Symbiosis

www.symbiosisonline.org
www.symbiosisonlinepublishing.com

Research Article Journal of Advanced Research in Biotechnology Open Access

An overview on the application of genus *Chlorella* in biotechnological processes

Cristiano José de Andrade[1*] and Lidiane Maria de Andrade[1]

[1]*Chemical Engineering Department of Polytechnic School of the University of São Paulo*

Received: 15 March, 2017; Accepted: 6 April, 2017; Published: 18 april, 2017

***Corresponding author:** *Dr. Andrade C. J, Chemical Engineering Department of Polytechnic School of the University of São Paulo, São Paulo, SP, Brazil. Tel no: + 55 (19) 98154-3393; E-mail: eng.crisja@gmail.com*

Abstract

Chlorella is a genus of green algae (single-cell) that shows spherical shape. The cultivation of microalgae, such as *Chlorella*, is promising due to technical easiness of this type of bioprocesses. The production of microalgae at industrial scale started in the early 1960s. The microalgae cultivation is aligned to the green chemistry concept. Thus, there is a strong trend that this type of bioprocess will be applied all over the world. The aim of this study was review some characteristics of genus *Chlorella*, and mainly the potential industrial applications of genus *Chlorella* and their biocompounds. Microalgae are able of two types of trophy: autotrophy and heterotrophy. The photoautotrophy organisms are used when the CO_2 fixation is the mainstream appeal, whereas heterotrophy and mixtrophy organisms can be mainly use for the production of microalgae biomass. Some of the main applications of genus *Chlorella* are: productions of biofuels (biodiesel, biomethane and biohydrogen), cosmetics (skin care), supplementary foods (polyunsaturated fatty acids), pigments (carotenoids and chlorophyll) and wastewater treatments (reduction of chemical oxygen demand and bioremediation).

Keywords: *Chlorella;* Biofuels; Supplementary Foods; Wastewater Treatments.

Introduction

Chlorella is a genus of green algae (single-cell) that shows spherical shape ≈ 2 to 10 µm (diameter) (Figure 1). Compared to others microalgae, *Chlorella* species present higher photosynthetic efficiency. In addition, it was predicted that 10,000 tons of proteins per year could be produced by 20 people staff (4-square kilometer) - *Chlorella* farm [1]. The production of microalgae at industrial scale started in the early 1960s in Japan, in which *Chlorella* species were applied as food additive. Then, in 1980s the microalgae cultivation spread out all over the world (e.g. USA, India, Israel and Australia) [2].

Microalgae are able of two types of trophy (nourishment) (i) autotrophy (phototrophy) and (ii) heterotrophy (phagotrophy). Autotrophy organisms absorb light in order to reduce CO_2 (to obtain energy). Photoautotrophy organisms require only inorganic minerals, in which a photoautotrophy obligate cannot grow in dark.

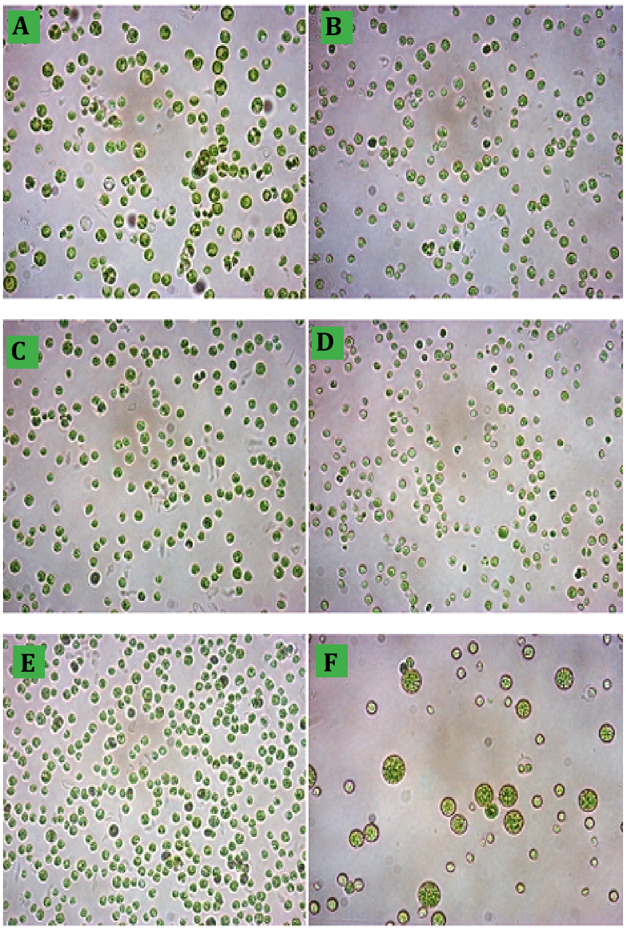

Figure 1: *Chlorella desiccata* (Cde), *Chlorella kessleri* (Cke), *Chlorella luteoviridis* (Clu), *Chlorella protothecoides* (Cpr), *Chlorella sorokiniana* and *Chlorella vulgaris* (Cv); respectively A, B, C, D, E and F (Source: Authors).

On the other hand, heterotrophic organisms obtain energy from organic compounds. Photoheterotrophic organisms use light for energy, nevertheless they cannot use CO_2 as sole carbon source, that is, they take organic compounds from environment to complete the carbon requirement (Table 1) [3].

Table 1: Trophic possibilities for the microalgae

	Inorganic carbon	Organic carbon	Light
Autotrophy	✓		✓
Heterotrophy†		✓	
Photoautotrophic††	✓		✓
Photoheterotrophic		✓	✓
Mixotrophic	✓	✓	✓
Auxotrophy*	✓	✓	

Last but not least, mixtrophy is other interesting type of microalgae's metabolism. Mixotrophy is the cultivation in which organic carbon and CO_2 are simultaneously assimilated (a mixture between autrotophy and heterotrophy). This metabolic pathway gives these microorganisms advantages over photosynthetic mode of cultivation. This metabolism is approximately the sum of specific growth rates of cells under heterotrophic and photoautrophic. In addition, mixotrophy growth allowed the cultivation in the dark (higher productivity) and higher cell concentration, for instance the maximum specific growth rate for *Chlorella vulgaris* 0.11 h^{-1} (photoautotrophy mode) and 0.098 h^{-1} (heterotrophic mode) and 0.198 h^{-1} (mixotrophic) [4-5].

Regarding culture media for the cultivation of microalgae, they are very simple (Table 2). In this sense, even similar one another, some compositions are widely used for the cultivation of genus *Chlorella*, for instance, Guillard's WC [6-7]; Bold [7-8].

Table 2: Culture media composition of culture media for microalgae cultivation

WC

$NaNO_3$ (g/L)	$CaCl_2 . 2H_2O$ (g/L)	$MgSO_4 . 7H_2O$ (g/L)	$NaHCO_3$ (g/L)	$Na_2SiO_3 . 9H_2O$ (g/L)	K_2HPO_4 (g/L)	NaEDTA . $2H_2O$ (g/L)	$FeCl_3 . 6H_2O$ (g/L)	$CuSO_4 . 5H_2O$ (g/L)	$ZnSO_4 . 7H_2O$ (g/L)	$CoCl_2 . 6H_2O$ (g/L)	$MnCl_2 . 4H_2O$ (g/L)	$Na_2MoO_4 . 2H_2O$ (g/L)	$MgSO_4$ (g/L)	Thiamine-HCl (µg/L)	Biotin (g/L)	Cyanocobalamin (g/L)
0.085	0.037	0.037	0.012	0.028	0.008	0.04	0.04	0.010	0.022	0.01	0.180	0.006	0.019	0.297	0.005	0.005

Bold

$NaNO_3$ (g/L)	$CaCl_2.2H_2O$ (g/L)	$MgSO_4.7H_2O$ (g/L)	K_2HPO_4 (g/L)	KH_2PO_4 (g/L)	NaCl (g/L)	NaEDTA (g/L)	$FeSO_4.7\,H_2O$ (g/L)	H_3BO_3 (g/L)	$ZnSO_4.7H_2O$ (g/L)	$MnCl_2.4H_2O$ (g/L)	MoO_3 (g/L)	$CuSO_4.5H_2O$ (g/L)	$Co(NO_3)_2.6H_2O$ (g/L)
0.25	0.026	0.075	0.075	0.175	0.025	0.0049	0.0049	0.0115	0.0088	0.00144	0.00071	0.00157	0.00048

Sorokin and Krauss [8]

KNO_3 (g /L)	KH_2PO_4 (g /L)	$MgSO_4.7H_2O$ (g/L)	$CaCl_2.2H_2O_2$ (g /L)	$FeSO_4.7H_2O$ (g /L)	EDTA (g /L)	H_3BO_4 (µg/L)	$MnCl_2.4H_2O$ (µg/L)	ZnSO4. $7H_2O$ (µg /L)	CuSO4. $5H_2O$ (µg /L)	Co(NO3)2.6H2O (µg/L)	MoO_3 (µg/L)
1.25	1.25	1.0	0.04	0.05	0.5	114	14	88	16	5	7

Citation: Cristiano José de Andrade, Lidiane Maria de Andrade.(2017) An overview on the application of genus *Chlorella* in biotechnological processes. J Adv Res Biotech 2(1):1-9.

Therefore, the culture media described in Table 2 can be used for photoautotrophy organisms, in which the 2 (CO_2) fixation is the mainstream appeal. In addition, the phototrophic production is the most effective in terms of net energy balance. Nevertheless, this bioprocess show higher variation and lower productivity - when compared with heterotrophic production [2]. In this regard, since atmospheric 2 (CO_2) does not supply enough carbon to achieve high rates of autotrophic microalgae production – (the diffusion of atmosphere 2 (CO_2) → aqueous phase ≈ 10 g/m.d); the use of bicarbonate-carbonate buffer (medium) can be useful because it provides 2 (CO_2) for photosynthesis as detailed below:

$2HCO_3^- \leftrightarrow CO_3^{2-} + H_2O + CO_2$

$HCO_3^- \leftrightarrow CO_2 + OH^-$

$CO_3^{2-} + H_2O \leftrightarrow 2OH^-$

Obviously, the pH of culture medium tends to become alkali, in which at high microalgae density, it reaches pH as high as 11 [3].

On the other hand, heterotrophy and mixtrophy organisms can be, mainly use for the production of microalgae biomass. In addition, compared to photoautotrophy system, the mixotrophy cultivation mode shows lower production cost due to the higher biomass and lipid productivity and the possibility in use low-cost culture media such as industrial wastes (culture medium is ≈ 80% of the total production cost) [5].

The cultivation of microalgae, either photoautotrophy or heterotrophy modes, plays already an important role in biobased economy (very aligned to green chemistry concept). It is worth noting that in 2050 the world population is estimated to reach 9 billion people, that is, the demand for commodities will increase exponentially, in which the sustainable production (food and energy). Microalgae are not only one the most promising waste converters and recyclers, but can be efficiently cultivated in places that are inhospitable for agriculture, which can provide proteins and lipids (food) or raw material for bioplastic [9].

Some of the main applications of *Chlorella* are described in more details below, such as productions of biofuels, cosmetics, supplementary foods, pigments, by wastewater treatments.

Biofuels

It is well-known that global climate change has increased due to the green house gas emissions from fossil fuels. Thus, alternative sources of energy need to be investigated and explored. Biofuel is a fuel that is produced by biological processes, for instance bioalcohols (ethanol, propanol, butanol), biodiesel, green diesel, biogas (biomethane, biohydrogen), etc.

Regarding microalgae-based biofuels, mainly biodiesel and biogas have been investigated. The lipids from microalgae can be extracted and then esterified with alcohol, which produce biodiesel, whereas biogas (biomethane and biohydrogen) is

Biodiesel

Biodiesel is a clean alternative fuel source that could replace fossil fuels. Usually, biodiesel is produced from raw oleaginous such as soybean and sunflower, which leads to issues in terms of deforestation, world hunger and land pollution. Thus, other sources of lipids should be investigated. In this sense, microalgae lipids are one of the most promising feedstock to produce biodiesel, since they do not compete with food crops and show high content of lipids (up to 75 wt%). However, the current high cost of biodiesel from microalgae makes its production at industrial scale infeasible [11-12]. Other advantage on the use of microalgae lipids to biodiesel production is the mixotroph metabolism of some microalgae (auto- and heterotrophy metabolisms), for instance glucose can be used by *Chlorella* protothecoides. In particular, the heterotrophy cultivation is cheaper, easier, feasible in colder climates and allows the use of agro-industrial wastes as substrates [12-13].

Veillette et al. [12] detailed the esterification of microalgae free fatty acids using Amberlyst-15 as a catalyst, in which a conversion higher as 84% was reached [12].

Biogas

As already mentioned the production of biogas from microalgae occurs by the anaerobic digestion of microalgae biomass by anaerobic bacteria. The anaerobic digestion encompasses 4 general steps (i) hydrolysis, fermentation, acetogenesis and methanogenesis. The composition of biogas is CH_4 (55–75%) and CO_2(25–45%) [10].

Jankowska et al. [10] compiled the biogas yields from microalgae, for instance, *C. kessleri* (0.335 L biogas/g.VS (65% CH_4) (0.218 L CH_4/gVS)); *C. vulgaris* (0.337 L CH_4/g.VS); *C. vulgaris* (0.180 L CH_4/g.CODin); *C. vulgaris* (0.156 L CH_4/g.COD); *C. vulgaris* ((0.364 LN biogas/g.VS) (62.6% CH_4) (0.228 LN CH_4/g.VS)); *C. vulgaris* ((0.366 L biogas/g.VS) (62.5% CH_4) (0.229 L CH_4/g.VS)); *C. vulgaris* (0.139 L CH_4/g.COD in) [10].

The biogas yield is highly affected by the specie of microalgae, type of pretreatment, presence of inhibitors of hydrogenesis or methanogenesis, organic loading, retention time, temperature, pH, substrate, etc. In this sense, as described by Choi et al. compared to others microorganisms, the cell walls of microalgae are more recalcitrant. Thus, the pretreatment (acid + thermal) of *C. vulgaris* was needed to increase the hydrolysis with consequent enhancement on the H_2 production [10, 14]

Cosmetics

Components of microalgae, typically *C. vulgaris* specie, are often used in cosmetics. One of the most interesting approaches on the applications of microalgae is in the cosmetic formulations. Microalgae have sun protection skills due to the presence of chlorophyll-a in its composition (light absorption) [15]. In addition, *Chlorella* extract is also used by the skin care industry, since some compounds from *Chlorella* extract have anti-aging, refreshing, regenerant, emollient and anti-irritant activities [15].

Microalgae of *Chlorella* genus can produce metabolites, such as sporopollenin and mycosporine-like amino acids, to protect themselves from ultra violet (UV) radiation (Table 3) [16].

Table 3: UV-Screening compounds produced by *Chlorella*

UV Screening Compound	Specie
Sporopollenin	*Chlorella fusca*
Mycosporine-Like Amino Acids	*Chlorella minutissima* *Chlorella sorokiniana*

In this sense, currently, there are some cosmetics microalgae-based commercially available. The world's first facial moisturizer, Sun *Chlorella* Cream® which is produced by Sun *Chlorella* Japanese Company, is based in *C. pyrenoidosa* extract. This facial moisturizer promotes the skin hydration and also aid the skin cell renewal. Other example is the Dermochlorella that is produced by the ProTec Ingredia French Company. Dermochlorella is produced from *C. vulgaris* extract, which shows firming, restructuring and eye contour effects besides it stimulates the synthesis of collagen. In addition, it decreases the morphology of stretch marks and reduces vascular imperfections.

Supplementary Food

Microalgae, in particular, genus *Chlorella,* can synthesize essential nutritional compounds. Microalgae composition is up to 50-70% protein (essential amino acids), 30% lipids (polyunsaturated fatty acids), up to 8-14% carotene and a fairly high concentration of vitamins B1, B2, B3, B6, B12, E, K, D, among others. Thus, *Chlorella* extract can be used as supplementary food as described below starch.

Starch

Microalgae under a wide range of conditions accumulate starch. Palacios et al. described an interesting system that integrated *Azospirillum brasilense* and *Chlorella sorokiniana. A. brasilense* is microalgae growth-promoting bacterium by mainly indole-3-acetic acid.

Human Nutrition

Microalgae have been used by humans for thousands years and the commercial large-scale of *Chlorella* genus started in the 1960's by the Japanese company Nohon *Chlorella*. In this sense, the human consumption of microalgae biomass is restricted to very few species, in which *Chlorella, Spirulina* and *Dunaliella* are the main genus produced at industrial scale. Microalgae biomass is usually marketed (human nutrition) as tablet or powder, in the health food market [2, 19-20].

Chlorella microalgae genus have positive impact on the health of humans due to their nutritional content (nutraceutical – proteins, lipids, pigments, carbohydrates), as shown in Table 4 (polyunsaturated fatty acids). In addition, *Chlorella* extract shows effects against renal failure and growth promotion of intestinal *Lactobacillus* (probiotic) [2]. In others interesting approaches, Ebrahimi-Mameghani et al. [21] studied the *C. vulgaris* supplementation on glucose homeostasis, insulin resistance and inflammatory biomarkers in patients with nonalcoholic fatty liver disease [21]. The authors indicated that 1,200 mg of *C. vulgaris* supplementation can effects on weight loss, serum glucose and enhanced the inflammatory biomarkers as well as liver function in non-alcoholic fatty liver disease patients, whereas, Cherng et al. [22]. proved that *Chlorella* contains a peptide known as *Chlorella*-11 (Val-Glu-Cys-Tyr-Gly-Pro-Asn-Arg-Pro-Gln-Phe) that showed activity against inflammation caused by lipopolysaccharides from Gram-negative bacteria [21-22].

Carbohydrates are also found in *Chlorella* extract as starch, sugar, glucose and other polysaccharides [17]. Fatty acids from microalgae have encompasses omega families such as oleic acid, linoleic acid, linolenic acid and especially arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which ones feature high added value as shown in Table 4.

Table 4: Fatty acids from microalgae.

Acids	Carbon Atoms	Chemical Formula	Omega Family
Palmitoleic	16	$C_{16}H_{30}O_2$	ω7
Oleic	18	$C_{18}H_{34}O_2$	ω9
Linoleic	18	$C_{18}H_{32}O_2$	ω6
α-Linolenic	18	$C_{18}H_{30}O_2$	ω3
γ-Linolenic	18	$C_{18}H_{30}O_2$	ω6
Homo γ-Linolenic	20	$C_{20}H_{34}O_2$	ω6
AA	20	$C_{20}H_{32}O_2$	ω6
EPA	20	$C_{20}H_{30}O_2$	ω3
DHA	22	$C_{22}H_{32}O_2$	ω3

Arachidonic acid from microalgae can be used as supplementary food when there is deficiency in linoleic acid or some difficulty to convert linoleic acid to Arachidonic acid [23-24].

"Regarding muscle growth, arachidonic acid repairs and promotes the growth of skeletal muscle tissue [25]. In addition, arachidonic acid is the most abundant fatty acid (20%) in the brain. Thus, the entire neurological health depends on the level of arachidonic acid [26-29]. Low content of arachidonic acid in the brain can contribute to diseases such as Alzheimer's and Bipolar disorder. Even more, arachidonic acid as supplementary food can has been shown to increase lean body mass, strength, resistance and present anti-inflammatory properties".

Eicosapentaenoic Acid (EPA)

In microalgae EPA is the precursor for prostaglandin-3, thromboxane-3, and leukotriene-5 group. EPA has the ability to reduce inflammation, decrease depression and suicidal behavior, schizophrenia and improves the chemotherapy response.

Citation: Cristiano José de Andrade, Lidiane Maria de Andrade.(2017) An overview on the application of genus *Chlorella* in biotechnological processes. J Adv Res Biotech 2(1):1-9.

Docosahexaenoic Acid (DHA)

Docosahexaenoic acid is an omega-3 fatty acid, that is, a primary structural component of the human brain, eye, cerebral cortex, skin, retina and heart health. Among the applications of DHA are: infant formulations, products for pregnant and nursing women, food and beverage products, dietary supplements, immune modulating effects and capacity to inhibit growth of human colon carcinoma cells, more than other omega-3.

Companies that produce omega fatty acids, mainly EPA and DHA, from microalgae, are shown in Table 5.

Table 5: Companies that market omega-3 from *Chlorella.*

Company	Location	Started
Live Fuels	USA	2006
Aurora Algae	USA	2006
Martek Biosciences	USA	2007
Blue Biotech International GmBH	Germany	2000
Photonz Corporation	New Zealand	2002
Ingrepro BV	The Netherlands	2001

The possibilities of human nutrition with microalgae are very wide. Microalgae for human nutrition can be incorporated into candies, beverages, snacks, pastas and juices formulations, in which the commercial applications are dominated by some genus including *Chlorella* [19]. Companies that market *Chlorella* for human nutrition are shown in Table 6.

Table 6: Companies that market *Chlorella* for human nutrition.

Company	Location	Started	Production (tons/ year)
Sun *Chlorella* Corporation	Japan	1969	NF
Yaeyama Shokusan Co Ltd.	Japan	1975	420
Maypro Industries Inc.	USA	1977	NF
Taiwan *Chlorella* Manufacturing Co ltd	Taiwan	1964	400
Far East Microalgae Ind Co., Ltd	Taiwan	1976	1000
Roquette Klötze GmbH & Co. KG	Germany	1995	130-150
Lotus Organics	Kazakhstan	2007	NF
Martek Biosciences Corporation	USA	2007	NF

*NF = Not Found

Animal Feed

Chlorella is one of the most frequently microorganisms used as animal nutrition which is directly consumed by larval (brief period), mollusks, penaeid shrimp or indirectly for live prey fed to small fish [2, 30]. In this sense, specific microalgae are feasible to be used as animal feed supplements, for instance, the supplementation animal nutrition by *Chlorella*, *Scenedesmus* and *Spirulina* has enhanced the immune response, improved fertility, better weight control, healthier skin and a lustrous coat [2].

Table 7: Composition of some species of *Chlorella* genus microalgae (% dried biomass) [31-33].

Specie	Protein	Carbohydrate	Lipid	Reference
Chlorella vulgaris	51-58	12-17	14-22	[17]
Chlorella calcitrans			14.6-16.4/39.8	
Chlorella emerson ii			25.0-63.0	
Chlorella protothecoides			14.6-57.8	
Chlorella pyrenoidosa	57	26	2.0	[34-35]
Chlorella sorokiniana			19.0-22.0	
Chlorella sp.			10.0-48.0	

Pigments

Microalgae pigments, carotenoids and chlorophyll, are often used by industries, such as food, nutraceutical, pharmaceutical, aquaculture, and cosmetic industry; as well by clinical/research laboratories (label for antibodies and receptors) [36].

Carotenoids

Carotenoids are pigments that have been draw attention due to their potential health benefits, in which microalgae are a natural source of carotenoids. They have a common C40 backbone structure of isoprene units; they are lypholilic and usually presented color such as red, orange or yellow. The carotenoids pigments can be divided in two groups: carotenes and xanthophylls [37].

Carotenes

The composition of carotenes contains only hydrocarbons and the common carotenes found in microalgae are lycopene and ß-carotene (Figure 2).

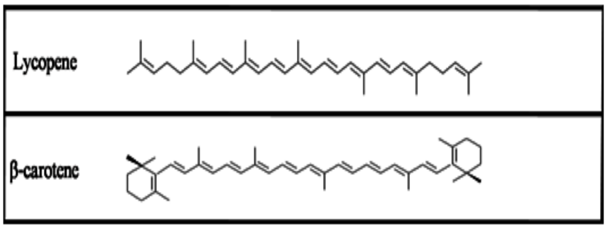

Figure 2: Chemical structure of carotenes from microalgae.

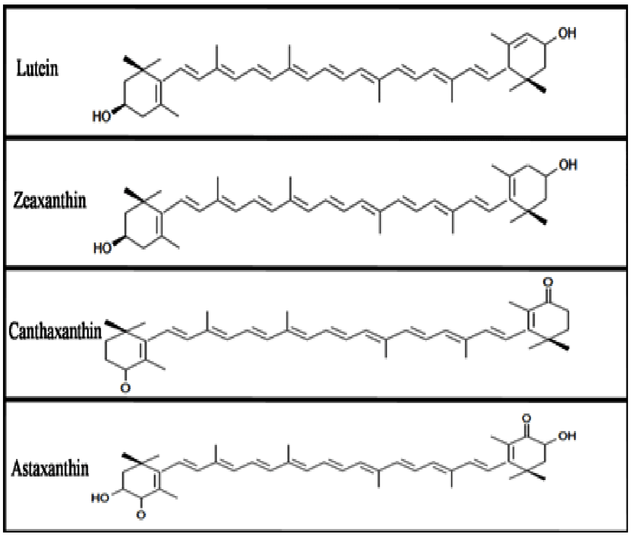

Figure 3: Chemical structure of xanthophylls from microalgae.

Xanthophylls

Xanthophylls are oxygenated derivatives of carotenes. The common carotenes found in microalgae are lutein, zeaxanthin, canthaxantin and astaxanthin and their chemical structures are shown in Figure 3.

The main carotenoids synthesize by *Chlorella* microalgae genus are mainly astaxanthin, ß-carotene, lutein, lycopen and canthaxantin. In general, carotenoids have an anti-oxidant property. Therefore they protect the cells from reactive radicals, prevent lipid oxidation, promote the stability and functionality of the photosynthetic machinery of cells [3].

Chlorophyll

Chlorella is also known as 'Emerald food' due to its high content (7% of biomass) of chlorophyll a [38]. Chlorophyll is a pigment present in microalgae that produce carbohydrates from carbon dioxide and water light through energy absorption (photosynthesis process – Calvin cycle). The chlorophylls present in *Chlorella* microalgae are: (i) chlorophyll-a - responsible for oxygenic photosynthesis, and (ii) chlorophyll-b - absorb energy to aid in photosynthesis process.

Chlorophyll can be recovered from microalgae biomass by organic solvent extraction. Chlorophyll has antioxidant and antimutagenic properties and can be used as additive in pharmaceutical, cosmetic products, and also as a natural food pigment. The Table 8 summarizes the health benefits by pigments produced by microalgae.

Table 8: Potential health human benefits by pigments produced by microalgae (Adapted from Gong and Bassi, [37]).

	Class	Pigment	Health Benefits	Reference
Carotenoids	**Carotenes**	**Lycopene**	Anti-cancer	[39]
			Cardiovascular health	
		ß-carotene	Anti-oxidant	[40]
			Prevents liber fibrosis	
	Xanthophylls	**Lutein**	Prevents cataract and age-related	[41]
			Cardiovascular health	[42]
		Canthaxanthin	Anti-oxidant	[43]
			Creates tan color	
		Astaxanthin	Strong anti-oxidant	[44]
			Anti-cancer	[45]
			Cardiovascular health	[46]

Wastewater Treatment

Generally, there are two subsequent treatments (i) for the sedimentation of materials; (ii) to oxidize the organic materials. Then, the wastewaters are disposal to aquatic environment [53]. Aquaculture systems - as wastewater treatment and recycling - have been draw attention, mainly due to their capacity to simultaneously solve environmental and sanitary issues. In addition, these processes can be economically feasible [54]. In this sense, aquaculture systems, in particular those that apply algae take advantages of oxygen production, which favors heterotrophic bacteria.

Hammouda et al. [54] tested microbial consortium comprised by *Chlorella* and *Scenedesmus* for the treatment of wastewater in both batch and continuous modes. The authors described a progressive and high reduction of chemical oxygen demand; 89% and 91.7%, in batch and continuous modes, respectively [54].

Murwanashyaka et al. [53] detailed a study that green microalgae *Chlorella sorokiniana* FACHB-275 was cultivated under both light and lightless conditions. The cultivation aimed to remediate wastewater under heterotrophic conditions. Preliminary results showed high tolerance from nitrogenous and phosphorous compounds. The authors described the relation between initial nutrient content and removal efficiency. The highest removal efficiency reached 99% (123.6 mg N/L and 26.8 mg P/L) [53].

Chlorella sp. was already tested to remediate aquaculture wastewater (fish farm) aerated with boiler flue gas wastewater. Thus, the authors proved that is feasible simultaneously reduce CO_2 emission and produce microalgae biomass [54-55].

Bioremediation

Microalgae efficiently absorb heavy metals, for instance *Chlorella vulgaris* absorbed Pb^{+2}, in which the highest adsorption rate was 15.4 mg/g.min [56-57].

Hammouda et al. [54] tested microbial consortium comprised by *Chlorella* and *Scenedesmus* for the treatment of wastewater. The system proved to be efficient on simultaneous removal of Fe (from 0.99 to 0.02 mg/L), Ni (from 0.661 to 0.15 mg/L) and Cr (from 0.4 to 0.09 mg/L) [54].

Perspective, Advantages and Drawbacks

The microalgae cultivation is an eco-friendly and renewable process, which is aligned to the green chemistry concept, in particular when the microalgae cultivation is integrated to flue gases (source of carbon). Thus, there is a strong trend that this type of bioprocess will be applied all over the world. One of the main advantages of microalgae cultivation, over other bioprocesses, is its versatility, for instance lipids (biodiesel); lipids (polyunsaturated fatty acids – human nutrition and animal feed), pigments, proteins (peptide known as Chlorella-11), among others [2,36].

Among the advantages of microalgae cultivation are: (i) seasonality - microalgae are cultivable throughout the year, which gives the microalgae cultivation advantages over all oilseed crops; (ii) microalgae cultivation is a submerged bioprocess, nevertheless, it needs less water than agricultural land crop; (iii) microalgae cultivation does not compete with production of food, since brackish water on non-arable land can be used; (iv) microalgae have fast growth (≈ 3.5 h generation time) and high oil concentration (20-50% - dry weight of biomass); (iv) it is an environmentally friendly process (green chemistry concept), since occurs the biofixation of CO_2 (1 kg of dry microalgae biomass utilize ≈ 1.83 kg of CO_2); (v) the nutrients can be obtained from wastewater, in particular nitrogen and phosphorus (↓ production cost and waste treatment), (vi) simultaneous production of valuable products (e.g. proteins and lipids) [2].

Although there are several potential microalgae applications, in particular those using genus *Chlorella,* there is still room for improvement, for instance, the production efficiency must be increased three times and production costs must be reduced ten times, whereas Brennan and Owende, pointed out the main challenges on microalgae biofuel technology, including (i) to achieve high photosynthetic efficiency on continuous production mode; (ii) the cultivation using single species of microalgae (not susceptible to contamination); (iii) few industrial scale plants are current in operation (lack of knowledge), (iv) integration between flue gases and microalgae cultivation [2,9].

Conclusion

The microalgae cultivation is one of the most promising bioprocesses due to its technical easiness and versatility. Very likely, the microalgae cultivation will be applied all over the world. Among the potential applications of genus *Chlorella,* the production of biofuels, in particular biodiesel, the supplementation of foods (polyunsaturated fatty acids) and wastewater treatments (reduction of chemical oxygen demand) are the most feasible ones.

References

1. Zuñiga C, Li C-T, Huelsman T, Levering J, Zielinski DC, McConnell BO, et al. Genome-scale metabolic model for the green alga *Chlorella vulgaris* UTEX 395 accurately predicts phenotypes under autotrophic, heterotrophic, and mixotrophic growth conditions. Plant Physiol. 2016;172:589-602. doi.org/10.1104/pp.16.00593
2. Brennan L, Owende P. Biofuels from microalgae - A review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sust Energ Rev. 2010;14:557-577. doi.org/10.1016/j.rser.2009.10.009
3. Grobbelaar JU. Algal nutrition. Mineral nutrition. Richmond A, editor. Handbook of microalgal culture: biotechnology and applied phycology. Oxford:Blackwell Science;2004.
4. Lee Y-K. Algal nutrition. Heterotrophic carbon nutrition. Richmond A,

editor. Handbook of microalgal culture: biotechnology and applied phycology. Oxford:Blackwell Science;2004.

5. Abreu AP, Fernandes B, Vicente A A, Teixeira J, Dragone G. Mixotrophic cultivation of *Chlorella vulgaris* using industrial dairy waste as organic carbon source. Bioresource Technol. 2012;118:61-66. doi.org/10.1016/j.biortech.2012.05.055
6. Guillard RRL. Culture of phytoplankton for feeding marine invertebrates. Smith WL and Chantey MH, editors. Culture of marine invertebrate animals. Plenum Publishers, New York;1975.
7. Andersen R. Algal culturing technique. San Diego: Elservier;2005.
8. Sorokin C, Krauss RW. The effect of light intensity on the growth rates of green algae. Plant Physiol.1958;33:109-113.
9. Wolkers H, Barbosa M, Kleinegris D, Bosma R, Wijffels RH. Large-scale sustainable cultivation of microalgae for the production of bulk commodities. 2011 Available at: www.groenegrondstoffen.nl/downloads/Boekjes/12Microalgae_UK.pdf
10. Jankowska E, Sahu AK, Oleskowicz-Popiel P. Biogas from microalgae: Review on microalgae's cultivation, harvesting and pretreatment for anaerobic digestion. Renew Sust Energ Rev. 2016.75:692-709. doi.org/10.1016/j.rser.2016.11.045
11. Karimi M. Exergy-based optimization of direct conversion of microalgae biomass to biodiesel. J Clean Prod. 2017;141: 50-55. doi.org/10.1016/j.jclepro.2016.09.032
12. Veillette M, Giroir-Fendler A, Faucheux N, Heitz M. Esterification of free fatty acids with methanol to biodiesel using heterogeneous catalysts: From model acid oil to microalgae lipids. Chem Eng J. 2017; 308:101-109. doi.org/10.1016/j.cej.2016.07.061.
13. EL-Sheekh MM, Bedaiwy MY, Osman ME. Ismail MM. Mixotrophic and heterotrophic growth of some microalgae using extract of fungal-treated wheat bran. Int J Recycl Org Waste Agricult. 2012;1:12. doi:10.1186/2251-7715-1-12
14. Choi J-M, Han S-K, Kim J-T, Lee C-Y. Optimization of combined (acid + thermal) pretreatment for enhanced dark fermentative H2 production from *Chlorella vulgaris* using response surface methodology. Int Biodeter Biodegr. 2016;108: 191-197. doi.org/10.1016/j.ibiod.2015.06.013
15. Stolz, Patrick, Obermayer, Barbara. Manufacturing microalgae for skin care. Cosmet Toiletries. 2005;120(3):99.
16. Priyadarshani I, Rath B. Commercial and industrial applications of micro alae - A review. J. Algal Biomass Utln. 2012;3(4):89-100.
17. Becker W. Microalgae in human and animal nutrition. Richmond A. editor, Handbook of microalgal culture. Oxford: Blackwell Science;2004.
18. Palacios OA, Choix FJ, Bashan Y, De-Bashan LE. Influence of tryptophan and indole-3-acetic acid on starch accumulation in the synthetic mutualistic *Chlorella sorokiniana* - *Azospirillum brasilense* system under heterotrophic conditions. Res Microbiol. 2016;167(5): 367-379. doi.org/10.1016/j.resmic.2016.02.005
19. Spalaore P, Cassan-Joannis C, Duran E, Isambert A. Commercial Applications of Microalgae. J Biosci Bioeng. 2006;101(2):87-96. doi.org/10.1263/jbb.101.87
20. Iwamoto H. Industrial production of microalgal cell-mass and secondary products - major industrial species - *Chlorella*. Richmond A. editor, Handbook of microalgal culture. Oxford: Blackwell Science;2004.
21. Ebrahimi-Mameghani M, Sadeghi Z, Farhangi MA, Vaghef-Mehrabany E, Aliashrafi, S. Glucose homeostasis, insulin resistance and in flammatory biomarkers in patients with non-alcoholic fatty liver disease: Beneficial effects of supplementation with microalgae *Chlorella vulgaris*: A double-blind placebo-controlled randomized clinical trial. Clin Nutr. 2016. doi.org/10.1016/j.clnu.2016.07.004
22. Cherng JY, Liu CC, Shen CR, Lin HH, Shih MF. Beneficial effects of *Chlorella*-ll peptide on blocking LPS-induced macrophage activation and alleviating thermal injury-induced inflammation in rats. I J Immunopath Ph. 2010;23:811-820. doi.org/ 10.1177/039463201002300316
23. MacDonald ML, Rogers QR, Morris JG. Nutrition of the domestic cat, a mammalian carnivore. Annu Rev Nutr. 1984;4: 521-562. doi:10.1146/annurev.nu.04.070184.002513
24. Rivers JP, Sinclair AJ, Craqford MA. Inability of the cat to desaturate essential fatty acids. Nature. 1975;258:171-173. doi:10.1038/258171a0
25. Trappe TA, Liu SZ. Effects of prostaglandins and COX-inhibiting drugs on skeletal muscle adaptations to exercise. J Appl Physiol. 2013;115(6):909-19. doi:10.1152/japplphysiol.00061.2013
26. Crawford MA and Sinclair, AJ. Nutritional influences in the evolution of mammalian brain. In: Lipids, malnutrition & the developing brain. Ciba Found Symp.1971:267-292
27. Rapoport SI. Arachidonic acid and the brain. J Nutr. 2008;138(12):2515-20.
28. Ormes Jacob. Effects of arachidonic acid supplementation on skeletal muscle mass, strength, and power. NSCA ePoster Gallery. National Strength and Conditioning Association. 2014. Available at: https://forum.bodybuilding.nl/topics/effects-of-arachidonic-acid-supplementation-on-skeletal-muscle-mass-strength-and-power.370627/
29. Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, et al. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. Circulation. 2009;119(6):902–907. doi.org/10.1161/CIRCULATIONAHA.108.191627
30. Muller-Feuga A. The role of microalgae in aquaculture: situation and trends. J Applied Phycol. 2000;12(3-5):527-534. doi.org/10.1023/A:1008106304417
31. Yu X, Zhao P, He C, Li J, Tang X, Zhou J, et al. Isolation of a novel strain of Monoraphidium sp. and characterization of its potential application as biodiesel feedstock. Bioresource Technol. 2013;121:256-262. doi.org/10.1016/j.biortech.2012.07.002
32. Hu G, Fan Y, Zhang L, Yuan C, Wang J, Li W, et al. Enhanced lipid productivity and photosynthesis efficiency in a *Desmodesmus sp.* mutant indued by heavy carbon ions. 2013;8:e60700. doi.org/10.1371/journal.pone.0060700
33. Chisti Y. Biodiesel from microalgae. Biotechnol Adv. 2007;25:294-306.

Citation: Cristiano José de Andrade, Lidiane Maria de Andrade.(2017) An overview on the application of genus *Chlorella* in biotechnological processes. J Adv Res Biotech 2(1):1-9.

doi.org/ 10.1016/j.biotechadv.2007.02.001

34. Um BH and Kim YS. Review: A chance for Korea to advance algal-biodiesel technology. J Ind Eng Chem. 2009;15:1-7. doi.org/10.1016/j.jiec.2008.08.002
35. Sydney EB, Sturm W, Carvalho JC, Thomaz-Soccol V, Larroche C, Pandey A, Soccol CR. Potential carbon dioxide fixation by industrially important microalgae. Bioresource Technol. 2010;101: 5892-5896. doi.org/10.1016/j.biortech.2010.02.088
36. Begum H, Yusoff FM, Banerjee S, Khatoon H, Shariff M. Availability and utilization of pigments from microalgae. Crit Rev Food Sci Nutr. 2016;56(13):2209-2222. doi.org/ 10.1080/10408398.2013.764841
37. Gong M, Bassi A. Carotenoids from microalgae: A review of recent developments. Biotechnol Adv. 2016;34(8):1396-1412. doi.org/10.1016/j.biotechadv.2016.10.005
38. Bewicke D, Potter B. Chlorella: The emerald food. Ronin Publishing: Berckley- CA,2009.
39. Viuda-Martos M, Sanchez-Zapata E, Sayas-Barberá E, Sendra E, Pérez-Álvarez JA, Fernández-López J. Tomato and tomato byproducts. Human health benefits of lycopene and its application to meat products: a review. Crit Rev Food Sci Nutr. 2014; 54(8): 1032–1049. doi.org/10.1080/10408398.2011.623799
40. Virtamo J, Taylor PR, Kontto J, Männistö S, Utriainen M, Weinstein SJ, Huttunen J, Albanes D. Effects of α-tocopherol and β-carotene supplementation on cancer incidence and mortality: 18-year postintervention follow-up of the alpha-tocopherol, beta-carotene cancer prevention study. Int J Cancer. 2014;135(1):178–185. doi.org/10.1002/ijc.28641
41. Manayi A, Abdollahi M, Raman T, Nabavi SF, Habtemariam S, Daglia M, et al. Lutein and cataract: from bench to bedside. Crit Rev Biotechnol. 2016;36(5):829. doi.org/10.3109/07388551.2015.1049510
42. Vijayapadma V, Ramyaa P, Pavithra D, Krishnasamy R. Protective effect of lutein against benzo(a)pyrene-induced oxidative stress in human erythrocytes. Toxicol Ind Health. 2014;30(3):284-293. doi.org/10.1177/0748233712457439
43. Zhang W, Wang J, Wang J, Liu T. Attached cultivation of *Haematococcus pluvialis* for astaxanthin production. Bioresour Technol. 2014; 158: 329-335. doi.org/10.1016/j.biortech.2014.02.044
44. Fasano E, Serini S, Mondella N, Trombino S, Celleno L, Lanza P, et al. Antioxidant and anti-inflammatory effects of selected two human immortalized keratinocyte lines. Biomed Res Int. 2014:2014; 1–11. doi.org/10.1155/2014/327452
45. Li J, Zhu D, Niu J, Shen S, Wang G. An economic assessment of astaxanthin production by large scale cultivation of *Haematococcus pluvialis*. Biotechnol Adv. 2011;29(6):568-574. doi.org/10.1016/j.biotechadv.2011.04.001
46. Park JS, Chyun JH, Kim YK, Line LL, Chew BP. Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. Nutr Metab. 2010;7:18. doi.org/10.1186/1743-7075-7-18
47. Stenblom EL, Montelius C, Östbring K, Håkansson M, Nilsson S, Rehfeld JF, Erlanson-Albertsson C. Supplementation by thylakoids to a high carbohydrate meal decreases feelings of hunger, elevates CCK levels and prevents postprandial hypoglycaemia in overweight women. Appetite. 2013;68:118-123. doi.org/10.1016/j.appet.2013.04.022
48. Jubert C, Mata J, Bench G, Dashwood R, Pereira C, Tracewell W, et al. Effects of chlorophyll and chlorophyllin on low-dose aflatoxin B(1) pharmacokinetics in human volunteers. Cancer Prev Res (Phila). 2009;2:1015-22. doi.org/10.1158/1940-6207.CAPR-09-0099
49. Shaughnessy DT, Gangarosa LM, Schliebe B, Umbach DM, Xu Z, MacIntosh B, et al. Inhibition of fried meat-induced colorectal DNA damage and altered systemic genotoxicity in humans by crucifera, chlorophyllin, and yogurt. PLoS One. 2011;6(4):e18707. doi.org/10.1371/journal.pone.0018707
50. Zhang YL, Guan L, Zhou PH, Mao LJ, Zhao ZM, Li SQ, et al. The protective effect of chlorophyllin against oxidative damage and its mechanism. Zhonghua Nei Ke Za Zhi. 2012;51(6):466-470.
51. Maekawa LE, Lamping R, Marcacci S, Maekawa MY, Nassri MRG, Koga-Ito CY. Antimicrobial activity of chlorophyll-based solution on *Candida albicans* and *Enterococcus faecalis.* Revista Sul-brasileira de Odontologia. 2007;4:36-40.
52. Miret S, Tascioglu S, van der Burg M, Frenken L, Klaffke W. In vitro bioavailability of iron from the heme analogue sodium iron chlorophyllin. J Agric Food Chem. 2010;58(2):1327-1332. doi.org/10.1021/jf903177q
53. Murwanashyaka T, Shen L, Ndayambaje JD, Wang Y, He N, Lu Y. Kinetic and transcriptional exploration of *Chlorella sorokiniana* in heterotrophic cultivation for nutrients removal from wastewaters. Algal Res. 2016. doi.org/10.1016/j.algal.2016.08.002
54. Hammouda O, Gaber A, Abdel-Raouf N. Microalgae and wastewater treatment. Ecotox Environ Safe, 1995;31:205-210. doi.org/10.1006/eesa.1995.1064
55. Kuo C-M, Jian J-F, Lin T-H, Chang Y-B, Wan X-H, Lai J-T, et al. Simultaneous microalgal biomass production and CO_2 fixation by cultivating *Chlorella* sp. GD with aquaculture wastewater and boiler flue gas. Bioresource Technol. 2016;221:241-250. doi.org/10.1016/j.biortech.2016.09.014
56. Aksu Z, Sag Y, Kutsal T. A comparative study of the adsorption of chromium(V1) ions to *C. vulgaris* and *Z. ramigera.* Environ Technol Lett. 1990;11:3340. doi.org/10.1080/09593339009384836
57. Shah M. Microbial Community Structure of Activated Sludge As Investigated With DGGE. J Adv Res Biotech. 2016;1(1): 7

Citation: Cristiano José de Andrade, Lidiane Maria de Andrade.(2017) An overview on the application of genus *Chlorella* in biotechnological processes. J Adv Res Biotech 2(1):1-9.