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Nutrient cycling in meat processing industry by microalgae-based processes

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ABSTRACT

The nutrient cycling from microalgae-based processes is a potential technological route to be applied in wastewater treatment plants. The pollutants conversion in parallel with microalgal sludge formation results in a renewable feedstock for bioproducts production that potentially reduces the cost of wastewater treatment. The experiments have been performed in a bubble column bioreactor, operating at 25ºC, pH 7.5, 100 mg/L of initial inoculum, absence of light and flow rate per unit volume (Q/V) of 1.0 VVM (volume of air per volume of culture per minute). Thereby, the kinetic parameters of cell growth, substrate consumption, analysis of microalgal sludge composition and the biodiesel quality properties have been realized. The nutrient cycling by *Phormidium autumnale* in wastewater has presented high removal efficiencies of pollutants. The generated microalgal sludge shows predominance of saturated fatty acids, indicates the potential of its use as a suitable lipid input for biodiesel synthesis. The lipid-extracted microalgae (LEM) showed high content of free amino acids, minerals and pigments. The heterotrophic microalgae cultivation in wastewater has demonstrated to be capable of removing pollutants and, simultaneously, producing biofuel and being a potential source for animal feeding from microalgal sludge, contributing, therefore, to the multi-purpose microalgal bioprocess development.

Keywords: Microalgae; Cyanobacteria; Wastewater; Waste valorization; Biodiesel; Animal feed

1. Introduction

Safeguard water resources policies have influenced the development of wastewater treatment systems and their management, focusing on energy consumption and sustainable performance of these industrial processes in recent decades. One direction towards renovating the wastewater treatment into a more sustainable one is to recover the resources that it holds, such as water, nutrients (e.g. C, N and P) and energy [1].

Nutrient cycling by microalgae emerges as a promising technology because it balances sustainable vectors by reused pollutants, like carbon, nitrogen and phosphorus, present in wastewater generated by the industry. Thus, they create a biomass that one can extract a great variety of bioproducts with substantial added value [2].

Together, wastewater treatment and valuable algal biomass production enhances environmental and economic benefits from this process [3,4]. According to Brennan and Owende [5], the combination of these processes will be the most conceivable commercial application in the short term; and it is probably one of the most sustainable ways to produce bioenergy and bio-products.

As an effort to reduce the cost of microalgal bioproducts, the biorefinery approaches have arise [6,7]. Biorefinery is a totally integrative and multifunctional process that uses raw material to generate a spectrum of different products in a sustainable way, such as biofuel and animal feeding. The objective of this study is to use comprehensively all the raw material components and to improve the resource flow in order to reduce the resource loss [8].

The fatty acids composition is crucial to the single-cell oil production, since it directly influences on the biodiesel quality. Some microalgae are acknowledged because they

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produce high amounts of lipids and they can be used as a mean of bioprocessing, in order to produce alternative oils for biofuel manufacturers [9,10]. The choice for oil accumulation through the manipulation of environmental culture conditions has a great potential in the single-cell oil production [11].

Furthermore, many non-lipid portions of microalgal biomass can be processed into wide ranges of bioproducts. Proteins and minerals in microalgal tissues can be used as food or feeding. Microalgae are the ideal source for the production of chemicals, human nutrition products, pharmaceuticals and cosmetics [8].

Phormidium is a genus of single-cell blue green algae that belongs to the cyanobacteria. Its filamentous can measure about 3–4 μm diameter. Several species live in limiting environments. These blue green algae show considerable potential as biocatalysts in environmental biotechnology processes because of their robustness and simple nutritional requirements [12,13].

Therefore, this study aims to develop a microalgae-based process through nutrient cycling of meat processing industry wastewater, with emphasis on biodiesel and animal feed manufacturing.

2. Material and methods

2.1. Microorganism and culture conditions

A monoculture of *Phormidium autumnale* has been originally isolated from the Cuatro Cienegas desert (26°59′N, 102°03′W-Mexico). Stock cultures have been propagated and remained in solidified agar-agar (20 g/L) containing synthetic BG11 medium [14] with the following composition (mg/L): K_2HPO_4 (30.0), $MgSO_4$ (75.0), $CaCl_2{\cdot}2H_2O$ (36.0), ammonium citrate and iron (0.6), Na_2 EDTA (1.0), NaCl (0.72), $NaNO_3$ (15.0), citric acid (0.6), Na_2CO_3 (1500.0), trace metals [H_3BO_3 (2.8), $MnCl_2{\cdot}4H_2O$ (1.8), $ZnSO_4{\cdot}7H_2O$ (0.22), $Na_2MoO_4{\cdot}2H_2O$ (0.39), $CoSO_4{\cdot}6H_2O$ (0.04). The incubation conditions were the following: 20°C, photon flux density of 15 μmol/m²/s and photo period of 12 h.

2.2. Wastewater

The poultry and swine slaughterhouse wastewater used in the experiments has been obtained from an industry located in the State of Santa Catarina, Brazil (27°14′02″S, 52°01′40″W). It has been collected from the discharge point of an equalization tank (after fine screen and rotary sieve) over the period of one year, and it has been analyzed according to its pH, COD, N-TKN, $P\text{-}PO_4^{-3}$, TS, SS, VS, and FS, following the Standard Methods for the Examination of Water and Wastewater [15]. Table 1 shows the average composition of the wastewater, in a one-year sampling. The C/N and N/P ratio has been calculated through COD, N-TKN, and $P\text{-}PO_4^{-3}$.

2.3. Bioreactor configuration

Measurements have been made in a bubble column bioreactor. The system has been built by using borosilicate glass. It has an external diameter of 12.5 cm and height of 16 cm, resulting in a height/diameter (h/D) ratio equals to 1.28 and a nominal working volume of 2.0 L. The reactor dispersion system has been composed of a 2.5 cm diameter air diffuser located inside the bioreactor. The air flow has been monitored by a flow meter (KI-Key Instruments®, Trevose-PA, USA) and the inlet and outlet of gases have worked through filtering units, which have been created by using a polypropylene membrane with a pore diameter 0.22 μm and total diameter 50 mm (Millex FG®, Billerica-MA, USA).

Table 1
Average composition of wastewater

Parameter	Value
pH	5.9 ± 0.05
COD (mg/L)	4100 ± 874
N-TKN (mg/L)	128.5 ±12.1
$P\text{-}PO_4^{-3}$ (mg/L)	2.84 ± 0.2
TS (mg/L)	3.8 ± 2.7
FS (mg/L)	0.9 ± 0.3
VS (mg/L)	2.9 ± 1.4
SS (mg/L)	1.9 ± 0.8
C/N	31.9 ± 1.2
N/P	45.2 ± 2.6

n = 54

2.4. Obtaining the kinetic data

Experiments have been conducted in a batch bioreactor. The bioreactor has been fed with 2.0 L of previously sterilized wastewater (15 psi/121°C). The experimental conditions were initial cell concentration of 100 mg/L, pH adjusted to 7.6, temperature of 25°C, flow rate per unit volume of 1.0 VVM, C/N ratio of 31.9 and absence of light.

2.5. Sampling and analytical methods for wastewater

Samples have been collected at regular intermissions of 12 h and characterized by chemical oxygen demand (COD), total nitrogen (N-TKN), total phosphorus ($P\text{-}PO_4^{-3}$) and cell biomass.

The chemical oxygen demand, total nitrogen and total phosphorus have been determined according to the methodology described in Standard Methods for the Examination of Water and Wastewater [15]. Cell biomass has been gravimetrically evaluated by wastewater filtering through a 0.45 μm membrane filter (Millex FG®, Billerica-MA, USA), drying at 60°C until it reaches constant weight.

External contamination has been monitored by the heterotrophic plate count method, according to Maroneze et al. [12].

The experiments were performed in duplicate, and kinetic data refer to the average of four repetitions.

2.6. Kinetic parameters

The cell growth and substrate consumption data have been used to calculate the biomass productivity ($P_X = dX/$

dt, mg/L/h); maximum specific growth rate ($\ln(X/X_0) = \mu_{max} \cdot t$, 1/h); consumption rates of COD, N-TKN and $P\text{-}PO_4^{-3}$ ($r_S = dS/dt$, mg/L/h) and removal efficiencies of COD, N-TKN and $P\text{-}PO_4^{-3}$ ($RE = (S_0 - S)/(S_0)$, %), where X is the cell biomass at time t = t (mg/L), X_0 is the initial cell biomass (mg/L), t is time (h), S is the final concentration of COD, N-TKN and $P\text{-}PO_4^{-3}$ (mg/L) and S_0 is the initial concentration of COD, N-TKN and $P\text{-}PO_4^{-3}$ (mg/L).

2.7. Sampling and analytical methods for microalgal sludge

2.7.1. Centesimal composition

Microalgal sludge chemical composition has been characterized according to AOAC [16]. Carbohydrate concentration has been determined by differentiation. Total lipid has been extracted by Bligh and Dyer modified method [17].

2.7.2. Fatty acid profile

Hartman and Lago method [18] has been used to saponify and esterify (methylation reaction) the dried lipid extract in order to obtain the fatty acid methyl esters (biodiesel). Fatty acid composition has been determined using a VARIAN 3600 CX gas chromatograph (Varian, Palo Alto, CA, USA) equipped with FID and a fused silica capillary column (SP 2560 Supelco), 100 m × 0.25 mm id, film thickness 0.20 µm. The fatty acid methyl esters (FAMEs) have been identified by comparing the retention times with external patterns (Supelco, Bellefonte, PA, USA). They have also been quantified by their area normalization using Varian Star 4.51 software.

2.7.3. Free amino acids

Amino acids have been determined by a Beckman 7300 (Beckman Instruments, Palo Alto, CA, USA) auto analyzer from hydrolysates obtained by the hydrolysis of 15–25 mg sample with 2.0 ml 6.0 N HCl in an evacuated sealed tube at 110°C for 24 h. The identification and quantification of the amino acids have been analyzed by comparison, according to external patterns (Sigma–Aldrich, St. Louis, MO, USA).

2.7.4. Minerals

The minerals chemical composition has been determined as described by Mesko et al. [19]. The solid samples have been exposed firstly to an acidic digestion; then, they have been heated up to 6000–8000 K in order to vaporize and ionize metallic compounds to be quantified. The ions have been detected and analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

2.7.5. Pigments

The carotenoids and chlorophylls have been analyzed through a high performance liquid chromatography HPLC (Shimadzu, Kyoto, Japan) equipped with quaternary pumps (model LC-20AD), online degasser, and injection valve 20 µL loop (Rheodyne, Rohnert Park, CA, USA). The equipment has been connected in series to a photodiode array detector (model SPD-M20A) and a mass spectrometer with an ion-trap analyzer and an atmospheric pressure chemical ionization (APCI) source (model Esquire 4000, Bruker Daltonics, Bremen, Germany). The carotenoid separation has been performed on a C30 YMC column (5 µm, 250 × 4.6 mm) (Waters, Wilmington, DE, USA). The mobile phase consisted in a mixture of methanol and methyl tert-butyl ether. The chlorophyll separation has been performed on a C18 NST column (5 µm, 150 × 4.4 mm) (Nano Separation Technologies, São Paulo, Brazil). The quantification process of total carotenoids and total chlorophyll has been conducted according to Rodrigues et al. [20].

When it comes to phycobiliproteins, freeze-dried biomass (1.0 ± 0.2 g) has been extracted with 50 mM sodium phosphate buffer pH 6.8 in a mortar with a pestle followed by filtration. The filtrate containing phycocyanin (C-PC) has been kept on. The phycocyanin extract recovery (%) has been calculated according to Soni et al. [21], and the pigment purity has been assessed by calculating the ratio and the absorbance of total protein (280 nm), phycoerythrin (C-PE) (540 nm) and allophycocyanin (C-APC) (650 nm). The phycobiliproteins amount has been calculated according to Bennett and Bogorad [22]. The UV–Vis absorption of C-PC and fluorescence emission and excitation spectra have been obtained through a SpectraMax M5 (Molecular Devices Corp, Los Angeles, CA, USA).

2.7.6. Antioxidant capacity

The antioxidant capacity of lipophilic extracts (carotenoids and chlorophylls) has been carried out according to Rodrigues et al. [23]. The antioxidant capacity of analyzing the hydrophilic extracts (phycobiliproteins) have been carried out according to the oxygen radical absorbance capacity (ORAC) method [24].

2.7.7. Biodiesel quality

The biodiesel quality properties (ester content, EC; cetane number, CN; iodine value, II; degree of unsaturation, DU; saponification value, SV; long-chain saturated factor, LCSF; cold filter plugging point, CFPP; cloud point, CP; allylic position equivalents, APE; bisallylic position equivalents, BAPE; oxidation stability, OS; higher heating value, HVV; kinematic viscosity, µ and kinematic density, ρ) have been calculated by Biodiesel Analyzer© 1.1 software [25].

2.8. Statistical analysis

Analysis of variance (one-way ANOVA) and Tukey's test ($p < 0.05$) have been made in this study. The analyses have been performed using Statistica 10 software (StatSoft, Tulsa-OK, USA).

3. Results and discussion

3.1. Nutrient cycling by microalgae-based process

The heterotrophic microalgal metabolism can simultaneously convert the main pollutants (C, N and P) presented

in wastewater in a single step. It's represents a considerable potential operational cost reduction [26]. Table 2 and Fig. 1 show the kinetic parameters of cell growth and substrate consumption for microalgal sludge grown in poultry and swine slaughterhouse wastewater. The microalgal heterotrophic culture has demonstrated high removal efficiencies of COD (97.5%), N-TKN (87.5%) and P-PO_4^{-3} (100%). Besides, it has presented maximum cellular concentration 1647 mg/L, average cellular productivity 9.0 mg/L. h and maximum specific growth rate 0.6 1/h. Thereby, the microalgal heterotrophic bioreactor has demonstrated to be able to treat this wastewater, not requiring additional unit operations to remove the pollutants, except the primary treatment. Additionally, substantial production of microalgal sludge occurs in this process.

Table 2
Kinetic parameters of cell growth and substrate consumption for microalgal sludge

Parameters	Value
X_{max} (mg/L)	1647 ± 120.2
P_X (mg/L·h)	9 ± 1.86
μ_{max} (1/h)	0.6 ± 0.1
r_{SCOD} (mg/L·h)	18.84 ± 4.54
$r_{SN\text{-}TKN}$ (mg/L·h)	0.96 ± 0.2
$r_{S\ P\text{-}PO4^{-3}}$ (mg/L·h)	0.072 ± 0.001
RE_{COD} (%)	97.57 ± 6.83
$RE_{N\text{-}TKN}$ (%)	87.5 ± 3.2
$RE_{P\text{-}PO4^{-3}}$ (%)	100 ± 1.1
HRT (h)	120

The removal mechanisms that enables these nutrient cycling is the respiration for organic matter, assimilation for nitrogen and phosphorylation for phosphorus [2]. Conversely, it should be considered that other mechanisms capable of removing nitrogen and phosphorus in this system are stripping, volatilization, adsorption, and sedimentation [27].

The aseptic procedures adopted have been suitable for preventing microbial contamination of the cultures (data not shown), since null results have been observed through the heterotrophic plate count method.

3.2. Centesimal composition of microalgal sludge

The nutrient cycling is inherent to the production of microalgal sludge. Thus, the chemical composition analysis of this bioproduct (Table 3) has showed that the proteins are

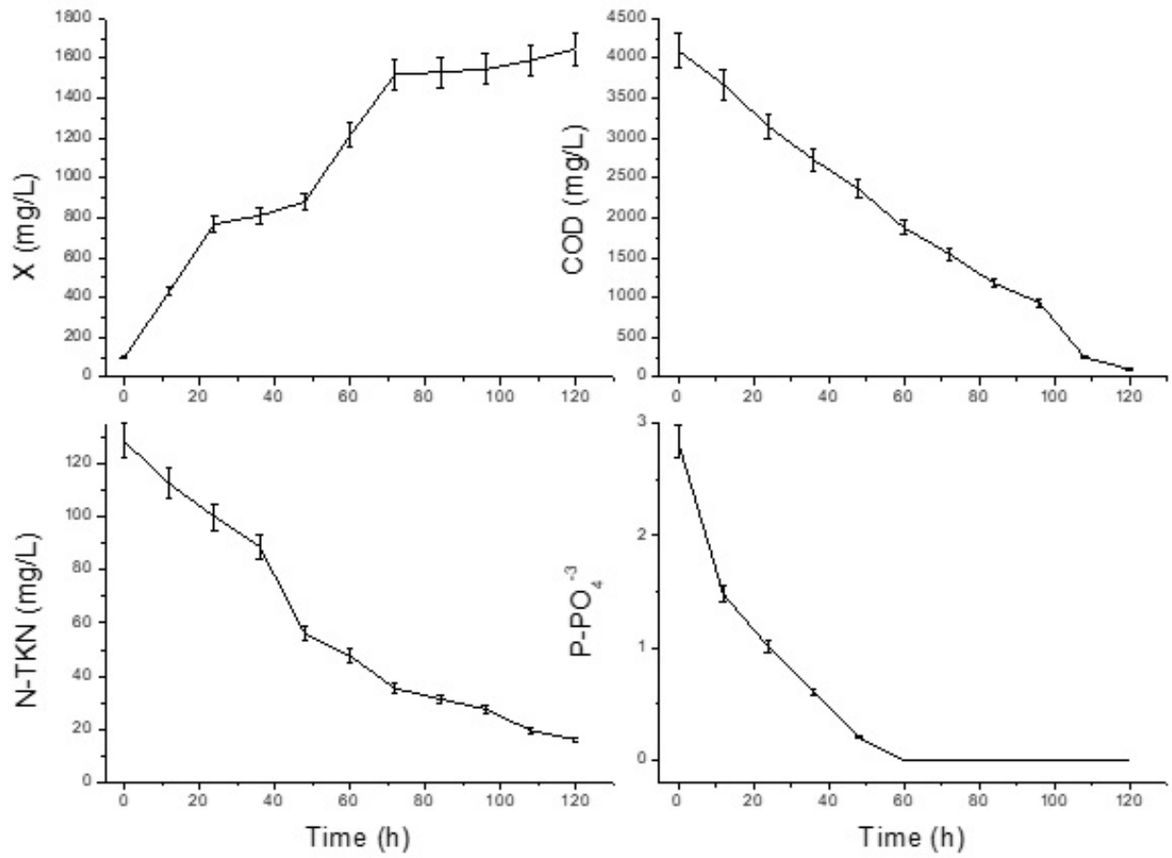

Fig. 1. Dynamics of cell biomass (X), organic carbon (COD), total nitrogen (N-TKN) and total phosphorus (P-PO_4^{-3}) in microalgal heterotrophic bioreactor.

Table 3
Centesimal composition of microalgal sludge produced in poultry and swine slaughterhouse wastewater

Constituent	Value (% dry weight)
Proteins	31.7 ± 0.9
Minerals	21.7 ± 0.2
Carbohydrates	15.9 ± 0.1
Lipids	15.4 ± 0.4
Moisture	15.3 ± 0.7

Table 4
Fatty acids profile of microalgal sludge

Fatty acid	Value (%)
C6:0	4.42 ± 0.2
C8:0	66.62 ± 0.4
C12:0	13.07 ± 0.2
C16:0	8.96 ± 0.5
C18:1n9c	6.91 ± 0.03
SFAs	93.08 ± 1.1
MUFAs	6.91 ± 0.6
PUFAs	ND

its major constituent (31.7%). On the other hand, concentrations of minerals (21.7%), carbohydrates (15.9%), lipids (15.4%) and moisture (15.1%) have also been observed in these conditions. These values show the potential for reusing microalgal sludge as a feedstock for several commercial products.

3.3. Single-cell oil as feedstock for biodiesel synthesis

3.3.1. Fatty acid profile of microalgal sludge

Table 4 shows the profile of fatty acids in the lipid fraction of microalgal sludge. There is a saturated fatty acid predominance (93.1%). The caprylic acid is the major fatty acid (66.6%), followed by lauric (13.1%), palmitic (8.9%) oleic acid (6.9%) and caproic acid (4.4%). It is normally expected that microalgae produce large amounts of polyunsaturated fatty acids (PUFAs) [28]. However, microalgae in heterotrophic cultivation tend to produce saturated and mono unsaturated fatty acids [29,30].

3.3.2. Biodiesel quality

Table 5 shows the quality parameters of biodiesel produced by microalgae *Phormidium autumnale*.

The biodiesel produced from single-cell oil contains: ester content of 99.8%, cetane number of 60.51, iodine value of 6.21 gI_2/100g, degree of unsaturation of 6.91%, saponification value of 349.81, long-chain saturated factor of 0.90%, cold filter plugging point at –13.65°C, cloud point at –0.28°C, allylic position equivalents of 6.91, bis-allylic position equivalents of 22.0, oxidation stability of 8.52 h, higher heating value of 34.96, kinematic viscosity of 0.22 mm^2/s, and kinematic density of 0.88 g/cm^3. All these parameters fulfill the limits established by U.S., European, and Brazilian patterns [32–34]. A high quality biodiesel derived from microalgal sludge has been verified and compared to soybean biodiesel [31]. These values are a direct consequence of the low rate of unsaturated fatty acids, present in single-cell oil, resulting in a high cetane number and a high oxidative stability [35]. These values indicate a potential use of microalgal sludge as a suitable lipid input for biodiesel manufacturing.

Table 5
Parameters for determining biodiesel quality

Properties	Microalgal sludge	Soybean [31]
EC (%)	99.8	96.9
CN	60.5	49.0
IV (gI_2/100 g)	6.2	128
DU (%)	6.9	143.8
SV	349.8	–
LCSF (%)	0.9	1.6
CFPP (°C)	–13.6	–5.0
CP (°C)	–0.28	–
APE	6.9	–
BAPE	–	–
OS (h)	–	1.3
HHV	34.9	–
μ (mm^2/s)	0.22	4.2
ρ (g/cm^3)	0.88	–

3.4. Lipid-extracted microalgae

3.4.1. Free amino acids content

The LEM microalgae are rich in protein and, therefore, in amino acids. Table 6 shows free amino acid content of microalgal sludge. Eighteen amino acids (tryptophan, lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine) have been detected and quantified.

In general, six amino acids out of the 18 ones have been responsible for more than 50% of the total concentration (Table 6): glutamic acid, aspartic acid, glycine, leucine, alanine and arginine. Although the major amino acids have been non-essential amino-acids as glutamic acid, aspartic acid and glycine, the product contains essential amino acids at percentages higher than 40% of the total content. Leucine has the highest concentration (5.16 g/16 gN). The microalgal sludge present some essential amino acids content (isoleucine, valine and threonine) higher than amino acid scoring patterns in FAO [36]. Therefore, they can be considered a potential protein source alternative.

3.4.2. Minerals

Table 7 shows the mineral element content in microalgal sludge. Twenty-nine different minerals have been

Table 6
Free amino acid content of microalgal sludge expressed as g/16 gN

Amino acids	Microalgal sludge (g/16gN)	FAO [36] (g/16gN)
Essential		
Lysine	3.96 ± 0.06	5.8
Methionine + Cystine	1.73 ± 0.00	2.5
Phenylalanine + Tyrosine	4.59 ± 0.01	6.3
Leucine	5.16 ± 0.03	6.6
Isoleucine	3.69 ± 0.03	2.8
Valine	3.94 ± 0.01	3.5
Threonine	3.75 ± 0.02	3.4
Tryptophan	0.92 ± 0.00	1.1
Non-essential		
Histidine	1.43 ± 0.01	–
Arginine	4.37 ± 0.03	–
Aspartic acid	7.00 ± 0.08	–
Serine	3.14 ± 0.03	–
Glutamic acid	7.54 ± 0.01	–
Proline	2.28 ± 0.06	–
Glycine	5.24 ± 0.02	–
Alanine	4.70 ± 0.01	–

found in microalgal sludge composition. Minerals analysis has showed that the main constituent of ash fraction was Na (103707 µg/g). Other minerals that showed high levels are the following: K (26042 µg/g), P (13876 µg/g), S (8629 µg/g), Ca (3302 µg/g), Mg (3302 µg/g) and Fe (1266 µg/g). One of the most important problems concerning microalgae for feeding is the elevated amounts of heavy metals contaminants (lead, cadmium, arsenic, mercury, chrome, manganese). However, toxic heavy metal values in microalgal sludge were lower than the recommended values, according to the Commission Regulation (EC) No 1881/2006, which sets maximum contaminant levels in foodstuffs [37]. The microalgal sludge also contains biologically important macro (Na, K, P, Ca, Mg) and micro minerals (Fe, Mn, Zn, Cu), indispensable for animal feeding [38].

3.4.3. Pigments content

The microalgae presents three natural pigments basic classes: carotenoids, chlorophylls and phycobiliproteins [39]. Table 8 shows the pigments characterization and scavenger capacity compared to peroxyl radicals, using hydrophilic and lipophilic extracts in microalgal sludge. The total carotenoid and total chlorophyll content was 714.3 ± 0.9 and 3400 ± 0.1 µg/g, respectively. In terms of phycobiliproteins, the results have showed 214000 ± 0.5µg/g. These natural pigments have an important role in the photosynthetic and pigmentation metabolism of microalgae, and they display several beneficial biological activities like antioxidant, anti-carcinogenic, anti-inflammatory and others [40–42].

Table 7
Mineral element content in microalgal sludge

Element	Microalgal sludge (µg/g)
Ag	6.40 ± 0,34
Al	111 ± 11
As	< 5.97
Ba	11 ± 1
Be	<0.014
Bi	<6.90
Ca	3302 ± 201
Cd	<0.889
Co	<0.909
Cr	3.29 ± 0.24
Cu	42.6 ± 0.7
Fe	1266 ± 51
Li	<0.508
K	26042 ± 2349
Mg	3302 ± 145
Mn	120 ± 9
Mo	15.8 ± 2.5
Na	103757 ± 667
Ni	<1.32
P	13876 ± 258
Pb	<2.72
S	8629 ± 217
Sb	<12.6
Se	<5.89
Sn	<0.974
Sr	20 ± 1.7
Ti	4.49 ± 0.35
V	<0.101
Zn	71.9 ± 3.9

Table 8
Characterization of pigments and scavenger capacity against peroxyl radicals by hydrophilic and lipophilic extracts in microalgal sludge

Extract	Concentration µg/g (dry weight)	Antioxidant capacity	
		Hydrophilic[a]	Lipophilic[b]
Total carotenoids	714.3 ± 0.9	–	28.1 ± 1.3
Total chlorophylls	3400 ± 0.1	–	84.9 ± 1.9
Total phycobiliproteins	214000 ± 0.5	237.4 ± 2.2	–

[a]micromoles of trolox equivalent per gram of microalgal sludge;
[b]α-tocopherol relative.

In terms of antioxidant capacity of pigments extracts, the carotenoid extract of microalgal sludge has been 28 times a more potent peroxyl radicals scavenger than α-tocopherol (Table 8). This value is higher than the one found on the expressive sources of bioactive compounds [24]. Another

class pigment assessed by the peroxyl radicals scavenger capacity for lipophilic extracts was the chlorophyll. It is almost 85 times more potent than α-tocopherol. Finally, the in vitro scavenging capacity in contrast to peroxyl radicals of phycobiliproteins extract from microalgal sludge was 237.4 $\mu mol_{trolox}/g$, which is about 10 times higher than that the one found in commercial microalgae [43].

This pigments work as natural antioxidants to remove harmful free radicals and they are produced through normal cellular activity and environmental stressors. So, it maintains the immune cells structural integrity. Therefore, the antioxidant capacity presented in LEM microalgae (Table 8) might play an important role to animal health by increasing their immunity [39]. A compromised immune system will increase animal morbidity and mortality rates and, consequently, decrease animal production efficiency [44].

3.5. Multi-purpose microalgal bioprocess

Multi-purpose bioprocess systems that utilize microalgae for treating wastewater, producing biofuels and potential animal feed are an attractive alternative to microalgae-based systems aimed exclusively at biofuels production [45,46]. The remaining portions of microalgae not used in biofuel production (carbohydrates, protein, minerals and unextracted lipids) can be a prospective coproduct that may be available in excess of oil production wasting. The use of LEM microalgae as a coproduct requires the evaluation of industries to determine its suitability.

Fig. 2 shows a nutrient cycling strategy based in poultry and swine slaughterhouse wastewater. Taking as basis for calculation 1 m³ wastewater, it is possible to obtain, in a batch process, 279 g [biomass]/d, 88.5 g [proteins]/d, 60.5 g [minerals]/day, 42.9 g [lipids]/d and 44.3 g [carbohydrates]/d.

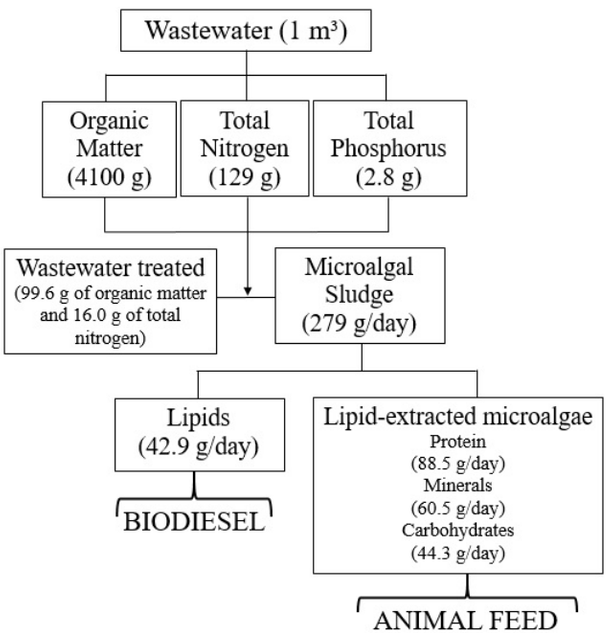

Fig. 2. Nutrient cycling by microalgae-based-processes.

Biorefining may be considered a good opportunity for industry, since microalgal biodiesel presents potential, as evidenced by its qualitative properties, and the remaining biomass fractions can be viable sources of protein, minerals and pigments supplement in animal formulations. Besides, in this calculation basis there are conversions of 4100 g into organic material, 129 g of total nitrogen and 2.8 g of total phosphorus, allowing proper issuance of wastewater to the receptor water bodies.

4. Conclusion

This study has demonstrated the feasibility of microalgae cultivation in wastewater for the sustainable production of microalgal sludge. Moreover, this opportunity to produce biofuel and potential animal feed from *Phormidium autummnale* biomass contributes to the development of a multi-purpose microalgal bioprocess concept, which enables the successful transition to a biobased economy.

Symbols

Q/V	—	Flow rate per unit volume
VVM	—	Volume of air per volume of wastewater per minute
LEM	—	Lipid-extracted microalgae
COD	—	Chemical oxygen demand (mg/L)
N-TKN	—	Total nitrogen (mg/L)
$P\text{-}PO_4^{-3}$	—	Total phosphorus (mg/L)
TS	—	Total solids (mg/L)
SS	—	Suspended solids (mg/L)
VS	—	Volatile solids (mg/L)
FS	—	Fixed solids (mg/L)
C/N	—	Carbon/nitrogen ratio
N/P	—	Nitrogen/phosphorous ratio
h/D	—	Height/diameter ratio
Px	—	Biomass productivity (mg/L)
μmax	—	Maximum specific growth rate (1/h)
rs	—	Consumption rates (mg/L·h)
RE	—	Removal efficiency (%)
X	—	Cell biomass at time t = t (mg/L)
X_0	—	Initial cell biomass (mg/L)
t	—	Time (h)
S	—	Final concentration (mg/L)
S_0	—	Initial concentration (mg/L)
HRT	—	Hydraulic retention time (h)
ORAC	—	Oxygen radical absorbance capacity
EC	—	Ester content (%)
CN	—	Cetane number
II	—	Iodine value ($gI_2/100g$)
DU	—	Degree of unsaturation (%)
SV	—	Saponification value
LCSF	—	Long-chain saturated factor (%)
CFPP	—	Cold filter plugging point (°C)
CP	—	Cloud point (°C)
APE	—	Allylic position equivalents
BAPE	—	Bisallylic position equivalents
OS	—	Oxidation stability (h)
HVV	—	Higher heating value
m	—	Kinematic viscosity (mm^2/s)
ρ	—	Kinematic density (g/cm^3)

PUFAs — Polyunsaturated fatty acids
SFAs — Saturated fatty acids
MUFAs — Monounsaturated fatty acids
ND — Not detected

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