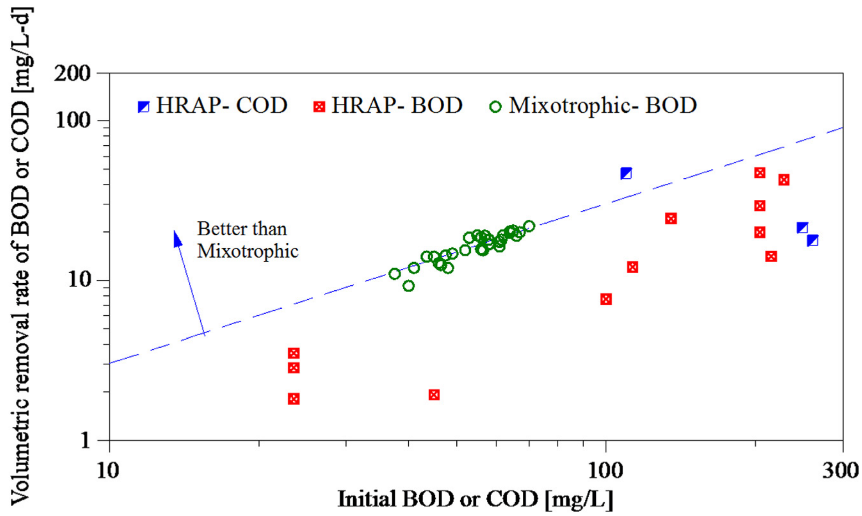

Fig. 4. Volumetric removal rates of BOD_5 (or COD) as a function of initial BOD_5 (or COD) concentrations. Dashed line represents correlation between the two for the mixotrophic process utilizing *G. sulphuraria*; $r^2 = 0.993$; n = 30. Details of the data sources are included in Table SI-1 in the Supplement section.

earlier, volatilization is the most significant abiotic mechanism of N reduction in the liquid; this loss increases with pH and temperature. As much as 90% of the ammonia will be lost by volatilization in summer when the pH can reach 10.5 and the temperature can reach 20 °C [13]. Since the pH in HRAPs is typically > 9, significant amounts of ammonia are lost to the atmosphere; in fact, several studies have reported loss of N by volatilization exceeding bio uptake [14,15]. In contrast, as the operating pH in the mixotrophic algal system utilizing *G. sulphuraria* is 4.0, most of the N-reduction reported here is attributed to algal uptake. Maximizing algal uptake can increase biomass production, a desirable outcome if downstream energy- and nutrient-recovery are integrated with wastewater treatment [16]. Atmospheric pollution by ammonia emission is also minimized at this low pH.

3.3.3. Phosphates reduction

Volumetric removal rates of PO_4 from our studies (30 data points from 30 cycles) are compared with similar results compiled/calculated from the literature for HRAPs from 22 literature reports. Details of the data sources are included in the Supplement section. Some reports had reported total phosphates (TP) instead of PO_4; we have listed the reported TP values in those cases. VRR values for the 22 cases are plotted against their respective initial PO_4 (or TP) concentrations in Fig. 6. As can be noted from Fig. 6, VRR found in our studies varied linearly with the initial concentration over the range of concentrations encountered during the run, with a correlation coefficient of $r^2 = 0.995$; n = 30. Since all but two of the 22 data points representing HRAPs fall below this correlation line, it follows that the mixotrophic process affords better removal of phosphates than HRAPs; in the case of the two outliers, the reported data were in terms of TP rather than of PO_4. Average volumetric removal rate of PO_4 in our mixotrophic system ($1.40 \pm 0.57\ mg\ L^{-1}\ d^{-1}$) was found to be greater than the average of the reported values for the photoautotrophic HRAPs (significance level of 0.05).

As mentioned earlier, VRR of P is also calculated from the initial and final liquid phase concentrations of PO_4. As such, the above values include removal of PO_4 by algal uptake as well as by other abiotic mechanisms. Abiotic reduction of P in the liquid phase could be due to two mechanisms: chemical precipitation with polyvalent cations such as calcium; or by physical adsorption to biomass and calcium carbonate crystals, both of which increase with pH [13]. Since the pH in HRAPs is typically > 9, removal of P by precipitation contributes significantly to VRR. As the operating pH in the mixotrophic algal system utilizing *G. sulphuraria* is < 4.0, most of the P-reduction reported here is associated with algal uptake. Maximizing algal uptake can increase biomass production, a desirable outcome if downstream energy- and nutrient-recovery are integrated with wastewater treatment.

3.3.4. Pathogen reduction

Pathogen reduction in HRAPs has been evaluated in terms of reductions of *Escherichia coli*, total coliform, and fecal coliform. A small-

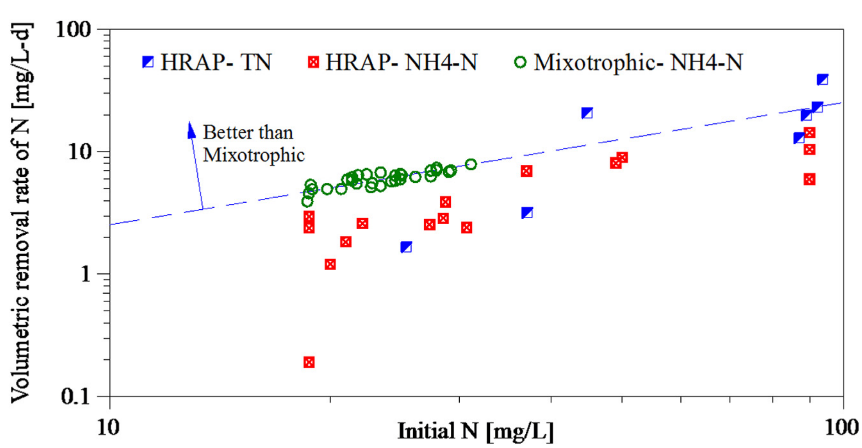

Fig. 5. Volumetric removal rates of NH_4-N (or TH) as a function of initial NH_4-N (or TN) concentrations. Dashed line represents correlation between the two for the mixotrophic process utilizing *G. sulphuraria*; $r^2 = 0.991$; n = 30. Details of the data sources are included in Table SI-2 in the Supplement section.

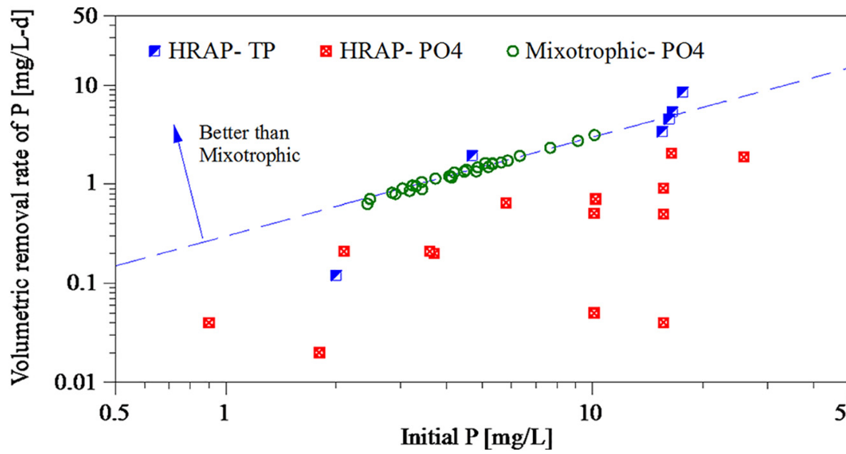

Fig. 6. Volumetric removal rates of PO_4 (or TP) as a function of initial PO_4 (or TP) concentrations. Dashed line represents correlation between the two for the mixotrophic process utilizing *G. sulphuraria*; $r^2 = 0.995$; n = 30. Details of the data sources are included in Table SI-3 in the Supplement section.

scale batch experiment reported 9 log reduction of *E. coli* from sewage effluent from a biological WWT plant diluted 1:1 with tap water [34]. Log reductions of total coliform, fecal coliform and fecal streptococci from settled sewage in a pilot scale HRAP have been reported as 1.08, 3.66 and 2.98, respectively [35]. Continuous operation of HRAPs at pilot-scale and full-scale has been reported [36,37].

Bacterial removal observed in algal systems has been attributed to the synergistic effect of multiple factors such as pH, temperature, photooxidation, dissolved oxygen, attachment to biomass, sedimentation, and algal biomass. Hamouri et al. reported higher log removal of 3.19 of fecal coliform in an HRAP (3-day HRT) during summer when the pH rose above 9 [35]. Deactivation by oxygen radicals produced by sunlight at dissolved oxygen levels above 10 mg L^{-1} has also been recognized as an important factor [35]. Ruas et al. evaluated the effect of CO_2 addition on pathogen removal in an HRAP (180-L, 5-h HRT) and reported log removal values of 0.3, 2.2 and 2.5 for enterococci, *E. coli* and *P. aeruginosa* respectively [38]. Laboratory studies by Ansa et al. concluded that long sunlight wavelengths, in the range of 380–780 nm, could damage fecal coliform in the presence of sensitizers such as humic substances [39]. Maintaining high pH and low algal concentrations can increase this photooxidation [39]. Fecal coliform reduction of 1.3–1.7 log units recorded in a semi-continuous bioreactor utilizing immobilized *S. obliquus* cells at a retention time of 35 h has been attributed to the high pH of 10 [40].

Similar factors as above were found to cause bacterial inactivation recorded in the mixotrophic system employing *G. sulphuraria*; the key difference being the acidic pH (2.0 to 4.0) in the latter versus the alkaline pH (> 9.0) in the HRAP systems. Even though algal toxins are believed to have the potential to cause bacterial inactivation [41], our toxicity studies had indicated that the *G. sulphuraria* system is free of any bacterial toxicants [42]. Elevated dissolved oxygen levels in our system (5.1 to 7.8 mg L^{-1}; average = 6.2 ± 0.5 mg L^{-1}) together with the photosensitizers in the primary effluent have the potential to produce reactive oxygen species during the photoperiod. Our results suggest that the dominant factor causing the high degree of bacterial inactivation recorded in the *G. sulphuraria* system is the unique acidic culture condition [42]. This feature can be highly beneficial in reducing downstream disinfection demand and hence, the potential for formation of disinfection byproducts.

4. Conclusions

This study summarized three algal metabolic pathways and the potential for their application in wastewater treatment systems. Based on the laboratory studies reported in the literature over the past two decades, mixotrophic systems can be seen to have several advantages over the traditional photoautotrophic systems. Laboratory and outdoor pilot scale results from our mixotrophic wastewater treatment system utilizing an extremophile, *Galdieria sulphuraria*, document its ability to remove biochemical oxygen demand, nutrients, and pathogens in primary effluent in a single step. Comparison of pilot scale results of this mixotrophic system with literature results on the classical high rate algal ponds showed its superior volumetric removal rates of biochemical oxygen demand, ammoniacal nitrogen, and phosphates. Based on its energy-and resource-efficient performance, the mixotrophic pathway utilizing *Galdieria sulphuraria* appears to have the potential for greener and sustainable wastewater treatment.

Acknowledgments

Support provided by City of Las Cruces Utilities Division in accommodating the algal testbed at the Las Cruces Wastewater Treatment Plant is acknowledged.

Funding

This study was supported in part by the NSF Engineering Research Center for Reinventing the Nation's Urban Water Infrastructure (ReNUWIt) award # EEC 1028968; the National Science Foundation award #IIA-1301346 Energize New Mexico (EPSCoR); the College of Engineering at NMSU; the Ed & Harold Foreman Endowed Chair; the City of Las Cruces Utilities; and the Las Cruces Wastewater Treatment Plant.

Declaration of authors' contribution

All authors whose names listed this manuscript certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

Conflict of interest statement

All authors whose names are listed in this manuscript certify that they have NO affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

Declaration of authors agreement to authorship and submission of the manuscript for peer review

All authors whose names are listed in this manuscript have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to Algal Research for peer review.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2019.101569.

References

[1] ASCE, ASCE's 2017 infrastructure report card | GPA: D+, https://www.infrastructurereportcard.org/, (2017) , Accessed date: 5 December 2018.
[2] P.L. McCarty, J. Bae, J. Kim, Domestic wastewater treatment as a net energy producer–can this be achieved? Environ. Sci. Technol. 45 (2011) 7100–7106.
[3] 820-R-14-006 EPA, Promoting Technology Innovations for Clean and Safe Water - Water Technology Innovation Blueprint—Version 2, (2014).
[4] P.D. Jenssen, L. Vråle, O. Lindholm, Sustainable wastewater treatment, Int. Conf. Nat. Resour. Environ. Manag. Environ. Saf. Heal., Kuching, Malaysia, 2007, pp. 27–29 www.ecosan.no , Accessed date: 17 February 2019.
[5] W.J. Oswald, C.G. Golueke, Biological transformation of solar energy, Adv. Appl. Microbiol. 2 (1960) 223–262.
[6] W.J. Oswald, The high-rate pond in waste disposal, Dev. Ind. Microbiol. 4 (1963) 112–125.
[7] P. Young, M. Taylor, H.J. Fallowfield, Mini-review: high rate algal ponds, flexible systems for sustainable wastewater treatment, World J. Microbiol. Biotechnol. 33 (2017) 117.
[8] Y. Shen, Carbon dioxide bio-fixation and wastewater treatment via algae photochemical synthesis for biofuels production, RSC Adv. 4 (2014) 49672–49722.
[9] J. Wang, H. Yang, F. Wang, Mixotrophic cultivation of microalgae for biodiesel production: status and prospects, Appl. Biochem. Biotechnol. 172 (2014) 3307–3329.
[10] R. Whitton, A. Le Mével, M. Pidou, F. Ometto, R. Villa, B. Jefferson, Influence of microalgal N and P composition on wastewater nutrient remediation, Water Res. 91 (2016) 371–378.
[11] A. Richmond, Handbook of Microalgal Culture: Biotechnology and Applied Phycology, Oxford, UK www.blackwellpublishing.com, (2004) , Accessed date: 17 February 2019.
[12] M.E. Martínez, S. Sánchez, J. M. Jiménez, F. El Yousfi, L. Muñoz, Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus, Bioresour. Technol. 73 (2000) 263–272.
[13] Y. Nurdogan, W.J. Oswald, Enhanced nutrient removal in high-rate ponds, Water Sci. Technol. 31 (1995) 33–43.
[14] J. García, R. Mujeriego, M. Hernández-Mariné, High rate algal pond operating strategies for urban wastewater nitrogen removal, J. Appl. Phycol. 12 (2000) 331–339.
[15] B. Picot, H. El Halouani, C. Casellas, S. Moersidik, J. Bontoux, Nutrient removal by high rate pond system in a Mediterranean climate (France), Water Sci. Technol. 23 (1991) 1535–1541.
[16] J.B.K. Park, R.J. Craggs, A.N. Shilton, Wastewater treatment high rate algal ponds for biofuel production, Bioresour. Technol. 102 (2011) 35–42.
[17] T. Ogawa, S. Aiba, Bioenergetic analysis of mixotrophic growth in Chlorella vulgaris and Scenedesmus acutus, Biotechnol. Bioeng. 23 (1981) 1121–1132.
[18] O. Perez-Garcia, F.M.E. Escalante, L.E. de-Bashan, Y. Bashan, Heterotrophic cultures of microalgae: metabolism and potential products, Water Res. 45 (2011) 11–36.
[19] M. Cecchin, S. Benfatto, F. Griggio, A. Mori, S. Cazzaniga, N. Vitulo, M. Delledonne, M. Ballottari, Molecular basis of autotrophic vs mixotrophic growth in Chlorella sorokiniana, Sci. Rep. 8 (2018) 6465.
[20] M. Wan, P. Liu, J. Xia, J.N. Rosenberg, G.A. Oyler, M.J. Betenbaugh, Z. Nie, G. Qiu, The effect of mixotrophy on microalgal growth, lipid content, and expression levels of three pathway genes in Chlorella sorokiniana, Appl. Microbiol. Biotechnol. 91 (2011) 835–844.
[21] F.J. Marquez, K. Sasaki, T. Kakizono, N. Nishio, S. Nagai, Growth characteristics of Spirulina platensis in mixotrophic and heterotrophic conditions, J. Ferment. Bioeng. 76 (1993) 408–410.
[22] T. Li, Y. Zheng, L. Yu, S. Chen, Mixotrophic cultivation of a Chlorella sorokiniana strain for enhanced biomass and lipid production, Biomass Bioenergy 66 (2014) 204–213.
[23] Z. Zhang, D. Sun, T. Wu, Y. Li, Y. Lee, J. Liu, F. Chen, The synergistic energy and carbon metabolism under mixotrophic cultivation reveals the coordination between photosynthesis and aerobic respiration in Chlorella zofingiensis, Algal Res. 25 (2017) 109–116.
[24] B. Cheirsilp, S. Torpee, Enhanced growth and lipid production of microalgae under mixotrophic culture condition: effect of light intensity, glucose concentration and fed-batch cultivation, Bioresour. Technol. 110 (2012) 510–516.
[25] M.C. Cerón Garcí, J.M. Fernández Sevilla, F.G. Acién Fernández, E. Molina Grima, F. García Camacho, Mixotrophic growth of Phaeodactylum tricornutum on glycerol: growth rate and fatty acid profile, J. Appl. Phycol. 12 (2000) 239–248.
[26] Y. Liang, N. Sarkany, Y. Cui, Biomass and lipid productivities of Chlorella vulgaris under autotrophic, heterotrophic and mixotrophic growth conditions, Biotechnol. Lett. 31 (2009) 1043–1049.
[27] K. Chojnacka, A. Noworyta, Evaluation of Spirulina sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures, Enzym. Microb. Technol. 34 (2004) 461–465.
[28] O. Perez-Garcia, Y. Bashan, Microalgal heterotrophic and mixotrophic culturing for bio-refining: from metabolic routes to techno-economics, Algal Biorefineries, Springer International Publishing, Cham, 2015, pp. 61–131.
[29] S.M. Henkanatte-Gedera, T. Selvaratnam, N. Caskan, N. Nirmalakhandan, W. Van Voorhies, P.J. Lammers, Algal-based, single-step treatment of urban wastewaters, Bioresour. Technol. 189 (2015) 273–278.
[30] S.M. Henkanatte-Gedera, T. Selvaratnam, M. Karbakhshravari, M. Myint, N. Nirmalakhandan, W. Van Voorhies, P.J. Lammers, Removal of dissolved organic carbon and nutrients from urban wastewaters by Galdieria sulphuraria: laboratory to field scale demonstration, Algal Res. 24 (2017) 450–456.
[31] T. Selvaratnam, A.K. Pegallapati, F. Montelya, G. Rodriguez, N. Nirmalakhandan, W. Van Voorhies, P.J. Lammers, Evaluation of a thermo-tolerant acidophilic alga, Galdieria sulphuraria, for nutrient removal from urban wastewaters, Bioresour. Technol. 156 (2014) 395–399.
[32] T. Selvaratnam, A.K. Pegallapati, H. Reddy, N. Kanapathipillai, N. Nirmalakhandan, S. Deng, P.J. Lammers, Algal biofuels from urban wastewaters: maximizing biomass yield using nutrients recycled from hydrothermal processing of biomass, Bioresour. Technol. 182 (2015) 232–238.
[33] H.M.K. Delanka-Pedige, S.P. Munasinghe-Arachchige, J. Cornelius, S.M. Henkanatte-Gedera, D. Tchinda, Y. Zhang, N. Nirmalakhandan, Pathogen reduction in an algal-based wastewater treatment system employing Galdieria sulphuraria, Algal Res. 39 (2019) 101423.
[34] S. Sebastian, K.V.K. Nair, Total removal of coliforms and E. coli from domestic sewage by high-rate pond mass culture of Scenedesmus obliquus, Environ. Pollut. Ser. A, Ecol. Biol. 34 (1984) 197–206.
[35] B. El Hamouri, K. Khallayoune, K. Bouzoubaa, N. Rhallabi, M. Chalabi, High-rate algal pond performances in faecal coliforms and helminth egg removals, Water Res. 28 (1994) 171–174.
[36] R. Craggs, D. Sutherland, H. Campbell, Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production, J. Appl. Phycol. 24 (2012) 329–337.
[37] M. García, F. Soto, J.M. González, E. Bécares, A comparison of bacterial removal efficiencies in constructed wetlands and algae-based systems, Ecol. Eng. 32 (2008) 238–243.
[38] G. Ruas, M.L. Serejo, P.L. Paulo, M.Á. Boncz, Evaluation of domestic wastewater treatment using microalgal-bacterial processes: effect of CO_2 addition on pathogen removal, J. Appl. Phycol. 30 (2018) 921–929.
[39] E.D.O. Ansa, H.J. Lubberding, H.J. Gijzen, Fecal Coliform Removal in Algal-based Domestic Wastewater Treatment Systems, (2008) (2008).
[40] A. Ruiz-Marin, L.G. Mendoza-Espinosa, T. Stephenson, Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater, Bioresour. Technol. 101 (2010) 58–64.
[41] E.D.O. Ansa, E. Awuah, A. Andoh, R. Banu, W.H.K. Dorgbetor, H.J. Lubberding, H.J. Gijzen, A review of the mechanisms of faecal coliform removal from algal and duckweed waste stabilization pond systems, Am. J. Environ. Sci. 11 (2015) 28–34.
[42] S.P. Munasinghe-Arachchige, H.M.K. Delanka-Pedige, S.M. Henkanatte-Gedera, D. Tchinda, Y. Zhang, N. Nirmalakhandan, Factors contributing to bacteria inactivation in the Galdieria sulphuraria-based wastewater treatment system, Algal Res. 38 (2019) 101392.