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Note

Treatment of cattle-slaughterhouse wastewater and the reuse of sludge for biodiesel production by microalgal heterotrophic bioreactors

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ABSTRACT: Microalgal heterotrophic bioreactors are a potential technological development that can convert organic matter, nitrogen and phosphorus of wastewaters into a biomass suitable for energy production. The aim of this work was to evaluate the performance of microalgal heterotrophic bioreactors in the secondary treatment of cattle-slaughterhouse wastewater and the reuse of microalgal sludge for biodiesel production. The experiments were performed in a bubble column bioreactor using the microalgae *Phormidium* sp. Heterotrophic microalgal bioreactors removed 90 % of the chemical oxygen demand, 57 % of total nitrogen and 52 % of total phosphorus. Substantial microalgal sludge is produced in the process (substrate yield coefficient of 0.43 mg_{sludge} $mg_{chemical\ oxygen\ demand}^{-1}$), resulting in a biomass with high potential for producing biodiesel (ester content of more than 99 %, cetane number of 55, iodine value of 73.5 g_{iodine} 100 g^{-1}, unsaturation degree of ~75 % and a cold filter plugging point of 5 ºC).
Keywords: bioreactor, agro-industrial wastewater, microalgae/cyanobacteria

Introduction

The high water consumption by slaughterhouses results in large volumes of wastewaters characterized by high organic load, due to the presence of blood, manure, fat, undigested stomach contents, and intestinal contents. The wastewater of slaughterhouses has a high pollution load (Mittal, 2006). These agro-industrial wastes have a high concentration of organic matter and are a suitable environment that favors microalgae heterotrophic cultivation, where nitrogen (N) and phosphorus (P) are usually found in favorable carbon/nitrogen (C/N) and nitrogen/phosphorous (N/P) ratios that support microalgal growth. Additionally, these wastewaters exhibit assimilated compounds and in most cases an absence of toxic compounds or growth inhibitors (Queiroz et al., 2013).

Cyanobacteria are prokaryote organisms that are widely distributed in nature. They are preferably photosynthetic, although some lines have a distinct ability to obtain energy from the consumption of organic substrates in the absence of light (Queiroz et al., 2007). *Phormidium* is a genus of single-cell blue green algae, belonging to the phylum cyanobacteria. It is filamentous, unbranched in shape and about 3 to 4 µm in diameter. Several species live in limiting environments such as thermal springs, desert soils, and polluted sites. This blue green algae shows considerable potential for being used as biocatalysts in environmental biotechnology processes because of its robustness and simple nutritional requirements (Cañizares-Villanueva et al., 1994; Al-Thukair et al., 2007; Guiry and Guiry, 2013).

The use of microalgae-based systems in secondary wastewater treatment has some interesting advantages when compared with conventional treatments, such as activated sludge and anaerobic systems. Microalgal heterotrophic bioreactors have been demonstrated to be the most cost-effective way to remove organic matter in wastewaters. These systems require low energy input, and microalgal sludge is a rich biomass that can be used for animal feeding and bioenergy production (Zepka et al., 2008). According to Queiroz et al. (2013), these bioreactors contribute to the partial removal of nitrogen and phosphorus of the wastewaters, thereby aiding tertiary wastewater treatment. Thus, the aim of this study was to evaluate the performance of microalgal heterotrophic bioreactors in the secondary treatment of cattle-slaughterhouse wastewater and the reuse of microalgal sludge for biodiesel production.

Materials and Methods

Microorganisms and culture media: the axenic cultures of *Phormidium* sp. were originally isolated from the Cuatro Cienegas desert, in Mexico (26º59' N, 102º03' W). Stock cultures were propagated and maintained in solidified agar-agar (20 g L^{-1}) containing synthetic BGN medium (Rippka et al., 1979) with the following composition (mg L^{-1}): K_2HPO_4 (3.0), $MgSO_4$ (75.0), $CaCl_2.2H_2O$ (36.0), ammonium citrate and iron (0.6), Na_2EDTA (1.0), NaCl (0.72), $NaNO_3$ (150.0), citric acid (0.6), Na_2CO_3 (15.0), H_3BO_3 (2.8), $MnCl_2.4H_2O$ (1.8), $ZnSO_4.7H_2O$ (0.22), $Na_2MoO_4.2H_2O$ (0.39), $CoSO_4.6H_2O$ (0.04). The incubation conditions were as follows: tem-

perature of 20 °C, a photon flux density of 15 µmol m^{-2} s^{-1}, and a photoperiod of 12 h.

Wastewater: cattle-slaughterhouse wastewater used in the experiments was obtained from an industrial plant located in Santa Maria, in the state of Rio Grande do Sul, Brazil (29°41′02″S, 53°48′25″W). It was collected from the discharge point of an equalization tank over a period of one year, and analyzed for pH, chemical oxygen demand (COD), total nitrogen (N-TKN), total phosphorus ($P\text{-}PO_4^{-3}$), total solids (TS), suspended solids (SS), volatile solids (VS), and fixed solids (FS) following the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The average composition of the wastewater was: pH = 7.0 ± 0.2, COD = 7,692.5 ± 5193.3 mg L^{-1}, N-TKN = 155.1 ± 80.1 mg L^{-1}, $P\text{-}PO_4^{-3}$ = 23.0 ± 10.1 mg L^{-1}, TS = 2,725 ± 645.8 mg L^{-1}, SS = 540.0 ± 212.3 mg L^{-1}, VS = 1,532.5 ± 565.9 mg L^{-1}, FS = 1,192.5 ± 821.8 mg L^{-1}, C/N ratio = 49.6 ± 9.4, and N/P = 6.7 ± 3.8. C/N and N/P were calculated using COD, N-TKN, and $P\text{-}PO_4^{-3}$ contents.

Bioreactor: measurements were made in a bubble column bioreactor. The system was made out of borosilicate glass and had an internal diameter of 15 cm and a height of 20 cm, resulting in a height/diameter (h/D) ratio equal to 1.33 and a nominal working volume of 3.5 L. The dispersion system of the reactor consisted of a 1.5 cm diameter air diffuser located inside the bioreactor.

Kinetic data in an experimental bioreactor: experiments were performed in a bioreactor operating under a batch regime, fed on 3.5 L of wastewater (previously sterilized, 103 kPa/121 °C). The experimental conditions were as follows: initial concentration of inoculum of 100 mg L^{-1} of *Phormidium* sp., temperature of 20 °C, pH adjusted to 7.6, aeration of 1 VVM (volume of air per volume of culture per minute), and absence of light. Samples were collected at regular intervals of 12 h for a total residence time of 168 h, and characterized the chemical oxygen demand (COD), total nitrogen (N-TKN), total phosphorus ($P\text{-}PO_4^{-3}$), and cellular concentration. The experiments were performed in triplicate, and kinetic data refer to the average of six repetitions. Data of cell growth and substrate consumption were used to calculate the following kinetic parameters: substrate yield coefficient ($Y_{X/S} = r_x r_c^{-1}$), consumption rates of COD, N-TKN and $P\text{-}PO_4^{-3}$ ($r_c = dC\ dt^{-1}$) and removal efficiency of COD, N-TKN, and $P\text{-}PO_4^{-3}$ ($RE = C\text{-}C_0\ C_0^{-1}$), where r_x is the sludge production rate (mg $L^{-1}\ d^{-1}$), r_c is the consumption rate of COD, N-TKN, and $P\text{-}PO_4^{-3}$ (mg L^{-1}), C is the final concentration of COD, N-TKN, and $P\text{-}PO_4^{-3}$ (mg L^{-1}), C_0 is the initial concentration of COD, N-TKN, and $P\text{-}PO_4^{-3}$ (mg L^{-1}) and t is the residence time (d).

Analytical methods: cell concentration was evaluated gravimetrically by filtering a known volume of culture medium through a 0.45 mm filter and drying at 60 °C for 24 h. External contamination was monitored by the heterotrophic plate count method (APHA, 2005). The procedure was conducted at the beginning and end of each experiment. The composition of the medium that was used consisted of glucose at 10 g L^{-1} and bacteriological agar at 20 g L^{-1} dissolved in a synthetic BGN medium. The culture medium was sterilized for 20 min at 121 °C and poured into sterile Petri dishes. An aliquot of 0.1 mL of the sample was transferred to a Petri dish through tips previously sterilized, and the homogenization of the sample surface was performed using a circular motion. The plates were incubated at 26 °C for seven days, and the counting was performed on the white surface, using the ratio of the number of colony forming units (CFU) to the volume of the samples to estimate the degree of contamination.

The wet biomass was dried on a tray dryer. The drying conditions were as follows: temperature of 60 °C, air speed of 1.5 m s^{-1} on a parallel flow, and material thickness of 7 mm (Jacob-Lopes et al., 2007). The centesimal composition of microalgal sludge (moisture, proteins, carbohydrates, and minerals) was performed based on the method described in AOAC (2000).

The lipid fraction was extracted from the biomass by the Bligh and Dyer (1959) method. The method of Hartman and Lago (1976) was used to saponify and esterify (methylation reaction) the dried lipid extract to obtain the fatty acid methyl esters-FAMEs (biodiesel). The fatty acid composition was determined using a gas chromatograph. The FAMEs were identified by comparison of the retention times with those of the standard (Supelco, Bellefonte, PA, USA) and quantified by area normalization using Varian Star 4.51 software.

The evaluated quality properties of the biodiesel were ester content (EC), cetane number (CN), iodine value (IV), degree of unsaturation (DU), and cold filter plugging point (CFPP), as determined according to the method proposed by Francisco et al. (2010).

Results and Discussion

The composition of the cattle-slaughterhouse wastewater is generally variable, as a function of the season and industrial processing type (Mittal, 2006). The cattle-slaughterhouse wastewater composition, that takes into consideration one year of sampling, indicated a high concentration of organic matter, nitrogen, and phosphorous, characteristic of food processing wastewater, resulting in C/N (49.59 ± 9.4) and N/P (6.74 ± 3.8) ratios that are suitable for microalgae growth.

The microalgal heterotrophic bioreactor applied to the treatment of cattle-slaughterhouse wastewater removed 2.7, 68.5, and 0.01 mg $L^{-1}\ d^{-1}$ of organic matter (COD), total nitrogen (N-TKN) and total phosphorus ($P\text{-}PO_4^{-3}$), respectively, resulting simultaneous conversions in the order of 90 % of COD, ~57 % of N-TKN, and ~52 % of $P\text{-}PO_4^{-3}$ (Table 1). Moreover, the substrate yield coefficient ($Y_{X/S}$), expressed by the conversion of

organic matter (COD) into microalgal sludge, indicated that 43 % of organic matter is converted into microalgal biomass. The remainder, according to Perez-Garcia et al. (2011), is related mainly to other carbon metabolites that are generated, in parallel, under these conditions, such as carbon dioxide. The metabolic route that controls these reactions is via the oxidative pentose-phosphate pathway. Respiration in the dark serves as the exclusive source of energy for maintenance and biosynthesis, besides providing the carbon that is required as building blocks for biosynthesis. Several organic compounds can be metabolized by these microorganisms. Heterotrophic cyanobacteria possess structurally specific mechanisms for the active transport of the organic growth substrate into the cell, enabling various technological applications of this metabolic route (Fay, 1983).

The substantial removal of nutrients evidenced in the process is a characteristic of the heterotrophic microalgal metabolism that simultaneously converts these pollutants in a single bioreactor, relieving the tertiary wastewater treatment. The nitrogen removal in the heterotrophic microalgal bioreactor, however, cannot be exclusively attributed to N bioconversion. Other mechanisms capable of eliminating N in intensively aerated microalgal systems are non-biological, such as air stripping, ammonia volatilization, absorption, and sedimentation (Queiroz et al., 2007). On the other hand, phosphorus removal in these systems is related to microalgal uptake, chemical precipitation and biosorption by microalgal biomass (Vieira et al., 2012).

The aseptic procedures adopted were suitable for preventing microbial contamination of the cultures (data not shown), since null results were observed by the heterotrophic plate count method. However, the external contamination by other heterotrophic microorganisms is one the main limitations of this technology. The maintenance of a monoculture in full-scale is prohibitively expensive and technically difficult to operate. In this sense, improving microalgae culture stability is a challenge to be surmounted before the industrial application of microalgal heterotrophic bioreactors in wastewater treatment facilities (Jacob-Lopes et al., 2013).

The biomass growth associated with pollutant removal makes the microalgal sludge, a co-product of the process, amenable to management. The chemical composition of microalgal sludge (Table 2) indicated levels of protein (32 %), lipids (15 %), carbohydrates (16 %), minerals (22 %) and moisture (15 %). In addition, the fatty acids profile of the oil extracted from the microalgal sludge showed a predominance of monounsaturated (~63 %), saturated (~31 %), and polyunsaturated (~7 %) fatty acids.

This composition qualifies this sludge as a potential source of single-cell proteins and single-cell oils to feed biorefineries (Queiroz et al., 2013). Comparatively, the two main commercial constituents of the microalgal sludge (proteins and lipids) correspond to ~79 and 77 %, respectively, of the average chemical composition of soybean, which is currently one of the main sources of protein and lipids worldwide (Berk, 1992).

Predominantly monounsaturated oils are the most suitable for biodiesel synthesis (Knothe et al., 2005). The biodiesel produced from microalgal sludge has the following fuel properties: an ester content of 99 %, a cetane number of 55, an iodine value of 73.5 gl_2 100 g^{-1}, a degree of unsaturation of ~75 %, and a cold filter plugging point of 5 ºC (Table 3). All these parameters

Table 1 – Kinetic parameters for the microalgal heterotrophic bioreactor in the cattle-slaughterhouse wastewater treatment.

Parameter	Value
$r_{c(COD)}$ (mg L^{-1} d^{-1})	68.5 ± 2.39
$r_{c(N\text{-}TKN)}$ (mg L^{-1} d^{-1})	2.7 ± 0.04
$r_{c(P\text{-}PO4^{-3})}$ (mg L^{-1} d^{-1})	0.01 ± 0.00
$RE_{(COD)}$ (%)	90.0 ± 3.15
$RE_{(N\text{-}TKN)}$ (%)	57.2 ± 0.85
$RE_{(P\text{-}PO4^{-3})}$ (%)	52.3 ± 0.68
$Y_{X/S}$ (mg_{sludge} mg_{COD}^{-1})	0.43 ± 0.00

Table 2 – Composition of the microalgal sludge, obtained after the separation of the wastewater.

General components	Percent (% wt)	Fatty acids	Percent (% wt)
Proteins	31.4 ± 0.81	C14:1	17.7 ± 0.21
Carbohydrates	15.9 ± 0.23	C16:1	15.2 ± 0.24
Lipids	15.4 ± 0.49	C17:0	8.8 ± 0.07
Minerals	21.7 ± 0.86	C18:0	13.5 ± 0.01
Moisture	15.2 ± 0.28	C18:1n9c	27.3 ± 0.46
		C18:2n6c	5.3 ± 0.01
		Minority	12.2 ± 0.11
		Saturated	30.7 ± 0.61
		Monounsaturated	62.5 ± 0.40
		Polyunsaturated	6.7 ± 0.09

Table 3 – Properties of biodiesel produced from microalgal sludge and its comparison with the standards used in US (ASTM 6751), Europe (EN 14214) and Brazil (ANP 255).

Fuel properties	Microalgae sludge	Soybean[a]	ASTM 6751	EN 14214	ANP 255
EC (%)	99.8 ± 0.80	96.9	-	min 96.5	-
CN	55.0 ± 0.66	49.0	min 47	min 51	min 45
IV (g_{iodine} 100 g^{-1})	73.5 ± 1.02	128.0	-	max 120	-
DU (%)	74.6 ± 0.89	143.8	-	-	-
CFPP (°C)	5.0 ± 0.10	-5.0	-	-	-

[a]Knothe (2005).

comply with the limits established by US (ASTM, 2002), European (EN, 2003), and Brazilian National Petroleum Agency (ANP, 2003) standards, in addition to being comparable to soybean biodiesel. These results indicate the potential for the exploitation of this feedstock for biofuel production.

Conclusion

Heterotrophic microalgal bioreactors are a technology high in potential for the secondary treatment of cattle-slaughterhouse wastewater, presenting high COD removal efficiencies and amelioration of total nitrogen and total phosphorus contents. Substantial microalgal sludge is produced by the biological conversion of the wastes, resulting in a biomass with potential to produce biodiesel with excellent characteristics.

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